

IMIDAZO[4,5-d]PYRIDAZINE COMPOUNDS AND CONJUGATES
THEREOF, THEIR PREPARATION, AND THEIR THERAPEUTIC APPLICATIONS

5 **FIELD OF THE INVENTION**

The present disclosure relates to new imidazo[4,5-d]pyridazine compounds (also named payloads), to new imidazo[4,5-d]pyridazine conjugates, to pharmaceutical compositions comprising these conjugates and to their therapeutic uses, for instance as TLR7
10 and/or 8 agonists. The present disclosure also relates to processes for preparing these payloads and conjugates.

BACKGROUND OF THE INVENTION

Over the last decade, immunotherapy, mainly immune checkpoint inhibitor (ICI)
15 has led to impressive clinical responses in patients. However, the percentage of ICI-responding patients remains low. It is noteworthy that ICI efficacy correlates with an “inflamed” tumor microenvironment (TME) and patients with “non-inflamed” tumors tend to respond poorly to ICI.

Therapeutic activation of TLR (Toll-like Receptor) such as TLR7 and/or 8 shows
20 potential for immunotherapy, by converting non-inflamed or poorly inflamed tumors into immunologically inflamed tumors and (re)initiating T-cell mediated anti-tumor immune response. The innate immune system contains several families of germline-encoded pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). These receptors recognize danger signals from either released intra-cellular components termed damage-associated molecular pattern (DAMP) or microbial components termed pathogen-associated
25 molecular patterns (PAMPs). The PAMPs are highly conserved molecular structures on a wide range of pathogens such as viruses, fungi, bacteria, and parasites. TLR7 and TLR8 are both located within endo-lysosomes and play an important role in the immune response during viral infection by their ability to recognize single stranded RNA PAMPs, as well as
30 synthetic small molecules. Their stimulation leads to intracellular signaling and downstream activation of genes coding, among others for co-stimulatory molecules, pro-inflammatory cytokines and type I interferons. The activation of TLR such as TLR7 and/or TLR8, by an

agonist can induce secretion of type I interferons such as IFN α and IFN β , tumor necrosis factor (TNF α) and interleukins such as IL6, IL12, which are important actors in the initiation of innate and adaptative immunity. For example, the activation of TLR7 may preferentially stimulate immune stimulating cytokines release such as IFN α and IP10 whereas the activation of TLR8 may preferentially stimulate pro-inflammatory cytokines release such as IL6 and TNF α . The secretion of these cytokines, associated with the expression of co-stimulatory molecules, are known to induce the maturation of dendritic cells, monocytes and macrophages, facilitating the presentation of antigen and the stimulation of the adaptive immune response.

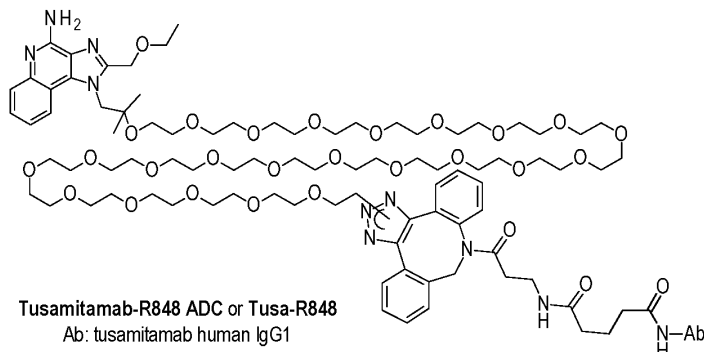
Various small molecules agonists of TLR7 (commonly named TLR7 agonists) and/or agonists of TLR8 (commonly named TLR8 agonists) have already been described as potent antitumoral compounds. For example, imidazoquinoline compounds such as Imiquimod (R-837, a TLR7 agonist) and Resiquimod (R-848, also named R848, a TLR7 and 8 agonist, as named TLR7/8 agonist) are developed for local administrations (topical and/or intratumoral) due to tolerability issues in systemic administration. Current intra-tumoral delivery approaches demonstrate poor tumor retention, limiting anti-tumor benefit and promoting treatment-related adverse events. For example, the Seven and Eight BDB-001, an intravenously administered TLR7/8 agonist in combination with Pembrolizumab in advanced solid tumors, shows activities but also treatment-related adverse events in around 78% of the enrolled patients under monotherapy (Journal of Clinical Oncology 2021 39:15_suppl, 2512-2512).

TLR7 and/or 8 agonists conjugated to antibody for intravenous tumor-targeted delivery have recently been described. Some conjugates have been under development and discontinued mainly due to not favorable therapeutical index or toxicity.

WO2018/009916 discloses imidazoquinoline or imidazopyrimidine compounds as TLR7 and/or 8 agonists for immunoconjugates. BDC-1001, an imidazoquinoline TLR7/8 agonist conjugated to an anti-Her2 antibody with a non-cleavable linker showed a safe profile with biomarkers consistent with the mechanism of action in phase 1 but has been discontinued in phase 2 due to low efficacy.

WO2023/057564 discloses imidazoquinoline R848 conjugates with non-cleavable linkers. All disclosed ADCs (antibody-drug conjugates), especially a R848 agonist conjugated to an anti-CEACAM5 antibody with a PEG24 linker (Tusamitamab-R848 ADC,

also named in the present disclosure Tusa-R848), have been discontinued in pre-clinical stage due to a narrow therapeutic index. The formula of Tusa-R848 is as follows:



5 WO2017/072662 discloses pyrrolo-pyrimidine compounds as TLR7 agonists for immunoconjugates. NJH395 (a TLR7 agonist conjugated to an anti-HER2 antibody) has been discontinued in Phase 1 for toxicity reasons (low grade CRS, meningitis/gism, hepatotoxicity).

10 SBT-6050 (a TLR8 agonist conjugated to an anti-HER2 antibody) and SBT-6290 (a TLR8 agonist conjugated to an anti-Nectin4 antibody) have been also stopped in phase 1 for toxicity (low grade CRS, hypotension).

Therefore, there remains a need for new immunotherapies based on TLR7 and/or 8 agonists with less adverse - events and/or better therapeutic index for the treatment of diseases, in particular cancer.

15 The purpose of the disclosure is to provide new immune-stimulating compounds suitable for systemic delivery, well tolerated and presenting less adverse-effects.

The present disclosure describes new TLR7 and/or 8 payloads compounds suitable for conjugation with an antibody and immunostimulant-conjugates thereof.

20 The present disclosure describes also immunostimulant-conjugate with a particular cytokine profile.

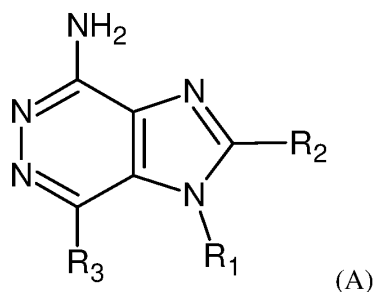
SUMMARY OF THE INVENTION

25 The present disclosure relates to a compound (also named payload or compound/payload in the present text) of formula (I) or a pharmaceutically acceptable salt thereof:

A-L1-RCG1 (I)

wherein:

- **A** is a drug unit of formula (A)



5 wherein:

R₁ represents:

- a hydrogen atom, or
- a group selected from:

10 a)

- a (C₁-C₆)alkyl- group;
- a hydroxy-(C₁-C₆)alkyl- group;
- a NH₂-(C₁-C₆)alkyl- group;
- a NH-(C₁-C₆)alkyl-(C₁-C₆)alkyl- group;

15 - a N((C₁-C₆)alkyl)₂-(C₁-C₆)alkyl- group;

- a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group;

b)

20 - a phenyl(C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from:

- b1) - a (C₁-C₆)alkoxy- group;
- b2) - a hydroxyl group;
- b3) a -C(O)-H group; and

25 b4) - a (C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from:

- b4.1) - a hydroxyl group; and

b4.2.) - a $\text{-NR}_4\text{R}_5$ group wherein R_4 and R_5 , being independently from each other selected from:

b4.2.1) - a hydrogen atom;

b4.2.2) - a $(\text{C}_1\text{-C}_{16})$ alkyl- group;

5 b4.2.3) - a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ group with n being an integer from 1 to 30, a $(\text{C}_1\text{-C}_6)$ alkoxy $(\text{C}_1\text{-C}_6)$ alkyl- group, or a $(\text{C}_1\text{-C}_6)$ alkoxy $(\text{C}_1\text{-C}_6)$ alkoxy $(\text{C}_1\text{-C}_6)$ alkyl- group;

b4.2.4) - a $(\text{C}_1\text{-C}_6)$ alkyl- $\text{S(O)}_2\text{-}$ group;

b4.2.5) - a $(\text{C}_1\text{-C}_6)$ alkyl- NH-C(O)- group;

b4.2.6) - a $(\text{C}_1\text{-C}_{16})$ alkyl- C(O)- group;

10 b4.2.7) - a $(\text{C}_1\text{-C}_{16})$ alkyl- O-C(O)- group;

b4.2.8) - a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-C(O)-}$ group with n being an integer from 1 to 30;

b4.2.9) - a $(\text{C}_3\text{-C}_{10})$ cycloalkyl- group being unsubstituted or substituted by at least one substituent selected from:

- a hydroxyl group; and

15 - a $(\text{C}_1\text{-C}_6)$ alkyl- group; or

b4.2.10) - a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising from one to four heteroatoms selected from oxygen, nitrogen, sulfur, -S(O)- and $\text{-SO}_2\text{-}$;

b4.2.11) - a phenyl- C(O)- group;

b4.2.12) - a $(\text{C}_1\text{-C}_6)$ alkoxy-phenyl- $(\text{C}_1\text{-C}_6)$ alkyl- O-C(O)- group;

20 b4.2.13) - a $(\text{C}_1\text{-C}_{16})$ alkyl- C(O)-NH-phenyl- $(\text{C}_1\text{-C}_6)$ alkyl- O-C(O)- group;

b4.2.14) - a $(\text{C}_1\text{-C}_{16})$ alkyl- O-C(O)- $(\text{C}_1\text{-C}_6)$ alkyl- group;

or R_4 and R_5 form together with the nitrogen atom to which they are attached a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, said $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group being
25 unsubstituted or substituted by at least one substituent selected from a $(\text{C}_1\text{-C}_6)$ alkyl- group, and a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ group with n being an integer from 1 to 30;

c) - a $(\text{C}_3\text{-C}_{10})$ cycloalkyl $(\text{C}_1\text{-C}_6)$ alkyl- group being unsubstituted or substituted by at least one substituent selected from -NH_2 and a $\text{NH}_2\text{-(C}_1\text{-C}_6)$ alkyl- group;

30

d) - a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl $(\text{C}_1\text{-C}_6)$ alkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group and a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30; and

- 5 e) - a (C₅-C₁₀)membered heteroaryl(C₁-C₆)alkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

said heteroaryl being unsubstituted or substituted by at least one substituent selected from:

- a (C₁-C₆)alkyl- group;
- a NH₂-(C₁-C₆)alkyl- group and
- 10 - a cyano group;

f) - a (C₃-C₁₀)membered heterocycloalkyl-NH-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;

15

g) - a (C₃-C₁₀)membered heterocycloalkyl-N(C(O)-(C₁-C₆)alkyl)-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;

- 20 **R₂** represents a halogen atom,

or a group selected from:

- a (C₁-C₆)alkyl- group;
- a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group;
- 25 - a (C₁-C₆)alkylthio- group;
- a (C₁-C₆)alkylthio(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-S(O)- group;
- a (C₁-C₆)alkyl-S(O₂)- group;
- a (C₁-C₆)alkyl-S(O)-(C₁-C₆)alkyl- group;
- 30 - a (C₁-C₆)alkyl-S(O₂)-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkoxy- group;
- a (C₁-C₆)alkoxy(C₁-C₆)alkyl- group;

- a (C₁-C₆)haloalkoxy(C₁-C₆)alkyl- group;
- a (C₃-C₅)cycloalkyl-O-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-NH-(C₁-C₆)alkyl- group;
- a ((C₁-C₆)alkyl)₂-N-(C₁-C₆)alkyl- group;
- 5 - a (C₁-C₆)alkyl-NH- group; and
- a ((C₁-C₆)alkyl)₂N- group;

R₃ represents:

- a deuterium atom;
 - 10 - a hydrogen atom
- or a group selected from:

a)

- a (C₁-C₆)alkyl- group;
- 15 - a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group; and
- a (C₁-C₆)alkylthio- group;

b)

- 20 - a -OR₆ group wherein R₆ is selected from:
 - a hydrogen atom;
 - a (C₁-C₆)alkyl- group;
 - a CH₃-[O-(CH₂)_n]- group with n being an integer from 1 to 30;
 - a (C₂-C₆)alkenyl- group;
 - 25 - a (C₂-C₆)alkynyl- group;
 - a (C₃-C₁₀)cycloalkyl- group;
 - a phenyl group;
 - a phenyl(C₁-C₆)alkyl- group; and
 - a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms
 - 30 selected from oxygen, nitrogen, sulfur, -S(=O)- and -S(=O)₂-;

c)

- a $\text{-NR}_7\text{R}_8$ group wherein R_7 and R_8 being, independently from each other, selected from:

- a hydrogen atom;
- a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ with n being an integer from 1 to 30;
- a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group unsubstituted or substituted by

5 - a $(\text{C}_5\text{-C}_{10})$ membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur; or

 - a phenyl group being unsubstituted or substituted by at least one substituent selected from:

 - a cyano group and

10 - a $\text{NR}_9\text{R}_{10}\text{-(C}_1\text{-C}_6)\text{alkyl-}$ group wherein:

R_9 and R_{10} being, independently from each other, selected from:

- a hydrogen atom;
- a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group;
- a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ with n being an integer from 1 to 30, or

15 R_9 and R_{10} together form with the nitrogen atom to which they are attached a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,
said $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group, and a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ group with n
20 being an integer from 1 to 30;

or R_7 and R_8 form together with the nitrogen atom to which they are attached a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

25 said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from:

- a phenyl group and
- a hydroxy $(\text{C}_1\text{-C}_6)\text{alkyl-phenyl-}$ group;

30 d)

 - a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur;

e)

- a (C₅-C₁₀)membered heteroaryl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

5 said (C₅-C₁₀)membered heteroaryl- group being unsubstituted or substituted by at least one (C₁-C₆)alkyl- group;

f)

- a (C₆-C₁₀)membered aryl- group; and

10

g)

- a (C₃-C₁₀)cycloalkyl- group;

- **L1** represents a linker; and

15 - **RCG1** represents a reactive chemical group that is reactive towards a chemical group present on a binding agent, for instance on a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance on a targeting agent for instance on a cell-binding agent such as an antibody such as a monoclonal antibody or a VHH antibody.

20 The present disclosure further relates to a compound (also named conjugate or compound/conjugate in the present text) of formula (II) or a pharmaceutically acceptable salt thereof:

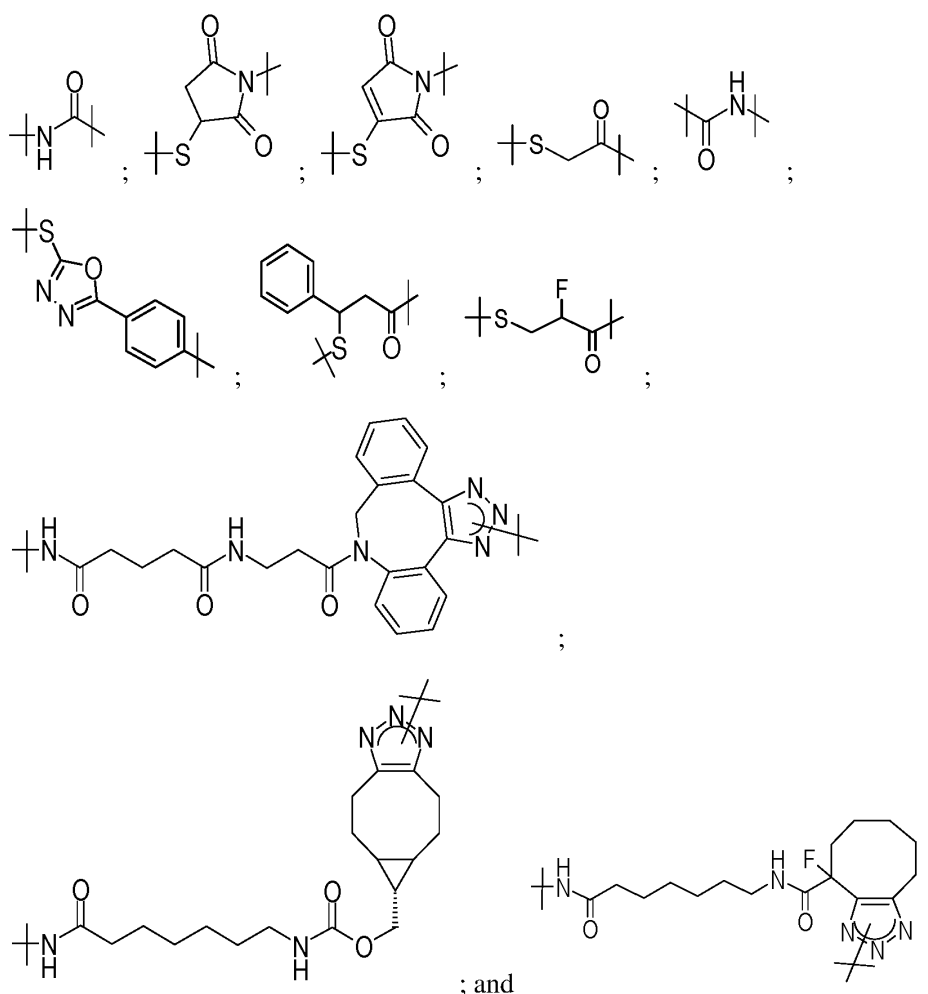
A-L1-G-BA (II)

25 wherein:

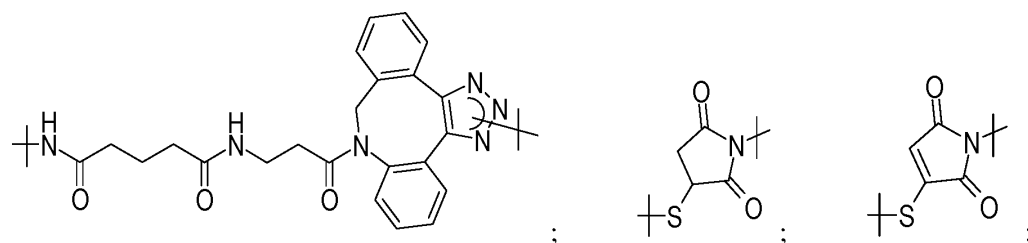
A, and **L1** are as defined in the present disclosure;

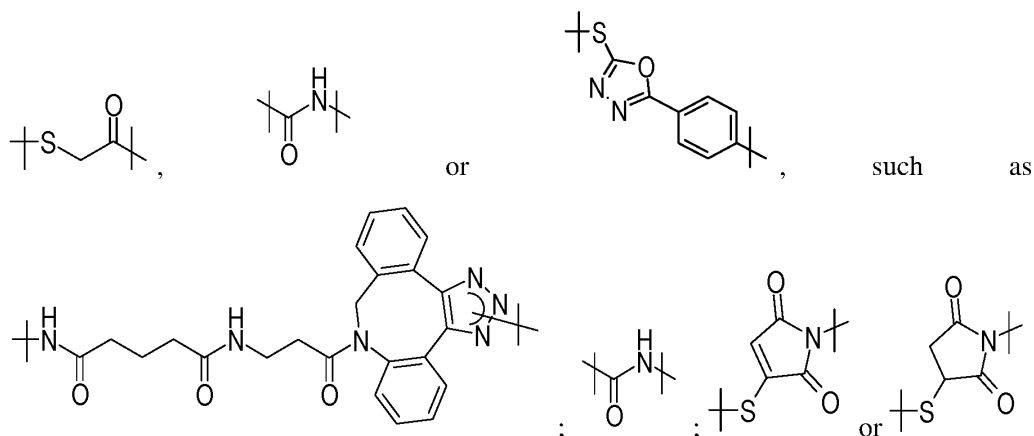
BA represents a binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance **BA** is a targeting agent for instance a cell-binding agent such as an antibody, such as a monoclonal antibody or a VHH antibody;

30 **G** represents the product of reaction between RCG1 as defined in the present disclosure and RCG2, a reactive group present on the binding agent, for instance G is selected from the group consisting of:



- 5 for instance, the left side of the G groups being linked to the binding agent, for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance a targeting agent for instance a cell-binding agent such as an antibody (Ab) such as a monoclonal antibody or a VHH antibody, for instance an antibody (Ab), and the right side of the G groups being linked to L1, for instance G represents the following groups:





The compounds of formulae (I) and (II) may be present as well under tautomer forms. Indeed, it is to be understood that the present disclosure encompasses all isomers of formulae (I) and (II) and their pharmaceutically acceptable derivatives, including all geometric, tautomeric and mixtures thereof.

In the context of the present disclosure, the compounds of formula (I) include the compounds of formula (II) and (I2) as defined in the present disclosure.

In the context of the present disclosure, the compounds of formula (II) include the compounds of formula (II1) and (II2) as defined in the present disclosure.

The compound/payload of formula (I) may exist in the form of bases or addition salts with acids or bases, in particular pharmaceutically acceptable salts.

Pharmaceutically acceptable salts of the compound/payload of formula (I) do form part of the disclosure.

The compound/payload of formula (I) may exist in the form of bases, acids, zwitterion or of addition salts with acids or bases. Such addition salts, bases, acids and zwitterion form part of the disclosure. Hence, the disclosure relates, inter alia, to the compound/payload of formula (I), or to pharmaceutically acceptable salts thereof.

These salts may be prepared with pharmaceutically acceptable acids or bases, although the salts of other acids or bases useful, for example, for purifying or isolating the compound/payload of formula (I), also form part of the disclosure.

The present disclosure also relates to processes for the preparation of the compounds/conjugates of formula (II) in accordance with the present disclosure.

Thus, according to one specific embodiment, the disclosure relates to a process for preparing a compound/conjugate of formula (II) as defined in the present disclosure comprising at least the steps of:

(i) placing in contact and leaving to react:

- 5 - an, optionally buffered, aqueous solution of a binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance a targeting agent for instance a cell-binding agent such as an antibody (Ab) such as a monoclonal antibody or a VHH antibody, comprising a reactive RCG2 group, optionally modified by means of a modifying agent,

10 and

- a solution of a compound/payload of formula (I) as defined in the present disclosure, comprising a reactive RCG1 group,

RCG1 groups of compound/payload of formula (I) being reactive towards the RCG2 groups of the binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance a targeting agent for instance a cell-binding agent such as an antibody (Ab) such as a monoclonal antibody or a VHH antibody to form the G group by a covalent bond and forming the compound/conjugate of formula (II);

(ii) and then optionally separating the compound/conjugate of formula (II) formed in step (i) from the unreacted compound/payload of formula (I) and/or from the unreacted binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance a targeting agent for instance a cell-binding agent such as an antibody (Ab) such as a monoclonal antibody or a VHH antibody and/or from any aggregates that may have been formed.

25 Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use as a medicine.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use in therapy, especially as a TLR7 and/or 8 agonists.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use to induce high amount of immune stimulating cytokines with low or no pro-inflammatory cytokines, for use to stimulate the cytokine induction of IFN α and IP10 with low or no stimulation of IL6 and TNF α , in particular wherein IFN α / TNF α ratio is higher than 1.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use in the prevention and/or treatment of a disease or a disorder that may benefit of an activation of the immune system for instance for use in the prevention and/or treatment of a cell-proliferative disease, a cancer, a chronic myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer (for example an external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic keratosis, a basal cell carcinoma, a cutaneous T-cell lymphoma), an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy (for example an allergic rhinitis or a respiratory allergy), an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, for instance in the prevention and/or in the treatment of cancers.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use in the prevention and/or treatment of a cancer.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use as anticancer agents.

Another subject matter of the instant disclosure is a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, for use in an anti-tumoral vaccine.

Another subject matter of the instant disclosure is a method of preventing and/or treating a disease or a disorder that may benefit of an activation of the immune system, for instance of preventing and/or treating a cell-proliferative disease, a cancer, a chronic

myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer (for example an external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic keratosis, a basal cell carcinoma, a cutaneous T-cell lymphoma), an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy (for example an allergic rhinitis or a respiratory allergy), an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, which comprises administering to a subject in need thereof, for instance a human, a therapeutically effective amount of a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof.

10 The present disclosure also relates, in another aspect, to a method of preventing a disease or a disorder that may benefit of an activation of the immune system, for instance of preventing a cell-proliferative disease, a cancer, a chronic myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer (for example an external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic
15 keratosis, a basal cell carcinoma, a cutaneous T-cell lymphoma), an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy (for example an allergic rhinitis or a respiratory allergy), an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, in a patient in need thereof, for instance a human, which comprises immunizing said patient with a vaccine comprising a compound/conjugate of formula (II) in
20 accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof.

 The present disclosure also relates, in another aspect, to an anti-tumoral vaccine.

 The present disclosure further relates to the use of a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below
25 definitions/lists, or a pharmaceutically acceptable salt thereof, for the manufacture of an antitumoral vaccine and/or of a medicament for preventing and/or treating a disease or a disorder that may benefit of an activation of the immune system, for instance for preventing and/or treating a cell-proliferative disease, a cancer, a chronic myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer (for example an
30 external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic keratosis, a basal cell carcinoma, a cutaneous T-cell lymphoma), an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy (for example an allergic

rhinitis or a respiratory allergy), an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, for instance a cancer.

Another subject matter of the instant disclosure is a medicament comprising as active principle an effective dose of a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof.

Another subject matter of the instant disclosure is a medicament comprising a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof.

Another subject matter of the instant disclosure is a pharmaceutical composition comprising as active principle an effective dose of a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

Another subject matter of the instant disclosure is a pharmaceutical composition comprising a compound/conjugate of formula (II) in accordance with the disclosure selected from the above and below definitions/lists, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

Definitions

In the context of the present disclosure, the terms below have the following definitions unless otherwise mentioned throughout the instant specification:

- a “**halogen atom**”: a fluorine, a chlorine, a bromine or an iodine atom, and for example a fluorine and a chlorine atom;
- a “**hydroxyl group**”: a “-OH” group;
- an “**oxo group**”: a “=O” group;
- a **nitro** group: a “-NO₂” group;
- a “**cyano group**”: a “-C≡N” group;
- a “**(C_x-C_y)alkyl**” group: a linear or branched saturated hydrocarbon-based aliphatic group comprising from x to y carbon atoms, for example from 1 to 6 carbon atoms, or from 1 to 16 carbon atoms. By way of examples, mention may be made of, but not limited to: methyl,

ethyl, propyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, *tert*-butyl, pentyl, isopentyl, hexyl, isohexyl, octyl, nonyl, decyl groups, and the like;

- a “(C_x-C_y)alkenyl” group: a linear or branched hydrocarbon-based aliphatic group comprising at least one unsaturation (double bond) and comprising, from x to y carbon atoms (x being an integer of at least 2), for example from 2 to 6 carbon atoms. By way of examples, mention may be made of, but not limited to: ethenyl, propenyl, butenyl, pentenyl, hexenyl groups, and the like;

- a “(C_x-C_y)alkynyl” group: a linear or branched hydrocarbon-based aliphatic group comprising at least one triple bond and comprising from x to y carbon atoms (x being an integer of at least 2) for example from 2 to 6 carbon atoms. By way of examples, mention may be made of, but not limited to: ethynyl, propynyl, butynyl, pentynyl, hexynyl groups, and the like;

- a “(C_x-C_y)alkoxy” group: an -O-alkyl group where the alkyl group is as previously defined. For example, a (C₁-C₆)alkoxy group. By way of examples, mention may be made of, (but not limited to: methoxy, ethoxy, propoxy, isopropoxy, linear, secondary or tertiary butoxy, isobutoxy, pentoxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, decyloxy groups, and the like;

- a “(C_x-C_y)haloalkoxy” group: an -O-alkyl group where the alkyl group is as previously defined and is further substituted by at least one halogen atom as previously defined. For example, a (C₁-C₆)haloalkoxy group. By way of examples, mention may be made of but not limited to: chloromethoxy, fluoromethoxy, dichloromethoxy, 2-fluoropropoxy groups, and the like;

- a “(C_x-C_y)alkylthio” group: an -S-alkyl group where the alkyl group is as previously defined. For example, a (C₁-C₆)alkylthio group. By way of examples, mention may be made of but not limited to: methylthio, ethylthio, propylthio, isopropylthio, linear, secondary or tertiary butylthio, isobutylthio, pentylthio, hexylthio, heptylthio, octylthio, nonylthio, decylthio groups, and the like;

- a “(C₃-C₁₀)cycloalkyl” group or a “(C₃-C₅)cycloalkyl” group: a cyclic alkyl group comprising, unless otherwise mentioned, from 3 to 10 carbon atoms (noted “(C₃-C₁₀)cycloalkyl group”) or from 3 to 5 carbon atoms (noted “(C₃-C₅)cycloalkyl group”), saturated or partially unsaturated and unsubstituted or substituted. By way of examples, mention may be made of, but not limited to: cyclopropyl, cyclobutyl, cyclopentyl,

cyclobutenyl, cyclopentenyl, cyclohexenyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl groups and the like;

- a “**(C₃-C₁₀)heterocycloalkyl**” group: a monocyclic alkyl group comprising, unless otherwise mentioned, from 3 to 10 carbon atoms (noted “a (C₃-C₁₀) membered heterocycloalkyl group”) and comprising 1 to 4 heteroatoms selected from oxygen, nitrogen, sulfur, -S(O)-, and -SO₂- (in other terms, one heteroatom replaces one carbon atom). Such heterocycloalkyl group may be saturated or partially saturated and unsubstituted or substituted. By way of examples of heterocycloalkyl groups, mention may be made of, but not limited to: piperazine, morpholino, pyrrolidine, tetrahydropyran, thietane dioxide, piperidine, thiolane, thiolane oxide, thiolane dioxide, dihydrofuran, tetrahydrofuran, azetidine, oxetane, thietane, 2H-pyrrole, 1H-, 2H- or 3H-pyrroline, tetrahydrothiophene, oxadiazole and for example 1,3,4-oxadiazole or 1,3,5-oxadiazole, thiadiazole and for example 1,3,4-thiadiazole, isoxazoline, 2- or 3-pyrazoline, pyrroline, pyrazolidine, imidazoline, imidazolidine, thiazolidine, isooxazoline, isoxazolidine, dioxalane, oxathiazole, oxathiadiazole, dioxazole groups and the like;

- a “**(C₅-C₁₀)heteroaryl**” group means: a cyclic aromatic group comprising from 5 to 10 carbon atoms and comprising from 1 and 4 heteroatoms selected from nitrogen, oxygen and sulfur (noted “a (C₅-C₁₀) membered heteroaryl group”) (in other terms, one heteroatom replaces one carbon atom). Such heteroaryl group may be unsubstituted or substituted. By way of examples of 5 to 10-membered heteroaryl groups, mention may be made of, but not limited to: pyridine, furan, pyrrole, thiophene, pyrazole, oxazole, isoxazole, triazole, tetrazole, oxadiazole, furazan, thiazole, isothiazole, thiadiazole, imidazole, pyrimidine, pyridazine, triazine groups and the like;

- a “**(C₆-C₁₀)aryl**” group: a cyclic aromatic group comprising from 6 to 10 carbon atoms (noted “a (C₆-C₁₀) membered aryl group”). Such aryl group may be unsubstituted or substituted. By way of examples of 6 to 10-membered aryl groups, mention may be made of, but not limited to: phenyl, naphthyl groups, and the like;

- a “**deuterium** atom” (D or ²H) is a stable, non-radioactive isotope of hydrogen and has an atomic weight of 2.0144;

- a “**hydroxyl protecting group**” means: Ethers, Silyl Ethers, Esters, carbonates, carbamates etc, such as Acetyl (Ac), Benzoyl (Bz), Benzyl (Bn), β-methoxyethoxymethyl ether (MEM), dimethoxytrityl, [bis-(4-methoxyphenyl)phenylmethyl] (DMT), methoxymethyl ether

- (MOM), methoxytrityl [(4-methoxyphenyl)diphenylmethyl] (MMT), *p*-methoxybenzyl ether (PMB), *p*-methoxyphenyl ether (PMP), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), tetrahydrofuran (THF), trityl (triphenylmethyl, Tr), silyl ether (for instance trimethylsilyl (TMS), *tert*-butyldimethylsilyl (TBDMS), tri-isopropylsilyloxymethyl (TOM), and triisopropylsilyl (TIPS) ethers), methyl ethers, ethoxyethyl ethers (EE), for instance acetyl (Ac), benzyl (Bn), β -methoxyethoxymethyl ether (MEM), dimethoxytrityl, [bis-(4-methoxyphenyl)phenylmethyl] (DMT), *p*-methoxybenzyl ether (PMB), tetrahydropyranyl (THP), trityl (triphenylmethyl, Tr), trimethylsilyl (TMS), and *tert*-butyldimethylsilyl (TBDMS). (see the manual Greene's protective groups in organic synthesis», P.G.M. WUTS and T.W. GREENE, fourth edition, 1807 Wiley 207, Wiley Interscience);
- an **“amino protecting group”** means: carbamates, amides, alkyls, enamines, imides, imines, etc, such as carbobenzyloxy (Cbz) group, *p*-methoxybenzyl carbonyl (Moz or MeOZ) group, *tert*-butyloxycarbonyl (BOC) group, 9-fluorenylmethyloxycarbonyl (Fmoc) group, acetyl (Ac) group, benzoyl (Bz) group, benzyl (Bn) group, carbamate group, *p*-methoxyphenyl (PMP) group, tosyl (Ts) group, *p*-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), Troc (trichloroethyl chloroformate) group, other sulfonamides, for instance carbobenzyloxy (Cbz) group, *tert*-butyloxycarbonyl (BOC) group, 9-fluorenylmethyloxycarbonyl (Fmoc) group, acetyl (Ac) group, benzoyl (Bz) group, *p*-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), Troc (trichloroethyl chloroformate) group. (see the manual Greene's protective groups in organic synthesis», P.G.M. WUTS and T.W. GREENE, fourth edition, 1807 Wiley 207, Wiley Interscience);
- a **“carboxylic acid protecting group”** means : Esters, silyl esters, amides, hydrazides, etc such as methyl esters, benzyl esters, *tert*-butyl esters, allyl esters, esters of 2,6-disubstituted phenols (e.g. 2,6-dimethylphenol, 2,6-diisopropylphenol, 2,6-di-*tert*-butylphenol), silyl esters, orthoesters, oxazoline, for instance, methyl esters and benzyl esters. (see the manual Greene's protective groups in organic synthesis», P.G.M. WUTS and T.W. GREENE, fourth edition, 1807 Wiley 207, Wiley Interscience);
- an **“aldehyde protecting group”** or a ketone protecting group (also named carbonyl protecting groups) means: acetals and ketals, dithio acetals and ketals, substituted hydrazones, oximes, etc, such as acetals and ketals, acylals and dithianes. (see the manual

Greene's protective groups in organic synthesis», P.G.M. WUTS and T.W. GREENE, fourth edition, 1807 Wiley 207, Wiley Interscience);

- a **“leaving group”** means: an atom or group of atoms that detaches from the main or residual part of a substrate during a reaction or elementary step of a reaction;

5 - a **“zwitterion”** means: a globally neutral molecule with a positive and a negative electrical charge and having an acid group and a basic group;

- **“room temperature”** (also named rt) in the present disclosure means a temperature ranging from 18°C to 30°C, for example from 18°C to 25°C;

- A **“self-immolative linker”** or **“self-immolative spacer”** is a covalent assembly tailored
10 to correlate the cleavage of two chemical bonds after activation of a protective part in a precursor (A.G. Gavriel *et al.*, *Polym. Chem.* **2022**, *13*, 3188; A. Alouane *et al.*, *Angew. Chem. Int. Ed.* **2015**, *54*, 7492 – 7509). For instance among the self-immolative linkers suitable for the present disclosure may be cited a *-C(O)-O-(CR₁₄R₁₅)-aryl-NR₁₆-** group, *-C(O)-O-(CR₁₄R₁₅)-heteroaryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-**, a *-
15 C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-aryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, a *-CR₁₅R₁₄-aryl-NR₁₆-** group or a *-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, aryl being a (C₆-C₁₀) membered aryl group and heteroaryl being a (C₅-C₁₀) membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, for instance a *-C(O)-O-CH₂-
20 phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)_w radical; R₁₄, R₁₅, R₁₆, R₁₇ and R₁₈ being a hydrogen atom or a (C₁-C₆)alkyl group;
- **“PEG”**: polyethylene glycol, PEG_n means, a -(CH₂-CH₂-O)_n portion wherein n represents an integer from 1 to 30, for instance from 2 to 29, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,
25 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28 and 29;

- **“TLR”**: the terms "Toll-like receptor" and "TLR" refer to any member of a family of highly conserved mammalian proteins which recognize pathogen-associated molecular patterns and act as key signaling elements in innate immunity. TLR polypeptides share a characteristic structure that includes an extracellular domain that has leucine-rich repeats, a
30 transmembrane domain, and an intracellular domain that is involved in TLR signaling;

- **“TLR7”**: the terms "Toll-like receptor 7" and "TLR7" refer to nucleic acids or polypeptides sharing at least 70%; at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, or more sequence identity to a publicly available TLR7 sequence, e.g., GenBank accession number AAZ99026 for human TLR7 polypeptide, or GenBank accession number AAK62676 for murine TLR7 polypeptide;

- “**TLR8**”: the terms “Toll-like receptor 8” and “TLR8” refer to nucleic acids or polypeptides sharing at least 70%; at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more sequence identity to a publicly available TLR8 sequence, e.g., GenBank accession number AAZ95441 for human TLR8 polypeptide, or GenBank accession number AAK62677 for murine TLR8 polypeptide;

- “**TLR agonist**”: it is a substance that binds, directly or indirectly, to a TLR (e.g., TLR7 and/or TLR8) to induce TLR signaling. Any detectable difference in TLR signaling can indicate that an agonist stimulates or activates a TLR. Signaling differences can be manifested, for example, as changes in the expression of target genes, in the phosphorylation of signal transduction components, in the intracellular localization of downstream elements such as NF- κ B, in the association of certain components (such as IRAK) with other proteins or intracellular structures, or in the biochemical activity of components such as kinases (such as MAPK). TLR7 agonists more effectively induces IFN α and IFN-regulated immune-stimulating cytokines such as IP10. TLR8 agonists more effectively induce pro-inflammatory cytokines related to NF- κ B-pathway such as TNF α and IL6;

- “**Immune response**”: An “immunological response” or “immune response” to an antigen or composition, as used herein, refers to the development in a subject of a humoral and/or cellular immune response to the antigen or composition;

Immune responses include innate and adaptive immune responses. Innate immune responses are fast-acting responses that provide a first line of defense for the immune system. In contrast, adaptive immunity uses selection and clonal expansion of immune cells having somatically rearranged receptor genes (e.g., T- and B-cell receptors) that recognize antigens from a given pathogen or disorder (e.g., a tumor), thereby providing specificity and immunological memory. Innate immune responses, among their many effects, lead to a rapid burst of inflammatory cytokines and activation of antigen-presenting cells (APCs) such as macrophages and dendritic cells. To distinguish pathogens from self-components, the innate immune system uses a variety of relatively invariable receptors that detect signatures from pathogens, known as pathogen-associated molecular patterns, or PAMPs. The mechanism behind this potentiation of the immune responses has been reported to involve pattern-

recognition receptors (PRRs), which are differentially expressed on a variety of immune cells, including neutrophils, monocytes, macrophages, dendritic cells, natural killer cells, B cells and some non-immune cells such as epithelial and endothelial cells. Engagement of PRRs leads to the activation of some of these cells and their secretion of cytokines and chemokines, as well as maturation and migration of other cells. In tandem, this creates an inflammatory environment that leads to the establishment of the adaptive immune response. PRRs include nonphagocytic receptors, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins, and receptors that induce phagocytosis, such as scavenger receptors, mannose receptors and β -glucan receptors. Dendritic cells are recognized as some of the most important cell types for initiating the priming of naive CD4⁺ helper T (T_H) cells and for inducing CD8⁺ T cell differentiation into killer cells. TLR signaling has been reported to play an important role in determining the quality of these helper T cell responses, for instance, with the nature of the TLR signal determining the specific type of TH response that is observed (e.g., T_H1 versus T_H2 response). A combination of antibody (humoral) and cellular immunity are produced as part of a T_H1 -type response, whereas a T_H2-type response is predominantly an antibody response ;

- A “**humoral immune response**” refers to an immune response mediated by antibody molecules, while a “cellular immune response” refers to an immune response mediated by T- lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells (“CTLs”). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A “cellular immune response” also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4⁺ and CD8⁺ T-cells;
- An “**immune response**” associated with TLR7 and/or TLR8 is an immune response which involves activation of TLR7 and/or TLR8 receptors. Activation of TLR7 and/or TLR8 receptors may be determined in vitro using methods such as those described in the Examples;

- “**Induce**”: “Induce” and variations thereof refer to any measurable increase in cellular activity. Induction of an immune response may include, for example, an increase in the production of a cytokine, an activation, proliferation, or maturation of a population of immune cells, and/or other indicator of increased immune function;

- 5 - A “**binding agent**”: a molecule with affinity for a biological target, such as a ligand, a protein, a polypeptide, an antibody such as a monoclonal antibody or a VHH antibody, a peptide, an oligonucleotide, an oligosaccharide for instance a targeting agent for instance a cell-binding agent. The function of the binding agent is to direct the biologically active compound, such as a TLR7 and/or 8 agonist compound towards the biological target;
- 10 - A “**cell binding agent**”: Cell binding agents may be of any kind presently known, or that become known and includes peptides and non-peptides. The cell binding agent may be any compound that can bind a cell, either in a specific or non-specific manner. Generally, these can be antibodies (especially monoclonal antibodies), lymphokines, hormones, growth factors, vitamins, nutrient-transport molecules (such as transferrin), or any other cell binding
- 15 molecule or substance.

More specific examples of cell binding agents that can be used include:

- (a) polyclonal antibodies;
- (b) monoclonal antibodies;
- (c) fragments of antibodies such as Fab, Fab1, and F(ab')₂, Fv (Parham, J. Immunol. 131:2895-2902 (1983); Spring et al. J. Immunol. 113:470-478 (1974); Nisonoff et al. Arch. Biochem. Biophys. 89:230-244 (1960));
- 20 (d) interferons (e.g. alpha, beta, gamma);
- (e) lymphokines such as IL2, IL3, IL4, IL6;
- (f) hormones such as insulin, TRH (thyrotropin releasing hormone), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens;
- 25 (g) growth factors and colony-stimulating factors such as EGF, TGF α , FGF, VEGF, G-CSF, M-CSF and GM-CSF (Burgess, Immunology Today 5:155-158 (1984)); or
- (h) transferrin (O'Keefe et al. J. Biol. Chem. 260:932-937 (1985)); and (i) vitamins, such as folate;

- A “cell permeable” compound: A membrane permeability of a compound is evaluated using an in vitro model of passive, transcellular permeation called PAMPA (for Parallel artificial membrane permeability assay). This PAMPA assay allows to evaluate permeability over pH, from pH 6.5 to pH 7.4. A compound is considered as: “poorly or low permeable” with a PAMPA below 25; “moderate permeable” between 26 and 300; and “high permeable” above 300. A “non-cell permeable compound” is defined by a PAMPA below 25, as well as a differential activity in THP1 DUAL reporter cell assay between the free drug and the drug conjugated to a mAb: a non-cell permeable drug is not active at 500 nM as free drug and active at the nM range conjugated to a mAb;
- 10 - A “**targeting agent**”: a targeting agent refers to a structure that has a selective affinity for a target molecule relative to other non-target molecules. A targeting moiety binds to a target molecule. A targeting moiety may include, for example, an antibody, a peptide, a ligand, a receptor, or a binding portion thereof the target biological molecule may be a biological receptor or other structure of a cell such as a tumor antigen. A targeting moiety is often
- 15 specific for a particular cell surface antigen, so as to target an immune-stimulatory compound to a target cell or disease site ;
- An “**antibody**”: an antibody with affinity for a biological target, such as a monoclonal antibody. The function of the antibody is to direct the biologically active compound such as a TLR7 and/or 8 agonist compound towards the biological target. The antibody may be
- 20 monoclonal, polyclonal or multispecific; it may also be an antibody fragment; it may also be a murine, chimeric, humanized or human antibody. An “antibody” may be a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond (also referred to as a “full-length antibody”). The terms “conventional (or full-length) antibody” refers both to an
- 25 antibody comprising the signal peptide (or pro-peptide, if any), and to the mature form obtained upon secretion and proteolytic processing of the chain(s). There are two types of light chain, lambda (l) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains
- 30 or regions, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH)

chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from non-hypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated CDR1-L, CDR2-L, CDR3-L and CDR1-H, CDR2-H, CDR3-H, respectively. A conventional antibody antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

As used herein, the term “antibody” denotes both conventional (full-length) antibodies and fragments thereof, as well as single domain antibodies and fragments thereof, such as variable heavy chain of single domain antibodies. Fragments of (conventional) antibodies typically comprise a portion of an intact antibody, such as the antigen binding region or variable region of the intact antibody and retain the biological function of the conventional antibody. Examples of such fragments include Fv, Fab, F(ab')₂, Fab', dsFv, (dsFv)₂, scFv, sc(Fv)₂, VHH and diabodies;

By convention the numbering of the variable constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the immunoglobulin or antibody. The N-terminus of each heavy and light immunoglobulin chain is a variable region and at the C-terminus is a constant region; the C_{H3} and C_L domains actually comprise the carboxy-terminus of the heavy and light chain, respectively. Accordingly, the domains of a light chain immunoglobulin are arranged in a V_L-C_L orientation, while the domains of the heavy chain are arranged in the V_H-C_{H1}-hinge-C_{H2}-C_{H3} orientation.

Amino acid positions in a heavy chain constant region, including amino acid positions in the C_{H1}, hinge, C_{H2}, C_{H3}, and C_L domains, may be numbered according to the Kabat index

numbering system (see Kabat et al, in "Sequences of Proteins of Immunological Interest", U.S. Dept. Health and Human Services, 5th edition, 1991), the EU numbering (See Edelman, G.M. et al., Proc. Natl. Acad. USA, 63, 78-85 (1969). PMID: 5257969) or according to IMGT (See Lefranc, M.-P. et al., Dev. Comp. Immunol., 29, 185-203 (2005) PMID: 15572068). As used herein, the term " V_H domain" includes the amino terminal variable domain of an immunoglobulin heavy chain, and the term " V_L domain" includes the amino terminal variable domain of an immunoglobulin light chain.

As used herein, the term "C_H1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain that extends, *e.g.*, from about positions 114-223 in the Kabat numbering system (EU positions 118-215). The C_H1 domain is adjacent to the V_H domain and amino terminal to the hinge region of an immunoglobulin heavy chain molecule, and does not form a part of the Fc region of an immunoglobulin heavy chain.

As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the C_H1 domain to the C_H2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.* J. Immunol. 1998, 161 :4083).

As used herein, the term "C_H2 domain" includes the portion of a heavy chain immunoglobulin molecule that extends, *e.g.*, from about positions 244-360 in the Kabat numbering system (EU positions 231-340). The C_H2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two C_H2 domains of an intact native IgG molecule. In one embodiment, an antigen-binding protein of the current disclosure comprises a C_H2 domain derived from an IgG1 molecule (*e.g.* a human IgG1 molecule).

As used herein, the term "C_H3 domain" includes the portion of a heavy chain immunoglobulin molecule that extends approximately 110 residues from N-terminus of the C_H2 domain, *e.g.*, from about positions 361-476 of the Kabat numbering system (EU positions 341 -445). The C_H3 domain typically forms the C-terminal portion of the antibody. In some immunoglobulins, however, additional domains may extend from C_H3 domain to form the C-terminal portion of the molecule (*e.g.* the C_H4 domain in the μ chain of IgM and the ϵ chain of IgE). In one embodiment, an antigen-binding protein of the current disclosure

comprises a C_H3 domain derived from an IgG1 molecule (*e.g.* a human IgG1 molecule). As used herein, the term " C_L domain" includes the constant region domain of an immunoglobulin light chain that extends, *e.g.* from about Kabat position 107A-216. The C_L domain is adjacent to the V_L domain. In one embodiment, an antigen-binding protein of the
5 current disclosure comprises a C_L domain derived from a kappa light chain (*e.g.*, a human kappa light chain).

As used herein, the positions are defined according to EU numbering.

For example: A118 is at position 118 according to EU numbering, 114 according to Kabat numbering and 1 according to IMGT numbering; E152 is at position 152 according to EU
10 numbering, 150 according to Kabat numbering and 35 according to IMGT numbering; K274 is at position 274 according to EU numbering, 187 according to Kabat numbering and 44 according to IMGT; K414 is at position 414 according to EU numbering, 145 according to Kabat numbering and 74 according to IMGT numbering; and K360 is at position 360 according to EU numbering, 383 according to Kabat numbering and 20 according to IMGT
15 numbering;

- **"biological target"**: an antigen (or group of antigens) for instance located at the surface of cancer cells or stromal cells associated with this tumor; these antigens may be, for example, a growth factor receptor, an oncogene product or a mutated "tumor suppressant" gene product, an angiogenesis-related molecule or an adhesion molecule or a receptor increased
20 in tumor micro-environment;

- A **"conjugate"**: an antibody-drug conjugate or ADC, *i.e.*, a polypeptide such as an antibody (as defined above) to which is covalently attached via a linker at least one molecule of a TLR7 and/or 8 agonist. In the case of the present disclosure means compound of formulae (II), (II1) and (II2);

25 - A **"DAR"** (drug-to-antibody ratio): a number of TLR7 and/or 8 agonists attached via a linker to an antibody. DAR may be an average number or a precise number depending on the conjugation technology used. The DAR may be an average or precise DAR between 2 to 10. Preferentially, the DAR may be 2, 4 or 8;

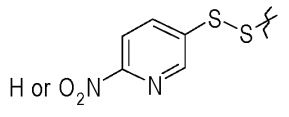
- A **"Fc receptor"**: a protein at the surface of certain immune cells such as B lymphocytes,
30 follicular dendritic cells, natural killer cells, macrophages, monocytes, neutrophils, eosinophils, basophils, human platelets, and mast cells and contributing to the protective

functions of the immune system. It may recognize the Fc (fragment crystallizable) region of an antibody;

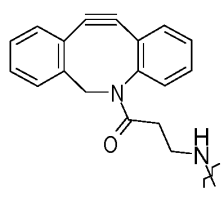
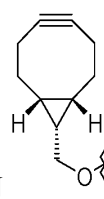
- A “**linker**”: a group of atoms or a single bond that can covalently attach a compound/payload of formula (I) to a binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance a targeting agent for instance a cell-binding agent such as an antibody, such as a monoclonal antibody or a VHH antibody in order to form a compound/conjugate of formula (II);
- A “**peptidic linker**”: a linker comprising amino acids;
- A “**PEGylated linker**”: a linker comprising PEG as defined above;
- 10 - A “**payload**”: a TLR7 and/or 8 agonist compound to which is covalently attached a linker.

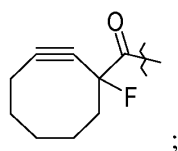
In the case of the present disclosure means compound of formulae (I), (II) and (I2).

- A “**modifying agent**”: chemical agent that is used to modify amino acid side chains of the antibody in order to introduce a reactive group;
- A “**reactive group**”: a group of atoms that can promote or undergo a chemical reaction;
- 15 - An “**activated disulfide**”: a group comprising at least one disulfide group prone to thiol-disulfide exchange as illustrated in Bioconjugate techniques, Second Edition by Greg T. Hermanson (ISBN (978-0-12-370501-3)). By way of examples of activated disulfide,

mention may be made of, but not limited to,  ;

- An “**activated C≡C**”: a group comprising at least one cycloalkyne C≡C bond as illustrated in Bioconjugate techniques, Second Edition by Greg T. Hermanson (ISBN (978-0-12-370501-3)). By way of examples of activated C≡C, mention may be made of, but not limited
- 20

to a cyclooctyne moiety, for instance a DBCO-amine  or BCN 

or MFCO  ;

- A “**cycloalkyne**”: a monocyclic or polycyclic ring which comprises at least one C≡C bond,
- 25 and from 3 to 16 carbon atoms and which can be unsubstituted or substituted;

- A “**heterocycloalkyne**”: a cycloalkyne group as defined above which comprises at least one to six heteroatoms selected from oxygen, nitrogen and sulfur.

BRIEF DESCRIPTION OF THE FIGURES

5 **Figure 1a** represents a graph which shows a comparison of the production of IFN α between three examples in accordance with the present disclosure, that is to say **Example 75**, **Example 103**, and **Example 105**; and one comparative assay (reference) named unconjugated mAb (monoclonal antibody) also called naked mAb in the present text. This graph comprises thus four columns which correspond respectively from the left to the right
10 to **Example 75**, **Example 103**, **Example 105** and unconjugated mAb. The x-axis corresponds to the concentration of ADC in nanomolar (nM) and the y-axis corresponds to the concentration of IFN α in pg/ml (see Example C below).

Figure 1b represents a graph which shows a comparison of the production of IP10 between
15 three examples in accordance with the present disclosure, that is to say **Example 75**, **Example 103**, and **Example 105**; and one comparative assay (reference) named unconjugated mAb (monoclonal antibody) also called naked mAb in the present text. This graph comprises thus four columns which correspond respectively from the left to the right
20 to **Example 75**, **Example 103**, **Example 105** and unconjugated mAb. The x-axis corresponds to the concentration of ADC in nM and the y-axis corresponds to the concentration of IP10 in pg/ml (see Example C below).

Figure 1c represents a graph which shows a comparison of the production of TNF α between
25 three examples in accordance with the present disclosure, that is to say **Example 75**, **Example 103**, and **Example 105**; and one comparative assay (reference) named unconjugated mAb (monoclonal antibody) also called naked mAb in the present text. This graph comprises thus four columns which correspond respectively from the left to the right
30 to **Example 75**, **Example 103**, **Example 105** and unconjugated mAb. The x-axis corresponds to the concentration of ADC in nM and the y-axis corresponds to the concentration of TNF α in pg/ml (see Example C below).

Figure 1d represents a graph which shows a comparison of the production of IL6 between three examples in accordance with the present disclosure, that is to say **Example 75**, **Example 103**, and **Example 105**; and one comparative assay (reference) named unconjugated mAb (monoclonal antibody) also called naked mAb in the present text. This graph comprises thus four columns which correspond respectively from the left to the right to **Example 75**, **Example 103**, **Example 105** and unconjugated mAb. The x-axis corresponds to the concentration of ADC in nM and the y-axis corresponds to the concentration of IL6 in pg/ml (see Example C below).

Figure 2 represents a graph which illustrates the evaluation of anti-tumoral activity. This graph comprises 11 (eleven) curves, namely 10 (ten) for 10 (ten) examples in accordance with the present disclosure and one for one comparative assay named Tusamitamab-R848 ADC. The x-axis corresponds to the concentration of the conjugates in nM and the y-axis corresponds to AUC (area under the curve) datas at 138 h (see Example D below). The 11 different curves are detailed below:

- The curve with the symbols  corresponds to Tusamitamab-R848 ADC (also named in the present disclosure Tusa-R848).
- The curve with the symbols  corresponds to Example 91.
- The curve with the symbols  corresponds to Example 99.
- 20 The curve with the symbols  corresponds to Example 19.
- The curve with the symbols  corresponds to Example 64.
- The curve with the symbols  corresponds to Example 15.
- The curve with the symbols  corresponds to Example 97.
- The curve with the symbols  corresponds to Example 21.
- 25 The curve with the symbols  corresponds to Example 107.
- The curve with the symbols  corresponds to Example 27.

The curve with the symbols  corresponds to Example 31.

Abbreviations

Ab: antibody; ACN, MeCN or CH₃CN: acetonitrile; AcOH: acetic acid; ADC: antibody-drug conjugate; ADIBO: azadibenzocyclooctyne; Ala: alanine; AP-1: activator protein-1; AUC: area under the curve; CDCl₃: deuterated chloroform; CEACAM5: cancer embryonic antigen cell adhesion molecule 5; CHCl₃: chloroform; Cit: citrulline, CO₂: carbon dioxide; conc.: concentrated; Cs₂CO₃: cesium carbonate; D₂O: deuterium oxide; DAR: drug-to-antibody ratio; DBCO: dibenzylcyclooctyne; DC: dendritic cell; DCC: N,N'-dicyclohexylcarbodiimide; DCM: dichloromethane; DHAA: dehydroascorbic acid; DIEA or DIPEA: N,N-diisopropylethylamine; DIC: N,N'-diisopropylcarbodiimide; DMA: dimethylacetamide; DMAP: 4-dimethylaminopyridine; DMF: N,N-dimethylformamide; DMSO: dimethylsulfoxide; DPBS: Dulbecco's phosphate-buffered saline; DSC: N,N'-disuccinimidyl carbonate; DTT: dithiothreitol; EC50: effective concentration 50; EDC.HCl: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDTA: ethylenediaminetetraacetic acid; ELSD: evaporative light scattering detector; ES: electrospray; Et₂O: diethyl ether; EtOAc: ethyl acetate; EtOH: ethanol; FBS: fetal bovine serum; Glu: glutamic acid; H₂O: water; H₂SO₄: sulfuric acid; HATU: 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate; HBTU: (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HCl: hydrochloric acid; HEK: human embryonic kidney; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HIC: hydrophobic interaction chromatography; HOAt: 1-hydroxy-7-azabenzotriazole; HOBt: hydroxybenzotriazole; HPLC: high pressure liquid chromatography; HRMS: high resolution mass spectrometry; IEC: ion exchange chromatography; IFN: interferon; i.e.: "id est", that is; IL: interleukin; IP10: interferon gamma-induced protein 10; iPr₂O: diisopropyl ether; iPrOH: isopropanol; ISG: interferon-stimulated gene; KCl: potassium chloride; K_D: dissociation constant; K₂HPO₄: dipotassium hydrogen phosphate; KH₂PO₄: potassium dihydrogen phosphate; LAR: linker-to-antibody ratio; LCMS: liquid chromatography mass spectrometry; LiOH: sodium hydroxide; mAb or MAb: monoclonal antibody; mCPBA: *meta*-chloroperoxybenzoic acid; MeOH: methanol; Me-THF or MeTHF: methyl

tetrahydrofuran; MgSO₄: magnesium sulfate; MS: mass spectrometry; MSD: Meso Scale Discovery; MTBE: methyl *tert*-butyl ether; N₂: azote; NaCl: sodium chloride; NaH: sodium hydride; NaHCO₃: sodium bicarbonate; Na₂HPO₄: disodium hydrogen phosphate; NaOH: sodium hydroxide; NaPi: sodium phosphate; Na₂SO₄: sodium sulfate; NBS: N-bromosuccinimide; nBuLi: n-butyllithium; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NH₂NH₂.H₂O: hydrazine hydrate; NH₃: ammonia; NH₄Cl: ammonium chloride; NH₄OH: ammonium hydroxide; NHS: N-hydroxysuccinimide; NMP: N-methyl-2-pyrrolidone; NMR: nuclear magnetic resonance; NT: not tested; OD: optical density; PBMC: peripheral blood mononuclear cells; PBS: phosphate buffer saline; PDL1: Programmed death-ligand 1; PEG: polyethylene glycol; PEG_n: polyethylene glycol comprising a number n (integer) of ethylene glycol units; PES: polyethersulfone; POCl₃: phosphoryl chloride; PODS: phenyloxadiazolyl methylsulfone; PS: polysorbate; PTFE: polytetrafluoroethylene; PVDF: polyvinylidene fluoride; RP: reverse phase; RPMI: Roswell Park Memorial Institute; rt or RT: room temperature; sat.: saturated; SCX: Strong Cation Exchange; SEAP: Secreted Embryonic Alkaline Phosphatase; SEC: size exclusion chromatography; SFC: supercritical fluid chromatography; SOCl₂: thionyl chloride; T: temperature; TAA: tumor-associated antigen; TCEP: tris(2-carboxyethyl)phosphine; TEA : triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TLC: thin-layer chromatography; TLR: Toll-like receptor; TMS: tetramethyl silane; t_R: retention time; UPLC: ultra-performance liquid chromatography; UV: ultraviolet; Val: valine; ~ : about.

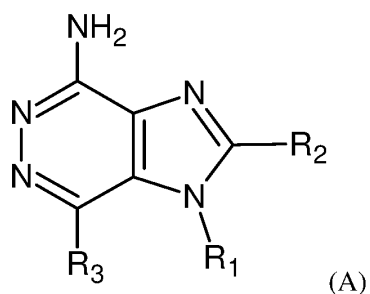
DETAILED DESCRIPTION OF THE INVENTION

The disclosure relates to a compound/payload of formula (I) or a pharmaceutically acceptable salt thereof:

25 A-L1-RCG1 (I)

wherein:

- **A** is a drug unit of formula (A)



wherein:

R₁ represents:

- 5 - a hydrogen atom, or
 - a group selected from:

a)

- a (C₁-C₆)alkyl- group;
 10 - a hydroxy-(C₁-C₆)alkyl- group;
 - a NH₂-(C₁-C₆)alkyl- group;
 - a NH-(C₁-C₆)alkyl-(C₁-C₆)alkyl- group;
 - a N((C₁-C₆)alkyl)₂-(C₁-C₆)alkyl- group;
 - a (C₂-C₆)alkenyl- group;
 15 - a (C₂-C₆)alkynyl- group;

b)

- a phenyl(C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from:

- 20 b1) - a (C₁-C₆)alkoxy- group;
 b2) - a hydroxyl group;
 b3) a -C(O)-H group; and
 b4) - a (C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from:
 25 b4.1) - a hydroxyl group; and
 b4.2) - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from:

- b4.2.1) - a hydrogen atom;
- b4.2.2) - a (C₁-C₁₆)alkyl- group;
- b4.2.3) - a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30, a (C₁-C₆)alkoxy(C₁-C₆)alkyl- group, or a (C₁-C₆)alkoxy(C₁-C₆)alkoxy(C₁-C₆)alkyl- group;
- 5 b4.2.4) - a (C₁-C₆)alkyl-S(O₂)- group;
- b4.2.5) - a (C₁-C₆)alkyl-NH-C(O)- group;
- b4.2.6) - a (C₁-C₁₆)alkyl-C(O)- group;
- b4.2.7) - a (C₁-C₁₆)alkyl-O-C(O)- group;
- b4.2.8) - a CH₃-[O-(CH₂)₂]_n-C(O)- group with n being an integer from 1 to 30;
- 10 b4.2.9) - a (C₃-C₁₀)cycloalkyl- group being unsubstituted or substituted by at least one substituent selected from:
- a hydroxyl group; and
- a (C₁-C₆)alkyl- group; or
- b4.2.10) - a (C₃-C₁₀)membered heterocycloalkyl- group comprising from one to four
- 15 heteroatoms selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-;
- b4.2.11) - a phenyl-C(O)- group;
- b4.2.12) - a (C₁-C₆)alkoxy-phenyl-(C₁-C₆)alkyl-O-C(O)- group;
- b4.2.13) - a (C₁-C₁₆)alkyl-C(O)-NH-phenyl-(C₁-C₆)alkyl-O-C(O)- group;
- b4.2.14) - a (C₁-C₁₆)alkyl-O-C(O)-(C₁-C₆)alkyl- group;
- 20 or R₄ and R₅ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, said (C₃-C₁₀)membered heterocycloalkyl- group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group, and a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30;
- 25 c) - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group;
- d) - a (C₃-C₁₀)membered heterocycloalkyl(C₁-C₆)alkyl- group comprising one to four
- 30 heteroatoms selected from oxygen, nitrogen, and sulfur,

said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group and a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30; and

- 5 e) - a (C₅-C₁₀)membered heteroaryl(C₁-C₆)alkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

said heteroaryl being unsubstituted or substituted by at least one substituent selected from:

- a (C₁-C₆)alkyl- group;
- a NH₂-(C₁-C₆)alkyl- group and
- 10 - a cyano group;

f) - a (C₃-C₁₀)membered heterocycloalkyl-NH-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;

15

g) - a (C₃-C₁₀)membered heterocycloalkyl-N(C(O)-(C₁-C₆)alkyl)-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;

- 20 **R₂** represents a halogen atom,

or a group selected from:

- a (C₁-C₆)alkyl- group;
- a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group;
- 25 - a (C₁-C₆)alkylthio- group;
- a (C₁-C₆)alkylthio(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-S(O)- group;
- a (C₁-C₆)alkyl-S(O₂)- group;
- a (C₁-C₆)alkyl-S(O)-(C₁-C₆)alkyl- group;
- 30 - a (C₁-C₆)alkyl-S(O₂)-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkoxy- group;
- a (C₁-C₆)alkoxy(C₁-C₆)alkyl- group;

- a (C₁-C₆)haloalkoxy(C₁-C₆)alkyl- group;
- a (C₃-C₅)cycloalkyl-O-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-NH-(C₁-C₆)alkyl- group;
- a ((C₁-C₆)alkyl)₂-N-(C₁-C₆)alkyl- group;
- 5 - a (C₁-C₆)alkyl-NH- group; and
- a ((C₁-C₆)alkyl)₂N- group;

R₃ represents:

- a deuterium atom;
 - 10 - a hydrogen atom
- or a group selected from:

a)

- a (C₁-C₆)alkyl- group;
- 15 - a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group; and
- a (C₁-C₆)alkylthio- group;

b)

- 20 - a -OR₆ group wherein R₆ is selected from:
 - a hydrogen atom;
 - a (C₁-C₆)alkyl- group;
 - a CH₃-[O-(CH₂)_n]- group with n being an integer from 1 to 30;
 - a (C₂-C₆)alkenyl- group;
 - 25 - a (C₂-C₆)alkynyl- group;
 - a (C₃-C₁₀)cycloalkyl- group;
 - a phenyl group;
 - a phenyl(C₁-C₆)alkyl- group; and
 - a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms
 - 30 selected from oxygen, nitrogen, sulfur, -S(=O)- and -S(=O)₂-;

c)

- a $\text{-NR}_7\text{R}_8$ group wherein R_7 and R_8 being, independently from each other, selected from:

- a hydrogen atom;

- a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ with n being an integer from 1 to 30;

- a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group unsubstituted or substituted by

5 - a $(\text{C}_5\text{-C}_{10})$ membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur; or

- a phenyl group being unsubstituted or substituted by at least one substituent selected from:

- a cyano group and

10 - a $\text{NR}_9\text{R}_{10}\text{-(C}_1\text{-C}_6)\text{alkyl-}$ group wherein:

R_9 and R_{10} being, independently from each other, selected from:

- a hydrogen atom;

- a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group;

- a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ with n being an integer from 1 to 30, or

15 R_9 and R_{10} together form with the nitrogen atom to which they are attached a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

said $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a $(\text{C}_1\text{-C}_6)\text{alkyl-}$ group, and a $\text{CH}_3\text{-[O-(CH}_2\text{)}_2\text{]}_n\text{-}$ group with n

20 being an integer from 1 to 30;

or R_7 and R_8 form together with the nitrogen atom to which they are attached a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

25 said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from:

- a phenyl group and

- a hydroxy $(\text{C}_1\text{-C}_6)\text{alkyl-phenyl-}$ group;

30 d)

- a $(\text{C}_3\text{-C}_{10})$ membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur;

e)

- a (C₅-C₁₀)membered heteroaryl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,

- 5 said (C₅-C₁₀)membered heteroaryl- group being unsubstituted or substituted by at least one (C₁-C₆)alkyl- group;

f)

- a (C₆-C₁₀)membered aryl- group; and

10

g)

- a (C₃-C₁₀)cycloalkyl- group;

- **L1** represents a linker; and

- 15 - **RCG1** represents a reactive chemical group that is reactive towards a chemical group present on a binding agent, for instance on a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance on a targeting agent for instance on a cell-binding agent such as an antibody such as a monoclonal antibody or a VHH antibody.

- 20 According to a particular embodiment, **L1** represents a cleavable linker or a non-cleavable linker.

According to one particular embodiment, **L1** is a cleavable linker which is a peptidic linker.

According to another particular embodiment, **L1** is a non-cleavable linker which is a

- 25 PEGylated linker.

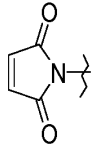
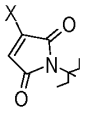
RCG1 is a reactive group chemical group which is well known by the skilled person in the art. **RCG1** is a tool for conjugating and as it is not a limitative radical, its list has not to be limited or restricted.

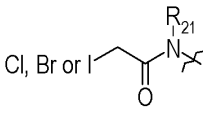
- 30 Examples of **RCG1** that may be mentioned include:

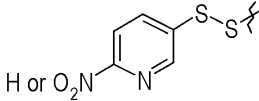
(i) a R_aZ_a-C(=O)- reactive group for which:

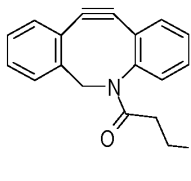
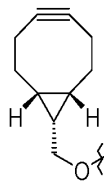
Z_a represents a single bond, -O- or -NH, such as -O-, and

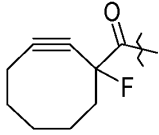
R_a represents a hydrogen atom, a $-(C_1-C_6)$ alkyl group, a $-(C_3-C_7)$ cycloalkyl group, a $-(C_2-C_6)$ alkenyl group, a $-(C_6-C_{10})$ aryl group, a $-(C_5-C_{10})$ heteroaryl group comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a $-(C_3-C_7)$ heterocycloalkyl group comprising 2 to 6 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, sulfur, $-S(O)-$ and $-SO_2-$, said $-(C_6-C_{10})$ aryl group, $-(C_5-C_{10})$ heteroaryl group and/or $-(C_3-C_7)$ heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a $-(C_1-C_6)$ alkyl group, a $-(C_1-C_6)$ alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

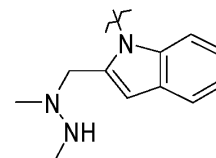
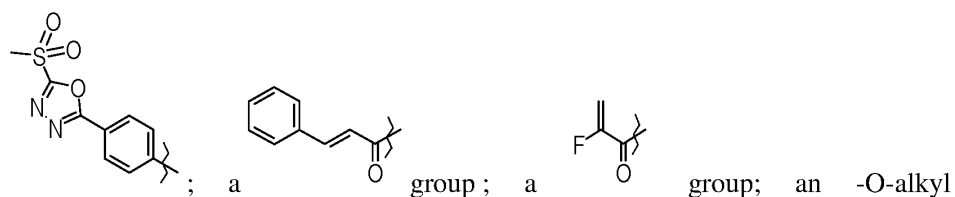
10 (ii) one of the following reactive groups: a maleimido  group; a substituted maleimido group such as  with $X = Br, O-C_6H_4-CN$; a haloacetamido

 group with R_{21} representing a hydrogen atom or a $-(C_1-C_6)$ alkyl group, such as a methyl group; $Cl-$; N_3- ; $HO-$; $HS-$; an activated disulfide such as

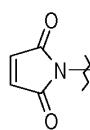
; H_2N- ; $HC\equiv C-$ or an activated $C\equiv C$ such as a cyclooctyne

15 moiety for instance a DBCO-amine  or BCN  or MFCO 

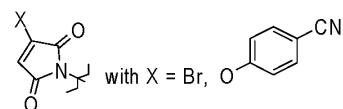
; a phenyloxadiazolyl methylsulfone group (PODS) such as



hydroxylamine or a Pictet-Spengler reaction substrate such as described in Agarwal P., *et al.*, *Bioconjugate Chem* **2013**, 24, 846-851, for instance

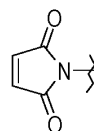
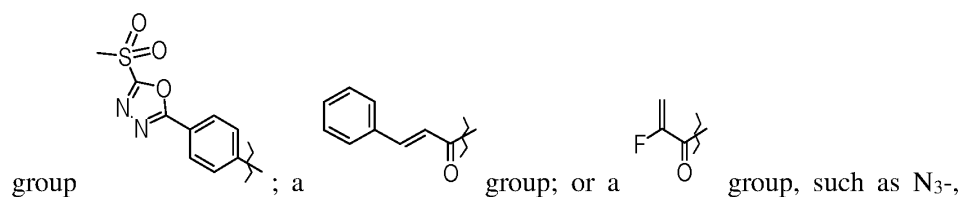


RCG₁ is N₃-, a maleimido group, a substituted maleimido group

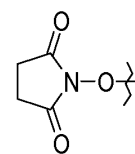


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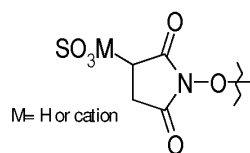
, a -COOH group, a phenyloxadiazolyl methylsulfone



a -COOH group, and a maleimido group.

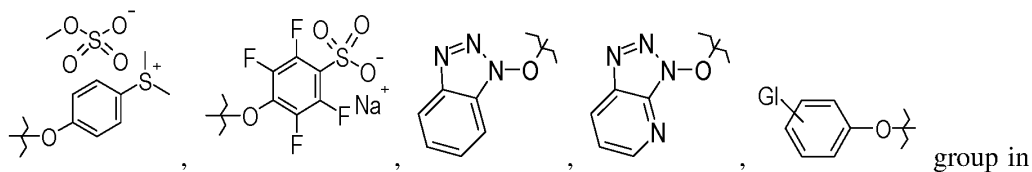


For instance, R_aZ_a- may represent HO-, CH₃O-, CH₂=CH-CH₂O-, (O-NHS),

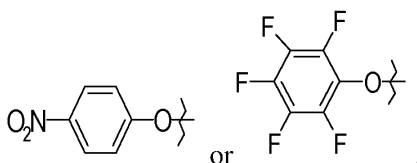


M= H or cation

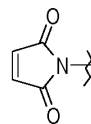
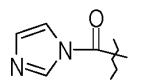
where cation represents for example sodium, potassium or cesium or



which **GI** represents at least one electroinductive group such as $-\text{NO}_2$ or a halogen atom, such as a fluorine atom (F). They may be, for example, the following groups:

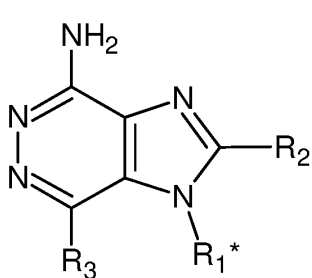


- 5 Another type of $\text{R}_a\text{Z}_a\text{-C(=O)-}$ group is the following:

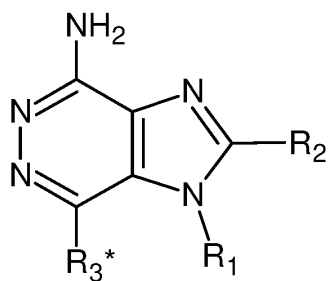


In particular, RCG_1 is N_3 -, $-\text{COOH}$, or a maleimido group.

- Among the compounds of formula (I) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2)



(A1)



(A2)

in which:

R₁, **R₂** and **R₃** are as defined in the present disclosure;

the single asterisk (*) indicates the site of covalent attachment to $-\text{L1-RCG}_1$;

- 15 **R₁*** represents:

- either a single bond, which means that $-\text{L1-RCG}_1$ is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R_1 as defined in the present disclosure, which means that -L1-RCG1 is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* R_1 ;

R_3^* represents:

5 - either a single bond, which means that -L1-RCG1 is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,

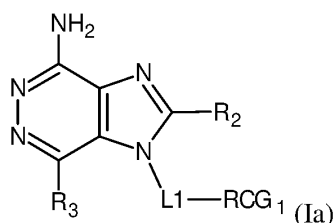
- or a divalent radical formed from R_3 as defined in the present disclosure, which means that -L1-RCG1 is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* R_3 .

10

According to one particular embodiment, **A** is of formula (A1).

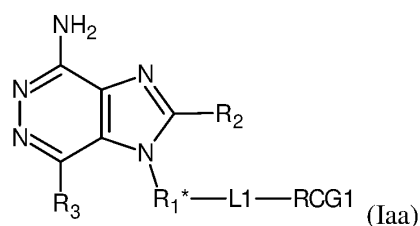
According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when R_1^* represents a single bond, the
15 compounds of formula (I) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ia):



wherein R_2 , R_3 , **L1** and **RCG1** are as defined in the present disclosure. This
20 means that in this particular embodiment, in the drug A, R_1 is a hydrogen atom.

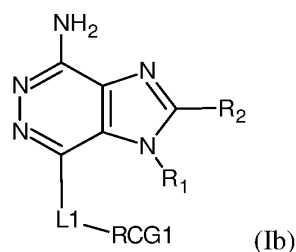
According to another particular embodiment, when R_1^* represents a divalent radical formed from R_1 as defined in the present disclosure, the compounds of formula (I) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the
25 following formula (Iaa):



wherein **R₂**, **R₃**, **L1** and **RCG1** are as defined in the present disclosure and **R₁*** is a divalent **R₁** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₁** is not a hydrogen atom.

5

According to another particular embodiment, when **R₃*** represents a single bond, the compounds of formula (I) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ib):

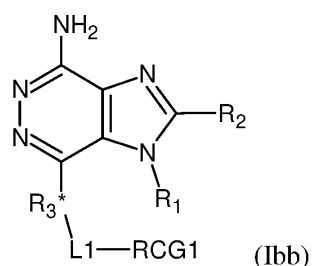


10

wherein **R₁**, **R₂**, **L1** and **RCG1** are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

According to another particular embodiment, when **R₃*** represents a divalent radical formed from **R₃** as defined in the present disclosure, the compounds of formula (I) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ibb):

15



wherein **R₁**, **R₂**, **L1** and **RCG1** are as defined in the present disclosure and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is not a hydrogen atom.

5 In the present disclosure, the  symbol on the bond denotes a covalent attachment site.

The drug A or a pharmaceutically acceptable salt thereof in accordance with the present disclosure which is comprised in the formula (I) and in the formula (II) of the present
10 disclosure, as well as processes for their preparation and their therapeutic uses, for instance as Toll-like receptor 7 agonists and/or Toll-like receptor 8 agonists are described in **PCT/EP2022/052387 (WO2022167438)**.

As described in **PCT/EP2022/052387 (WO2022167438)**, according to a particular embodiment, in the formula of drug A or a pharmaceutically acceptable salt
15 thereof, at least one of **R₁**, and **R₃** may be other than a hydrogen atom.

As described in **PCT/EP2022/052387 (WO2022167438)**, according to another particular embodiment, in the formula of drug A or a pharmaceutically acceptable salt thereof, **R₁** and **R₃** may be simultaneously other than a hydrogen atom.

20 Among the compounds of formula (A) which are described in **PCT/EP2022/052387 (WO2022167438)** and/or encompassed by claim 1 of **PCT/EP2022/052387 (WO2022167438)**, mention may be made for instance of the following compounds:

(1) 2-butyl-7-isopropoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-
25 amine;

(2) 2-butyl-N7-isopropyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4,7-diamine;

(3) 2-butyl-7-(isopropylthio)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

30 (4) 2-butyl-1-(4-methoxybenzyl)-7-(2-methoxyethoxy)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (5) 2-butyl-1-(4-methoxybenzyl)-7-propoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (6) 7-(allyloxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (7) 7-(sec-butoxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (8) 7-butoxy-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (9) 2-butyl-7-(cyclopentyloxy)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (10) 2-butyl-1-(4-methoxybenzyl)-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (11) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrrol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (12) (E)-2-butyl-1-(4-methoxybenzyl)-7-(3-methylbut-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (13) 2-butyl-7-isopentyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (14) 2-butyl-1-(4-methoxybenzyl)-7-(1H-pyrrol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (15) 2-butyl-7-(cyclopent-1-en-1-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (16) 2-butyl-7-cyclopentyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 25 (17) 2-butyl-1-(4-methoxybenzyl)-7-(prop-1-en-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (18) 2-butyl-7-isopropyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (19) 4-(aminomethyl)cyclohexylmethyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride;
- 30 (20) 4-(aminomethyl)cyclohexylmethyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;

- (21) 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (22) 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- 5 (23) 1-(((1S,3R)-3-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (and enantiomer);
- (24) 1-(4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)-2-methylpropan-2-ol;
- (25) Trans 1-(4-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-
10 imidazo[4,5-d]pyridazin-4-amine;
- (26) 1-((5-(aminomethyl)pyridin-2-yl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (27) 6-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)nicotinonitrile;
- 15 (28) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)acetamide;
- (29) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)undecanamide;
- (30) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-
20 1-yl)methyl)benzyl)pentanamide;
- (31) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)-3-(2-methoxyethoxy)propanamide;
- (32) 1-(((1S,3S)-3-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-]pyridazin-4-amine (and enantiomer);
- 25 (33) 1-(3-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (34) 4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzaldehyde;
- (35) (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-
30 yl)methyl)phenyl)methanol;
- (36) 2-butyl-1-(4-((cyclopropylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

- (37) 3-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-yl)methyl)benzyl)amino)thietane 1,1-dioxide;
- (38) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)-N-(1,1-dioxidothietan-3-yl)acetamide;
- 5 (39) 2-butyl-7-isopropoxy-1-(4-(((1-methylcyclobutyl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (40) 2-butyl-7-isopropoxy-1-(4-(piperazin-1-ylmethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (41) 2-butyl-N7,N7,1-trimethyl-1H-imidazo[4,5-d]pyridazin-4,7-diamine;
- 10 (42) 2-butyl-1-methyl-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (43) 2-butyl-N7-(4-((dimethylamino)methyl)benzyl)-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazin-4,7-diamine;
- (44) 2-butyl-N7,1-dimethyl-N7-(4-(morpholinomethyl)benzyl)-1H-
- 15 imidazo[4,5-d]pyridazine-4,7-diamine;
- (45) 4-(((4-amino-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazin-7-yl)(methyl)amino)methyl)benzonitrile;
- (46) N7-(4-(aminomethyl)benzyl)-2-butyl-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- 20 (47) 2-butyl-N7-(4-(((2-methoxyethyl)(methyl)amino)methyl)benzyl)-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (48) 2-butyl-7-ethoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (49) 2-butyl-1-(4-methoxybenzyl)-N7-(2-methoxyethyl)-1H-
- 25 imidazo[4,5-d]pyridazine-4,7-diamine;
- (50) 2-butyl-7-methoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (51) 2-butyl-7-cyclohexyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 30 (52) 7-(benzyloxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (53) 2-butyl-1-(4-methoxybenzyl)-N7-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (54) (S)-2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrofuran-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (55) 2-butyl-7-(furan-2-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazine-4-amine;
- (56) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrofuran-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (57) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydro-2H-pyran-4-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (58) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrothiophen-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (59) 2-butyl-1-(4-methoxybenzyl)-7-(tetrahydrofuran-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (60) 2-butyl-1-(4-methoxybenzyl)-7-(2-methylprop-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (61) 2-butyl-7-isobutyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (62) 2-butyl-1-(4-methoxybenzyl)-7-(thiophen-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (63) 2-butyl-1-(4-methoxybenzyl)-7-(thiophen-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (64) 2-butyl-1-(4-methoxybenzyl)-7-(1H-pyrrol-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- 25 (65) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1-oxide isomer A;
- (66) 2-butyl-7-(cyclohex-1-en-1-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (67) 2-butyl-7-(furan-3-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 30 (68) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1,1-dioxide;

- (69) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1-oxide isomer B;
- (70) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrrol-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- 5 (71) 2-butyl-1-(4-methoxybenzyl)-N7,N7-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (72) 2-butyl-1-(4-methoxybenzyl)-7-phenoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (73) 2-butyl-7-(2,5-dihydrofuran-3-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (74) 2-butyl-7-isopropoxy-1-(pyridin-3-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (75) 2-butyl-7-isopropoxy-1-(pyridin-2-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (76) 2-butyl-7-isopropoxy-1-(pyridin-4-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (77) 1-(5-aminopentyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (78) 2-butyl-7-isopropoxy-1-(2-(piperidin-4-yl)ethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (79) 2-butyl-7-isopropoxy-1-(2-(piperazin-1-yl)ethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (80) 1-benzyl-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- 25 (81) 2-butyl-1-(cyclohexylmethyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (82) 2-butyl-7-isopropoxy-1-(4-(((tetrahydro-2H-pyran-4-yl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (83) 2-butyl-7-isopropoxy-1-(4-((isopropylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride;
- 30 (84) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)heptanamide;

- (85) (4-(1-(4-amino-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazin-7-yl)pyrrolidin-3-yl)phenyl)methanol hydrochloride;
- (86) 2-butyl-7-isopropoxy-1-methyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- (87) N7-(4-(aminomethyl)benzyl)-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (88) 2-butyl-N7-isopropyl-1-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (89) 2-butyl-1-methyl-7-(3-phenylpyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (90) N7-benzyl-2-butyl-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (91) 2-butyl-1-methyl-N7-(4-((4-methylpiperazin-1-yl)methyl)benzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (92) 2-butyl-1-(4-methoxybenzyl)-7-phenyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- (93) 4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-ol;
- (94) 2-butyl-N7-(3-(furan-2-yl)propyl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (95) 2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (96) 2-butyl-1-(4-methoxybenzyl)-7-(1H-pyrazol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (97) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrazol-4-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (98) 2-butyl-N7-isopropyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (99) 2-butyl-7-(isopropylthio)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (100) (1R,3R)-3-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)cyclobutan-1-ol dihydrochloride salt;
- (101) 2-butyl-7-isopropoxy-1-(4-(pyrrolidin-1-ylmethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (102) 2-butyl-7-isopropoxy-1-(4-(morpholinomethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (103) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)propionamide;

(104) 2-butyl-7-isopropoxy-1-(4-(((2-methoxyethyl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

(105) 2-butyl-4-isopropoxy-3-[[4-[[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethylamino)methyl]phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine;

(106) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)benzamide;

(107) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)-3-methoxypropanamide;

(108) 2-butyl-7-isopropoxy-1-(4-(((2-(2-methoxyethoxy)ethyl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

(109) 2-butyl-1-(4-((hexylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

(110) 2-butyl-1-(4-((decylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

(111) ethyl (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate;

(112) 4-methoxybenzyl (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate;

(113) 1-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)-3-ethylurea;

(114) 4-acetamidobenzyl (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate;

(115) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)methanesulfonamide;

(116) 2-butyl-1-(4-((dimethylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

(117) tert-butyl (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)glycinate;

(118) 2-butyl-7-isopropoxy-1-(4-((methylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (119) tert-butyl 3-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)propanoate;
- (120) 1-(4-(5,8,11-trioxa-2-azadodecyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (121) 2-butyl-4-isopropoxy-3-[[4-[[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethylamino]methyl]phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine;
- (122) 2-butyl-4-isopropoxy-3-[[4-[[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]methyl]phenyl]
- 10 methyl]imidazo[4,5-d]pyridazin-7-amine di2,2,2-trifluoroacetate;
- (123) 2-butyl-4-isopropoxy-3-[[4-[[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]methyl]phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine di2,2,2-trifluoroacetate;
- 15 (124) 2-butyl-7-isopropoxy-1-(3-((methylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (125) 2-butyl-1-(3-((dimethylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (126) 2-butyl-1-(3-((cyclobutylamino)methyl)benzyl)-7-isopropoxy-1H-
- 20 imidazo[4,5-d]pyridazin-4-amine dihydrochloride salt;
- (127) 2-butyl-1-(3-((cyclopropylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride salt;
- (128) 2-butyl-7-isopropoxy-1-(3-((isopropylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride salt;
- 25 (129) 3-((3-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)thietane 1,1-dioxide;
- (130) 2-butyl-7-isopropoxy-1-(3-(((1-methylcyclobutyl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride salt;
- (131) 2-butyl-7-isopropoxy-1-(3-(piperazin-1-ylmethyl)benzyl)-1H-
- 30 imidazo[4,5-d]pyridazin-4-amine;
- (132) (E)-1-(4-(aminomethyl)benzyl)-2-butyl-7-(3-methylbut-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (133) 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopentyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- (134) 1-(4-(aminomethyl)benzyl)-2-butyl-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (135) 1-(4-(aminomethyl)benzyl)-2-butyl-7-(1H-pyrrol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (137) 1-(((1R,4R)-4-(aminomethyl)cyclohexyl)methyl)-2-butyl-7-((E)-3-methylbut-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine
- (138) 1-(((1R,4R)-4-(aminomethyl)cyclohexyl)methyl)-2-butyl-7-isopentyl-10 1H-imidazo[4,5-d]pyridazin-4-amine;
- (139) 1-(((1R,4R)-4-aminocyclohexyl)methyl)-2-butyl-7-((E)-3-methylbut-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (140) 1-(((1R,4R)-4-aminocyclohexyl)methyl)-2-butyl-7-isopentyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (141) 1-(((1R,4R)-4-aminocyclohexyl)methyl)-2-butyl-7-(1H-pyrrol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (142) 1-(((1R,4R)-4-(aminomethyl)cyclohexyl)methyl)-2-butyl-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (143) 3-[(4-aminocyclohexyl)methyl]-2-butyl-4-pyrrolidin-1-yl-imidazo[4,5-d]pyridazin-7-amine;
- 20 (144) 1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)imidazo[4,5-d]pyridazin-4-amine;
- (145) 1-[[4-(aminomethyl)phenyl]methyl]-2-butyl-7-pyrrolidin-3-yl-imidazo[4,5-d]pyridazin-4-amine;
- 25 (146) 3-(6-aminoheptyl)-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine dihydrochloride salt;
- (147) 2-butyl-4-isopropoxy-3-[6-(tetrahydropyran-4-ylamino)heptyl]imidazo[4,5-d]pyridazin-7-amine dihydrochloride salt;
- (148) N-[6-(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)heptyl]-N-tetrahydropyran-4-yl-acetamide;
- 30 (149) 3-(4-aminobutyl)-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine dihydrochloride salt;

- (150) 2-butyl-4-isopropoxy-3-[4-(tetrahydropyran-4-ylamino)butyl]imidazo[4,5-d]pyridazin-7-amine hydrochloride salt;
- (151) N-[4-(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)butyl]-N-tetrahydropyran-4-yl-acetamide;
- 5 (152) 2-butyl-3-[4-[(1,1-dioxothietan-3-yl)amino]butyl]-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine;
- (153) N-[4-(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)butyl]-N-(1,1-dioxothietan-3-yl)acetamide;
- (154) 2-butyl-3-[6-[(1,1-dioxothietan-3-yl)amino]hexyl]-4-isopropoxy-
- 10 imidazo[4,5-d]pyridazin-7-amine;
- (155) N-[6-(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)hexyl]-N-(1,1-dioxothietan-3-yl)acetamide hydrochloride salt;
- (156) 2-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]-2-methyl-propane-1,3-diol hydrochloride salt;
- 15 (157) 2-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]propane-1,3-diol hydrochloride salt;
- (158) 1-(4-(aminomethyl)benzyl)-7-isopropoxy-2-propyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- (159) 1-(4-(aminomethyl)benzyl)-2-(ethoxymethyl)-7-isopropoxy-1H-
- 20 imidazo[4,5-d]pyridazin-4-amine;
- (160) 1-(4-(aminomethyl)benzyl)-7-isopropoxy-2-methyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- (161) 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-7-amine;
- 25 (162) 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-2-propylsulfinyl-imidazo[4,5-d]pyridazin-7-amine;
- (163) 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-N2-propyl-imidazo[4,5-d]pyridazine-2,7-diamine;
- (164) 3-[[4-(aminomethyl)phenyl]methyl]-2-(ethylaminomethyl)-4-
- 30 isopropoxy-imidazo[4,5-d]pyridazin-7-amine;
- (165) 2-butyl-7-isopropoxy-1-(piperidin-4-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

(166) 3-[[4-(1-amino-1-methyl-ethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine;

(167) 2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)-1-methyl-imidazo[4,5-d]pyridazin-4-amine;

- 5 (168) 2-butyl-3-methyl-4-piperazin-1-yl-imidazo[4,5-d]pyridazin-7-amine; or
a pharmaceutically acceptable salt thereof.

Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the present disclosure, a group of compounds is composed of the compounds wherein:

R₁ represents:

- a (C₁-C₆)alkyl- group, in particular a methyl group;
- a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:

- a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted) or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:

- a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group, for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl-group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or

- a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

R₂ represents :

- a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;

- a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

R₃ represents :

- a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that is to say a -O-CH(CH₃)₂ group);
- a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperazinyl group; or
- 10 - a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group.

Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the present disclosure, a group of compounds is composed of the compounds for which the drug (or drug unit) **A** is a TLR7 agonist and in particular is selected from the group consisting of:

- (1) 2-butyl-7-isopropoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (2) 2-butyl-N7-isopropyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (3) 2-butyl-7-(isopropylthio)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (4) 2-butyl-1-(4-methoxybenzyl)-7-(2-methoxyethoxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 25 (5) 2-butyl-1-(4-methoxybenzyl)-7-propoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (6) 7-(allyloxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 30 (7) 7-(sec-butoxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (8) 7-butoxy-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (9) 2-butyl-7-(cyclopentyloxy)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (10) 2-butyl-1-(4-methoxybenzyl)-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (11) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrrol-3-yl)-1H-imidazo[4,5d] pyridazin-4-amine;
- (12) (E)-2-butyl-1-(4-methoxybenzyl)-7-(3-methylbut-1-en-1-yl)-1H-
10 imidazo[4,5-d]pyridazin-4-amine;
- (13) 2-butyl-7-isopentyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (14) 2-butyl-1-(4-methoxybenzyl)-7-(1H-pyrrol-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (15) 2-butyl-7-(cyclopent-1-en-1-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (16) 2-butyl-7-cyclopentyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (17) 2-butyl-1-(4-methoxybenzyl)-7-(prop-1-en-2-yl)-1H-imidazo[4,5-
20 d]pyridazin-4-amine;
- (18) 2-butyl-7-isopropyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (19) 4-(aminomethyl)cyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride;
- 25 (20) 4-(aminomethyl)cyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (25) Trans 1-(4-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo [4,5-d]pyridazin-4-amine;
- (26) 1-((5-(aminomethyl)pyridin-2-yl)methyl)-2-butyl-7-isopropoxy-1H-
30 imidazo[4,5-d]pyridazin-4-amine;
- (27) 6-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)nicotinonitrile;

- (28) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)acetamide;
- (29) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)undecanamide;
- 5 (30) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)pentanamide;
- (31) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)-3-(2-methoxyethoxy)propenamide;
- (34) 4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzaldehyde;
- 10 (35) (4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)phenyl)methanol;
- (37) 3-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)thietane 1,1-dioxide;
- 15 (48) 2-butyl-7-ethoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (49) 2-butyl-1-(4-methoxybenzyl)-N7-(2-methoxyethyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (50) 2-butyl-7-methoxy-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (51) 2-butyl-7-cyclohexyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (52) 7-(benzyloxy)-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 25 (53) 2-butyl-1-(4-methoxybenzyl)-N7-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (54) (S)-2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrofuran-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (55) 2-butyl-7-(furan-2-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 30 (56) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrofuran-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (57) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydro-2H-pyran-4-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (58) 2-butyl-1-(4-methoxybenzyl)-7-((tetrahydrothiophen-3-yl)oxy)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (59) 2-butyl-1-(4-methoxybenzyl)-7-(tetrahydrofuran-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (60) 2-butyl-1-(4-methoxybenzyl)-7-(2-methylprop-1-en-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (61) 2-butyl-7-isobutyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (62) 2-butyl-1-(4-methoxybenzyl)-7-(thiophen-3-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (63) 2-butyl-1-(4-methoxybenzyl)-7-(thiophen-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (64) 2-butyl-1-(4-methoxybenzyl)-7-(1H-pyrrol-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (65) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1-oxide isomer A;
- (66) 2-butyl-7-(cyclohex-1-en-1-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (67) 2-butyl-7-(furan-3-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (68) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1,1-dioxide;
- 25 (69) 3-((4-amino-2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-7-yl)oxy)tetrahydrothiophene 1-oxide isomer B;
- (70) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrrol-2-yl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (71) 2-butyl-1-(4-methoxybenzyl)-N7,N7-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- 30 (72) 2-butyl-1-(4-methoxybenzyl)-7-phenoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

- (73) 2-butyl-7-(2,5-dihydrofuran-3-yl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (75) 2-butyl-7-isopropoxy-1-(pyridin-2-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 5 (76) 2-butyl-7-isopropoxy-1-(pyridin-4-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (77) 1-(5-aminopentyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (78) 2-butyl-7-isopropoxy-1-(2-(piperidin-4-yl)ethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (79) 2-butyl-7-isopropoxy-1-(2-(piperazin-1-yl)ethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (80) 1-benzyl-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- 15 (81) 2-butyl-1-(cyclohexylmethyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (87) N7-(4-(aminomethyl)benzyl)-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (92) 2-butyl-1-(4-methoxybenzyl)-7-phenyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (94) 2-butyl-N7-(3-(furan-2-yl)propyl)-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (97) 2-butyl-1-(4-methoxybenzyl)-7-(1-methyl-1H-pyrazol-4-yl)-1H-imidazo[4,5-d]pyridazin-4-amine; and
- 25 a pharmaceutically acceptable salt thereof.

Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the present disclosure, a group of compounds is composed of the compounds for which the drug (or drug unit) **A** is a TLR7/8 agonist and in particular is selected from the group consisting of:

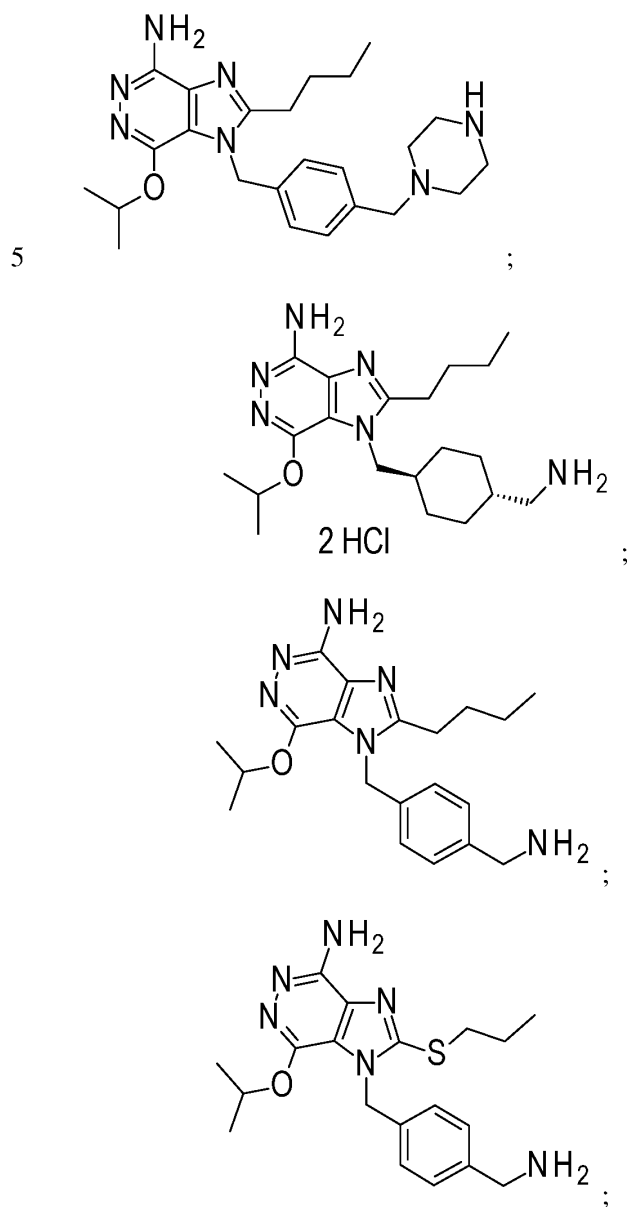
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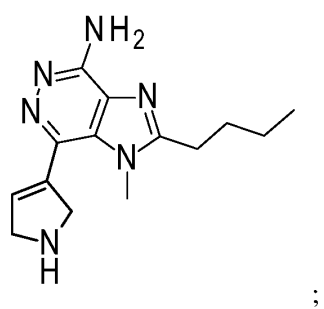
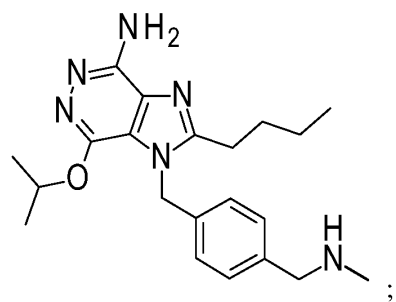
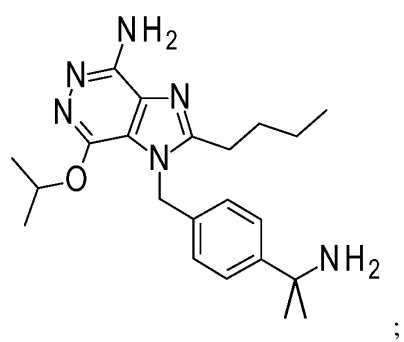
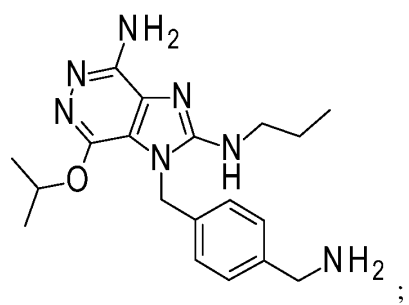
- (21) 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;

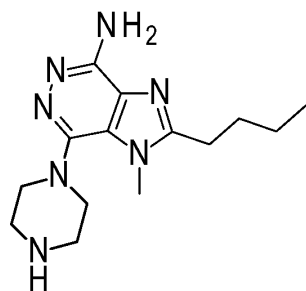
- (22) 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- (23) 1-(((1S,3R)-3-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (and enantiomer);
- 5 (24) 1-(4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)-2-methylpropan-2-ol;
- (32) 1-(((1S,3S)-3-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (and enantiomer);
- (33) 1-(3-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine 2,2,2-trifluoroacetate;
- 10 (36) 2-butyl-1-(4-((cyclopropylamino)methyl)benzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine;
- (39) 2-butyl-7-isopropoxy-1-(4-(((1-methylcyclobutyl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 15 (40) 2-butyl-7-isopropoxy-1-(4-(piperazin-1-ylmethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (41) 2-butyl-N7,N7,1-trimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (42) 2-butyl-1-methyl-7-(pyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (43) 2-butyl-N7-(4-((dimethylamino)methyl)benzyl)-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (44) 2-butyl-N7,1-dimethyl-N7-(4-(morpholinomethyl)benzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (45) 4-(((4-amino-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazin-7-yl)(methyl)amino)methyl)benzonitrile;
- 25 (46) N7-(4-(aminomethyl)benzyl)-2-butyl-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (47) 2-butyl-N7-(4-(((2-methoxyethyl)(methyl)amino)methyl)benzyl)-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- 30 (74) 2-butyl-7-isopropoxy-1-(pyridin-3-ylmethyl)-1H-imidazo[4,5-d]pyridazin-4-amine;

- (82) 2-butyl-7-isopropoxy-1-(4-(((tetrahydro-2H-pyran-4-yl)amino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine hydrochloride;
- (83) 2-butyl-7-isopropoxy-1-(4-((isopropylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine dihydrochloride;
- 5 (84) N-(4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)heptanamide;
- (85) (4-(1-(4-amino-2-butyl-1-methyl-1H-imidazo[4,5-d]pyridazin-7-yl)pyrrolidin-3-yl)phenyl)methanol hydrochloride;
- (86) 2-butyl-7-isopropoxy-1-methyl-1H-imidazo[4,5-d]pyridazin-4-amine;
- 10 (88) 2-butyl-N7-isopropyl-1-methyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (89) 2-butyl-1-methyl-7-(3-phenylpyrrolidin-1-yl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (90) N7-benzyl-2-butyl-N7,1-dimethyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- 15 (91) 2-butyl-1-methyl-N7-(4-((4-methylpiperazin-1-yl)methyl)benzyl)-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (95) 2-butyl-1-(4-methoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (98) 2-butyl-N7-isopropyl-1H-imidazo[4,5-d]pyridazine-4,7-diamine;
- (99) 2-butyl-7-(isopropylthio)-1H-imidazo[4,5-d]pyridazin-4-amine;
- 20 (118) 2-butyl-7-isopropoxy-1-(4-((methylamino)methyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine;
- (161) 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-7-amine;
- (163) 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-N2-propyl-imidazo[4,5-d]pyridazine-2,7-diamine;
- 25 (166) 3-[[4-(1-amino-1-methyl-ethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine;
- (167) 2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)-1-methyl-imidazo[4,5-d]pyridazin-4-amine;
- 30 (168) 2-butyl-3-methyl-4-piperazin-1-yl-imidazo[4,5-d]pyridazin-7-amine; and a pharmaceutically acceptable salt thereof.

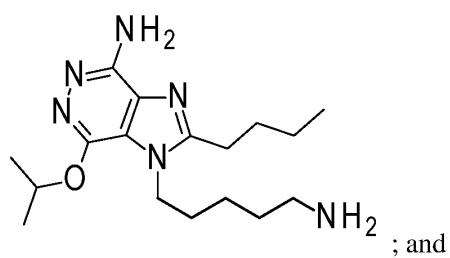
Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matters of the disclosure, a group of compounds is composed of the compounds for which the drug (or drug unit) **A** is selected from the group consisting of:



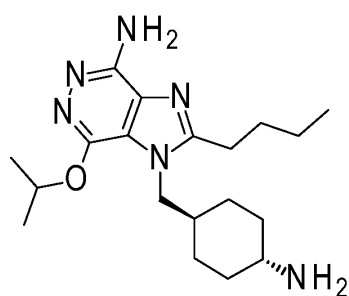




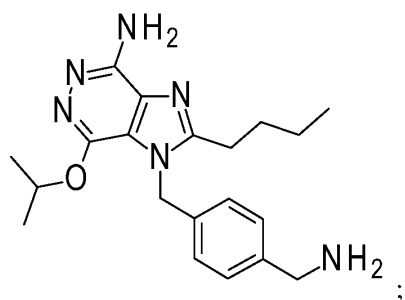
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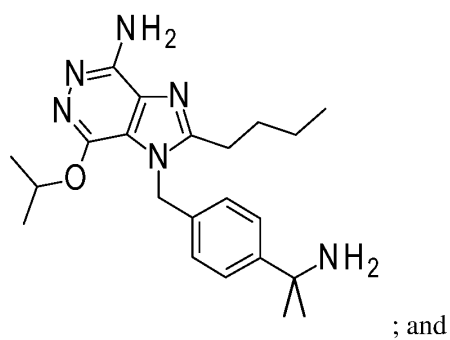
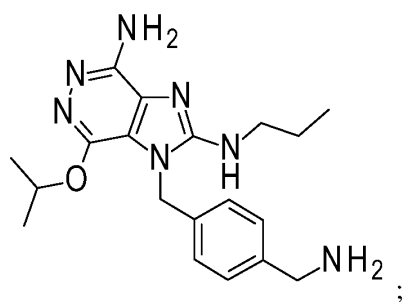
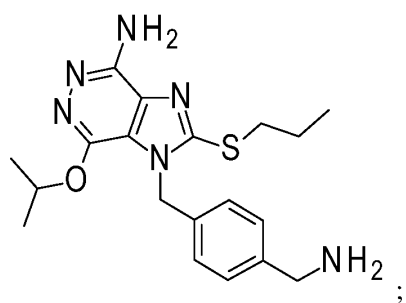


; and

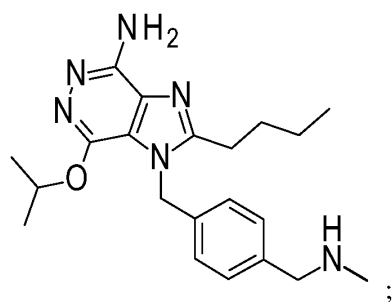


- 5 Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:

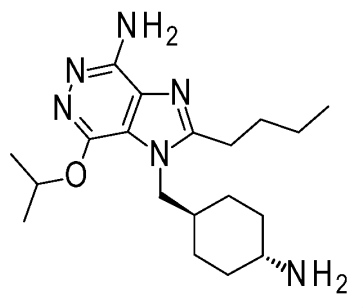
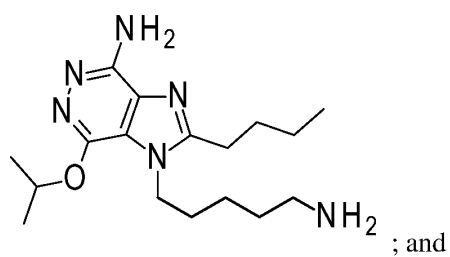
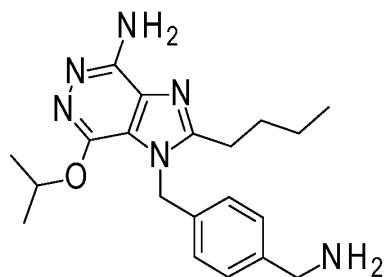




; and

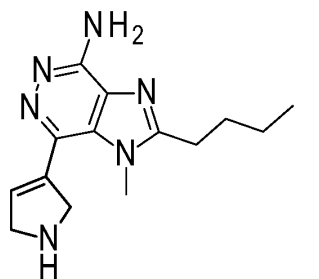


Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:

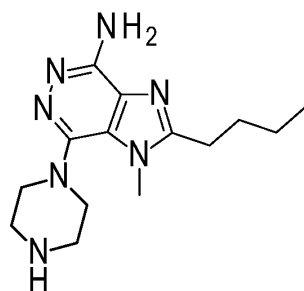


5 Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the present disclosure, a group of compounds is composed of the compounds for which the drug (or drug unit) **A** is further low or non – cell permeable, in particular the low or non - cell permeable drug unit **A** is defined by a PAMPA below 25.

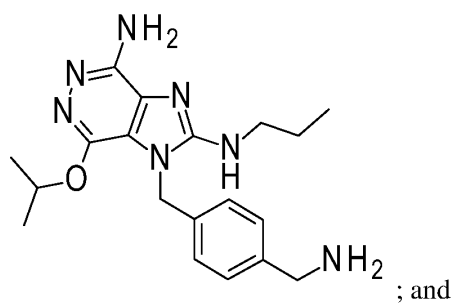
10 Among the compounds of formula (A), of formula (A1) or of formula (A2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:



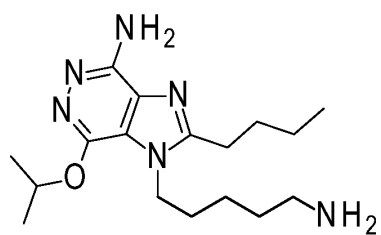
;



;



; and



5

Among the compounds of formula (I) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

A is of formula (A) in which:

R₁ represents:

10 - a (C₁-C₆)alkyl- group, in particular a methyl group;

- a $\text{NH}_2\text{-(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a $\text{NH}_2\text{-(CH}_2\text{)}_5\text{-}$ group;
- a $\text{phenyl(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a $\text{phenyl-CH}_2\text{-}$ group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:

5 - a $\text{(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a methyl group (or a $\text{-CH}_2\text{-}$ group if substituted) or an isopropyl group (or a $\text{-CH(CH}_3\text{)}_2\text{-}$ group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:

 - a $\text{-NR}_4\text{R}_5$ group wherein R_4 and R_5 , being independently from each other selected from: a hydrogen atom and a $\text{(C}_1\text{-C}_{16}\text{)alkyl-}$ group, in particular a methyl group,
10 for instance $\text{-NR}_4\text{R}_5$ is -NH_2 or $\text{-NH(CH}_3\text{)}$; or R_4 and R_5 form together with the nitrogen atom to which they are attached an unsubstituted $\text{(C}_3\text{-C}_{10}\text{)membered heterocycloalkyl-}$ group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted $\text{(C}_3\text{-C}_{10}\text{)membered heterocycloalkyl-}$ group comprising two nitrogen atoms, for instance a piperazinyl group; or

15 - a $\text{(C}_3\text{-C}_{10}\text{)cycloalkyl(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a cyclohexyl- $\text{CH}_2\text{-}$ group, being unsubstituted or substituted, in particular substituted on the $\text{(C}_3\text{-C}_{10}\text{)cycloalkyl-}$ group, by at least one substituent, in particular by one substituent, selected from -NH_2 and a $\text{NH}_2\text{-(C}_1\text{-C}_6\text{)alkyl-}$ group such as a $\text{NH}_2\text{-CH}_2\text{-}$ group;

R2 represents :

20 - a $\text{(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a $\text{-(CH}_2\text{)}_3\text{-CH}_3$ group;
 - a $\text{(C}_1\text{-C}_6\text{)alkylthio-}$ group, in particular a $\text{-S-(CH}_2\text{)}_2\text{-CH}_3$ group; or
 - a $\text{(C}_1\text{-C}_6\text{)alkyl-NH-}$ group, in particular a $\text{-NH-(CH}_2\text{)}_2\text{-CH}_3$ group; and

R3 represents :

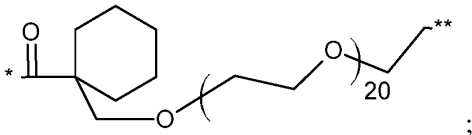
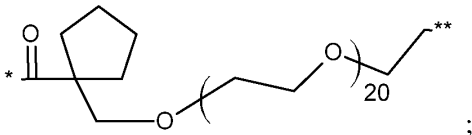
 - a -OR_6 group wherein R_6 is a $\text{(C}_1\text{-C}_6\text{)alkyl-}$ group, in particular a -O-isopropyl group (that
25 is to say a $\text{-O-CH(CH}_3\text{)}_2$ group);

 - a $\text{-NR}_7\text{R}_8$ group wherein R_7 and R_8 form together with the nitrogen atom to which they are attached a $\text{(C}_3\text{-C}_{10}\text{)membered heterocycloalkyl-}$ group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperazinyl group; or

30 - a $\text{(C}_3\text{-C}_{10}\text{)membered heterocycloalkyl-}$ group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for

instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group;

L1 represents:

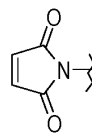
- 5 $^{*}\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-Cit-Val-C(O)-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-Cit-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;
- 10 $^{*}\text{-Ala-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;
- 15 $^{*}\text{-Cit-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-Ala-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_{24}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-(CH}_2\text{-CH}_2\text{-O)}_{23}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- 20 $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_{16}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_{12}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_8\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-C(CH}_3\text{)}_2\text{-CH}_2\text{-O-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- $^{*}\text{-C(O)-O-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
- 25 $^{*}\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
-  ;
-  ;
- $^{*}\text{-C(O)-(CH}_2\text{-CH}_2\text{-O)}_{24}\text{-CH}_2\text{-CH}_2\text{-NH-C(O)-CH}_2\text{-CH}_2\text{-}^{**}$;

*-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**; or

*-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**;

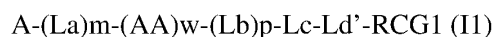
the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the -RCG1 group;

5 and



RCG1 is N₃-, a -COOH group, or a maleimido group .

According to a particular embodiment, the compounds of formula (I) or a pharmaceutically acceptable salt thereof are chosen among compounds of formula (II) and compounds of formula (I2):



wherein:

- **A** is as defined in formula (I);

- **La** represents a self immolative linker, for instance a *-C(O)-O-(CR₁₄R₁₅)-aryl-NR₁₆-** group, *-C(O)-O-(CR₁₄R₁₅)-heteroaryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-**, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-aryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, a *-CR₁₅R₁₄-aryl-NR₁₆-** group or a *-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, aryl being a (C₆-C₁₀) membered aryl group and heteroaryl being a (C₅-C₁₀) membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, for instance a *-C(O)-O-CH₂-phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)_w radical;

R₁₄, R₁₅, R₁₆, R₁₇ and R₁₈ being a hydrogen atom or a (C₁-C₆)alkyl group;

- **m** represents an integer from 0 to 1;

- (AA)_w represents a sequence of **w** amino acids **AA** connected together *via* peptide bonds;

w representing an integer ranging from 1 to 12, for instance from 1 to 6, such as 2 and 3;

AA denoting a natural or unnatural amino acid, of configuration D or L, chosen from: alanine (Ala), β -alanine, γ -aminobutyric acid, 2-amino-2-cyclohexylacetic acid, 2-amino-2-phenylacetic acid, arginine (Arg), asparagine (Asn), aspartic acid (Asp),
 5 citrulline (Cit), cysteine (Cys), α,α -dimethyl- γ -aminobutyric acid, β,β -dimethyl- γ -aminobutyric acid, glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), ϵ -acetyl-lysine (AcLys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan
 10 (Trp), tyrosine (Tyr), and valine (Val), for instance valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu) ;

- **Lb** represents a spacer, for instance a $^*-\text{C}(\text{O})-(\text{C}_1-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-^{**}$ group or a $^*-\text{C}(\text{O})-^{**}$ group, such as a $^*-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-^{**}$ group or a $^*-\text{C}(\text{O})-^{**}$ group, the single
 15 asterisk (*) indicating the site of covalent attachment to the (AA)_w radical and the double asterisk (**) indicating the site of covalent attachment to the Lc radical;

- **p** represents an integer from 0 to 1;

20 - **Lc** represents a spacer, for instance a $^*-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents an integer from 2 to 12, for instance from 3 to 7, such as 3 and 4 , the single asterisk (*) indicating the site of covalent attachment to the Lb radical if present (that is to say if p is 1) or directly to the (AA)_w radical if Lb is not present (that is to say if p is 0) and the double
 asterisk (**) indicating the site of covalent attachment to the Ld' radical;

25 - **Ld'** represents a spacer, for instance a $^*-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, a $^*-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{NH}-\text{C}(\text{O})-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group or a $^*-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, such as a $^*-(\text{CH}_2)_2-^{**}$ group, a $^*-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^*-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical
 30 and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical;
 and

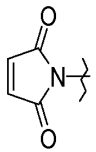
-**RCG1** represents a reactive chemical group that is reactive towards a chemical group present on a binding agent, for instance on a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance on a targeting agent for instance on a cell-binding agent such as an antibody such as a monoclonal antibody or a VHH antibody.

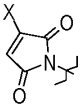
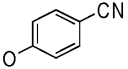
5 - For instance, **RCG1** represents:

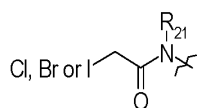
(i) a $R_aZ_a-C(=O)-$ reactive group for which:

Z_a represents a single bond, -O- or -NH, such as -O-, and

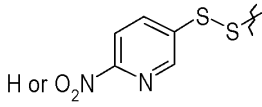
R_a represents a hydrogen atom, a $-(C_1-C_6)$ alkyl group, a $-(C_3-C_7)$ cycloalkyl group, a $-(C_2-C_6)$ alkenyl group, a $-(C_6-C_{10})$ aryl group, a $-(C_5-C_{10})$ heteroaryl group comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a $-(C_3-C_7)$ heterocycloalkyl group comprising 2 to 6 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-, said $-(C_6-C_{10})$ aryl group, $-(C_5-C_{10})$ heteroaryl group and/or $-(C_3-C_7)$ heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a $-(C_1-C_6)$ alkyl group, a $-(C_1-C_6)$ alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

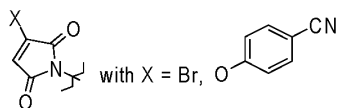
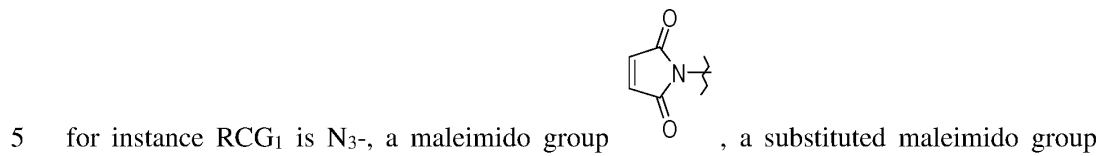
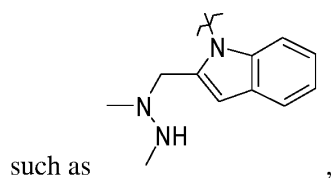
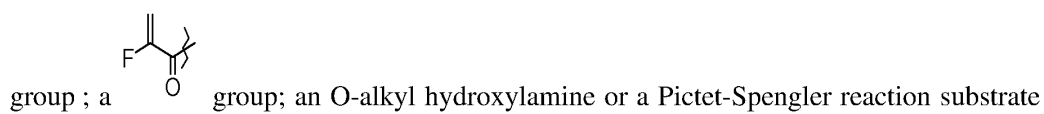
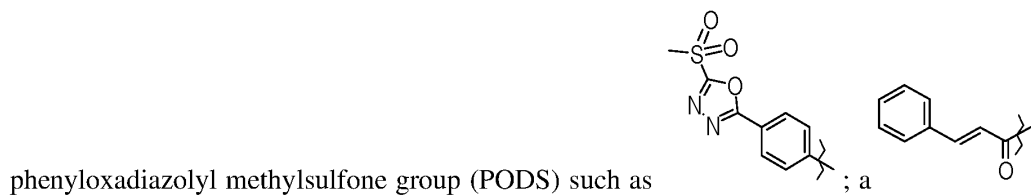
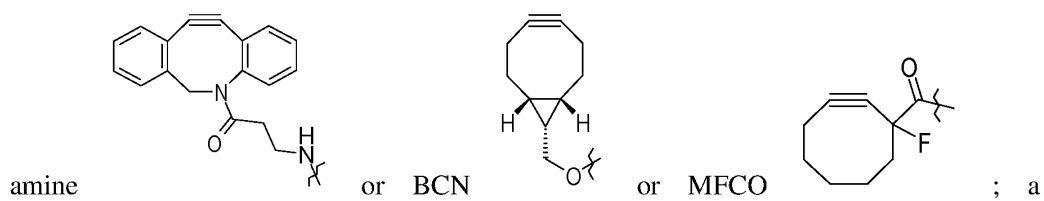
(ii) one of the following reactive groups: a maleimido  group; a

substituted maleimido group such as  with X = Br, ; a haloacetamido

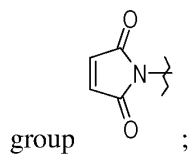
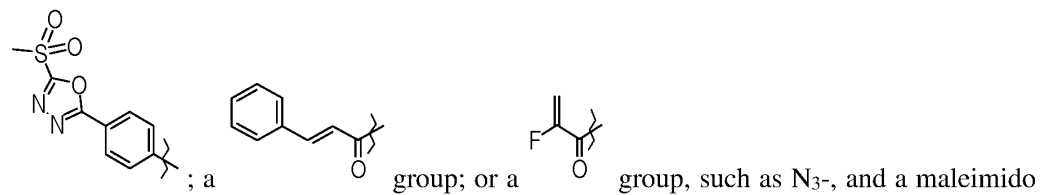


group with R_{21} representing a hydrogen atom or a (C_1-C_6) alkyl group, such

20 as a methyl group; Cl-; N₃-; HO-; HS-; an activated disulfide such as  H or O₂N-; H₂N-; HC≡C- or an activated C≡C such as a cyclooctyne moiety for instance a DBCO-



, a COOH group, a phenyloxadiazolyl methylsulfone group

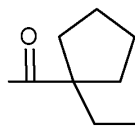


wherein

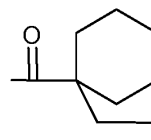
- **A** is as defined in formula (I);

- **P** represents a -C(O)- group, a -C(O)-O- group, a -S(O₂)- group, a -C(O)-C(CH₃)₂-CH₂-O-

group, a -C(O)-NH- group, a



group, or a



group;

5

- **o** represents an integer from 0 to 1;

- **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents an integer from 1 to 30, for instance from 8 to 25, such as 8, 12, 16, 20, 23 and 24, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical;

- **Ld** represents a spacer, for instance a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{NH}-\text{C}(\text{O})-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group or a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, such as a $^{*}-(\text{CH}_2)_2-^{**}$ group, a $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^{*}-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical; and

- **RCG1** represents a reactive chemical group that is reactive towards a chemical group present on a binding agent, for instance on a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance on a targeting agent for instance on a cell-binding agent such as an antibody such as a monoclonal antibody or a VHH antibody.

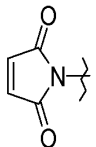
- For instance, **RCG1** represents:

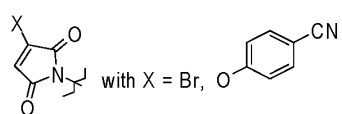
(i) a $\text{R}_a\text{Z}_a-\text{C}(=\text{O})-$ reactive group for which:

Z_a represents a single bond, -O- or -NH, such as -O-, and

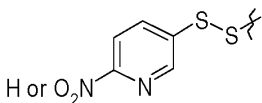
R_a represents a hydrogen atom, a $-(\text{C}_1-\text{C}_6)\text{alkyl}$ group, a $-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ group, a $-(\text{C}_2-\text{C}_6)\text{alkenyl}$ group, a $-(\text{C}_6-\text{C}_{10})\text{aryl}$ group, a $-(\text{C}_5-\text{C}_{10})\text{heteroaryl}$ group comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a $-(\text{C}_3-\text{C}_7)\text{heterocycloalkyl}$ group comprising 2 to 6 carbon atoms and 1 to 4

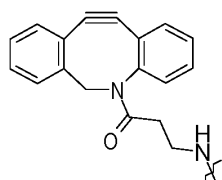
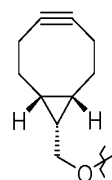
heteroatom(s) selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-, said -(C₆-C₁₀)aryl group, -(C₅-C₁₀)heteroaryl group and/or -(C₃-C₇)heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a -(C₁-C₆)alkyl group, a -(C₁-C₆)alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

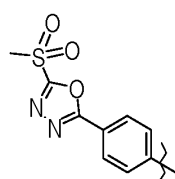
(ii) one of the following reactive groups: a maleimido  group; a

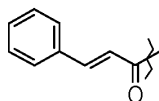
substituted maleimido group such as  with X = Br, O-C₆H₄-CN; a haloacetamido

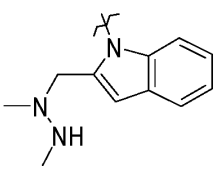
group with R₂₁ representing a hydrogen atom or a (C₁-C₆)alkyl group, such as a methyl group; Cl-; N₃-; HO-; HS-; an activated disulfide such as

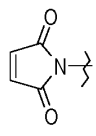
10 ; H₂N-; HC≡C- or an activated C≡C such as a cyclooctyne moiety

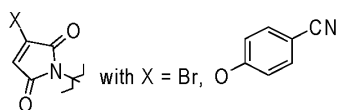
for instance a DBCO-amine  or BCN  or MFCO 

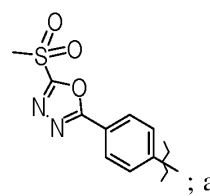
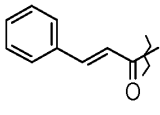
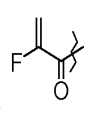
; a phenyloxadiazolyl methylsulfone group (PODS) such as 

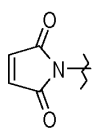
; a  group; a  group; an O-alkyl hydroxylamine or a Pictet-Spengler

reaction substrate such as ,

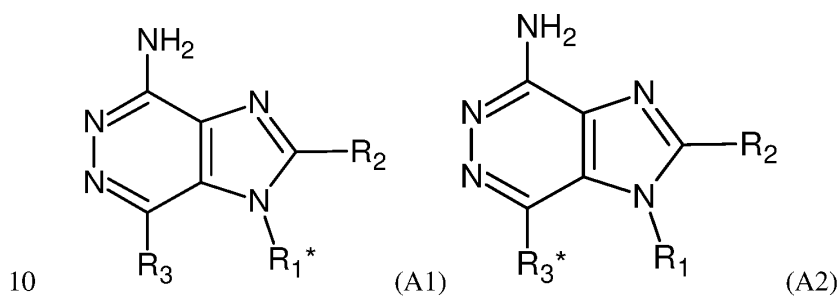
for instance RCG₁ is N₃-, a maleimido group , a substituted maleimido group



; a  group; or a  group, such as N₃-, a -COOH group,

5 and a maleimido group .

Among the compounds of formula (II) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2)



in which:

R₁, **R₂** and **R₃** are as defined in the present disclosure;

the single asterisk (*) indicates the site of covalent attachment to -(La)_m-(AA)_w-(Lb)_p-Lc-Ld'-RCG₁;

15 **R₁*** represents:

- either a single bond, which means that $-(La)m-(AA)w-(Lb)p-Lc-Ld'-RCG1$ is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R_1 as defined in the present disclosure, which means that $-(La)m-(AA)w-(Lb)p-Lc-Ld'-RCG1$ is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* R_1 ;

R_3^* represents:

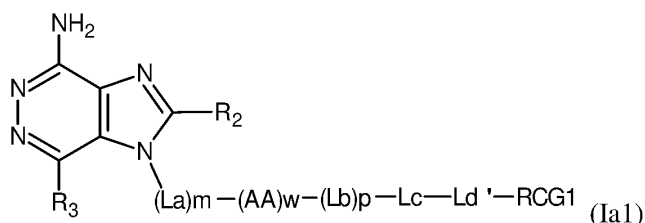
- either a single bond, which means that $-(La)m-(AA)w-(Lb)p-Lc-Ld'-RCG1$ is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R_3 as defined in the present disclosure, which means that $-(La)m-(AA)w-(Lb)p-Lc-Ld'-RCG1$ is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* R_3 .

According to one particular embodiment, **A** is of formula (A1).

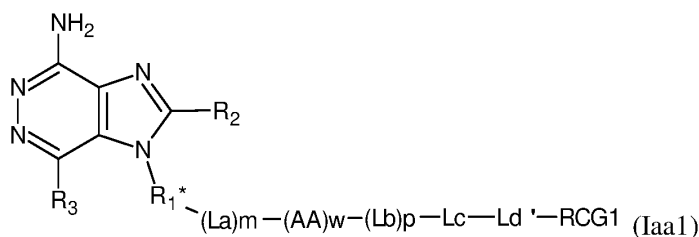
According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when R_1^* represents a single bond, the compounds of formula (I1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ia1):



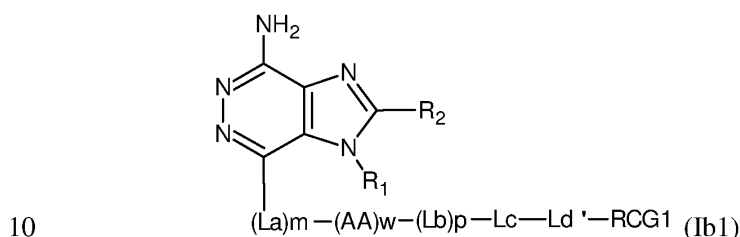
wherein **R₂**, **R₃**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'** and **RCG1** are as defined in the present disclosure. This means that in this particular embodiment, in the drug **A**, **R₁** is a hydrogen atom.

According to another particular embodiment, when R_1^* represents a divalent radical formed from R_1 as defined in the present disclosure, the compounds of formula (I1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Iaa1):



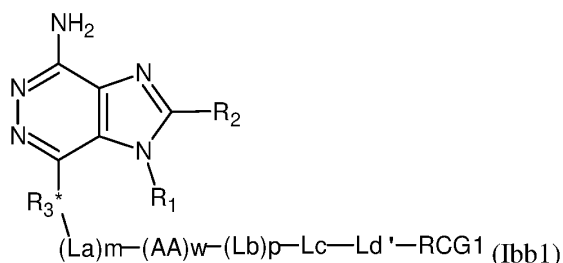
wherein **R₂**, **R₃**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'** and **RCG1** are as defined in the present disclosure and **R₁*** is a divalent **R₁** as defined in the present disclosure. This means
 5 that in this particular embodiment, in the drug A, **R₁** is not a hydrogen atom.

According to another particular embodiment, when **R₃*** represents a single bond, the compounds of formula (I1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ib1):



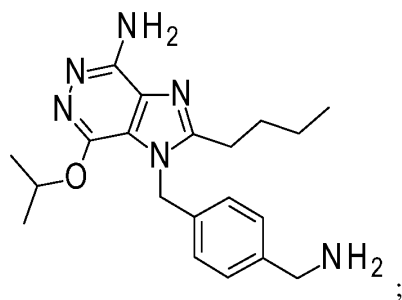
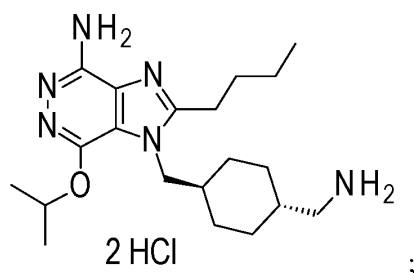
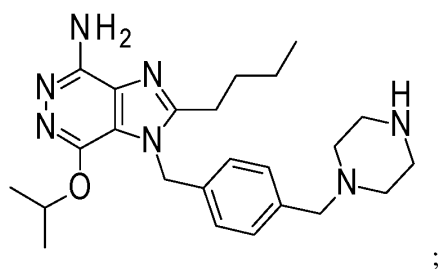
wherein **R₁**, **R₂**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'** and **RCG1** are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

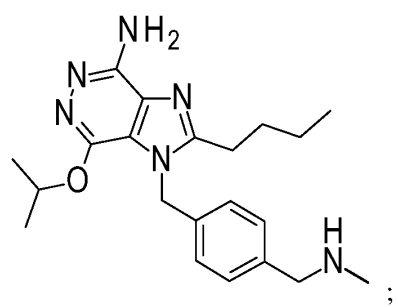
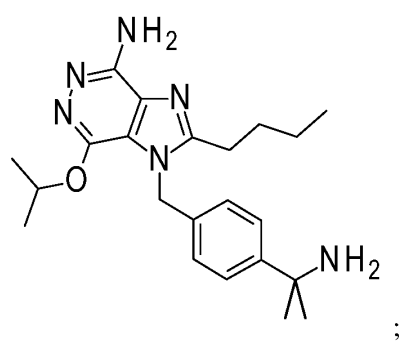
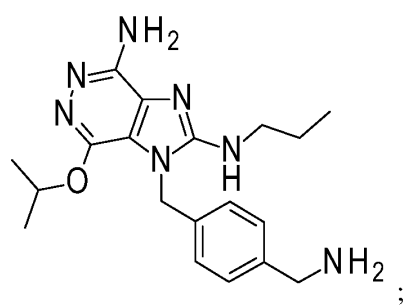
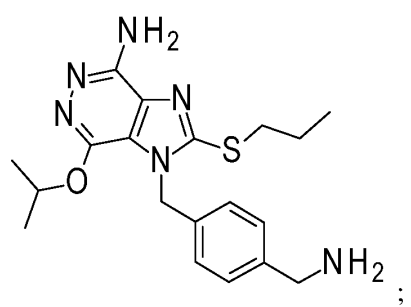
15 According to another particular embodiment, when **R₃*** represents a divalent radical formed from **R₃** as defined in the present disclosure, the compounds of formula (I1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ibb1):

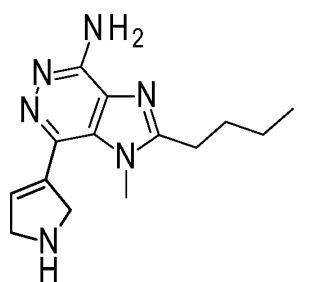


wherein **R₁**, **R₂**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'** and **RCG1** are as defined in the present disclosure and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular embodiment, in the drug **A**, **R₃** is not a hydrogen atom.

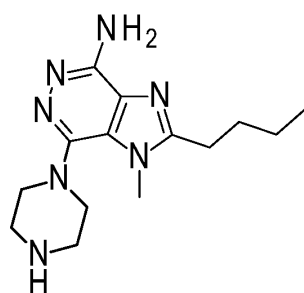
- 5 Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:



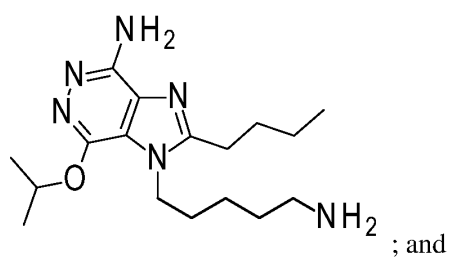




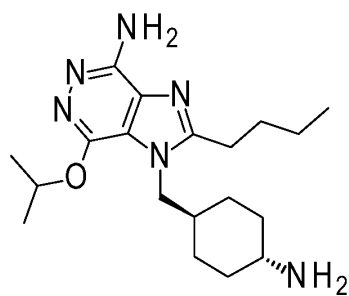
;



;

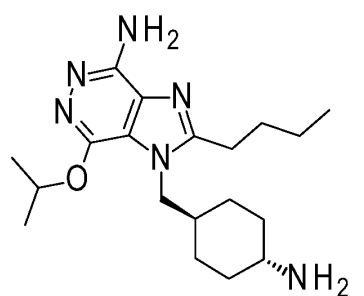
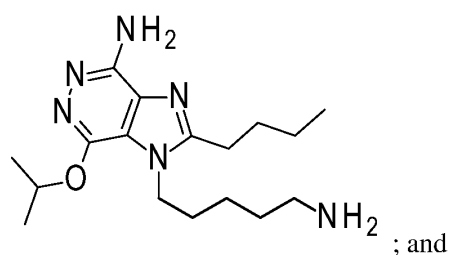
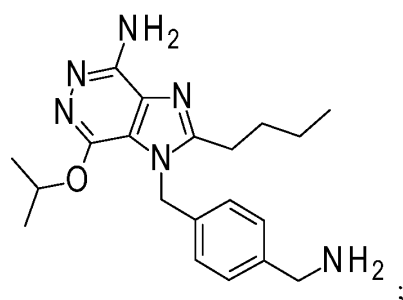


; and



5

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:



5

Among the compounds of formula (II) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **La** is a self-immolative linker. Self immolative linkers are well known for the person of the art. **La** is a covalent assembly tailored to correlate the cleavage of two chemical bonds after activation of a protective part in a precursor. Thus, its list has not to be limited or restricted.

For instance, **La** represents a $^*-C(O)-O-(CR_{14}R_{15})-aryl-NR_{16}-^{**}$ group, $^*-C(O)-O-(CR_{14}R_{15})-heteroaryl-NR_{16}-^{**}$ group, a $^*-C(O)-NR_{17}-(C_2-C_6)alkyl-NR_{18}-^{**}$, a $^*-C(O)-NR_{17}-(C_2-C_6)alkyl-NR_{18}-C(O)-O-CR_{15}R_{14}-aryl-NR_{16}-^{**}$ group, a $^*-C(O)-NR_{17}-(C_2-C_6)alkyl-NR_{18}-C(O)-O-CR_{15}R_{14}-heteroaryl-NR_{16}-^{**}$ group, a $^*-CR_{15}R_{14}-aryl-NR_{16}-^{**}$ group or a $^*-CR_{15}R_{14}-heteroaryl-NR_{16}-^{**}$ group, aryl being a (C_6-C_{10}) membered aryl group and heteroaryl being a (C_5-C_{10}) membered heteroaryl group comprising one to four heteroatoms

15

selected from oxygen, nitrogen and sulfur, the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)_w radical, R₁₄, R₁₅, R₁₆, R₁₇ and R₁₈ being a hydrogen atom or a (C₁-C₆)alkyl group.

5

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **La** represents a *-C(O)-O-CH₂-phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)_w radical.

10

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which in (AA)_w, **w** represents an integer from 1 to 6, and **AA** is chosen from valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu).

15

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which in (AA)_w, **w** represents an integer 2 or 3, and **AA** is chosen from valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu).

20

For instance, the amino acid sequence (AA)_w comprises a citrulline (Cit).

For instance, the amino acid sequence (AA)_w is chosen among Glu-Val-Cit; Glu-Val-Ala; Val-Cit or Val-Ala.

For instance, when the amino acid sequence (AA)_w is Glu-Val-Cit, the citrulline (Cit) is linked to La or to A (if La is absent, that is to say if m is 0) and the glutamic acid (Glu) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

25

For instance, when the amino acid sequence (AA)_w is Glu-Val-Ala, the alanine (Ala) is linked to La or to A (if La is absent, that is to say if m is 0) and the glutamic acid (Glu) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

For instance, when the amino acid sequence (AA)_w is Val-Cit, the citrulline (Cit) is linked to La or to A (if La is absent, that is to say if m is 0) and the valine (Val) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

30

For instance, when the amino acid sequence (AA)_w is Val-Ala, the alanine (Ala) is linked to L_a or to A (if L_a is absent, that is to say if m is 0) and the valine (Val) is linked to L_b or to L_c (if L_b is absent, that is to say if p is 0).

For instance, the amino acid sequence (AA)_w is Val-Cit.

5

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_b** represents a *-C(O)-(C₁-C₆)alkyl-C(O)-NH-** group or a *-C(O)-** group, the single asterisk (*) indicating the site of covalent attachment to the (AA)_w radical and the double asterisk (**) indicating the site of covalent attachment to the L_c radical.

10

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_b** represents a *-C(O)-(CH₂)₃-C(O)-NH-** group or a *-C(O)-** group, the single asterisk (*) indicating the site of covalent attachment to the (AA)_w radical and the double asterisk (**) indicating the site of covalent attachment to the L_c radical.

15

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-** group in which n represents an integer from 2 to 12, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

20

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-** group in which n represents an integer from 3 to 7, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

25

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-** group in which n represents an integer 3 or 4, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly

30

to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

5 Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_d'** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, a $^{*}-(C_2-C_6)alkyl-NH-C(O)-(C_2-C_6)alkyl-^{**}$ group or a $^{*}-(C_2-C_6)alkyl-C(O)-NH-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L_c radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

10 Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_d'** represents a $^{*}-(CH_2)_2-^{**}$ group, a $^{*}-(CH_2)_2-NH-C(O)-(CH_2)_2-^{**}$ group or a $^{*}-(CH_2)_2-C(O)-NH-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L_c radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

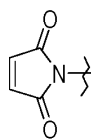
15 Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **m** represents an integer 1.

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **m** represents 0.

20 Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **p** represents an integer 1.

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **p** represents 0.

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **RCG1** represents N₃-, or a



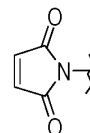
25 maleimido group .

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **L_d'** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L_c radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents N₃-.

30

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(C_2-C_6)alkyl-NH-C(O)-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of

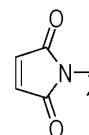
5 covalent attachment to the RCG1 radical, then **RCG1** represents a maleimido group



Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents N₃-.

Among the compounds of formula (I1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(CH_2)_2-NH-C(O)-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent

15 attachment to the RCG1 radical, then **RCG1** represents a maleimido group



All these sub-groups taken alone or in combination are part of the present disclosure.

20 According to a particular embodiment, the disclosure relates to a compound/payload of formula (I1) or a pharmaceutically acceptable salt thereof wherein **A** and **RCG1** are as defined in the present disclosure, and

La is $^{*}-C(O)-O-CH_2-para-phenyl-NH-^{**}$;

m is 1;

25 **AA** is selected from Ala, Val, Cit and Glu;

w is 2 or 3;

Lb is $^{*}-C(O)-(CH_2)_3-C(O)-NH-^{**}$;

p is 1;

Lc is $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_3-^{**}$; and

Ld' is $^{*}-(\text{CH}_2)_2-^{**}$ or $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$.

According to another particular embodiment, the disclosure relates to a
5 compound/payload of formula (I1) or a pharmaceutically acceptable salt thereof wherein
A and **RCG1** are as defined in the present disclosure;

m is 0;

AA is selected from Ala, Val and Cit;

w is 2;

10 Lb is $^{*}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-^{**}$ or $^{*}-\text{C}(\text{O})-^{**}$;

p is 1;

Lc is $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_3-^{**}$; and

Ld' is $^{*}-(\text{CH}_2)_2-^{**}$ or $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$.

15 According to another particular embodiment, the disclosure relates to a
compound/payload of formula (I1) or a pharmaceutically acceptable salt thereof wherein the
-(La)m-(AA)w-(Lb)p-Lc-Ld' - part is selected from the group consisting of:

$^{*}-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{para-phenyl}-\text{NH}-\text{Cit}-\text{Val}-\text{Glu}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-$
 $\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-^{**}$;

20 $^{*}-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{para-phenyl}-\text{NH}-\text{Ala}-\text{Val}-\text{Glu}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-$
 $\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-^{**}$;

$^{*}-\text{Cit}-\text{Val}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-^{**}$;

$^{*}-\text{Ala}-\text{Val}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-^{**}$;

25 $^{*}-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{para-phenyl}-\text{NH}-\text{Cit}-\text{Val}-\text{Glu}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-$
 $\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$;

$^{*}-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{para-phenyl}-\text{NH}-\text{Ala}-\text{Val}-\text{Glu}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-$
 $\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$;

$^{*}-\text{Cit}-\text{Val}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$;

$^{*}-\text{Ala}-\text{Val}-\text{C}(\text{O})-(\text{CH}_2)_3-\text{C}(\text{O})-\text{NH}-(\text{CH}_2-\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$,

30 and

$^{*}-\text{Cit}-\text{Val}-\text{C}(\text{O})-(\text{CH}_2-\text{CH}_2-\text{O})_3-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$,

the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

Among the compounds of formula (II) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

A is of formula (A) in which:

R₁ represents:

- a (C₁-C₆)alkyl- group, in particular a methyl group;
- a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- 10 - a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:
 - a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted) or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or
 - 15 substituted by at least one substituent, in particular by one substituent, selected from:
 - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group, for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl-
 - 20 group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or
 - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at
 - 25 least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

R₂ represents :

- a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;
- a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- 30 - a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

R₃ represents :

- a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that is to say a -O-CH(CH₃)₂ group);

- a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperazinyl group; or

- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group;

*-(La)m-(AA)w-(Lb)p-Lc-Ld'-** represents:

*-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-** ;

*-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

*-Cit-Val-C(O)-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;

*-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

*-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

*-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-** ;

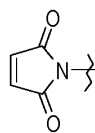
*-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;

*-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;

or

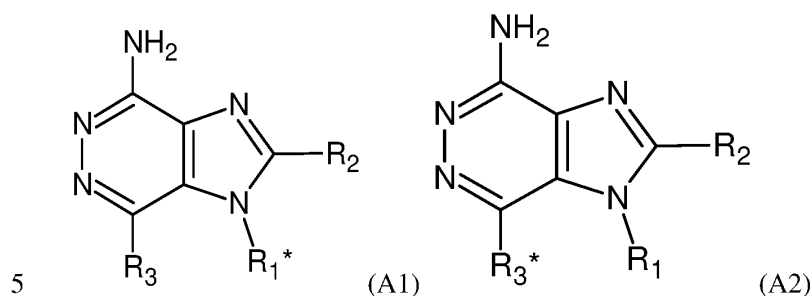
*-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**,

the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the -RCG1 group; and



RCG1 is N₃-, or a maleimido group

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2):



in which:

R₁, **R₂** and **R₃** are as defined in the present disclosure;

the single asterisk (*) indicates the site of covalent attachment to $-(P)o-L-Ld-RCG1$;

R₁* represents:

- 10 - either a single bond, which means that $-(P)o-L-Ld-RCG1$ is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,
- or a divalent radical formed from **R₁** as defined in the present disclosure, which means that $-(P)o-L-Ld-RCG1$ is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* **R₁**;

15 **R₃*** represents:

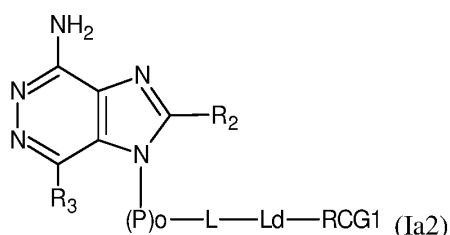
- either a single bond, which means that $-(P)o-L-Ld-RCG1$ is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,
- or a divalent radical formed from **R₃** as defined in the present disclosure, which means that $-(P)o-L-Ld-RCG1$ is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* **R₃**.
- 20

 According to one particular embodiment, **A** is of formula (A1).

 According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when **R₁*** represents a single bond, the compounds of formula (I2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ia2):

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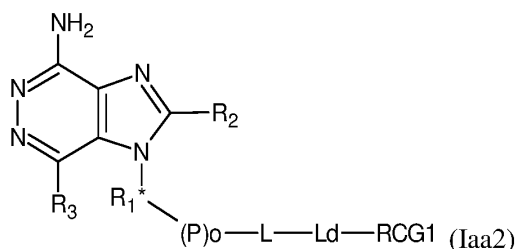
wherein **R₂**, **R₃**, **P**, **o**, **L**, **Ld** and **RCG1** are as defined in the present disclosure.

This means that in this particular embodiment, in the drug A, **R₁** is a hydrogen atom.

5

According to another particular embodiment, when **R₁^{*}** represents a divalent radical formed from **R₁** as defined in the present disclosure, the compounds of formula (I2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Iaa2):

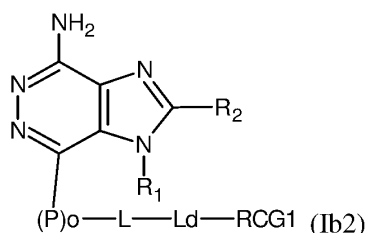
10



wherein **R₂**, **R₃**, **P**, **o**, **L**, **Ld** and **RCG1** are as defined in the present disclosure and **R₁^{*}** is a divalent **R₁** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₁** is not a hydrogen atom.

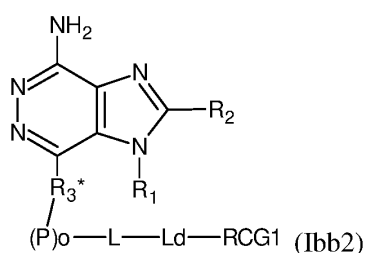
15

According to another particular embodiment, when **R₃^{*}** represents a single bond, the compounds of formula (I2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (Ib2):



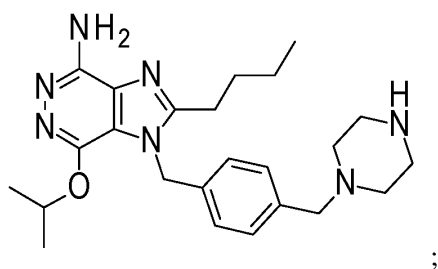
wherein **R₁**, **R₂**, **P**, **o**, **L**, **Ld** and **RCG1** are as defined in the present disclosure.
This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

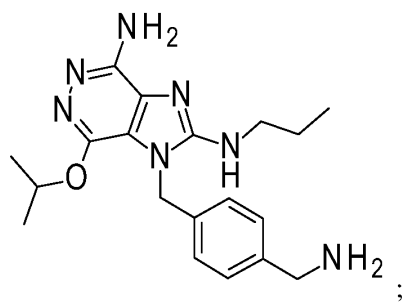
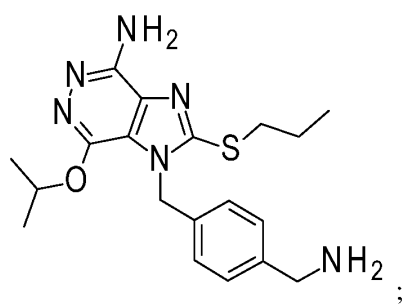
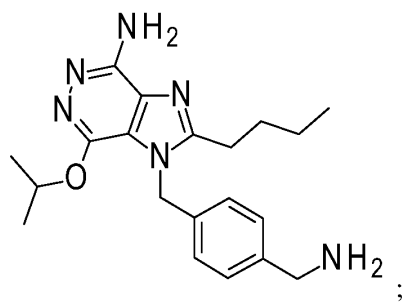
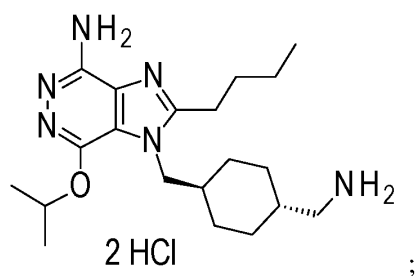
According to another particular embodiment, when **R₃*** represents a divalent radical formed
5 from **R₃** as defined in the present disclosure, the compounds of formula (I2) or a
pharmaceutically acceptable salt thereof in accordance with the present disclosure have the
following formula (Ibb2):

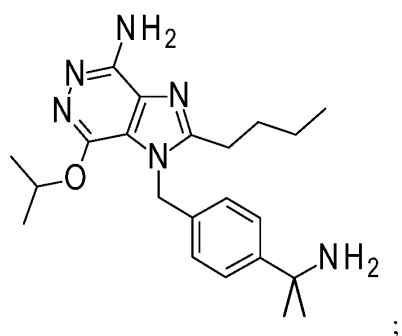


wherein **R₁**, **R₂**, **P**, **o**, **L**, **Ld** and **RCG1** are as defined in the present disclosure
10 and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular
embodiment, in the drug A, **R₃** is not a hydrogen atom.

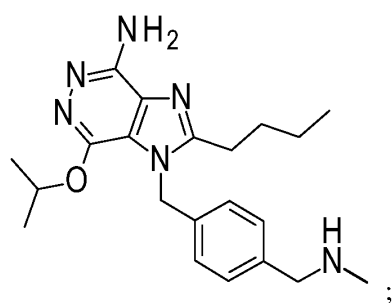
Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which **A** is selected from the group
15 consisting of:



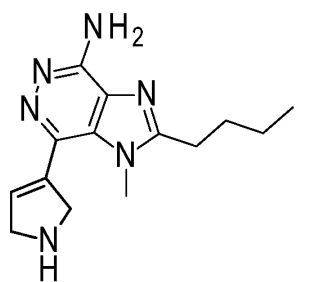




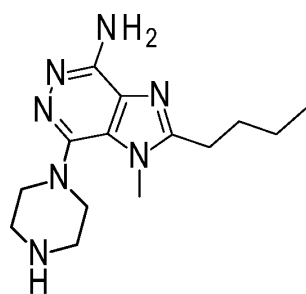
;



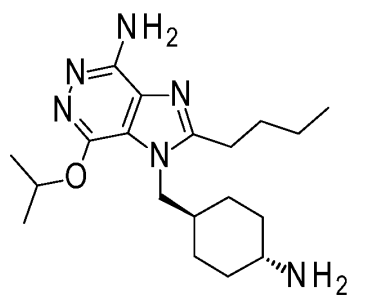
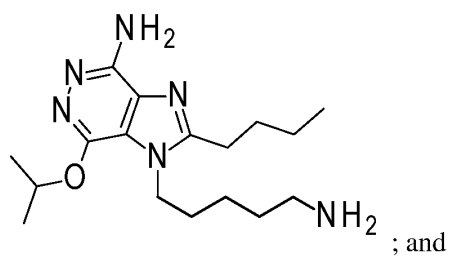
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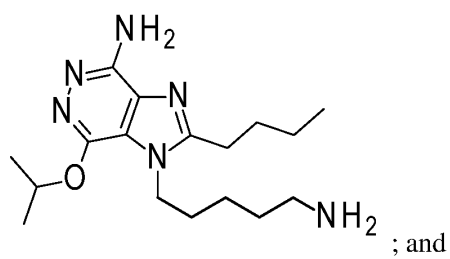
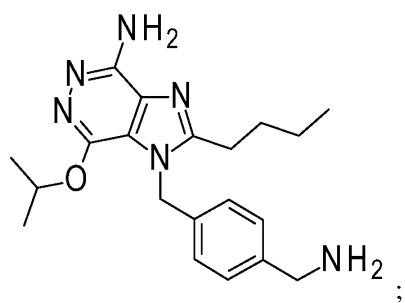
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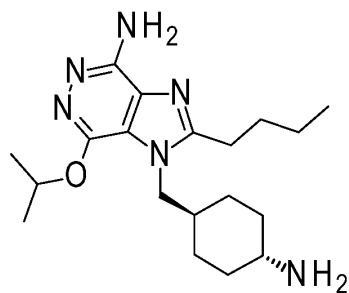


;



- Among the compounds of formula (I2) that are subject matter of the disclosure,
- 5 a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:





Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)- group.

5 Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-O- group.

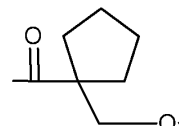
Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -S(O₂)- group.

10 Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-C(CH₃)₂-CH₂-
O- group.

Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-NH- group.

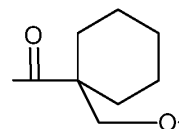
Among the compounds of formula (I2) that are subject matter of the disclosure,

15 a group of compounds is composed of the compounds for which P is a
group.



Among the compounds of formula (I2) that are subject matter of the disclosure,

a group of compounds is composed of the compounds for which P is a
group.



20 Among the compounds of formula (I2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which o is 0.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which o is 1.

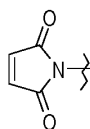
Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents an integer from 8 to 25, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents 8, 12, 16, 20, 23 or 24, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **Ld** represents a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{NH}-\text{C}(\text{O})-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group or a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **Ld** represents a $^{*}-(\text{CH}_2)_2-^{**}$ group, a $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^{*}-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **RCG1** represents N_3- ,



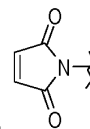
a -COOH group, or a maleimido group .

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents N_3- .

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents $-COOH$.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)alkyl-NH-C(O)-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of

covalent attachment to the RCG1 radical, then **RCG1** represents a maleimido group

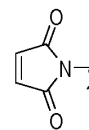


Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents N_3- .

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical, then **RCG1** represents $-COOH$.

Among the compounds of formula (I2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(CH_2)_2-NH-C(O)-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent

attachment to the L radical and the double asterisk (**) indicating the site of covalent



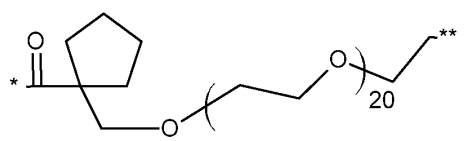
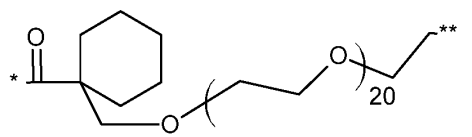
attachment to the RCG1 radical, then **RCG1** represents a maleimido group .

All these sub-groups taken alone or in combination are part of the present disclosure.

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According to a particular embodiment, the disclosure relates to a compound/payload of formula (I2) or a pharmaceutically acceptable salt thereof wherein the -(P)o-L-Ld- part is selected from the group consisting of:

- 10 *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-**;
- *-(CH₂-CH₂-O)₂₃-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₁₆-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₈-CH₂-CH₂-**;
- 15 *-C(O)-C(CH₃)₂-CH₂-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-NH-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;



- 20 *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**; and
- *-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**;

the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical.

25

Among the compounds of formula (I2) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

A is of formula (A) in which:

R₁ represents:

- 5 - a (C₁-C₆)alkyl- group, in particular a methyl group;
- a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:
- 10 - a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted) or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:
 - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group,
 - 15 for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or
- 20 - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

R₂ represents :

- 25 - a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;
- a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

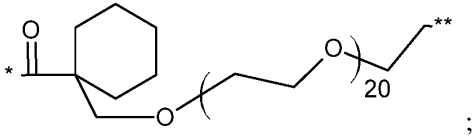
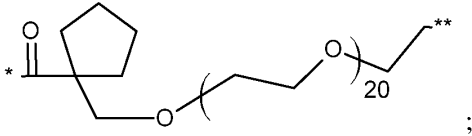
R₃ represents :

- a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that
- 30 is to say a -O-CH(CH₃)₂ group);
- a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms

selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperaziny group; or

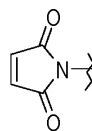
- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperaziny group;

*(P)o-L-Ld-** represents:

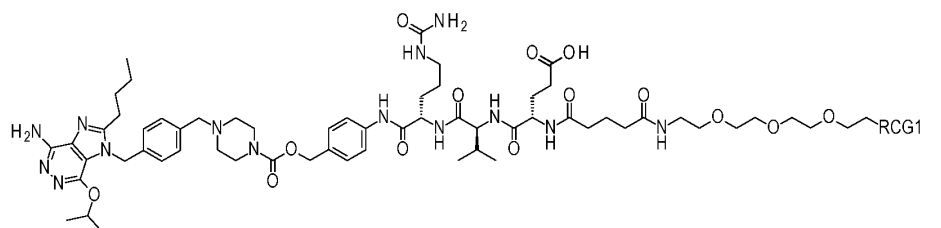
- *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-**;
 *-(CH₂-CH₂-O)₂₃-CH₂-CH₂-**;
 *-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
 *-C(O)-(CH₂-CH₂-O)₁₆-CH₂-CH₂-**;
 *-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-**;
 *-C(O)-(CH₂-CH₂-O)₈-CH₂-CH₂-**;
 *-C(O)-C(CH₃)₂-CH₂-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
 *-C(O)-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
 *-C(O)-NH-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
;
;
 *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**;
 *-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**; or
 *-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-NH-C(O)-CH₂-CH₂-**;

the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the -RCG1 group; and

- RCG1 is N₃-, a -COOH group or a maleimido group

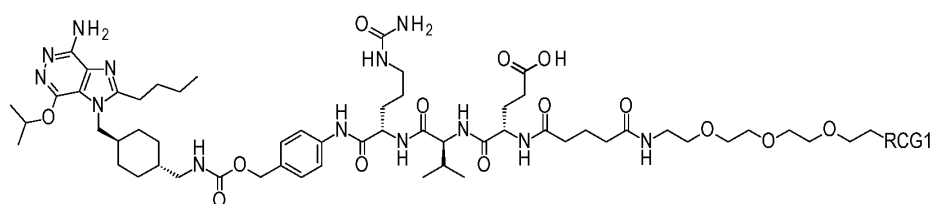


Among the compounds/payloads of formulae (I), (II) or (I2) or a pharmaceutically acceptable salt thereof that are subject matter of the present disclosure, mention may be made for instance to the following compounds:

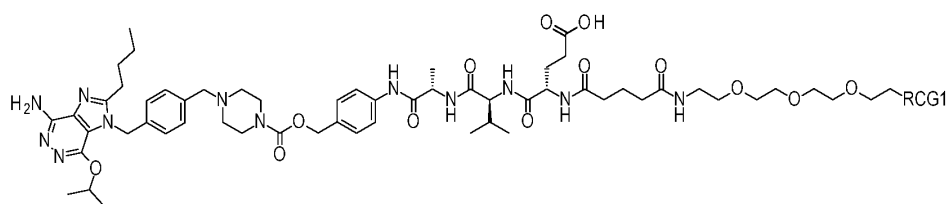


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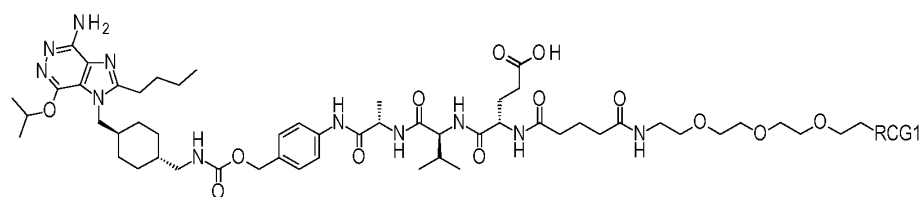
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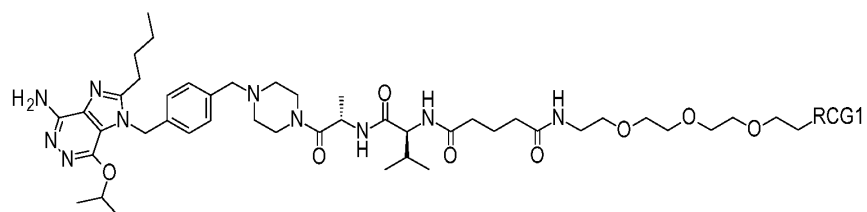


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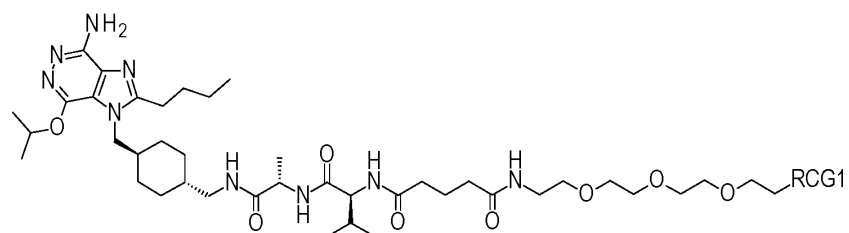


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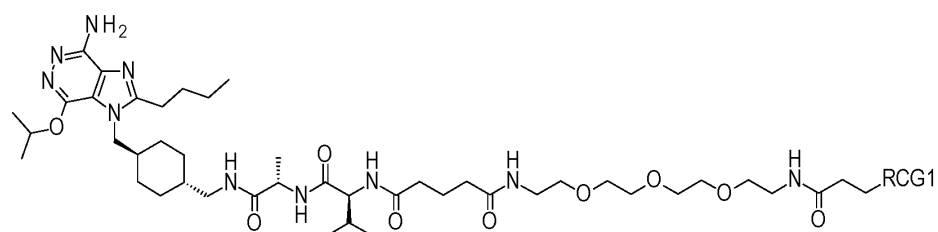
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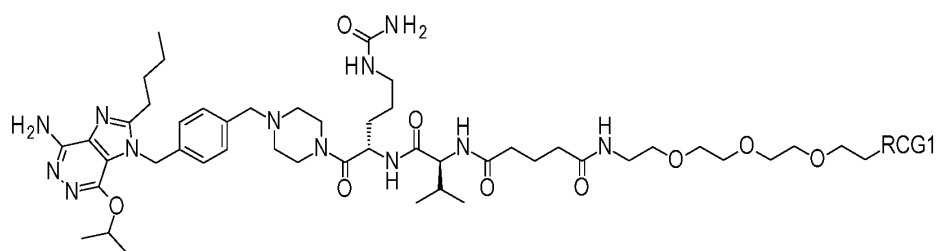
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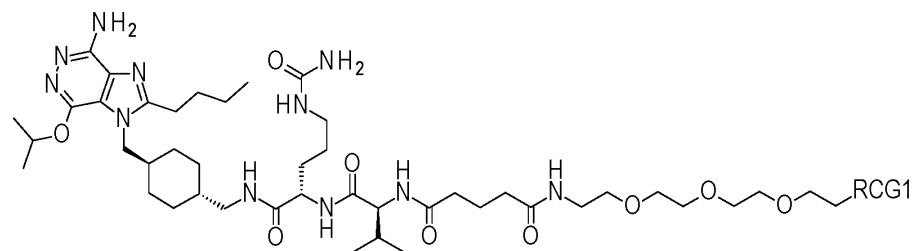


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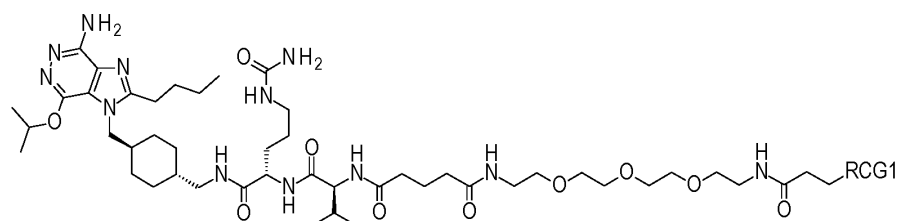


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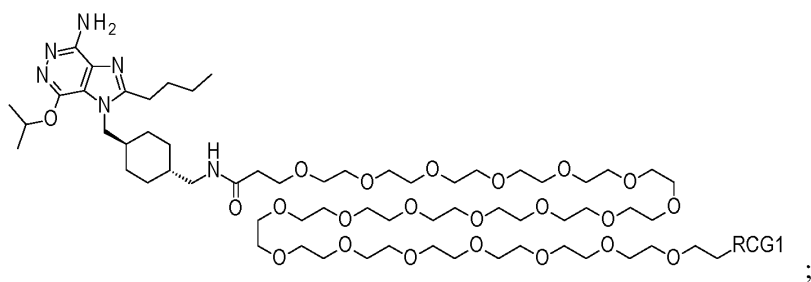
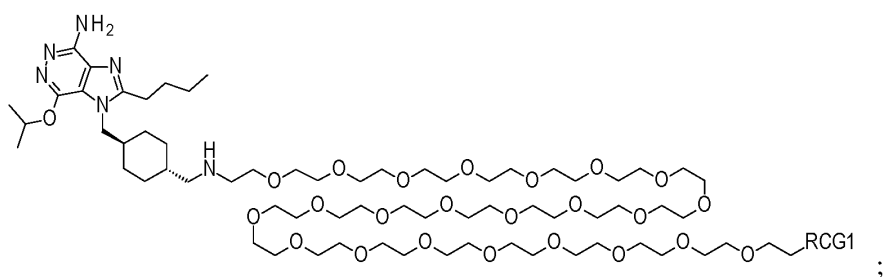
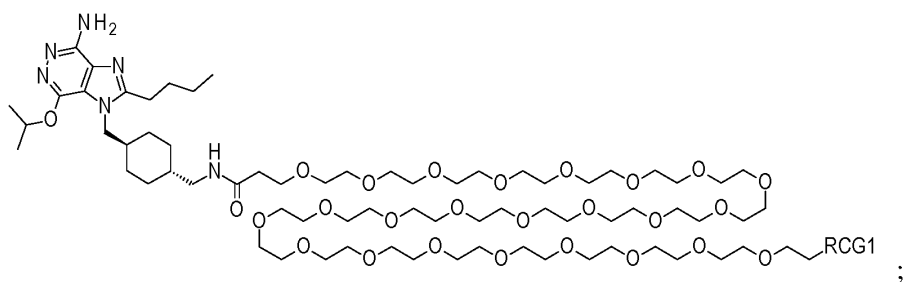
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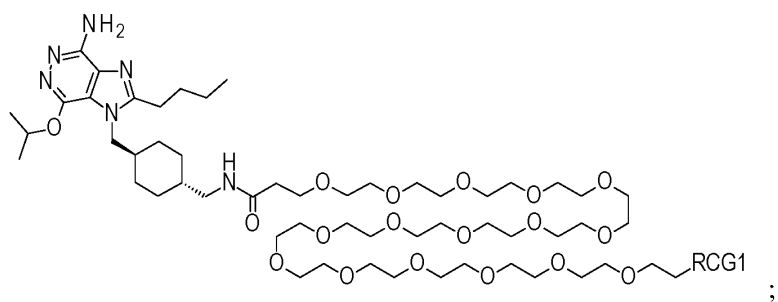
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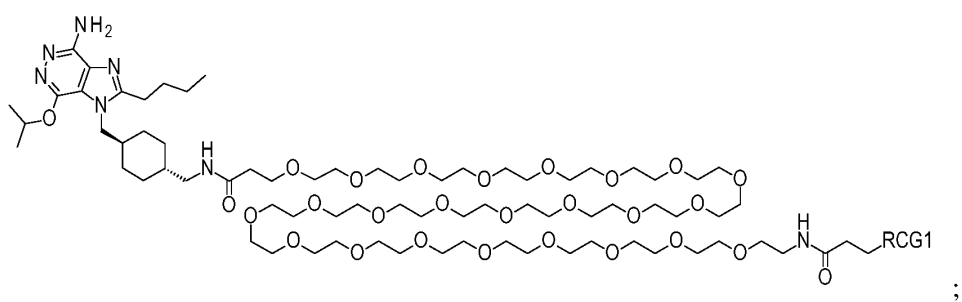
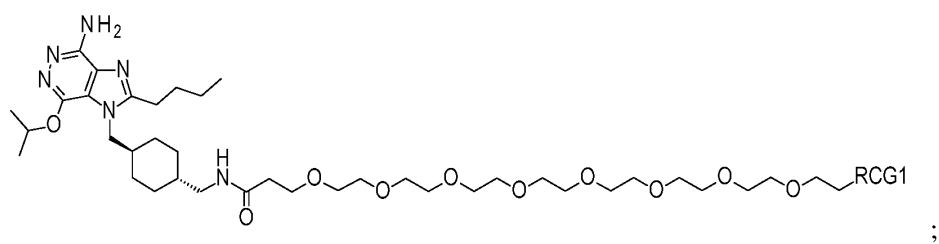
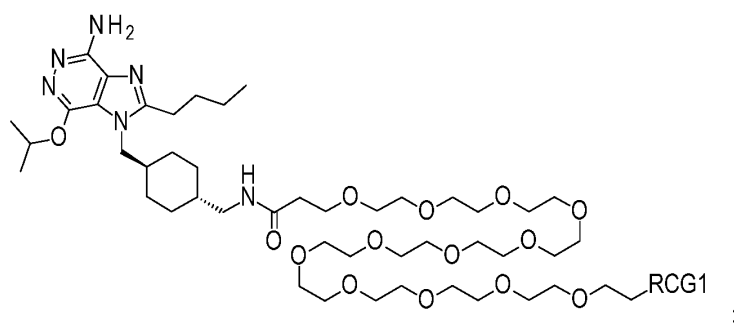


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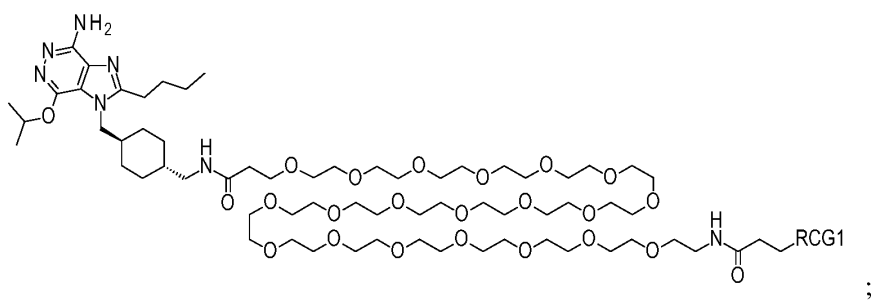


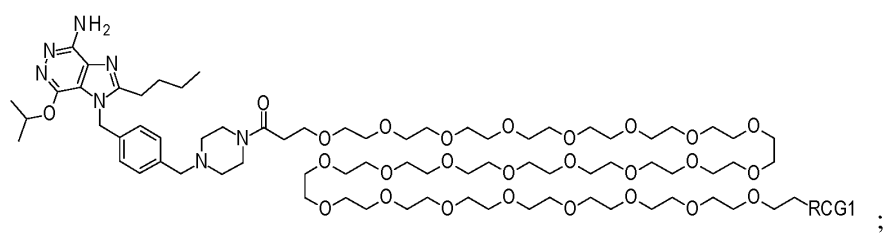
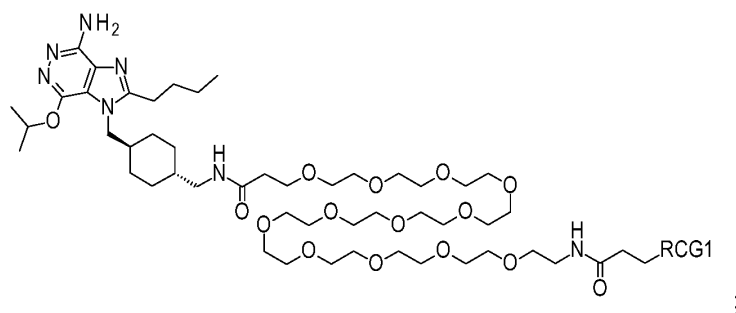
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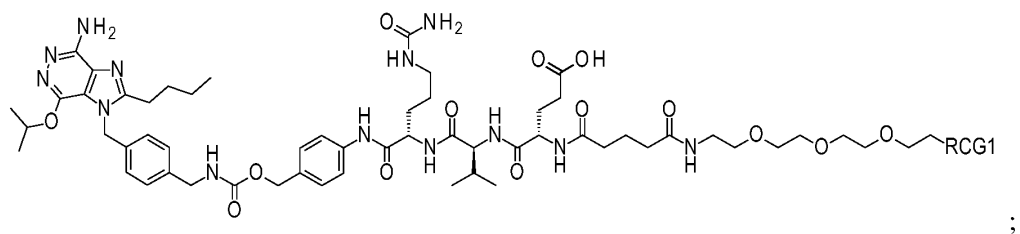
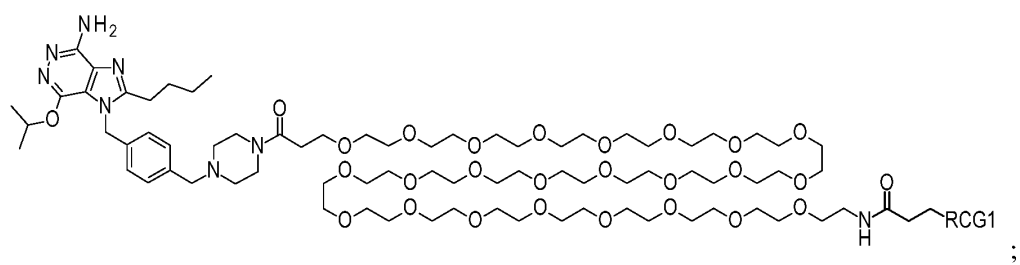
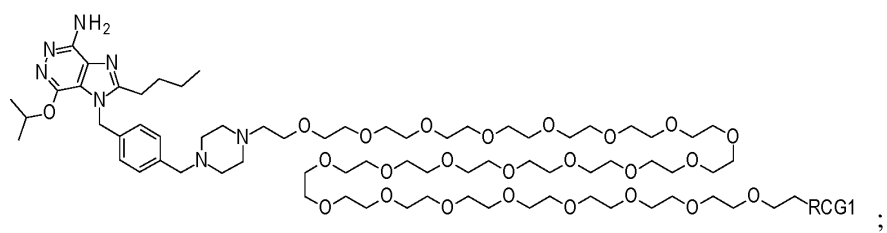


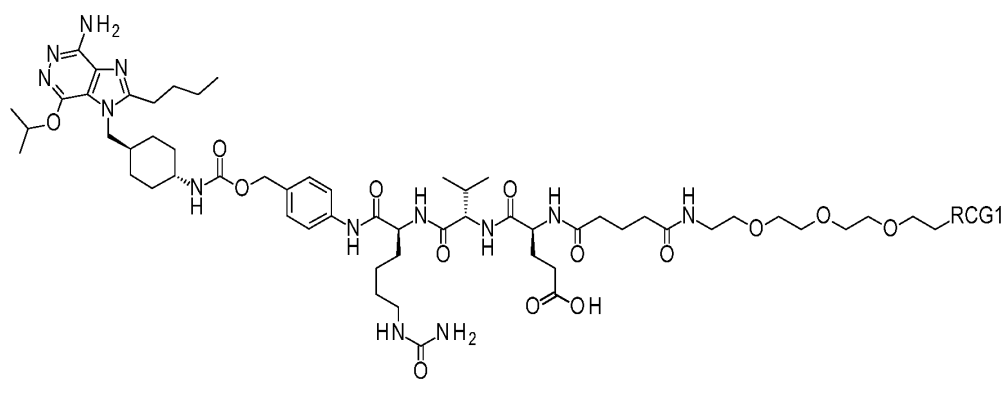
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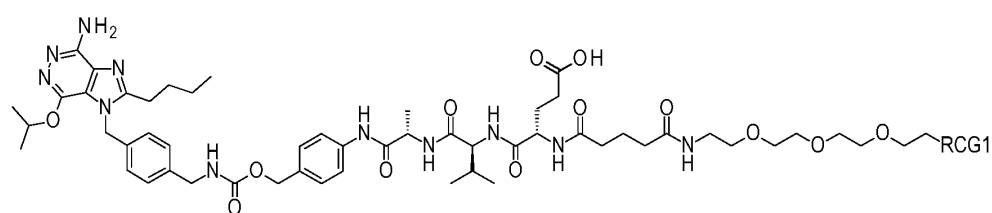


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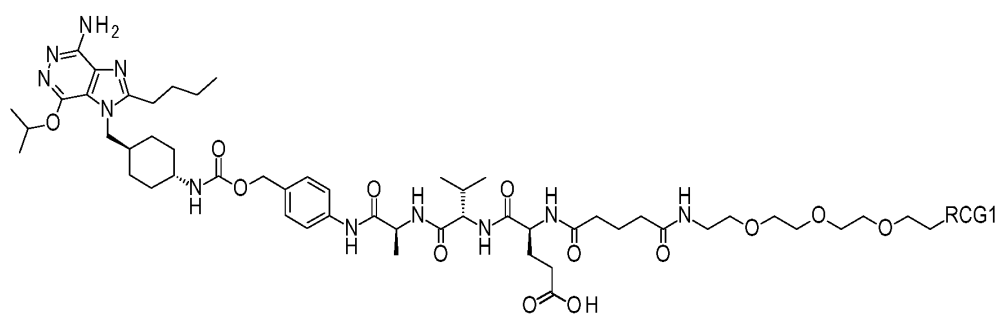




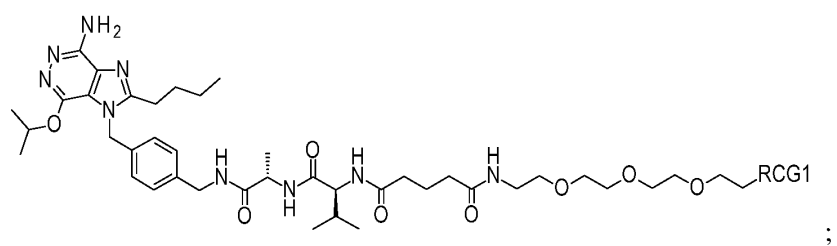
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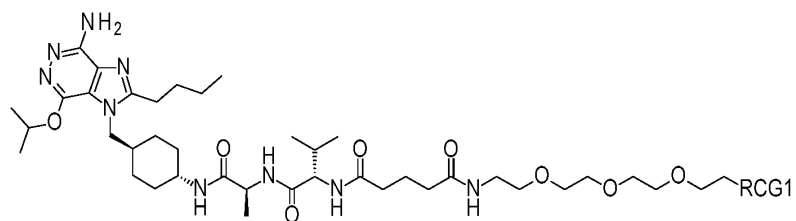
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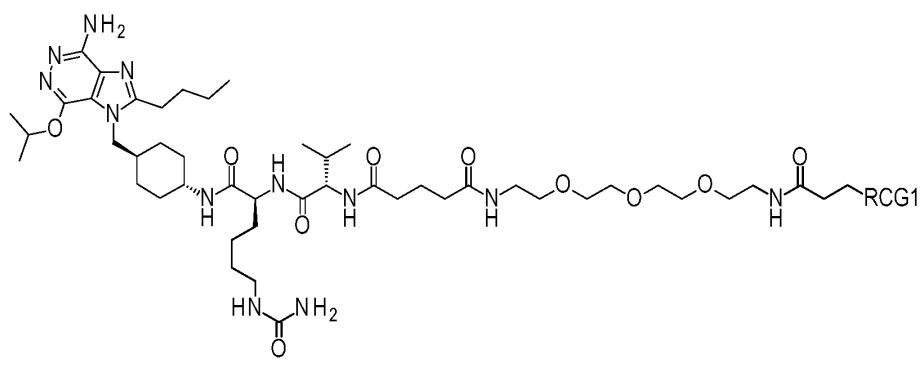
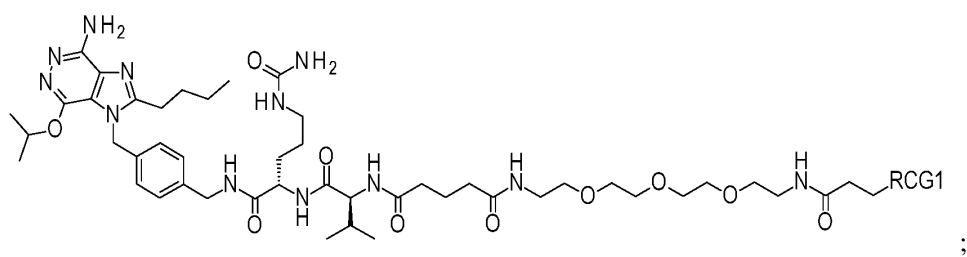
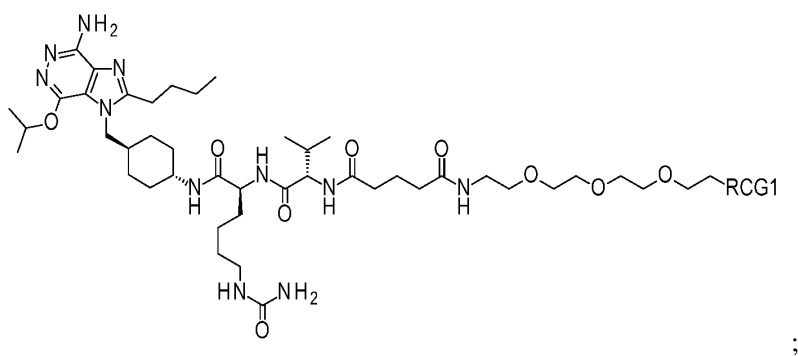
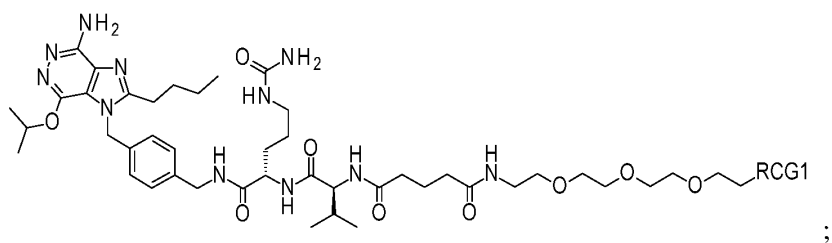
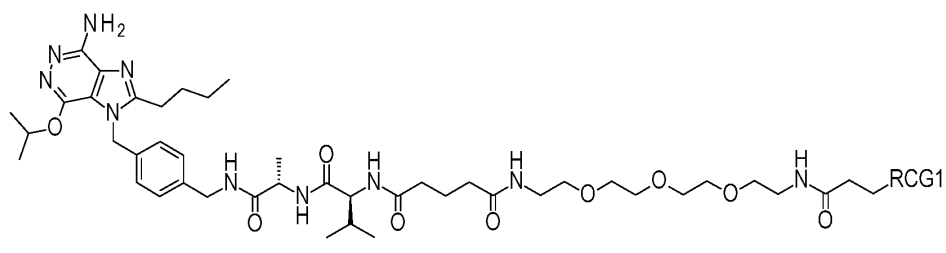
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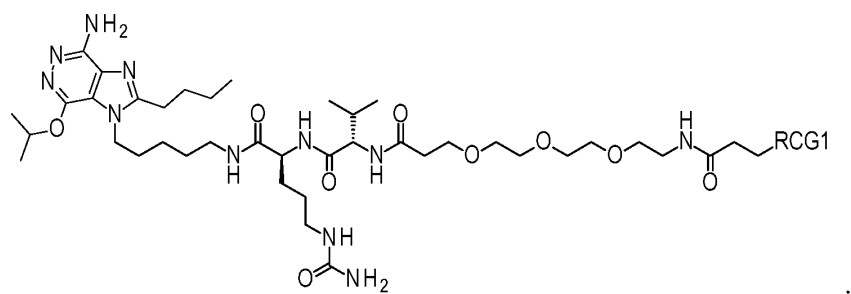
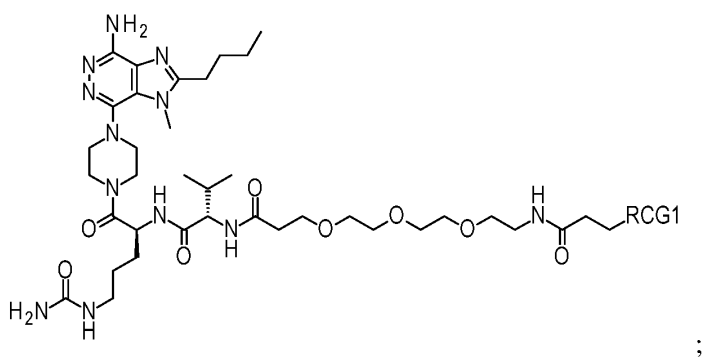
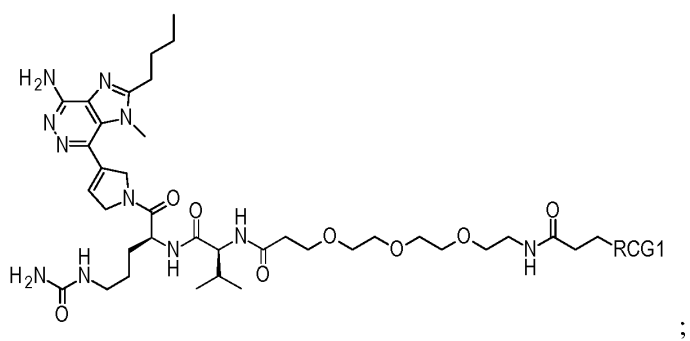
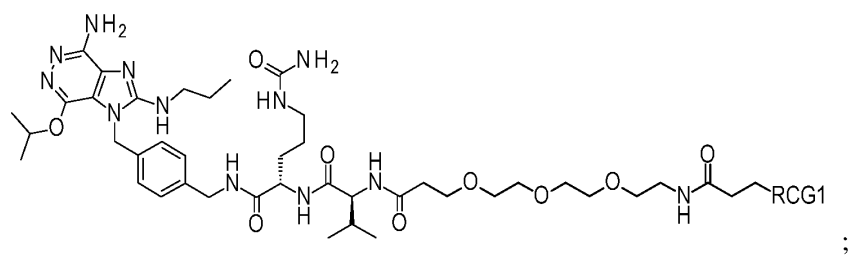
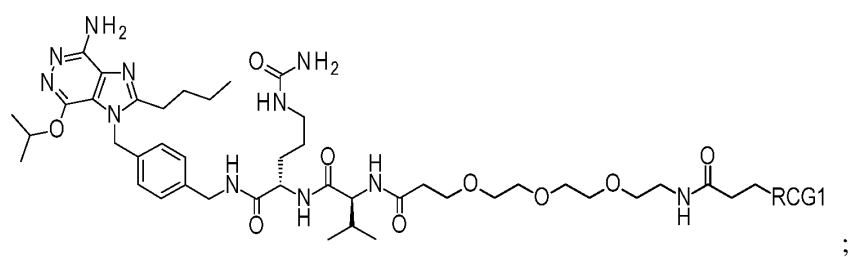


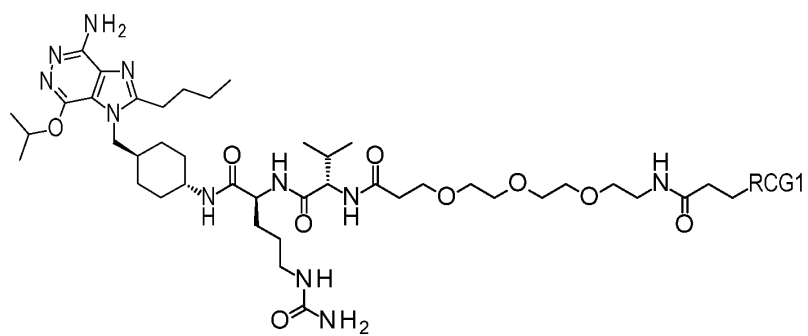
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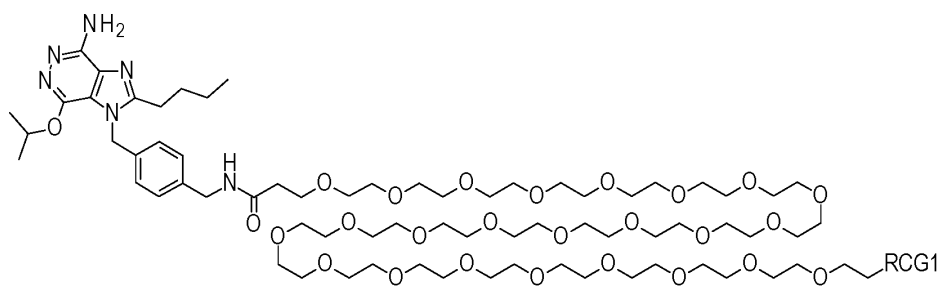
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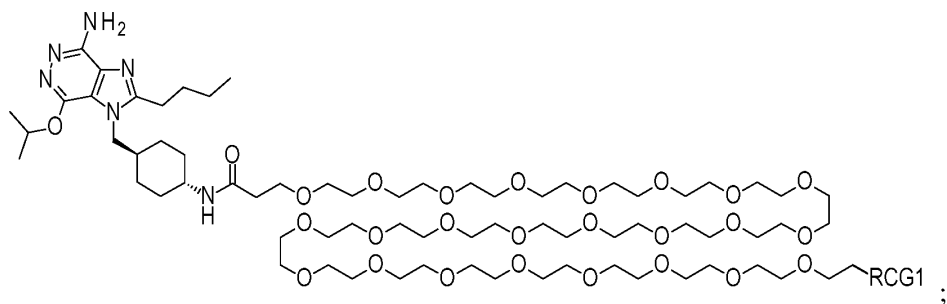




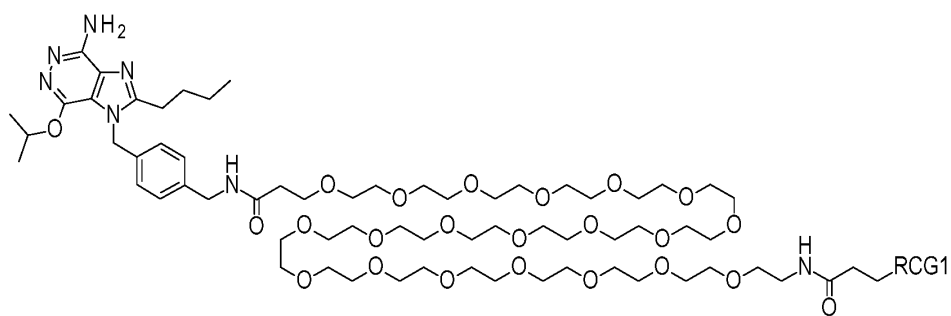
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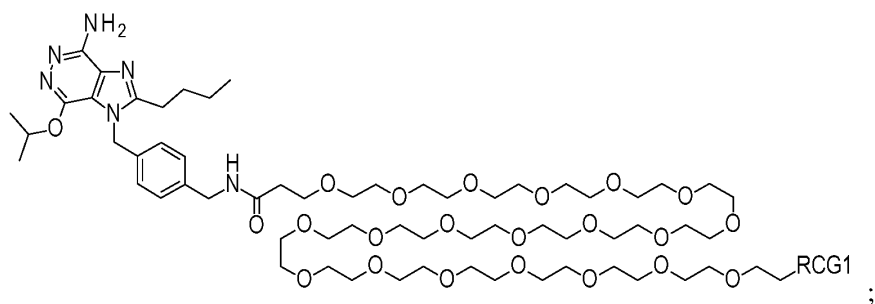
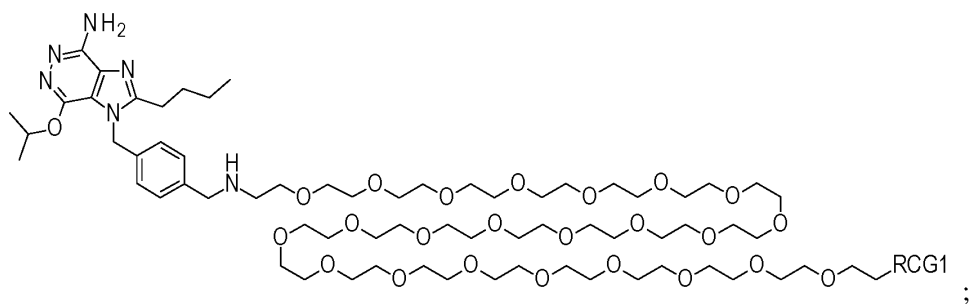
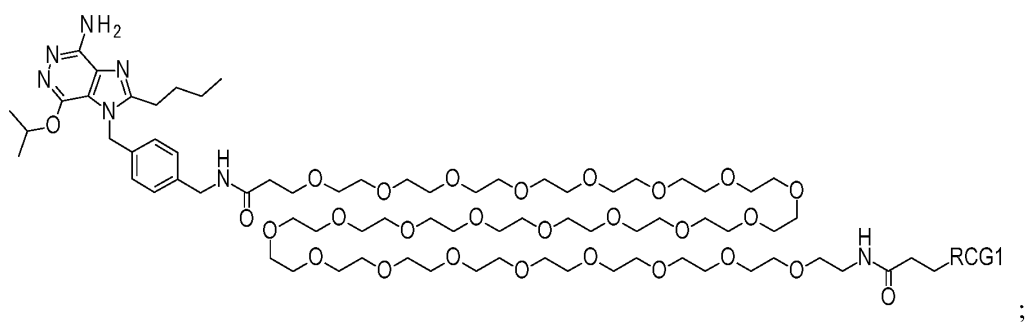
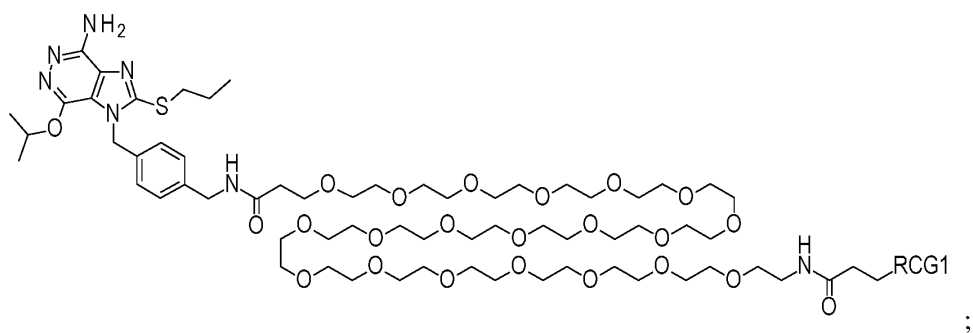
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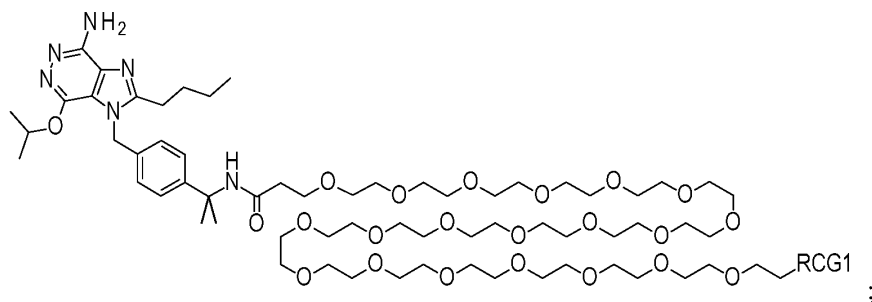
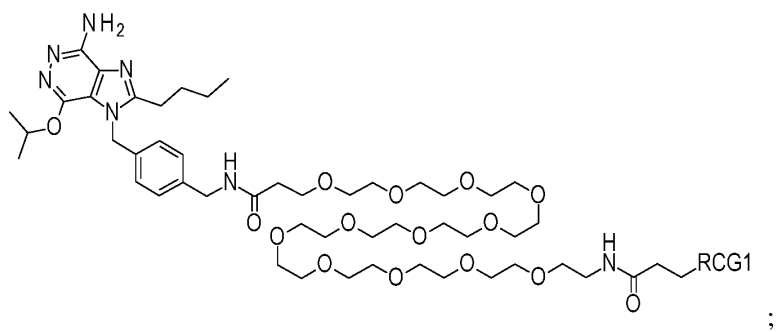
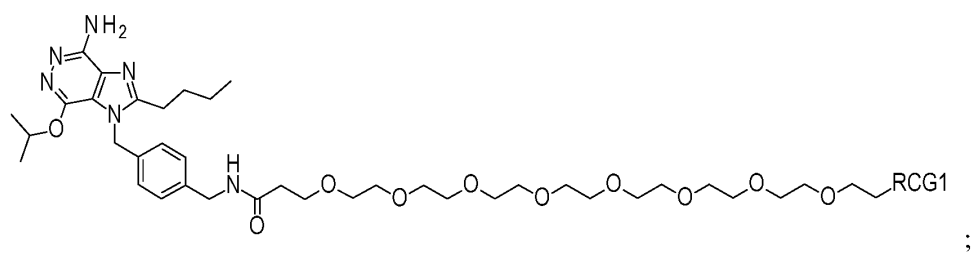
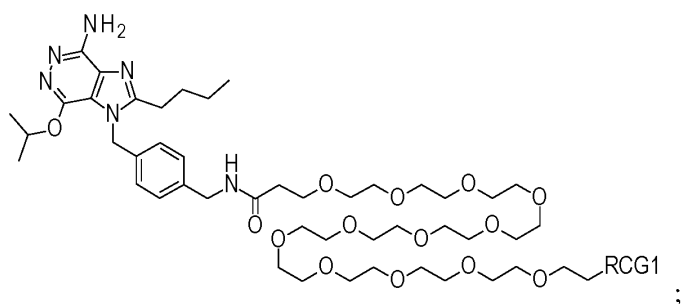
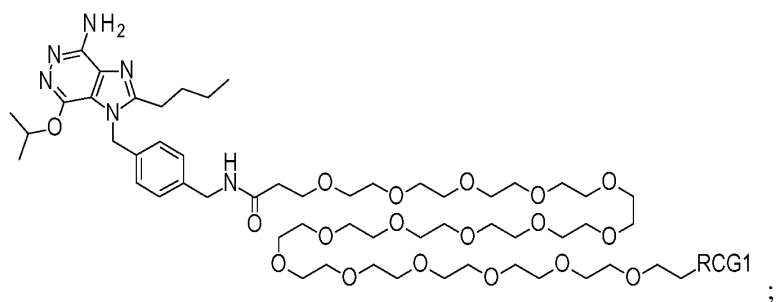


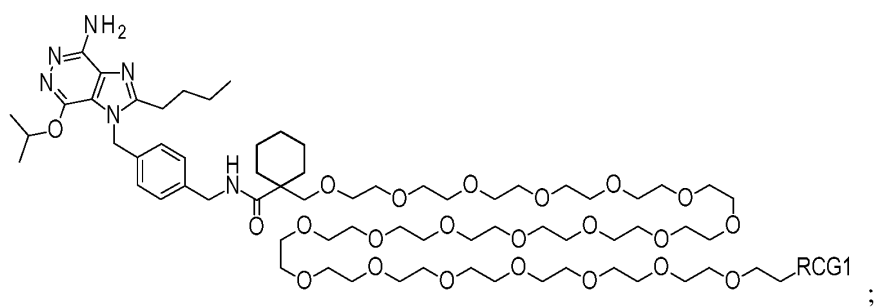
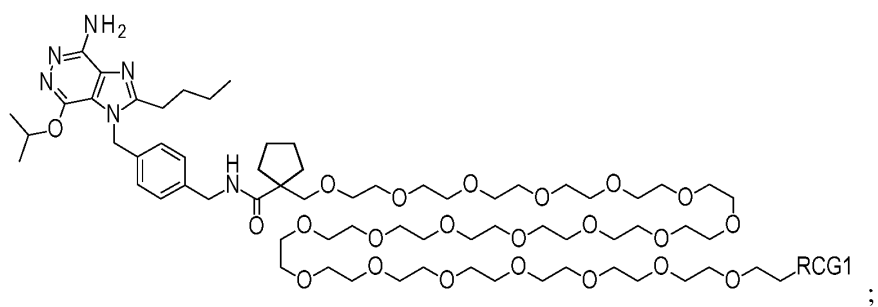
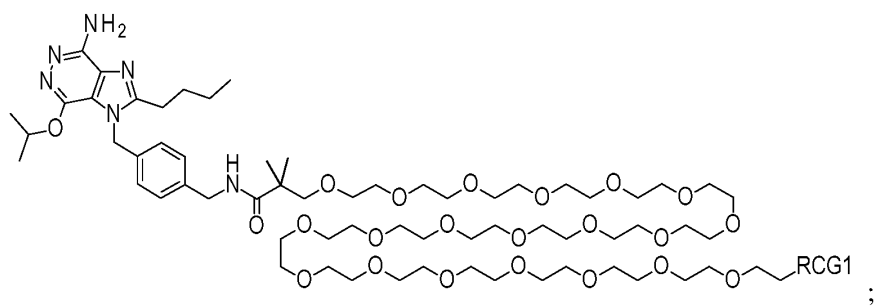
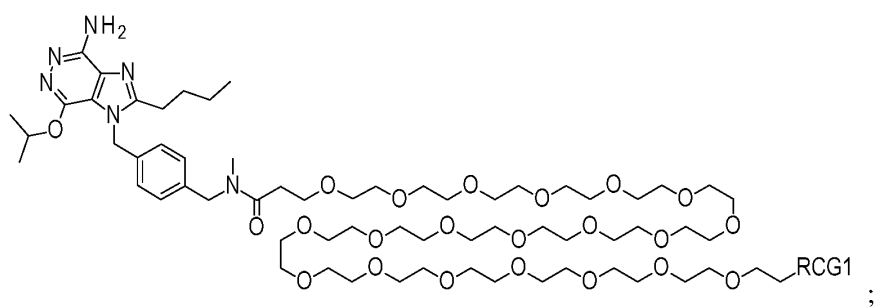
;

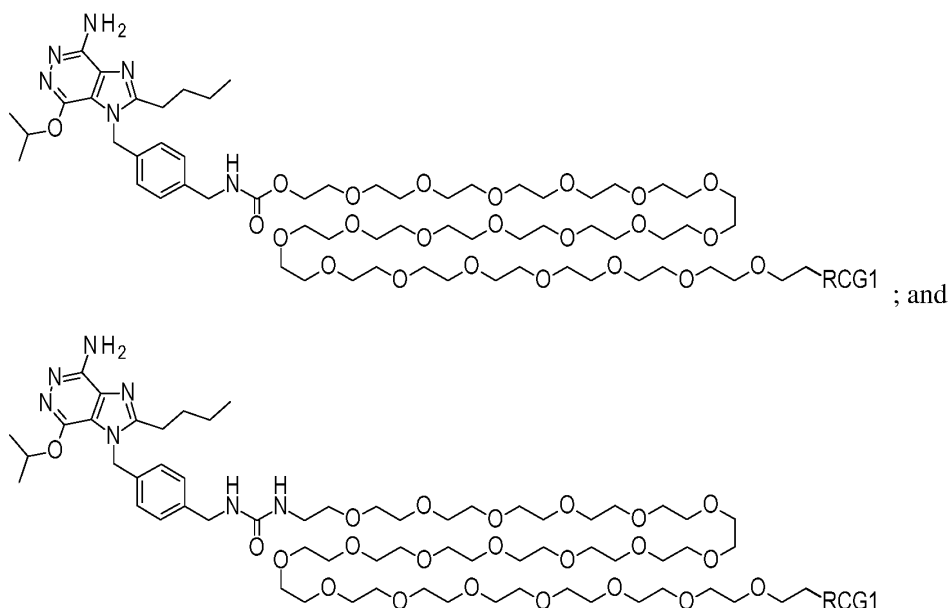


;









Among the compounds/payloads of formulae (I), (II) or (I2) or a pharmaceutically acceptable salt thereof that are subject matter of the present disclosure, mention may be made for instance to the following compounds:

(P1) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazine-1-carbonyl]oxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P2) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]carbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P3) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazine-1-carbonyl]oxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid;

(P26) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]carbamoyloxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid;

(P27) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]carbamoyloxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid;

(P28) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

(P29) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]amino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

(P30) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide;

(P31) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

(P32) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]amino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate;

(P33) N'-[(2S)-1-[[[(2S)-1-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate;

(P56) N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclopentanecarboxamide;

(P57) N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclohexanecarboxamide;

(P58) 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]carbamate;

(P59) 1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]urea;

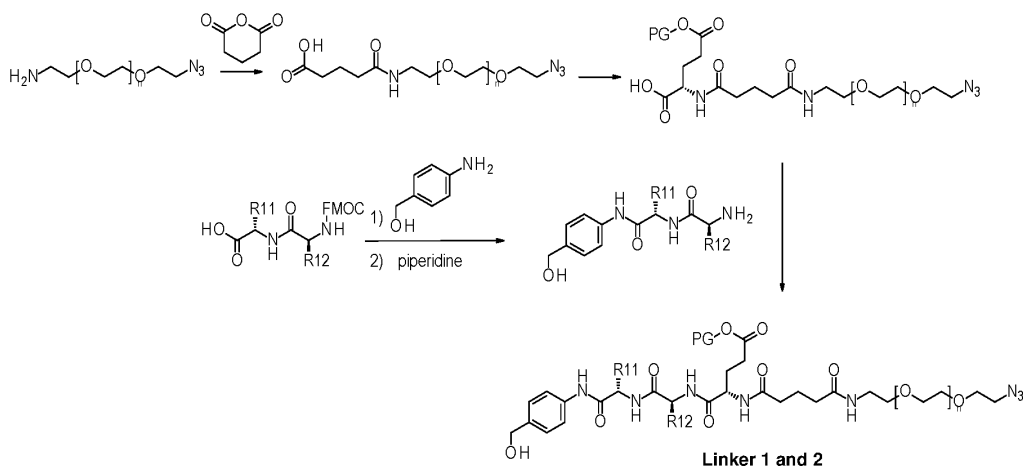
or a pharmaceutically acceptable salt thereof.

The compounds/payloads of formulae (I), (II) or (I2) or a pharmaceutically acceptable salt thereof according to the present disclosure can be prepared according to any process known by the skilled person, and for instance by the following processes.

25 Synthesis of the compounds/payloads of formulae (I) and (II):

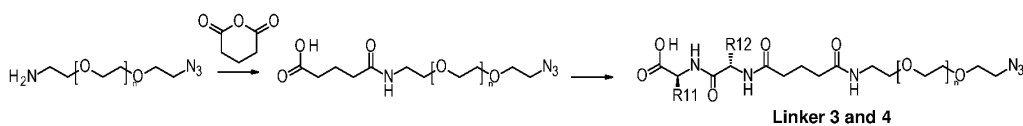
- Synthesis of linkers

a) Synthetic scheme of linker types 1 and 2

**Scheme 1**

In scheme 1, PG represents a carboxylic acid protecting group as defined in the present disclosure; n is as defined in the present disclosure; and R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure.

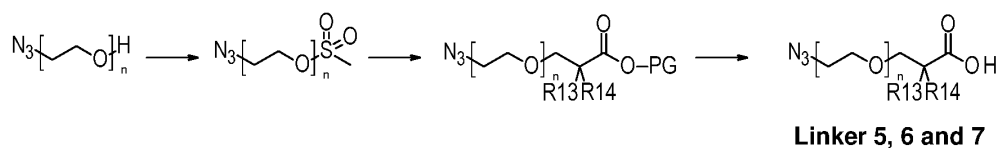
b) Synthetic scheme of linker types 3 and 4

**Scheme 2**

10

In scheme 2, R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure; and n is as defined in the present disclosure.

c) Synthetic scheme of linker types 5, 6 and 7

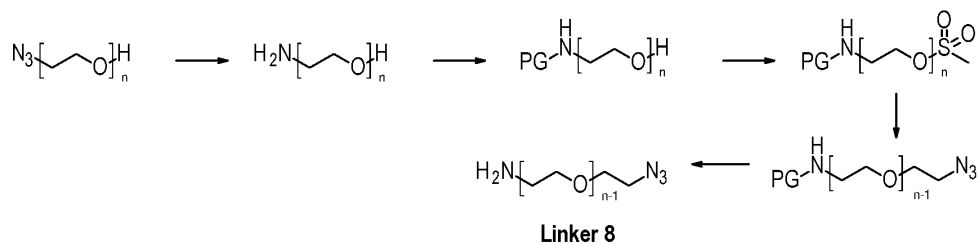
**Scheme 2a**

15

In scheme 2a, R13 and R14 represent, independently of each other, a $-(C_1-C_6)$ alkyl group or alternatively R13 and R14 form together with the carbon atom to which they are attached a

-(C₃-C₆)cycloalkyl group or a -(C₃-C₆)heterocycloalkyl group as defined in the present disclosure; and n is as defined in the present disclosure.

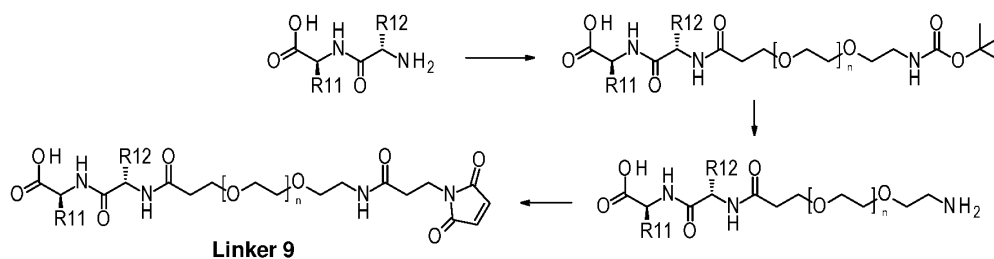
d) Synthetic scheme of linker type 8



Scheme 2b

In scheme 2b, n is as defined in the present disclosure.

e) Synthetic scheme of linker type 9



Scheme 2c

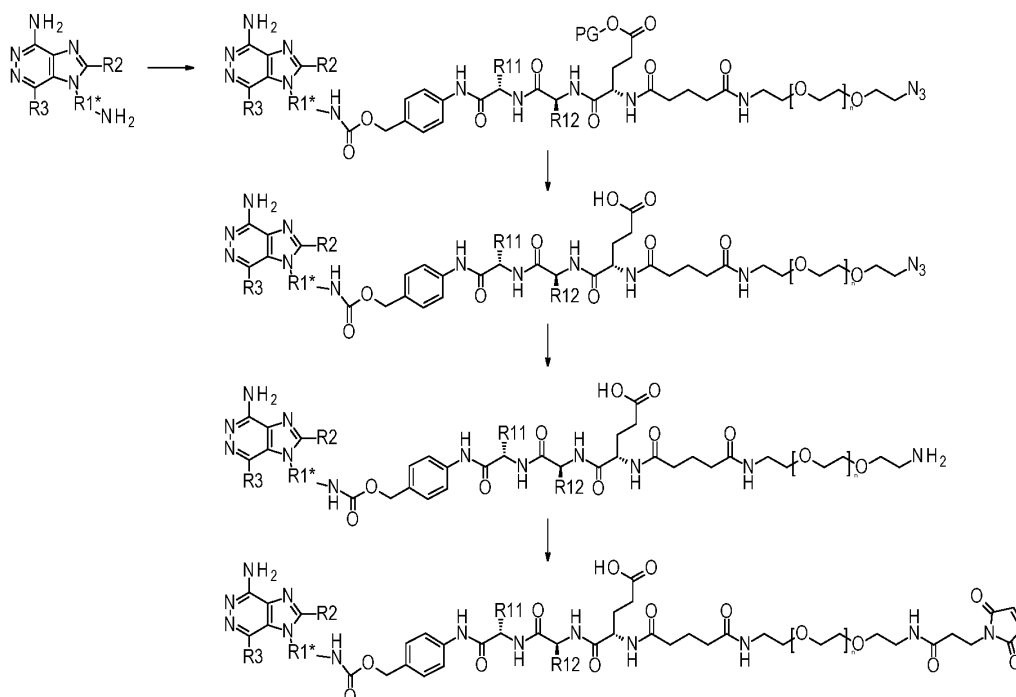
In scheme 2c, R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure; and n is as defined in the present disclosure.

15 - Synthesis of Payloads

Synthesis of the compounds/payloads of formulas (I) and (II)

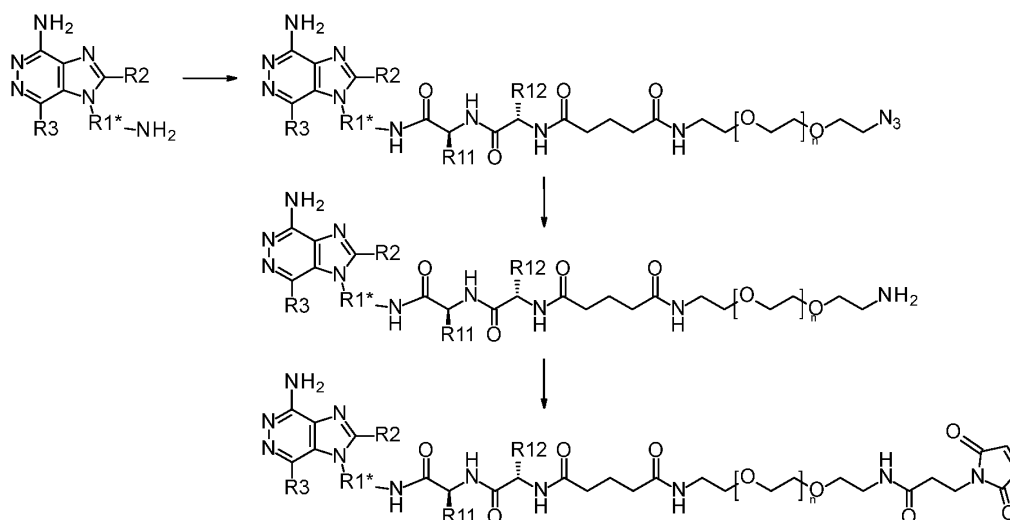
a) Synthetic scheme of payloads with CL (cleavable) linker types

1 and 2

**Scheme 3**

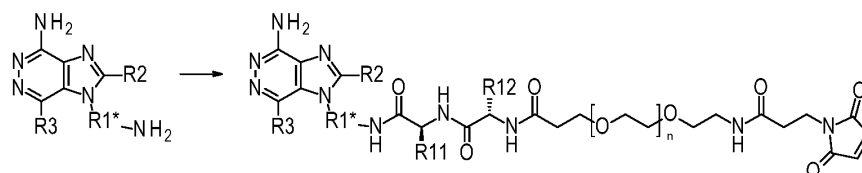
In scheme 3, $\text{-R}_1^*\text{-NH}_2$ corresponds to -R_1 as defined in the present disclosure provided that R_1 comprises a NH_2 group; R_2 and R_3 are as defined in the present disclosure; n is as defined in the present disclosure; R_{11} and R_{12} represent, independently of each other, the side chain of AA as defined in the present disclosure; and PG represents a carboxylic acid protecting group as defined in the present disclosure.

b) Synthetic scheme of payloads with CL linker types 3 and 4

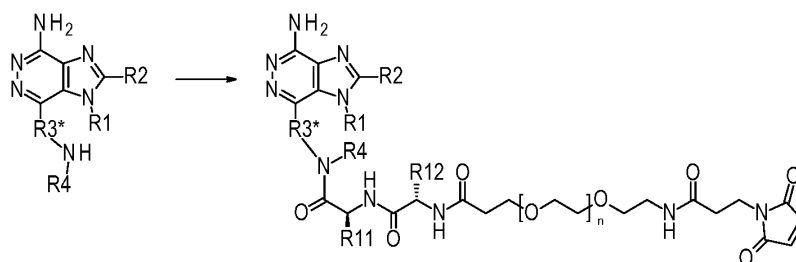
**Scheme 4**

In scheme 4, -R1*-NH₂ corresponds to -R1 as defined in the present disclosure provided that R1 comprises a NH₂ group; n is as defined in the present disclosure; R2 and R3 are as defined in the present disclosure; and R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure.

c) Synthetic scheme of payloads with CL linker type 9

**Scheme 4a**

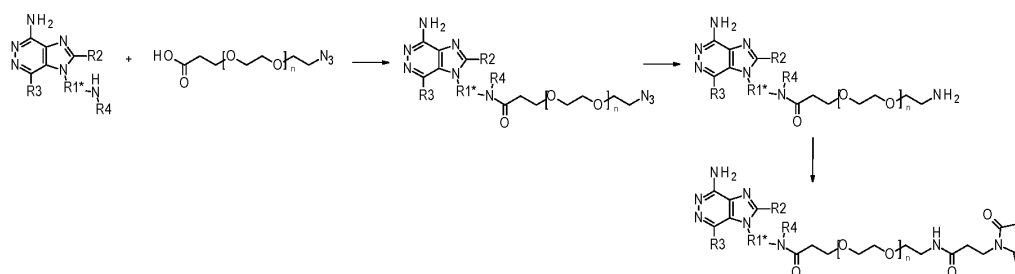
In scheme 4a, -R1*-NH₂ corresponds to -R1 as defined in the present disclosure provided that R1 comprises a NH₂ group; n is as defined in the present disclosure; R2 and R3 are as defined in the present disclosure; and R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure.

**Scheme 4b**

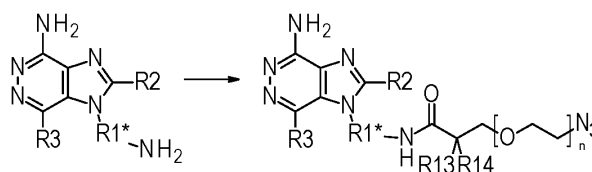
In scheme 4b, -R3*-NH-R4 corresponds to -R3 as defined in the present disclosure provided that R3 comprises a -NH-R4 group, R4 being as defined in the present disclosure; n is as defined in the present disclosure; R1 and R2 are as defined in the present disclosure; and R11 and R12 represent, independently of each other, the side chain of AA as defined in the present disclosure.

Synthesis of the compounds/payloads of formulas (I) and (I2):

10 a) Synthetic scheme of payloads with NCL (non - cleavable) amide linkers

**Scheme 1'**

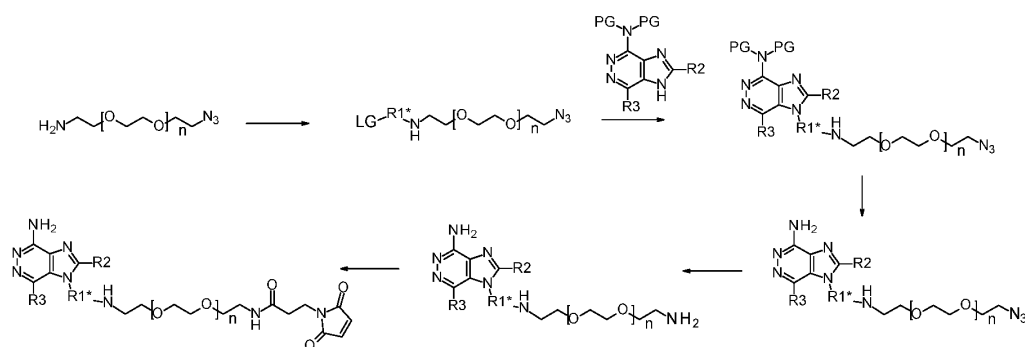
In scheme 1', -R1*-NH-R4 corresponds to -R1 as defined in the present disclosure, provided that -R1 comprises a -NH-R4 group, R4 being as defined in the present disclosure; n is as defined in the present disclosure; and R2 and R3 are as defined in the present disclosure.

**Scheme 1'a**

In scheme 1'a, -R1*-NH-R4 corresponds to -R1 as defined in the present disclosure,

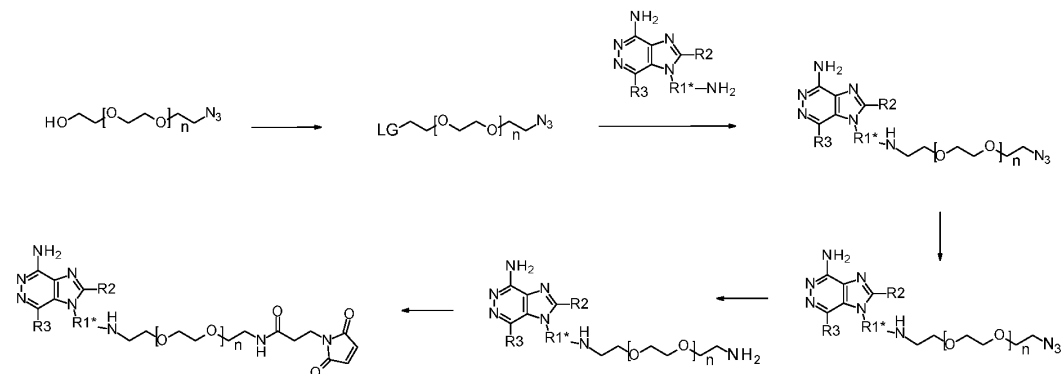
provided that -R1 comprises a -NH-R4 group, R4 being as defined in the present disclosure; n is as defined in the present disclosure; and R2 and R3 are as defined in the present disclosure.

5 **b) Synthetic scheme of payloads with NCL (non - cleavable) alkyl linkers**



Scheme 2'

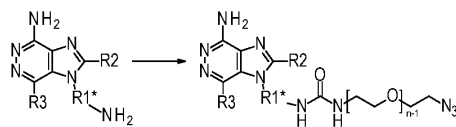
In scheme 2', -R1*-NH- corresponds to -R1 provided that R1 as defined in the present disclosure comprises a NH₂ group; n is as defined in the present disclosure; R2 and R3 are as defined in the present disclosure; LG represents a leaving group as defined in the present disclosure; and PG represents an amino protecting group as defined in the present disclosure.



Scheme 2''

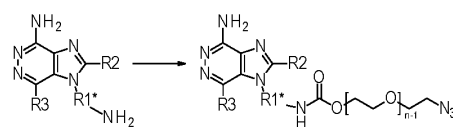
In scheme 2'', -R1*-NH- corresponds to -R1 as defined in the present disclosure provided that R1 comprises a NH₂ group; n is as defined in the present disclosure; R2 and R3 are as defined in the present disclosure; and LG represents a leaving group as defined in the present disclosure.

c) Synthetic scheme of payloads with NCL (non - cleavable) carbamate linkers

**Scheme 2''a**

In scheme 2''a, -R1*-NH₂ corresponds to -R1 as defined in the present disclosure, provided that -R1 comprises a -NH₂ group; n is as defined in the present disclosure; and R2 and R3 are as defined in the present disclosure.

d) Synthetic scheme of payloads with NCL (non-cleavable) urea linkers type 8

**Scheme 2''b**

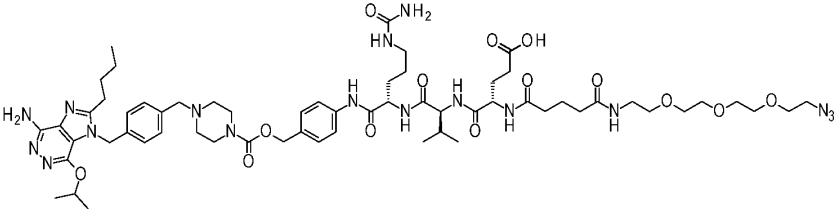
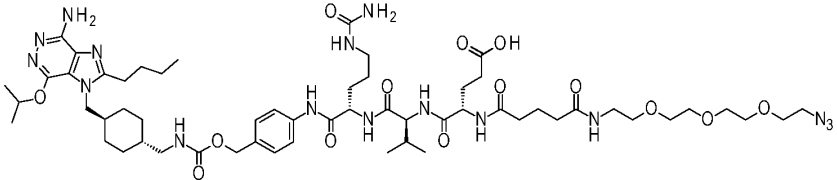
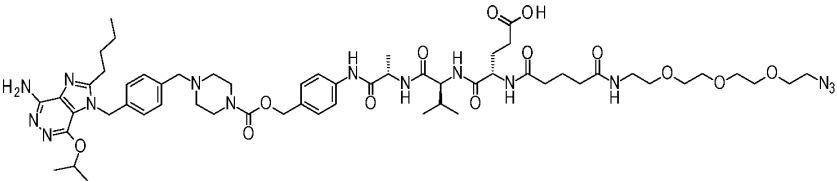
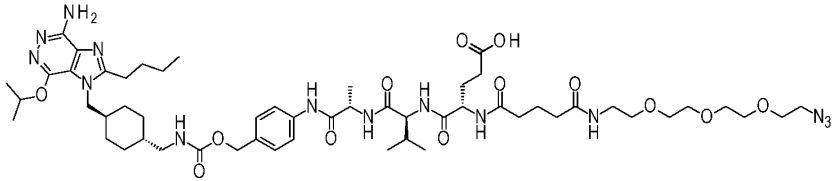
In scheme 2''b, -R1*-NH₂ corresponds to -R1 as defined in the present disclosure, provided that -R1 comprises a -NH₂ group; n is as defined in the present disclosure; and R2 and R3 are as defined in the present disclosure.

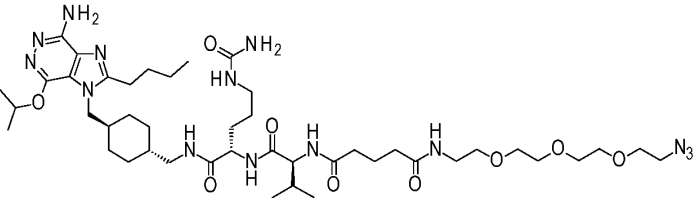
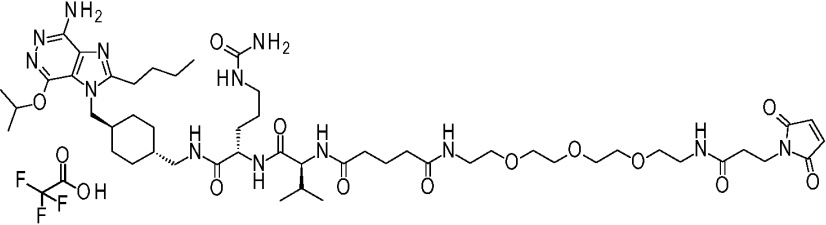
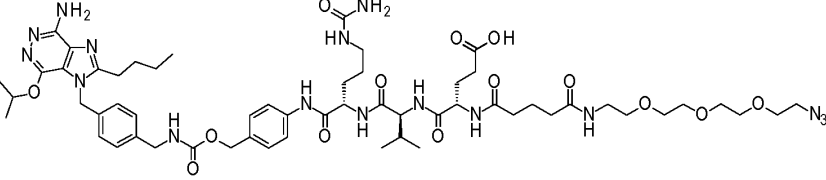
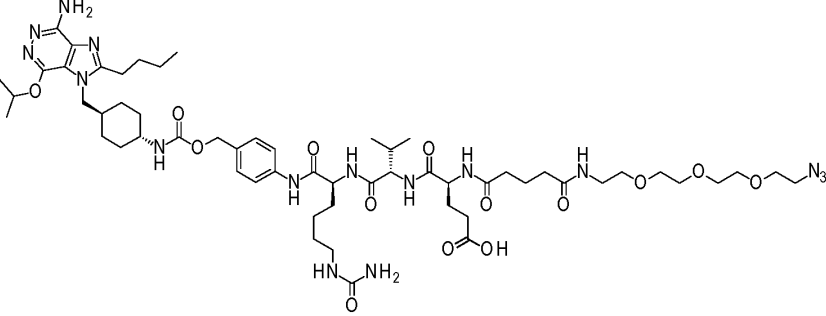
The specific compounds/payloads of formulae (I), (I1) and (I2) as defined in the present disclosure are indicated in Table 1 and Table 1' (number, and formula) and are further detailed hereafter. In Table 2 and Table 2', ¹H NMR, and liquid chromatography/mass spectra are also indicated.

The ¹H NMR of Table 2 and Table 2' is ¹H NMR Spectra (400 MHz, δ in ppm, DMSO-d₆ or CDCl₃) as defined in the Experimental part.

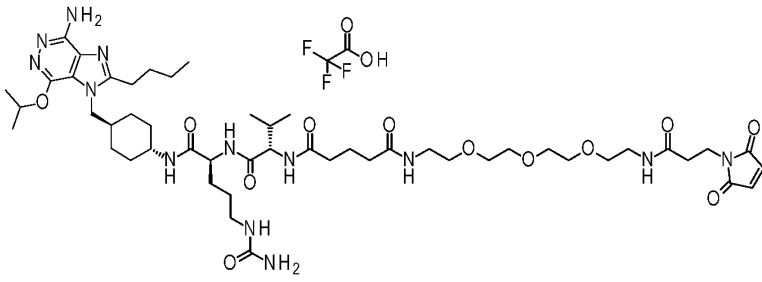
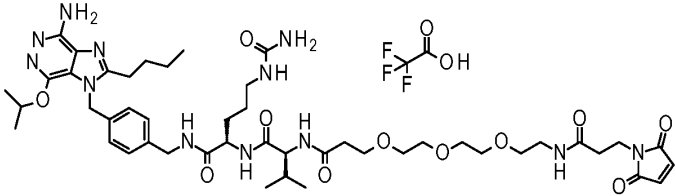
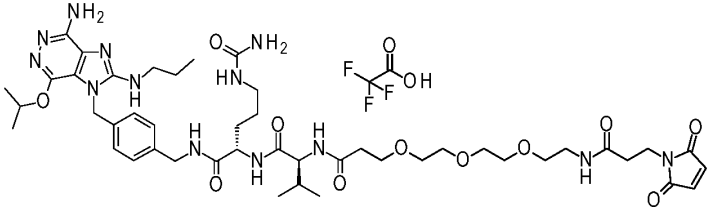
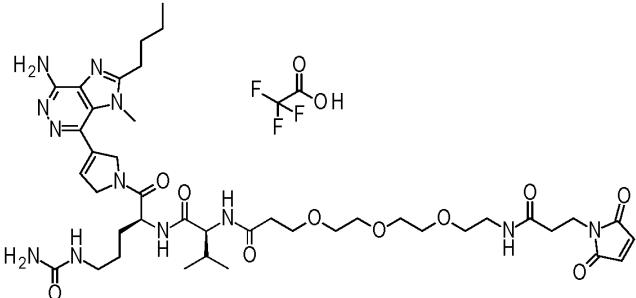
The liquid chromatography/mass spectra (LC/MS) of Table 2 and Table 2' were obtained according to one of the four methods described in the Experimental part.

Table 1:

Compound Number (P for payload)	Formula
P1	
P2 Example 1	
P3	
P4 Example 2	

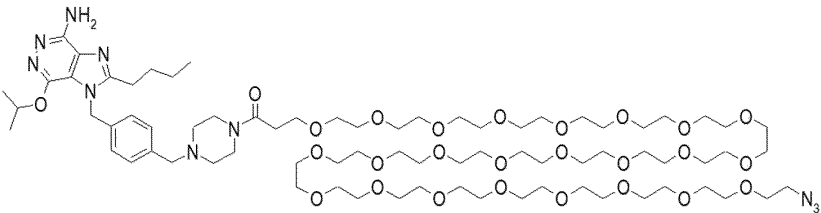
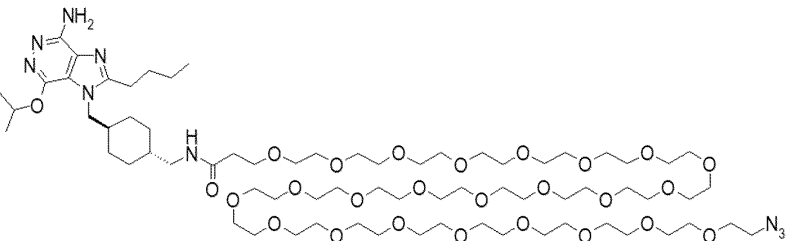
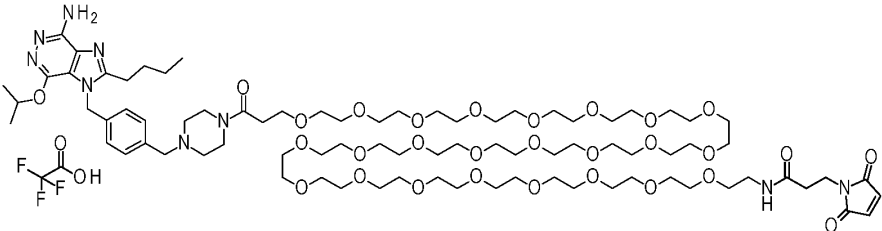
Compound Number (P for payload)	Formula
P9 Example 5	 Chemical structure of P9 Example 5: A complex molecule featuring a purine core substituted with an isopropoxy group, a propyl group, and a cyclohexylmethyl group. The cyclohexylmethyl group is linked via a chiral amide bond to a chain containing a guanidino group, a chiral amide, and a terminal azide group.
P10 Example 6	 Chemical structure of P10 Example 6: Similar to P9, but the terminal azide group is replaced by a pyridine ring substituted with a trifluoromethyl group and a carboxylic acid group.
P24 Example 41	 Chemical structure of P24 Example 41: The purine core is substituted with an isopropoxy group, a propyl group, and a benzyl group. The benzyl group is linked via a chiral amide bond to a chain containing a guanidino group, a chiral amide, and a terminal azide group.
P25	 Chemical structure of P25: The purine core is substituted with an isopropoxy group, a propyl group, and a cyclohexylmethyl group. The cyclohexylmethyl group is linked via a chiral amide bond to a chain containing a guanidino group, a chiral amide, and a terminal azide group. Additionally, there is a carboxylic acid group and a guanidino group on the chain.

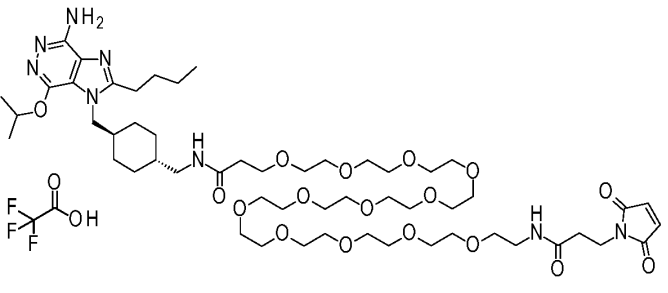
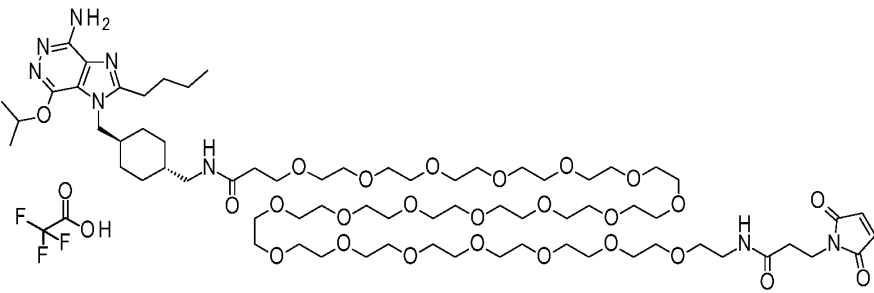
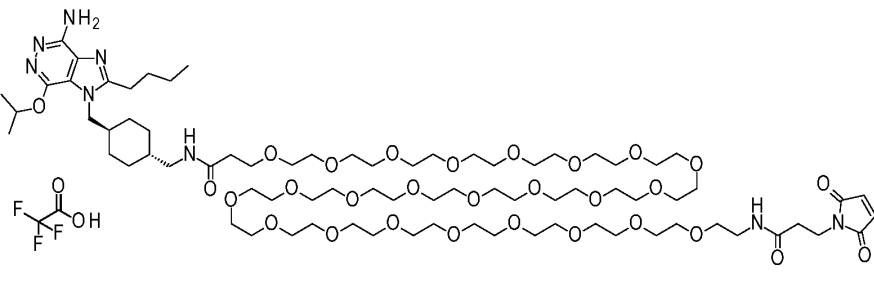
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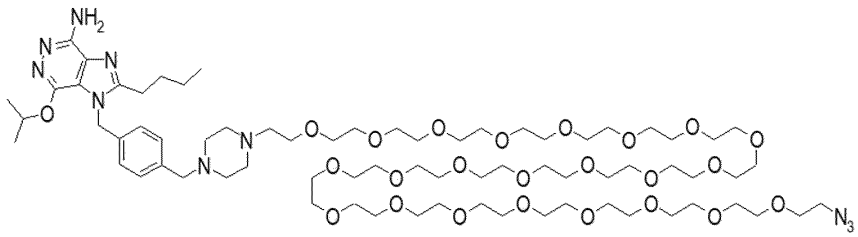
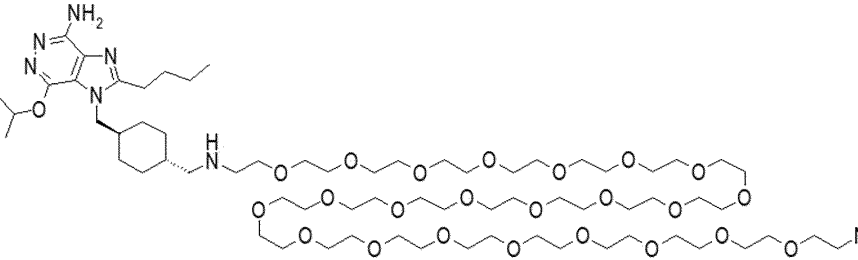
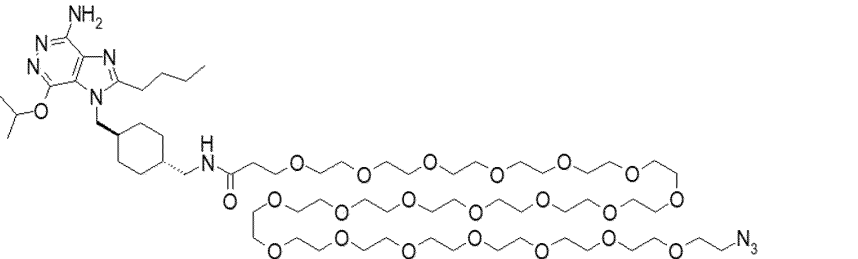
Compound Number (P for payload)	Formula
P34	 Chemical structure of P34: A complex molecule featuring a purine core substituted with an isopropoxy group and a propyl group. The purine is linked via a cyclohexylmethyl group to a chain containing an amide, a chiral center with a methyl group, and a primary amide. This chain is further linked to a long polyether chain (tetraethylene glycol) which terminates in a succinimide ring. A trifluoroacetic acid (TFA) molecule is shown as a counterion.
P35 Example 47	 Chemical structure of P35: A complex molecule featuring a purine core substituted with an isopropoxy group and a propyl group. The purine is linked via a benzyl group to a chain containing an amide, a primary amide, and a chiral center with a methyl group. This chain is further linked to a long polyether chain (tetraethylene glycol) which terminates in a succinimide ring. A trifluoroacetic acid (TFA) molecule is shown as a counterion.
P36	 Chemical structure of P36: A complex molecule featuring a purine core substituted with an isopropoxy group and a propyl group. The purine is linked via a benzyl group to a chain containing an amide, a primary amide, and a chiral center with a methyl group. This chain is further linked to a long polyether chain (tetraethylene glycol) which terminates in a succinimide ring. A trifluoroacetic acid (TFA) molecule is shown as a counterion.
P37 Example 48	 Chemical structure of P37: A complex molecule featuring a purine core substituted with an isopropoxy group and a propyl group. The purine is linked via a pyrrolidinylmethyl group to a chain containing an amide, a primary amide, and a chiral center with a methyl group. This chain is further linked to a long polyether chain (tetraethylene glycol) which terminates in a succinimide ring. A trifluoroacetic acid (TFA) molecule is shown as a counterion.

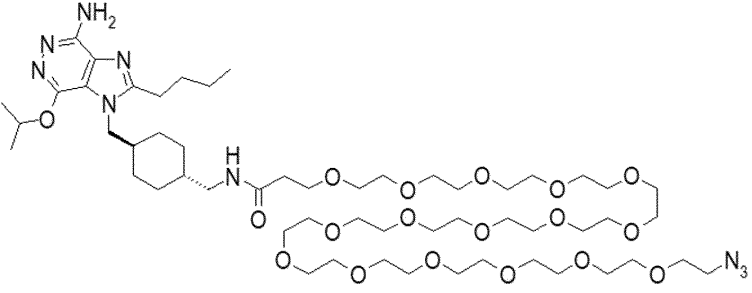
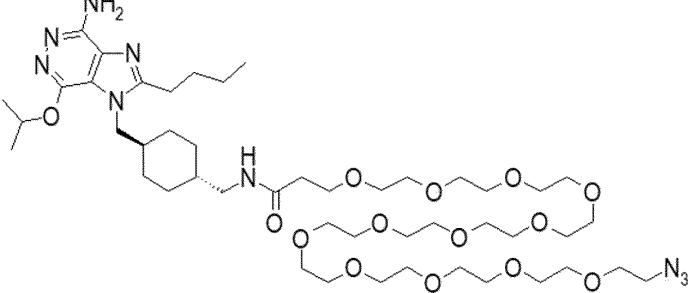
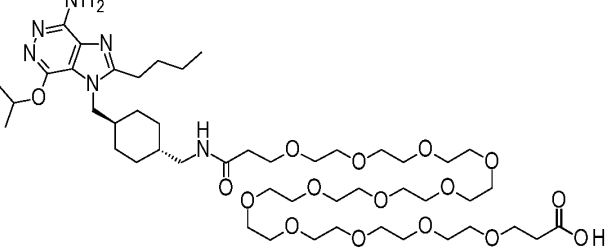
Compound Number (P for payload)	Formula
P38 Example 49	
P39	
P40	

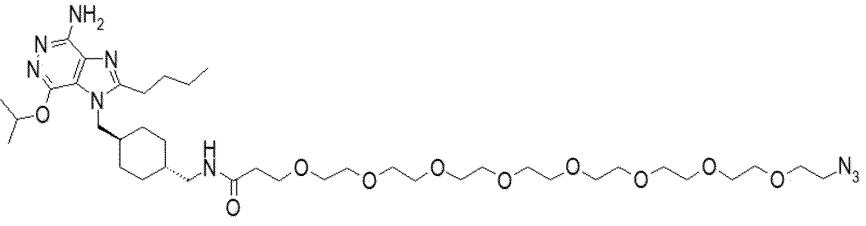
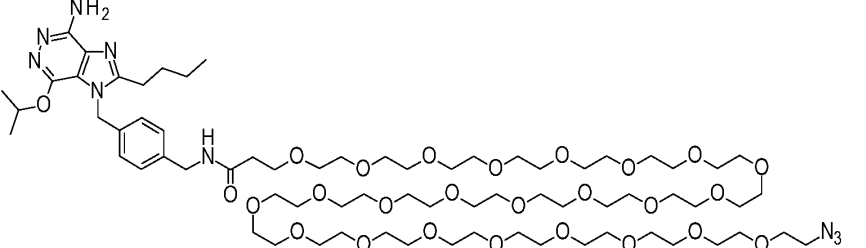
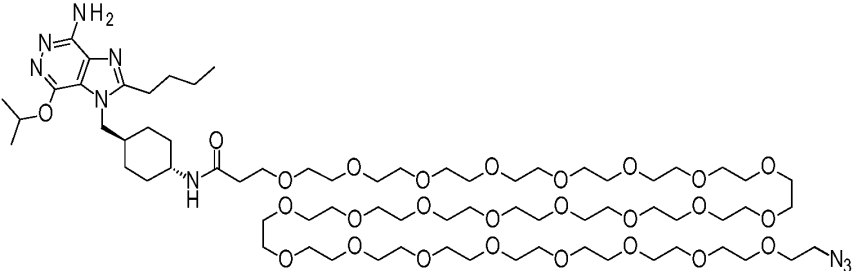
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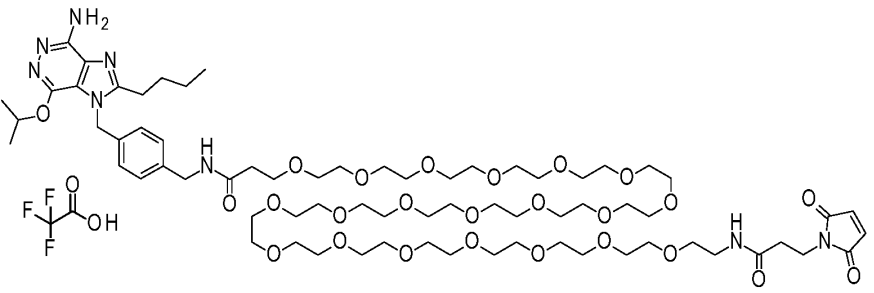
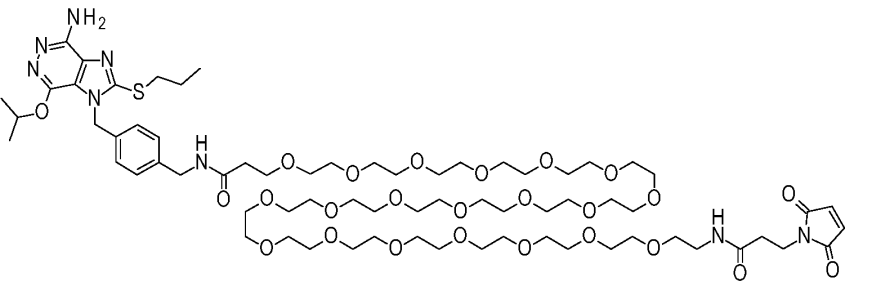
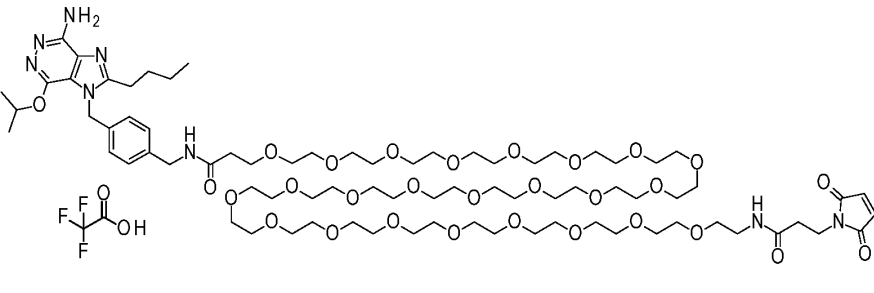
Compound Number (P for payload)	Formula
P11 Example7	
P12 Example 8	
P13 Example 9	

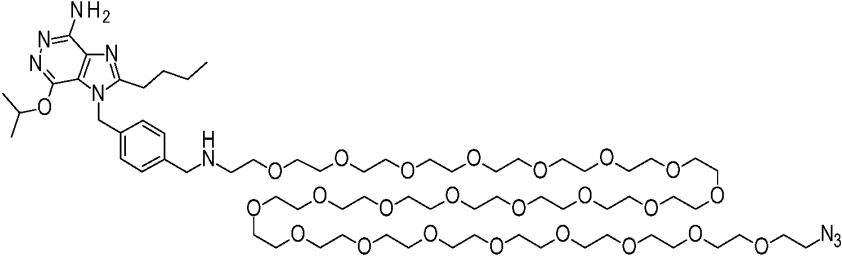
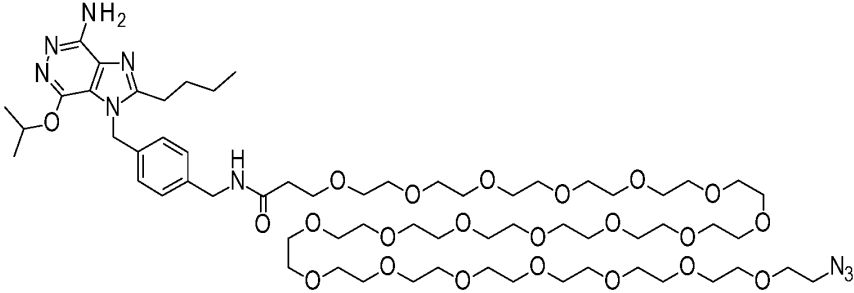
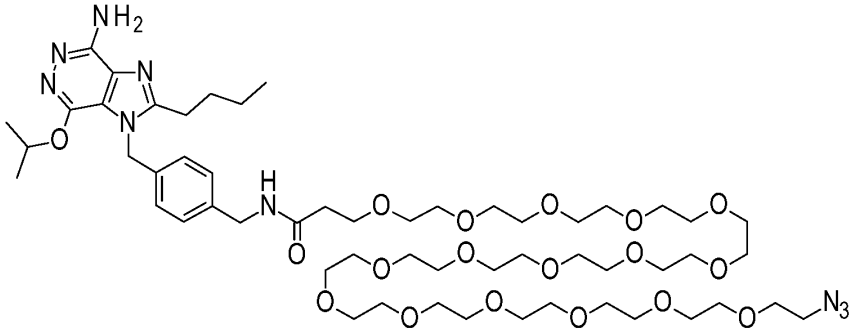
Compound Number (P for payload)	Formula
P14	 Chemical structure of P14: A complex molecule featuring a purine core. The purine has an amino group (NH₂) at position 6, an isopropoxy group at position 7, and a butyl group at position 2. The purine is linked via its N9 atom to a cyclohexyl ring. The cyclohexyl ring is further linked to a long, flexible polyether chain (containing 10 oxygen atoms). This chain is terminated by a succinimide ring. A trifluoromethyl group (CF₃) is attached to the polyether chain via an amide linkage.
P15 Example 10	 Chemical structure of P15: A complex molecule featuring a purine core. The purine has an amino group (NH₂) at position 6, an isopropoxy group at position 7, and a butyl group at position 2. The purine is linked via its N9 atom to a cyclohexyl ring. The cyclohexyl ring is further linked to a long, flexible polyether chain (containing 10 oxygen atoms). This chain is terminated by a succinimide ring. A trifluoromethyl group (CF₃) is attached to the polyether chain via an amide linkage.
P16	 Chemical structure of P16: A complex molecule featuring a purine core. The purine has an amino group (NH₂) at position 6, an isopropoxy group at position 7, and a butyl group at position 2. The purine is linked via its N9 atom to a cyclohexyl ring. The cyclohexyl ring is further linked to a long, flexible polyether chain (containing 10 oxygen atoms). This chain is terminated by a succinimide ring. A trifluoromethyl group (CF₃) is attached to the polyether chain via an amide linkage.

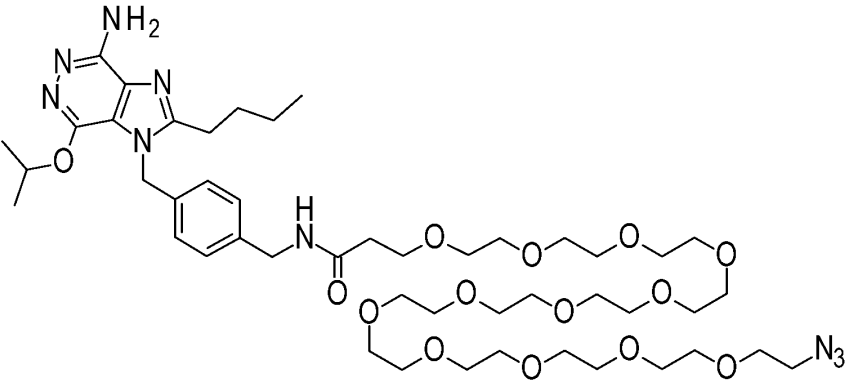
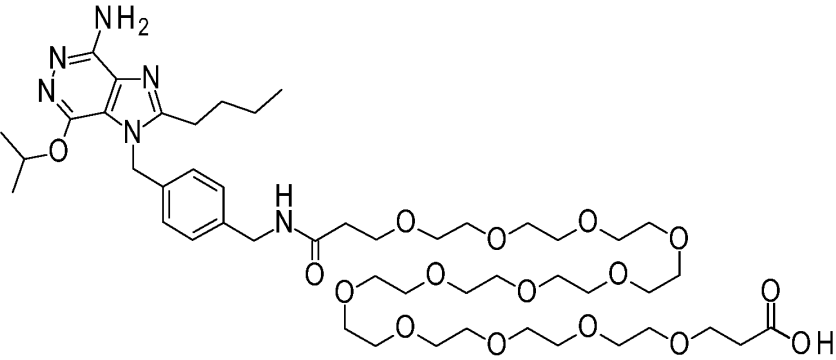
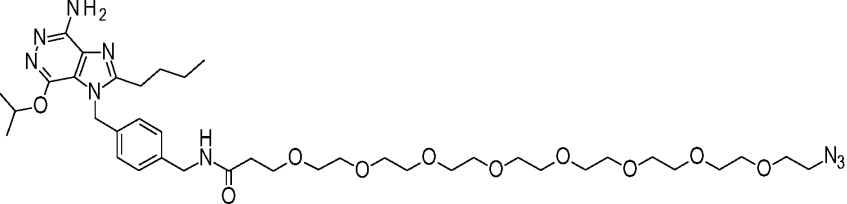
Compound Number (P for payload)	Formula
P17 Example 11b	 Chemical structure of P17 Example 11b: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its N1 atom to a benzyl group, which is connected to a piperazine ring. The piperazine ring is further linked to a long, branched polyether chain ending in an azide group (N₃).
P18 Example 11a	 Chemical structure of P18 Example 11a: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its N1 atom to a cyclohexyl group, which is connected to an amide group. The amide group is further linked to a long, branched polyether chain ending in an azide group (N₃).
P19	 Chemical structure of P19: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its N1 atom to a cyclohexyl group, which is connected to an amide group. The amide group is further linked to a long, branched polyether chain ending in an azide group (N₃).

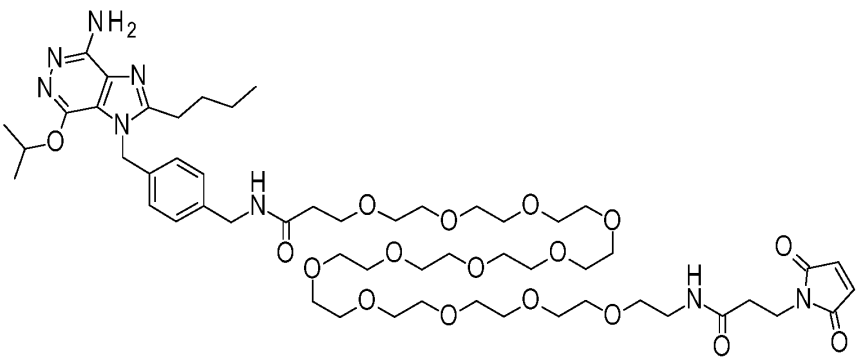
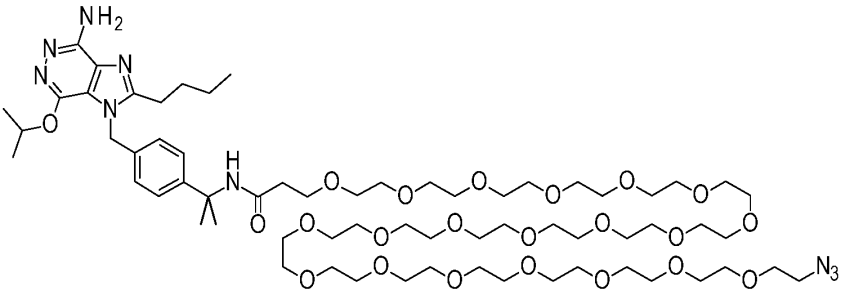
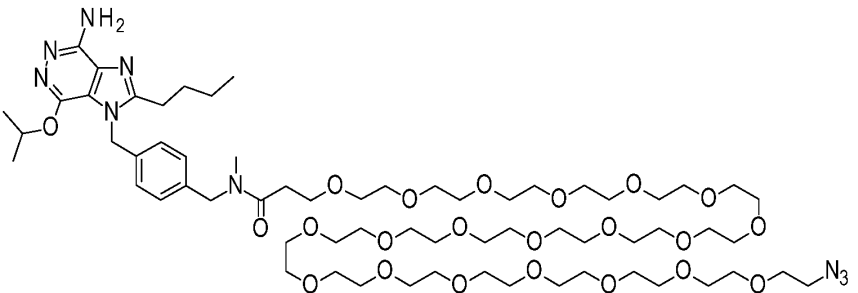
Compound Number (P for payload)	Formula
P20	 Chemical structure of P20: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 2, and a butyl group at position 9. The purine ring is connected via a methylene bridge to a cyclohexane ring. The cyclohexane ring is further connected via a methylene bridge to an amide group (NH-C=O). The amide group is connected to a long, branched polyether chain. The polyether chain consists of two parallel chains of ethylene glycol units (O-CH₂-CH₂-O) connected by a central chain of ethylene glycol units. The terminal group of the polyether chain is an azide group (N₃).
P21	 Chemical structure of P21: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 2, and a butyl group at position 9. The purine ring is connected via a methylene bridge to a cyclohexane ring. The cyclohexane ring is further connected via a methylene bridge to an amide group (NH-C=O). The amide group is connected to a long, branched polyether chain. The polyether chain consists of two parallel chains of ethylene glycol units (O-CH₂-CH₂-O) connected by a central chain of ethylene glycol units. The terminal group of the polyether chain is an azide group (N₃).
P22 Example 12	 Chemical structure of P22: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 2, and a butyl group at position 9. The purine ring is connected via a methylene bridge to a cyclohexane ring. The cyclohexane ring is further connected via a methylene bridge to an amide group (NH-C=O). The amide group is connected to a long, branched polyether chain. The polyether chain consists of two parallel chains of ethylene glycol units (O-CH₂-CH₂-O) connected by a central chain of ethylene glycol units. The terminal group of the polyether chain is a carboxylic acid group (COOH).

Compound Number (P for payload)	Formula
P23	 Chemical structure of compound P23: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its nitrogen at position 1 to a cyclohexyl ring. The cyclohexyl ring is further linked to a long polyether chain (10 repeating units of -CH₂-CH₂-O-) terminated with an azide group (N₃).
P41 Example 50	 Chemical structure of compound P41: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its nitrogen at position 1 to a benzyl group. The benzyl group is further linked to a long polyether chain (10 repeating units of -CH₂-CH₂-O-) terminated with an azide group (N₃).
P42	 Chemical structure of compound P42: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine is linked via its nitrogen at position 1 to a cyclohexyl ring. The cyclohexyl ring is further linked to a long polyether chain (10 repeating units of -CH₂-CH₂-O-) terminated with an azide group (N₃).

Compound Number (P for payload)	Formula
P43 Example 51	
P44 Example 52	
P45	

Compound Number (P for payload)	Formula
P46 Example 53	
P47	
P48	

Compound Number (P for payload)	Formula
P49	 Chemical structure of compound P49: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine ring is connected via a methylene group to a benzene ring, which is further connected via a methylene group to an amide linkage. The amide is connected to a long, branched polyether chain ending in an azide group (N₃).
P50 Example 54	 Chemical structure of compound P50: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine ring is connected via a methylene group to a benzene ring, which is further connected via a methylene group to an amide linkage. The amide is connected to a long, branched polyether chain ending in a carboxylic acid group (COOH).
P51	 Chemical structure of compound P51: A purine derivative with an amino group (NH₂) at position 6, an isopropoxy group at position 9, and a butyl group at position 2. The purine ring is connected via a methylene group to a benzene ring, which is further connected via a methylene group to an amide linkage. The amide is connected to a long, branched polyether chain ending in an azide group (N₃).

Compound Number (P for payload)	Formula
P52	
P53 Example 55	
P54 Example 56	

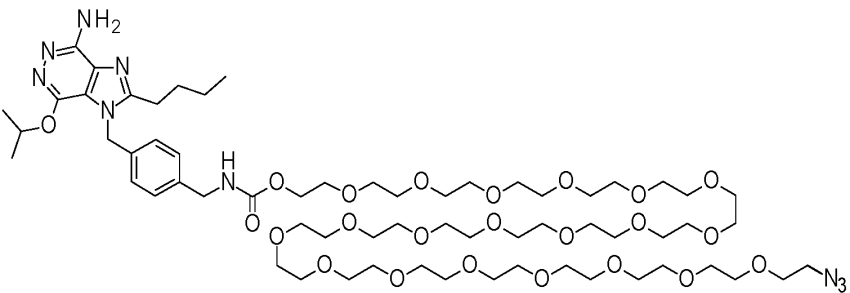
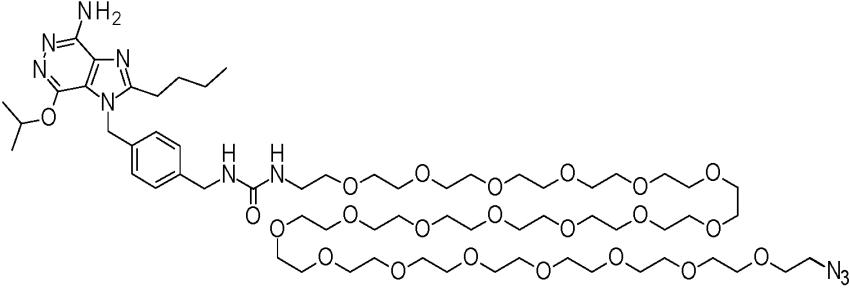
Compound Number (P for payload)	Formula
P58 Example 60	
P59 Example 61	

Table 2 :

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
P1	1284.5 [M-H] ⁻	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.79 - 0.86 (m, 9 H), 1.16 (d, J=6.3 Hz, 6 H), 1.25 - 1.49 (m, 4 H), 1.54 - 1.78 (m, 7 H), 1.82 - 1.92 (m, 1 H), 1.95 - 2.03 (m, 1 H), 2.06 (t, J=7.4 Hz, 2 H), 2.11 (td, J=7.3, 3.0 Hz, 2 H), 2.23 (br t, J=8.0 Hz, 2 H), 2.27 (br s, 4 H), 2.82 (t, J=7.7 Hz, 2 H), 2.91 - 3.05 (m, 2 H), 3.18 (q, J=5.8 Hz, 2 H), 3.27 - 3.35 (m hidden, 4 H), 3.36 - 3.41 (m, 4 H), 3.44 (s, 2 H), 3.47 - 3.56 (m, 8 H), 3.59 (t, J=4.9 Hz, 2 H), 4.20 (dd, J=8.2, 6.6 Hz, 1 H), 4.25 - 4.32 (m, 1 H), 4.33 - 4.42 (m, 1 H), 4.98 (s, 2 H), 5.21 - 5.34 (m, 1 H), 5.44 (br s, 2 H), 5.55 (s, 2 H), 5.91 - 6.13 (m, 3 H), 7.01 (d, J=7.9 Hz,

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
		2 H), 7.21 - 7.30 (m, 4 H), 7.57 (d, J=8.5 Hz, 2 H), 7.70 (br d, J=6.6Hz, 1 H), 7.83 (t, J=5.7 Hz, 1 H), 8.03 (d, J=7.9 Hz, 1 H), 8.17 - 8.36 (m, 1 H), 10.01 (s, 1 H)
P2 Example 1	1223.0	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.72 - 0.80 (m, 2 H), 0.82 (d, J=7 Hz, 3 H), 0.85 (d, J=7 Hz, 3 H), 0.92 (t, J=7 Hz, 3 H), 1.00 - 1.12 (m, 2 H), 1.35 (d, J=6 Hz, 6 H), 1.37 - 1.51 (m, 6 H), 1.60 - 1.91 (m, 12 H), 1.95 - 2.22 (m, 7 H), 2.77 - 2.86 (m, 4 H), 2.94 - 3.00 (m, 2 H), 3.18 (q, J=6 Hz, 2 H), 3.36 - 3.42 (m, 4 H), 3.47 - 3.57 (m, 8 H), 3.57 - 3.62 (m, 2 H), 4.10 (d, J=7 Hz, 2 H), 4.21 (dd, J=9, 6 Hz, 1 H), 4.24 - 4.34 (m, 2 H), 4.91 (s, 2 H), 5.38 (spt, J=6 Hz, 1 H), 5.56 - 5.79 (m, 2 H), 5.87 (br s, 2 H), 7.20 (br t, J=6 Hz, 1 H), 7.25 (d, J=9 Hz, 2 H), 7.58 (d, J=9 Hz, 2 H), 7.77 - 7.98 (m, 2 H), 8.05 - 8.25 (m, 1 H), 8.43 - 8.86 (m, 1 H), 10.01 (s, 1 H)
P3	1201.0	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.76 - 0.92 (m, 9 H) 1.16 (d, J=6 Hz, 6 H) 1.27 - 1.34 (m, 2 H) 1.30 (d, J=7 Hz, 3 H) 1.60 (m, 2 H) 1.65 - 1.78 (m, 3 H) 1.83 - 1.93 (m, 1 H) 1.94 - 2.02 (m, 1 H) 2.03 - 2.15 (m, 4 H) 2.23 (br t, J=8 Hz, 2 H) 2.27 (br s, 4 H) 2.82 (t, J=8 Hz, 2 H) 3.18 (m, 2 H) 3.32 - 3.35 (m, 6 H, hidden) 3.36 - 3.41 (m, 2 H) 3.43 (s, 2 H) 3.47 - 3.62 (m, 10 H) 4.19 (m, 1 H) 4.28 (m, 1 H) 4.37 (m, 1 H) 4.97 (s, 2 H) 5.30 (m, 1 H) 5.55 (s, 2 H) 5.98 (s, 2 H) 7.01 (d, J=8 Hz, 2 H) 7.23 - 7.30 (m, 4 H) 7.56 (d, J=9 Hz, 2 H) 7.68 (br d, J=9 Hz, 1 H) 7.84 (br t, J=4 Hz, 1 H) 7.99 - 8.13 (m, 1 H) 8.23 (br d, J=7 Hz, 1 H) 9.99 (br s, 1 H)
P4 Example 2	1223.6	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.69 - 0.82 (m, 2 H), 0.82 (d, J=7 Hz, 3 H), 0.86 (d, J=7 Hz, 3 H), 0.92 (t, J=7 Hz, 3 H), 1.07 (br q, J=12 Hz, 2 H), 1.30 (d, J=7 Hz, 3 H), 1.35 (d, J=6 Hz, 6 H), 1.37 - 1.44 (m, 2 H), 1.47 (br d, J=12 Hz, 2 H), 1.60 - 1.80 (m, 9 H), 1.88 (m, 1 H), 1.98 (m, 1 H), 2.06 (t, J=7 Hz, 2 H), 2.08 - 2.14 (m, 2 H), 2.18 - 2.26 (m, 2 H), 2.78 - 2.89 (m, 4 H), 3.18 (q, J=6 Hz, 2 H), 3.36 - 3.64 (m, 14 H), 4.10 (br d, J=7 Hz, 2 H), 4.19 (m, 1 H), 4.28 (m, 1 H), 4.37 (quin, J=7 Hz, 1 H), 4.91 (s, 2 H), 5.37 (m, 1 H), 5.94 (br s, 2 H), 7.20 (t, J=6 Hz, 1 H), 7.26 (d, J=8 Hz, 2 H), 7.56 (d, J=8 Hz, 2 H), 7.69 (d, J=9 Hz, 1 H), 7.85 (t, J=5 Hz, 1 H), 8.06 (br d, J=7 Hz, 1 H), 8.24 (d, J=7 Hz, 1 H), 9.98 (s, 1 H), 11.59 - 13.21 (m, 1 H)

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
P5	859.9	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 - 0.85 (m, 9 H), 1.13 (d, J=6.9 Hz, 3 H), 1.17 (d, J=6.0 Hz, 6 H), 1.21 - 1.34 (m, 2 H), 1.60 (quin, J=7.6 Hz, 2 H), 1.69 (quin, J=7.4 Hz, 2 H), 1.91 - 1.98 (m, 1 H), 2.05 (t, J=7.6 Hz, 2 H), 2.10 - 2.18 (m, 2 H), 2.19 - 2.32 (m, 4 H), 2.82 (t, J=7.6 Hz, 2 H), 3.18 (q, J=6.0 Hz, 2 H), 3.35 - 3.44 (m, 9 H), 3.46 - 3.60 (m, 11 H), 4.14 (dd, J=8.8, 6.3 Hz, 1 H), 4.67 (quin, J=7.0 Hz, 1 H), 5.27 - 5.33 (m, 1 H), 5.56 (s, 2 H), 5.91 (s, 2 H), 7.02 (d, J=7.9 Hz, 2 H), 7.26 (d, J=7.9 Hz, 2 H), 7.75 (d, J=8.8 Hz, 1 H), 7.81 (t, J=5.5 Hz, 1 H), 8.01 (d, J=7.6 Hz, 1 H)
P6 Example 3	859.9	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.76 - 0.86 (m, 8 H), 0.93 (t, J=7.4 Hz, 3 H), 1.05 (q, J=12.1 Hz, 2 H), 1.17 (d, J=7.0 Hz, 3 H), 1.32 - 1.53 (m, 5 H), 1.36 (d, J=6.0 Hz, 6 H), 1.64 - 1.81 (m, 7 H), 1.89 - 2.00 (m, 1 H), 2.05 (t, J=7.5 Hz, 2 H), 2.10 - 2.19 (m, 2 H), 2.79 - 2.94 (m, 4 H), 3.18 (q, J=5.9 Hz, 2 H), 3.35 - 3.43 (m, 4 H), 3.47 - 3.61 (m, 10 H), 4.06 - 4.16 (m, 3 H), 4.22 (quin, J=7.2 Hz, 1 H), 5.38 (m, 1 H), 5.80 (s, 2 H), 7.71 (t, J=5.8 Hz, 1 H), 7.75 - 7.82 (m, 2 H), 7.90 (d, J=7.5 Hz, 1 H)
P7 Example 4	984.5	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.73 - 0.85 (m, 8 H), 0.92 (t, J=7.4 Hz, 3 H), 0.99 - 1.12 (m, 2 H), 1.17 (d, J=7.3 Hz, 3 H), 1.33 - 1.49 (m, 6 H), 1.35 (d, J=6.0 Hz, 6 H), 1.64 - 1.79 (m, 6 H), 1.90 - 1.98 (m, 1 H), 2.05 (t, J=7.6 Hz, 2 H), 2.10 - 2.18 (m, 2 H), 2.27 - 2.35 (m, 2 H), 2.82 (t, J=7.7 Hz, 2 H), 2.84 - 2.93 (m, 2 H), 3.08 - 3.64 (m partially hidden, 18 H), 4.05 - 4.17 (m, 3 H), 4.22 (m, 1 H), 5.38 (m, 1 H), 5.85 (s, 2 H), 7.00 (s, 2 H), 7.72 (t, J=6.4 Hz, 1 H), 7.78 (d, J=8.2 Hz, 1 H), 7.83 (t, J=5.5 Hz, 1 H), 7.92 (d, J=7.9 Hz, 1 H), 7.97 - 8.11 (m, 1 H)
P8	1008.9	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 - 0.85 (m, 9 H) 1.17 (d, J=6.0 Hz, 6 H) 1.22 - 1.37 (m, 4 H) 1.38 - 1.52 (m, 1 H) 1.51 - 1.76 (m, 5 H) 1.93 (m, 1 H) 2.02 - 2.18 (m, 4 H) 2.18 - 2.31 (m, 4 H) 2.80 (m, 2 H) 2.92 (m, 2 H) 3.18 (m, 2 H) 3.34 - 3.57 (m, 18 H) 3.57 - 3.63 (m, 2 H) 4.16 (dd, J=8.9, 6.6 Hz, 1 H) 4.67 (m, 1 H) 5.26 - 5.37 (m, 3 H) 5.56 (s, 2 H) 5.77 - 5.97 (m, 3 H) 7.02 (m, J=8.3 Hz, 2 H) 7.26 (m, J=8.3 Hz, 2 H) 7.74 (d, J=8.8 Hz, 1 H) 7.80 (t, J=5.8 Hz, 1 H) 7.93 (d, J=8.0 Hz, 1 H)
P9 Example 5	945.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.67 - 0.86 (m, 2 H), 0.81 (m, 6 H), 0.92 (t, J=7 Hz, 3 H), 1.06 (q, J=12 Hz, 2 H), 1.20 - 1.53 (m, 9 H), 1.36 (d, J=6 Hz, 6 H), 1.53 - 1.84 (m, 7 H), 1.94 (m, 1 H), 2.05 (t, J=8 Hz, 2 H), 2.10 - 2.21 (m, 2 H), 2.77 - 2.96 (m, 6 H), 3.18 (q, J=6 Hz, 2 H), 3.36 - 3.67 (m, 14 H), 4.05 - 4.15 (m, 3 H), 4.19 (m, 1 H), 5.33 (s, 2 H), 5.38 (spt, J=6 Hz, 1 H), 5.82 (s, 2 H), 5.89 (t, J=6 Hz, 1 H), 7.63 - 7.91 (m, 4 H)

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
P10 Example 6	1070.3	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.69 - 0.86 (m, 2 H), 0.81 (t, J=6 Hz, 6 H), 0.93 (t, J=7 Hz, 3 H), 1.01 - 1.14 (m, 2 H), 1.20 - 1.63 (m, 10 H), 1.39 (d, J=6 Hz, 6 H), 1.64 - 1.73 (m, 4 H), 1.74 - 1.83 (m, 2 H), 1.94 (m, 1 H), 2.05 (t, J=8 Hz, 2 H), 2.11 - 2.19 (m, 2 H), 2.33 (t, J=7 Hz, 2 H), 2.84 - 2.99 (m, 6 H), 3.12 - 3.53 (m partially hidden, 16 H), 3.59 (t, J=7 Hz, 2 H), 4.03 - 4.32 (m, 4 H), 4.77 - 5.46 (m, 2 H), 5.27 (m, 1 H), 5.34 (s, 2 H), 5.90 (t, J=6 Hz, 1 H), 7.00 (s, 2 H), 7.72 - 7.81 (m, 2 H), 7.82 - 7.90 (m, 2 H), 8.00 t, J=5 Hz, 1 H), 11.62 - 12.70 (m, 1 H)
P24 Example 41	1217.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.76 - 0.86 (m, 8 H), 0.93 (t, J=7.4 Hz, 3 H), 1.05 (q, J=12.1 Hz, 2 H), 1.17 (d, J=7.0 Hz, 3 H), 1.32 - 1.53 (m, 5 H), 1.36 (d, J=6.0 Hz, 6 H), 1.64 - 1.81 (m, 7 H), 1.89 - 2.00 (m, 1 H), 2.05 (t, J=7.5 Hz, 2 H), 2.10 - 2.19 (m, 2 H), 2.79 - 2.94 (m, 4 H), 3.18 (q, J=5.9 Hz, 2 H), 3.35 - 3.43 (m, 4 H), 3.47 - 3.61 (m, 10 H), 4.06 - 4.16 (m, 3 H), 4.22 (quin, J=7.2 Hz, 1 H), 5.38 (m, 1 H), 5.80 (s, 2 H), 7.71 (t, J=5.8 Hz, 1 H), 7.75 - 7.82 (m, 2 H), 7.90 (d, J=7.5 Hz, 1 H)
P25	1209.6	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 - 0.87 (m, 9 H), 1.20 (d, J=6 Hz, 6 H), 1.27 - 1.37 (m, 2 H), 1.33 (d, J=7 Hz, 3 H), 1.56 - 1.66 (m, 2 H), 1.66 - 1.74 (m, 2 H), 1.74 - 1.83 (m, 2 H), 1.96 - 2.17 (m, 7 H), 2.79 (t, J=8 Hz, 2 H), 3.14 - 3.21 (m, 2 H), 3.22 - 3.63 (m, 14 H), 4.09 - 4.18 (m, 4 H), 4.35 (quin, J=7 Hz, 1 H), 4.93 (s, 2 H), 5.31 (m, 1 H), 5.55 (s, 2 H), 5.90 (s, 2 H), 7.02 (d, J=8 Hz, 2 H), 7.21 (d, J=8 Hz, 2 H), 7.25 (d, J=8 Hz, 2 H), 7.63 (d, J=8 Hz, 2 H), 7.74 (t, J=6 Hz, 1 H), 8.02 (d, J=9 Hz, 1 H), 8.18 (t, J=5 Hz, 1 H), 8.58 (d, J=7 Hz, 1 H), 9.08 (br d, J=5 Hz, 1 H), 10.40 (s, 1 H)
P26 Example 42	1131.3	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 - 0.87 (m, 9 H), 1.20 (d, J=6 Hz, 6 H), 1.27 - 1.37 (m, 2 H), 1.33 (d, J=7 Hz, 3 H), 1.56 - 1.66 (m, 2 H), 1.66 - 1.74 (m, 2 H), 1.74 - 1.83 (m, 2 H), 1.96 - 2.17 (m, 7 H), 2.79 (t, J=8 Hz, 2 H), 3.14 - 3.21 (m, 2 H), 3.22 - 3.63 (m, 14 H), 4.09 - 4.18 (m, 4 H), 4.35 (quin, J=7 Hz, 1 H), 4.93 (s, 2 H), 5.31 (m, 1 H), 5.55 (s, 2 H), 5.90 (s, 2 H), 7.02 (d, J=8 Hz, 2 H), 7.21 (d, J=8 Hz, 2 H), 7.25 (d, J=8 Hz, 2 H), 7.63 (d, J=8 Hz, 2 H), 7.74 (t, J=6 Hz, 1 H), 8.02 (d, J=9 Hz, 1 H), 8.18 (t, J=5 Hz, 1 H), 8.58 (d, J=7 Hz, 1 H), 9.08 (br d, J=5 Hz, 1 H), 10.40 (s, 1 H)
P27	1223.6	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.82 (d, J=7 Hz, 3 H), 0.86 (d, J=7 Hz, 3 H), 0.93 (t, J=7 Hz, 3 H), 0.99 - 1.11 (m, 2 H), 1.11 - 1.21 (m, 2 H), 1.30 (d, J=7 Hz, 3 H), 1.36 (d, J=6 Hz, 6 H), 1.37 - 1.43 (m, 2 H), 1.46 (br d, J=11 Hz, 2 H), 1.64 - 1.91 (m, 10 H), 2.00 (m, 1 H), 2.05 (br t, J=7 Hz, 2 H), 2.08 - 2.13 (m, 2 H), 2.15 - 2.22 (m, 2 H), 2.83 (t, J=8 Hz, 2 H), 3.14 - 3.21 (m, 2 H), 3.20 - 3.65 (m partially hidden, 14 H), 4.10 (d, J=6 Hz, 2 H), 4.18 (t, J=7 Hz, 1 H), 4.24 (m, 1 H), 4.36 (m, 1 H), 4.90 (br s, 2 H), 5.38 (spt, J=6 Hz, 1 H), 5.84 (br

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
		s, 2 H), 7.10 (d, J=7 Hz, 1 H), 7.25 (d, J=8 Hz, 2 H), 7.57 (d, J=8 Hz, 2 H), 7.75 (br d, J=7 Hz, 1 H), 7.86 - 7.97 (m, 1 H), 8.16 - 8.37 (m, 2 H), 10.08 (br s, 1 H)
P28 Example 43	853.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.73 - 0.81 (m, 6 H), 0.84 (t, J=7.3 Hz, 3 H), 1.16 - 1.37 (m, 11 H), 1.59 - 1.71 (m, 4 H), 1.92 (m, 1 H), 2.04 (t, J=7.5 Hz, 2 H), 2.08 - 2.24 (m, 2 H), 2.71 - 2.84 (m, 2 H), 3.15 - 3.20 (m, 2 H), 3.36 - 3.67 (m, 14 H), 4.13 (dd, J=8.6, 6.6 Hz, 1 H), 4.19 - 4.30 (m, 3 H), 5.32 (quin, J=6.1 Hz, 1 H), 5.54 (s, 2 H), 5.91 (s, 2 H), 7.00 (d, J=8.1 Hz, 2 H), 7.19 (d, J=8.1 Hz, 2 H), 7.79 (d, J=8.8 Hz, 1 H), 7.82 (t, J=5.6 Hz, 1 H), 8.00 (d, J=7.1 Hz, 1 H), 8.30 (t, J=5.9 Hz, 1 H)
P29	845.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.76 - 0.88 (m, 6 H); 0.93 (t, J=7.3 Hz, 3 H); 0.98 - 1.27 (m, 7 H); 1.37 (d, J=6.1 Hz, 6 H); 1.35 - 1.56 (m, 4 H); 1.60 - 1.82 (m, 8 H); 1.83 - 2.22 (m, 5 H); 2.83 (t, J=7.6 Hz, 2 H); 3.12 - 3.21 (m, 2 H); 3.35 - 3.64 (m, 14 H); 3.91 - 4.23 (m, 4 H); 5.35 - 5.45 (m, 1 H); 5.82 (s, 2 H); 7.50 - 7.59 (m, 1 H); 7.72 - 7.92 (m, 3 H); 8.14 - 8.26 (m, 1 H)
P30 Example 44	978.1	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.71 - 0.93 (m, 9 H), 1.12 - 1.45 (m, 11 H), 1.55 - 1.77 (m, 4 H), 1.90 - 1.98 (m, 1 H), 1.98 - 2.07 (m, 2 H), 2.09 - 2.19 (m, 2 H), 2.30 - 2.37 (m, 2 H), 2.73 - 2.82 (m, 2 H), 3.17 (m, 4 H), 3.30 - 3.54 (m partially hidden, 12 H), 3.56 - 3.65 (m, 2 H), 4.13 (dd, J=9, 7 Hz, 1 H), 4.19 - 4.39 (m, 3 H), 5.32 (quin, J=6 Hz, 1 H), 5.54 (s, 2 H), 5.89 (s, 2 H), 6.94 - 7.06 (m, 4 H), 7.19 (d, J=8 Hz, 2 H), 7.76 (d, J=9 Hz, 1 H), 7.80 (br t, J=6 Hz, 1 H), 7.96 - 8.04 (m, 2 H), 8.28 (br t, J=6 Hz, 1 H)
P31 Example 45	939.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 (d, J=6 Hz, 6 H), 0.84 (t, J=7 Hz, 3 H), 1.22 (d, J=6 Hz, 6 H), 1.25 - 1.41 (m, 4 H), 1.45 - 1.56 (m, 1 H), 1.56 - 1.77 (m, 5 H), 1.93 (m, 1 H), 2.05 (t, J=7 Hz, 2 H), 2.09 - 2.21 (m, 2 H), 2.82 (t, J=8 Hz, 2 H), 2.92 (q, J=6 Hz, 2 H), 3.18 (q, J=6 Hz, 2 H), 3.29 - 3.66 (m partially hidden, 14 H), 4.07 - 4.32 (m, 4 H), 5.24 (spt, J=6 Hz, 1 H), 5.35 (br s, 2 H), 5.58 (s, 2 H), 5.92 (t, J=5 Hz, 1 H), 6.85 - 7.30 (m, 2H) 7.03 (d, J=8 Hz, 2 H), 7.19 (d, J=8 Hz, 2 H), 7.79 (d, J=9 Hz, 1 H), 7.84 (t, J=6 Hz, 1 H), 7.94 (d, J=8 Hz, 1 H), 8.36 (t, J=6 Hz, 1 H)
P32	931.4	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.80 - 0.84 (m, 6 H), 0.94 (t, J=7.3 Hz, 3 H), 1.00 - 1.12 (m, 2 H), 1.13 - 1.25 (m, 2 H), 1.24 - 1.35 (m, 2 H), 1.39 - 1.46 (m, 3 H), 1.42 (d, J=6.3 Hz, 6 H), 1.48 - 1.62 (m, 3 H), 1.67 - 1.82 (m, 7 H), 1.92 - 1.99 (m, 1 H), 2.05 (t, J=7.6 Hz, 2 H), 2.14 (td, J=7.4, 3.2 Hz, 2 H), 2.88 - 2.95 (m, 4 H), 3.17 (q, J=5.9 Hz, 2 H), 3.30 - 3.64 (m partially hidden, 15 H), 4.02 - 4.17 (m, 2 H), 4.20 (d, J=7.3 Hz, 2 H), 5.24 (m, 1 H), 5.34 (br s, 2 H), 5.89 (br t, J=5.5 Hz, 1 H), 7.64 (d, J=8.2 Hz, 1 H), 7.79 (br d, J=8.2 Hz, 2 H), 7.83 (t, J=5.7 Hz, 1 H), 8.50 (s, 2 H), 13.61 (s, 1 H)

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
P33 Example 46	1064.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.74 - 0.80 (m, 6 H), 0.84 (t, J=7 Hz, 3 H), 1.24 (d, J=6 Hz, 6 H), 1.25 - 1.42 (m, 5 H), 1.42 - 1.55 (m, 1 H), 1.56 - 1.74 (m, 4 H), 1.88 - 1.98 (m, 1 H), 2.05 (t, J=8 Hz, 2 H), 2.09 - 2.22 (m, 2 H), 2.33 (t, J=7 Hz, 2 H), 2.85 - 2.96 (m, 4 H), 3.10 - 3.21 (m, 4 H), 3.31 - 3.52 (m partially hidden, 12 H), 3.56 - 3.62 (m, 2 H), 4.13 (dd, J=8, 7 Hz, 1 H), 4.17 - 4.34 (m, 3 H), 5.15 (spt, J=6 Hz, 1 H), 5.25 - 5.45 (m, 2 H), 5.63 (s, 2 H), 5.86 - 5.97 (m, 1 H), 7.00 (s, 2 H), 7.06 (d, J=8 Hz, 2 H), 7.21 (d, J=8 Hz, 2 H), 7.79 (d, J=9 Hz, 1 H), 7.84 (t, J=5 Hz, 1 H), 7.93 (d, J=8 Hz, 1 H), 8.01 (t, J=6 Hz, 1 H), 8.36 (t, J=6 Hz, 1 H), 8.58 (br s, 2 H), 13.69 (s, 1 H)
P34	1056.9	¹ H NMR (400 MHz, D ₂ O) δ ppm 0.89 - 0.97 (m, 9 H); 1.08 - 1.33 (m, 4 H); 1.35 - 1.95 (m, 15 H); 1.45 (d, J=6.3 Hz, 6 H); 1.97 - 2.08 (m, 1 H); 2.23 (t, J=7.6 Hz, 2 H); 2.32 (td, J=7.4, 2.3 Hz, 2 H); 2.46 - 2.51 (m, 2 H); 2.96 (t, J=7.6 Hz, 2 H); 3.07 (t, J=6.8 Hz, 2 H); 3.27 - 3.32 (m, 2 H); 3.33 - 3.37 (m, 2 H); 3.54 (t, J=5.5 Hz, 1 H); 3.52 - 3.61 (m, 1 H); 3.59 (t, J=5.4 Hz, 2 H); 3.61 - 3.69 (m, 8 H); 3.72 - 3.80 (m, 2 H); 4.01 (d, J=7.4 Hz, 1 H); 4.12 - 4.23 (m, 1 H); 4.27 (br d, J=6.9 Hz, 2 H); 5.24 - 5.36 (m, 1 H); 6.84 (s, 2 H)
P35 Example 47	979.3	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.73 - 0.81 (m, 6 H); 0.84 (t, J=7.4 Hz, 3 H); 1.24 (d, J=6.1 Hz, 6 H); 1.26 - 1.42 (m, 4 H); 1.43 - 1.55 (m, 1 H); 1.56 - 1.73 (m, 3 H); 1.86 - 1.99 (m, 1 H); 2.28 - 2.50 (m partially hidden, 4 H); 2.83 - 2.99 (m, 4 H); 3.14 (q, J=5.8 Hz, 2 H); 3.36 (t, J=5.8 Hz, 2 H); 3.41 - 3.52 (m, 8 H); 3.53 - 3.64 (m, 4 H); 4.12 - 4.35 (m, 4 H); 5.09 - 5.21 (m, 1 H); 5.22 - 5.52 (m, 2 H); 5.63 (s, 2 H); 5.87 - 5.97 (m, 1 H); 7.00 (s, 2 H); 7.06 (d, J=8.3 Hz, 2 H); 7.21 (d, J=8.3 Hz, 2 H); 7.84 (d, J=8.6 Hz, 1 H); 7.96 (d, J=7.9 Hz, 1 H); 8.01 (br t, J=5.5 Hz, 1 H); 8.35 (t, J=5.9 Hz, 1 H); 8.60 (br s, 2 H); 13.74 (br s, 1 H)
P36	980.6	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.75 - 0.81 (m, 6 H); 0.88 (t, J=7.4 Hz, 3 H); 1.22 (d, J=6.1 Hz, 6 H); 1.25 - 1.41 (m, 1 H); 1.42 - 1.55 (m, 1 H); 1.56 - 1.69 (m, 3 H); 1.86 - 1.99 (m, 1 H); 2.28 - 2.50 (m, 4 H); 2.92 (br q, J=6.3 Hz, 2 H); 3.14 (q, J=5.9 Hz, 2 H); 3.26 - 3.64 (m, 16 H); 4.11 - 4.30 (m, 4 H); 5.08 (quin, J=6.1 Hz, 1 H); 5.25 - 5.39 (m, 2 H); 5.41 (s, 2 H); 5.86 - 5.94 (m, 1 H); 6.99 (s, 2 H); 7.08 (d, J=8.3 Hz, 2 H); 7.19 (d, J=8.3 Hz, 2 H); 7.61 (t, J=5.6 Hz, 1 H); 7.82 (d, J=8.5 Hz, 1 H); 7.93 (d, J=7.9 Hz, 1 H); 7.96 - 8.01 (m, 1 H); 8.02 (s, 2 H); 8.31 (t, J=6.1 Hz, 1 H); 13.48 (s, 1 H)
P37 Example 48	883.2	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.77 - 0.87 (m, 6 H); 0.95 (t, J=7.4 Hz, 3 H); 1.29 - 1.50 (m, 4 H); 1.51 - 1.63 (m, 1 H); 1.64 - 1.74 (m, 1 H); 1.74 - 1.85 (m, 2 H); 1.90 - 2.00 (m, 1 H); 2.26 - 2.54 (m partially hidden, 4 H); 2.92 - 3.04 (m, 4 H); 3.11 - 3.18 (m, 2 H); 3.29 - 3.74 (m partially hidden, 14 H); 3.83 - 3.88 (m, 3 H); 4.19 - 4.28 (m, 1 H); 4.31 - 5.03 (m, 5 H); 5.06 - 5.60 (m, 2 H); 5.89 - 6.00 (m, 1 H);

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise stated in the cell	¹ H NMR
		6.50 (br s, 1 H); 6.99 (s, 2 H); 7.74 - 7.84 (m, 1 H); 7.96 - 8.04 (m, 1 H); 8.17 (br d, J=6.3 Hz, 1 H); 8.68 - 9.05 (m, 2 H); 14.41 - 14.81 (m, 1 H)
P38 Example 49	900.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.77 - 0.88 (m, 6 H); 0.95 (t, J=7.3 Hz, 3 H); 1.31 - 1.58 (m, 5 H); 1.59 - 1.71 (m, 1 H); 1.79 (quin, J=7.6 Hz, 2 H); 1.90 - 2.04 (m, 1 H); 2.27 - 2.50 (m, 4 H); 2.88 - 3.23 (m, 6 H); 3.36 (t, J=5.9 Hz, 1 H); 3.43 - 3.53 (m, 8 H); 3.53 - 4.18 (m, 12 H); 4.02 (s, 3 H); 4.22 (br dd, J=8.8, 6.6 Hz, 1 H); 4.70 - 4.79 (m, 1 H); 5.04 - 5.70 (m, 2 H); 5.87 - 6.00 (m, 1 H); 7.00 (s, 2 H); 7.82 (d, J=8.9 Hz, 1 H); 8.01 (br t, J=5.5 Hz, 1 H); 8.10 (br d, J=7.6 Hz, 1 H); 8.61 (br s, 2 H); 13.93 (br s, 1 H)
P39	945.7	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.79 - 0.86 (m, 6 H); 0.94 (t, J=7.3 Hz, 3 H); 1.23 - 1.36 (m, 6 H); 1.37 - 1.64 (m, 4 H); 1.40 (d, J=6.2 Hz, 6 H); 1.69 - 1.83 (m, 4 H); 1.91 - 1.99 (m, 1 H); 2.30 - 2.49 (m, 4 H); 2.90 - 3.11 (m, 6 H); 3.12 - 3.17 (m, 2 H); 3.35 (t, J=5.9 Hz, 2 H); 3.44 - 3.52 (m, 8 H); 3.54 - 3.63 (m, 4 H); 4.11 - 4.20 (m, 2 H); 4.27 - 4.33 (m, 2 H); 5.15 - 5.70 (m, 2 H); 5.23 (spt, J=6.2 Hz, 1 H); 5.91 - 5.95 (m, 1 H); 7.00 (s, 2 H); 7.79 (t, J=5.6 Hz, 1 H); 7.82 - 7.88 (m, 2 H); 8.02 (br t, J=5.6 Hz, 1 H); 8.51 - 8.57 (m, 3 H)
P40	972.0	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.76 - 0.87 (m, 6 H); 0.94 (t, J=7.4 Hz, 3 H); 0.98 - 1.65 (m, 12 H); 1.42 (d, J=6.1 Hz, 6 H); 1.67 - 1.86 (m, 5 H); 1.88 - 2.03 (m, 1 H); 2.25 - 2.53 (m partially hidden, 4 H); 2.85 - 2.97 (m, 4 H); 3.14 (q, J=5.9 Hz, 2 H); 3.36 (t, J=5.9 Hz, 2 H); 3.45 - 3.50 (m, 10 H); 3.51 - 3.91 (m partially hidden, 3 H); 4.08 - 4.18 (m, 2 H); 4.20 (br d, J=7.1 Hz, 2 H); 5.18 - 5.28 (m, 1 H); 5.24 - 5.55 (m, 2 H); 5.83 - 5.94 (m, 1 H); 6.99 (s, 2 H); 7.61 (d, J=7.9 Hz, 1 H); 7.75 - 7.88 (m, 2 H); 7.98 (br t, J=5.4 Hz, 1 H); 8.51 (br s, 2 H); 13.65 (br s, 1 H)

Table 2' :

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise mentioned	¹ H NMR
P11 Example 7	1592.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.82 (t, J=7 Hz, 3 H), 1.17 (d, J=6 Hz, 6 H), 1.26 - 1.38 (m, 2 H), 1.53 - 1.66 (m, 2 H), 2.18 - 2.37 (m, 4 H), 2.52 - 2.54 (m partially hidden, 2 H), 2.74 - 2.86 (m, 2 H), 3.36 - 3.42 (m, 4 H), 3.43 - 3.71 (m, 100 H), 5.31 (quin, J=6 Hz, 1 H), 5.56 (s, 2 H), 5.89 (s, 2 H), 7.02 (d, J=8 Hz, 2 H), 7.26 (d, J=8 Hz, 2 H)
P12 Example 8	1528.8	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.73 - 0.84 (m, 2 H), 0.92 (t, J=7.3 Hz, 3 H), 1.00 - 1.12 (m, 2 H), 1.36 (d, J=6.1 Hz, 6 H), 1.34 - 1.50 (m, 5 H), 1.67 - 1.80 (m, 5 H), 2.28 (t, J=6.5 Hz, 2 H), 2.79 - 2.89 (m, 4 H), 3.36 - 3.75 (m, 98 H), 4.11 (d, J=7.1 Hz, 2 H), 5.38 (spt, J=6.0 Hz, 1 H), 5.84 (s, 2 H), 7.76 (t, J=5.6 Hz, 1 H)
P13 Example 9	1717	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7 Hz, 3 H), 1.19 (d, J=6 Hz, 6 H), 1.28 - 1.37 (m, 2 H), 1.60 - 1.69 (m, 2 H), 2.33 (t, J=8 Hz, 2 H), 2.50 (m hidden, 2 H), 2.90 (t, J=8 Hz, 2 H), 3.11 - 3.18 (m, 2 H), 3.37 - 3.76 (m partially hidden, 106 H), 3.94 - 4.58 (m, 2 H), 5.13 (quin, J=6 Hz, 1 H), 5.69 (s, 2 H), 7.00 (s, 2 H), 7.18 - 7.26 (m, 2 H), 7.41 - 7.50 (m, 2 H), 7.99 (t, J=5 Hz, 1 H), 8.60 (s, 2 H), 13.75 (br s, 1 H)
P14	1125.3	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 (br q, J=11 Hz, 2 H), 0.93 (t, J=7 Hz, 3 H), 1.08 (br q, J=11 Hz, 2 H), 1.27 - 1.48 (m, 4 H), 1.41 (d, J=6 Hz, 6 H), 1.52 (br d, J=11 Hz, 2 H), 1.71 (br d, J=11 Hz, 2 H), 1.81 (qt, J=7 Hz, 2 H), 2.22 - 2.36 (m, 4 H), 2.87 (t, J=6 Hz, 2 H), 2.92 (t, J=8 Hz, 2 H), 3.15 (q, J=6 Hz, 2 H), 3.26 - 3.72 (m, 50 H), 4.19 (d, J=7 Hz, 2 H), 5.23 (m, 1 H), 6.99 (s, 2 H), 7.75 (t, J=6 Hz, 1 H), 7.98 (t, J=5 Hz, 1 H), 8.50 (s, 2 H), 13.55 (s, 1 H)
P15 Example 10	1477.8	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.78 (br q, J=12 Hz, 2 H), 0.93 (t, J=7 Hz, 3 H), 1.08 (br q, J=12 Hz, 2 H), 1.28 - 1.47 (m, 4 H), 1.41 (d, J=6 Hz, 6 H), 1.52 (br d, J=11 Hz, 2 H), 1.70 (br d, J=11 Hz, 2 H), 1.79 (quin, J=8 Hz, 2 H), 2.29 (t, J=6 Hz, 2 H), 2.33 (t, J=7 Hz, 2 H), 2.87 (br t, J=6 Hz, 2 H), 2.92 (t, J=8 Hz, 2 H), 3.15 (q, J=6 Hz, 2 H), 3.34 - 3.78 (m, 82 H), 4.19 (d, J=7 Hz, 2 H), 5.23 (spt, J=6 Hz, 1 H), 7.00 (s, 2 H), 7.76 (t, J=6 Hz, 1 H), 7.99 (t, J=5 Hz, 1 H), 8.41 - 8.78 (m, 2 H), 13.59 - 13.78 (m, 1 H)
P16	1697.6 [M+HCO O] ⁻	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.71 - 0.83 (m, 2 H), 0.93 (t, J=7 Hz, 3 H), 1.03 - 1.16 (m, 2 H), 1.30 - 1.47 (m, 4 H), 1.41 (d, J=6 Hz, 6 H), 1.52 (br d, J=11 Hz, 2 H), 1.70 (br d, J=11 Hz, 2 H), 1.79 (quin, J=8 Hz, 2 H), 2.28 (t, J=6 Hz, 2 H), 2.32 (t, J=7 Hz, 2 H), 2.87 (t, J=6 Hz, 2 H), 2.92 (t, J=8 Hz, 2 H), 3.14 (q, J=6 Hz, 2 H), 3.31 - 3.73 (m, 98 H), 4.19 (d, J=7 Hz, 2 H), 5.23 (spt, J=6 Hz, 1 H), 7.00 (s, 2 H), 7.76 (t, J=6 Hz, 1 H), 7.99 (t, J=5 Hz, 1 H), 8.51 (br s, 2 H), 13.64 (s, 1 H)
P17	1564.9 [M+HCO O] ⁻	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.81 (t, J=7.3 Hz, 3 H), 1.17 (d, J=6.1 Hz, 6 H), 1.26 - 1.41 (m, 2 H), 1.48 - 1.69 (m, 2 H), 2.22 - 2.55 (m partially hidden, 8 H), 2.81 (t, J=7.5 Hz, 2 H), 3.36 - 3.75 (m

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise mentioned	¹ H NMR
Example 11b		partially hidden, 98 H), 5.22 - 5.38 (m, 1 H), 5.55 (s, 2 H), 5.88 (s, 2 H), 7.00 (d, J=8.1 Hz, 2 H), 7.24 (d, J=8.1 Hz, 2 H)
P18 Example 11a	1456.4	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.65 - 0.82 (m, 4 H), 0.92 (t, J=7.4 Hz, 3 H), 1.01 - 1.14 (m, 4 H), 1.36 (d, J=6.3 Hz, 6 H), 1.35 - 1.50 (m, 5 H), 1.70 - 1.84 (m, 5 H), 2.31 (t, J=6.5 Hz, 2 H), 2.57 - 2.61 (m, 2 H), 2.82 (t, J=7.7 Hz, 2 H), 3.36 - 3.74 (m, 91 H), 4.11 (d, J=6.9 Hz, 2 H), 5.34 - 5.44 (m, 1 H), 5.83 (s, 2 H)
P19	1352.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.68 - 0.84 (m, 2 H) 0.93 (t, J=7.3 Hz, 3 H) 0.98 - 1.14 (m, 2 H) 1.30 - 1.53 (m, 5 H) 1.36 (d, J=6.1 Hz, 6 H) 1.61 - 1.82 (m, 5 H) 2.28 (t, J=6.4 Hz, 2 H) 2.77 - 2.92 (m, 4 H) 3.25 - 3.35 (m hidden, 2H) 3.36 - 3.42 (m, 2 H) 3.42 - 3.63 (m, 78 H) 4.11 (br d, J=7.1 Hz, 2 H) 5.38 (spt, J=6.1 Hz, 1 H) 5.89 (br s, 2 H) 7.76 (t, J=5.8 Hz, 1 H)
P20	1176.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.69 - 0.86 (m, 2 H) 0.93 (t, J=7.4 Hz, 3 H) 1.00 - 1.16 (m, 2 H) 1.28 - 1.56 (m, 5 H) 1.36 (d, J=6.2 Hz, 6 H) 1.61 - 1.83 (m, 5 H) 2.28 (t, J=6.4 Hz, 2 H) 2.76 - 2.91 (m, 4 H) 3.35 - 3.41 (m, 2 H) 3.42 - 3.65 (m, 64 H) 4.11 (br d, J=7.1 Hz, 2 H) 5.37 (spt, J=6.2 Hz, 1 H) 5.95 (br s, 2 H) 7.74 (t, J=5.7 Hz, 1 H)
P21	1000.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.68 - 0.85 (m, 2 H) 0.93 (t, J=7.3 Hz, 3 H) 0.99 - 1.14 (m, 2 H) 1.30 - 1.53 (m, 5 H) 1.36 (d, J=6.1 Hz, 6 H) 1.62 - 1.83 (m, 5 H) 2.28 (t, J=6.4 Hz, 2 H) 2.78 - 2.93 (m, 4 H) 3.35 - 3.42 (m, 2 H) 3.43 - 3.64 (m, 48 H) 4.11 (br d, J=7.1 Hz, 2 H) 5.38 (quin, J=6.1 Hz, 1 H) 5.89 (br s, 2 H) 7.74 (br t, J=5.7 Hz, 1 H)
P22 Example 12	1003.6	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.69 - 0.86 (m, 2 H) 0.93 (t, J=7 Hz, 3 H) 1.00 - 1.15 (m, 2 H) 1.27 - 1.55 (m, 5H) 1.36 (d, J=6 Hz, 6 H) 1.65 - 1.81 (m, 5 H) 2.28 (t, J=6 Hz, 2 H) 2.43 (t, J=6 Hz, 2 H) 2.78 - 2.91 (m, 4 H) 3.40 - 3.64 (m, 48 H) 4.11 (br d, J=7 Hz, 2 H) 5.38 (spt, J=6 Hz, 1 H) 5.92 (s, 2 H) 7.74 (t, J=6 Hz, 1 H)
P23	824.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.66 - 0.85 (m, 2 H), 0.93 (t, J=7.3 Hz, 3 H), 0.99 - 1.17 (m, 2 H), 1.29 - 1.55 (m, 5 H), 1.36 (d, J=6.1 Hz, 6 H), 1.64 - 1.86 (m, 5 H), 2.28 (t, J=6.4 Hz, 2 H), 2.78 - 2.92 (m, 4 H), 3.35 - 3.64 (m, 34 H), 4.11 (br d, J=7.1 Hz, 2 H), 5.38 (spt, J=6.1 Hz, 1 H), 5.94 (br s, 2 H), 7.74 (t, J=5.7 Hz, 1 H)
P41 Example 50	1522.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.85 (t, J=7 Hz, 3 H) 1.22 (d, J=6 Hz, 6 H) 1.26 - 1.40 (m, 2 H) 1.64 (quin, J=8 Hz, 2 H) 2.30 - 2.40 (m, 2 H) 2.79 (t, J=8 Hz, 2 H) 3.36 - 3.73 (m, 98 H) 4.23 (d, J=6 Hz, 2 H) 5.33 (spt, J=6Hz, 1 H) 5.56 (s, 2 H) 5.91 (s, 2 H) 7.03 (d, J=8 Hz, 2 H) 7.21 (d, J=8 Hz, 2 H) 8.31 (t, J=6Hz, 1 H)

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise mentioned	¹ H NMR
P42	1514.9	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.94 (t, J=7 Hz, 3 H), 0.98 - 1.11 (m, 2 H), 1.13 - 1.27 (m, 2 H), 1.38 (d, J=6 Hz, 6 H), 1.40 - 1.54 (m, 3 H), 1.67 - 1.85 (m, 4 H), 2.25 (t, J=7 Hz, 2 H), 2.46 - 2.50 (m hidden, 4 H), 2.84 (t, J=7 Hz, 2 H), 3.33 - 3.36 (m partially hidden, 1 H), 3.40 (t, J=5 Hz, 2 H), 3.43 - 3.65 (m, 94 H), 4.04 - 4.20 (m, 2 H), 5.41 (dt, J=12, 6 Hz, 1 H), 5.82 (s, 2 H), 7.65 (br d, J=8 Hz, 1 H)
P43 Example 51	1471.8	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7 Hz, 3 H), 1.24 (d, J=6 Hz, 6 H), 1.33 (sxt, J=7 Hz, 2 H), 1.64 (quin, J=8 Hz, 2 H), 2.27 - 2.39 (m, 4 H), 2.88 (t, J=8 Hz, 2 H), 3.14 (q, J=7 Hz, 2 H), 3.36 (t, J=6 Hz, 2 H), 3.41 - 3.54 (m, 76 H), 3.56 - 3.66 (m, 4 H), 4.24 (d, J=6 Hz, 2 H), 5.16 (spt, J=6 Hz, 1 H), 5.63 (s, 2 H), 7.00 (s, 2 H), 7.08 (d, J=8 Hz, 2 H), 7.22 (d, J=8 Hz, 2 H), 7.92 - 8.04 (m, 1 H), 8.32 (t, J=6 Hz, 1 H), 8.55 (s, 2 H), 13.71 (s, 1 H)
P44 Example 52	1489.9	Not determined.
P45	1648.1	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7 Hz, 3 H), 1.24 (d, J=6 Hz, 6 H), 1.28 - 1.38 (m, 2 H), 1.59 - 1.70 (m, 2 H), 2.29 - 2.39 (m, 4 H), 2.88 (t, J=8 Hz, 2 H), 3.14 (q, J=6 Hz, 2 H), 3.35 (m hidden, 2 H), 3.45 - 3.52 (m, 92 H), 3.56 - 3.63 (m, 4 H), 4.24 (d, J=6 Hz, 2 H), 5.16 (quin, J=6 Hz, 1 H), 5.63 (s, 2 H), 7.00 (s, 2 H), 7.08 (d, J=8 Hz, 2 H), 7.22 (d, J=8 Hz, 2 H), 8.00 (t, J=5 Hz, 1 H), 8.33 (t, J=6 Hz, 1 H), 8.58 (br s, 2 H), 13.70 (br d, J=2 Hz, 1 H)
P46 Example 53	1450.6	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.3 Hz, 3 H), 1.19 (d, J=6.1 Hz, 6 H), 1.26 - 1.40 (m, 2 H), 1.60 (quin, J=7.5 Hz, 2 H), 2.73 (br t, J=7.7 Hz, 2 H), 3.40 (t, J=5 Hz, 2 H), 3.45 - 3.62 (m, 90 H), 3.67 (s, 2 H), 3.73 (t, J=6.4 Hz, 2 H), 4.08 - 4.24 (m, 2 H), 4.89 - 5.12 (m, 1 H), 5.44 (s, 2 H), 7.01 (d, J=8.3 Hz, 2 H), 7.29 (d, J=7.8 Hz, 2 H)
P47	1347.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.21 (d, J=6.1 Hz, 6 H); 1.26 - 1.39 (m, 2 H); 1.63 (quin, J=7.6 Hz, 2 H); 2.33 - 2.37 (m, 2 H); 2.75 - 2.83 (m, 2 H); 3.30 - 3.73 (m, 82 H); 4.23 (d, J=5.9 Hz, 2 H); 5.27 - 5.38 (m, 1 H); 5.55 (s, 2 H); 5.91 (s, 2 H); 7.02 (d, J=8.2 Hz, 2 H); 7.20 (d, J=8.2 Hz, 2 H); 8.30 (t, J=6.1 Hz, 1 H)
P48	1170.4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.3 Hz, 3 H); 1.22 (d, J=6.1 Hz, 6 H); 1.27 - 1.39 (m, 2 H); 1.64 (quin, J=7.6 Hz, 2 H); 2.35 (t, J=6.4 Hz, 2 H); 2.79 (t, J=7.7 Hz, 2 H); 3.25 - 3.72 (m partially hidden, 66 H); 4.23 (d, J=5.9 Hz, 2 H); 5.25 - 5.38 (m, 1 H); 5.55 (s, 2 H); 5.95 - 6.13 (m, 2 H); 7.02 (d, J=8.1 Hz, 2 H); 7.20 (d, J=8.1 Hz, 2 H); 8.28 (t, J=5.8 Hz, 1 H)

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise mentioned	¹ H NMR
P49	994.3	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.22 (d, J=6.1 Hz, 6 H); 1.28 - 1.38 (m, 2 H); 1.63 (quin, J=7.6 Hz, 2 H); 2.35 (t, J=6.2 Hz, 2 H); 2.75 - 2.83 (m, 2 H); 3.24 - 3.67 (m partially hidden, 50 H); 4.23 (d, J=5.9 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.55 (s, 2 H); 5.92 - 6.14 (m, 2 H); 7.02 (d, J=8.1 Hz, 2 H); 7.20 (d, J=8.1 Hz, 2 H); 8.30 (t, J=5.9 Hz, 1 H)
P50 Example 54	459.3	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7 Hz, 3 H) 1.21 (d, J=6 Hz, 6 H) 1.32 (m, 2 H) 1.63 (quin, J=8 Hz, 2 H) 2.35 (t, J=6 Hz, 2 H) 2.43 (t, J=6 Hz, 2 H) 2.79 (t, J=8 Hz, 2 H) 3.41 - 3.69 (m, 48 H) 4.23 (d, J=6 Hz, 2 H) 5.32 (spt, J=6 Hz, 1 H) 5.55 (s, 2 H) 5.96 (s, 2 H) 7.02 (d, J=8 Hz, 2 H) 7.20 (d, J=8 Hz, 2 H) 8.28 (t, J=6 Hz, 1 H)
P51	818.3	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.3 Hz, 3 H); 1.21 (d, J=6.2 Hz, 6 H); 1.32 (m, 2 H); 1.63 (quin, J=7.6 Hz, 2 H); 2.35 (t, J=6.4 Hz, 2 H); 2.75 - 2.82 (m, 2 H); 3.24 - 3.65 (m partially hidden, 34 H); 4.23 (d, J=5.9 Hz, 2 H); 5.27 - 5.39 (m, 1 H); 5.55 (s, 2 H); 5.88 (s, 2 H); 7.02 (d, J=8.2 Hz, 2 H); 7.20 (d, J=8.2 Hz, 2 H); 8.28 (t, J=5.9 Hz, 1 H)
P52	560.3	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7 Hz, 3 H) 1.24 (d, J=6 Hz, 6 H) 1.28 - 1.40 (m, 2 H) 1.60 - 1.70 (m, 2 H) 2.28 - 2.40 (m, 4 H) 2.88 (t, J=8 Hz, 2 H) 3.14 (q, J=6 Hz, 2 H) 3.36 (t, J=6 Hz, 2 H) 3.43 - 3.54 (m, 44 H) 3.55 - 3.64 (m, 4 H) 4.24 (d, J=6 Hz, 2 H) 5.16 (spt, J=6 Hz, 1 H) 5.63 (s, 2 H) 7.00 (s, 2 H) 7.07 (d, J=8 Hz, 2 H) 7.22 (d, J=8 Hz, 2 H) 7.99 (br t, J=5 Hz, 1 H) 8.32 (t, J=6 Hz, 1 H) 8.51 (br s, 2 H) 13.55 - 13.78 (m, 1 H)
P53 Example 55	1374.2	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ in ppm 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.1 Hz, 6 H); 1.25 - 1.39 (m, 2 H); 1.47 (s, 6 H); 1.56 - 1.68 (m, 2 H); 2.27 - 2.37 (m, 2 H); 2.78 - 2.85 (m, 2 H); 3.33 - 3.74 (m, 82 H); 5.24 - 5.39 (m, 1 H); 5.53 (s, 2 H); 5.89 (s, 2 H); 6.98 (d, J=8.4 Hz, 2 H); 7.27 (d, J=8.4 Hz, 2 H); 7.99 (s, 1 H)
P54 Example 56	1360.5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.1 Hz, 6 H); 1.28 - 1.40 (m, 2 H); 1.61 - 1.72 (m, 2 H); 2.56 (t, J=6.5 Hz, 2 H); 2.75 - 2.81 (m, 2 H); 2.84 (br s, 3 H); 3.31 - 3.75 (m, 82 H); 4.48 (br s, 2 H); 5.26 - 5.40 (m, 1 H); 5.49 (br s, 2 H); 5.55 (s, 2 H); 7.03 (br d, J=8.1 Hz, 2 H); 7.17 (d, J=8.1 Hz, 2 H)
P55 Example 57	1419 [M+HCO O]	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.3 Hz, 3 H); 1.06 (s, 6 H); 1.21 (d, J=6.1 Hz, 6 H); 1.27 - 1.39 (m, 2 H); 1.58 - 1.67 (m, 2 H); 2.74 - 2.83 (m, 2 H); 3.35 - 3.64 (m, 82 H); 4.23 (d, J=6.0 Hz, 2 H); 5.32 (quin, J=6.2 Hz, 1 H); 5.54 (s, 2 H); 5.87 (s, 2 H); 7.00 (d, J=8.3 Hz, 2 H); 7.18 (d, J=8.3 Hz, 2 H); 7.91 (t, J=5.9 Hz, 1 H)
P56 Example 58	1401.3	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.3 Hz, 3 H); 1.20 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.44 - 1.57 (m, 6 H); 1.58 - 1.69 (m, 2 H); 1.86 - 1.95 (m, 2 H); 2.75 - 2.82 (m, 2 H); 3.36 - 3.65 (m, 80 H); 3.43 (s, 2 H); 4.24 (d, J=6.2 Hz, 2 H); 5.32 (spt, J=6.1 Hz,

Compound Number (P for payload)	LCMS [M+H] ⁺ unless otherwise mentioned	¹ H NMR
		1 H); 5.54 (s, 2 H); 5.89 (s, 2 H); 7.00 (d, J=8.0 Hz, 2 H); 7.19 (d, J=8.0 Hz, 2 H); 7.95 (t, J=6.1 Hz, 1 H)
P57 Example 59	1416.4	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.1 Hz, 6 H); 1.22 - 1.52 (m, 10 H); 1.59 - 1.66 (m, 2 H); 1.86 - 1.94 (m, 2 H); 2.76 - 2.80 (m, 2 H); 3.35 - 3.65 (m, 80 H); 4.25 (d, J=6.1 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.90 (s, 2 H); 7.00 (d, J=8.5 Hz, 2 H); 7.20 (d, J=8.2 Hz, 2 H); 7.94 (br t, J=6.1 Hz, 1 H)
P58 Example 60	1362.3 [M+HCO O] ⁻	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.57 - 1.68 (m, 2 H); 2.75 - 2.82 (m, 2 H); 3.34 - 3.70 (m, 78 H); 4.02 - 4.07 (m, 2 H); 4.13 (br d, J=6.1 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.55 (s, 2 H); 5.90 (s, 2 H); 7.02 (d, J=8.1 Hz, 2 H); 7.20 (d, J=8.1 Hz, 2 H); 7.73 (t, J=6.1 Hz, 1 H)
P59 Example 61	1317.2	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 0.84 (t, J=7.4 Hz, 3 H); 1.21 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.59 - 1.70 (m, 2 H); 2.76 - 2.82 (m, 2 H); 3.11 - 3.17 (m, 2 H); 3.22 - 3.68 (m partially hidden, 78 H); 4.15 (d, J=6.0 Hz, 2 H); 5.32 (quin, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.89 (s, 2 H); 5.94 (t, J=5.7 Hz, 1 H); 6.37 (t, J=5.8 Hz, 1 H); 7.02 (d, J=8.0 Hz, 2 H); 7.19 (d, J=8.0 Hz, 2 H)

The present disclosure further relates to a compound/conjugate of formula (II) or a pharmaceutically acceptable salt thereof:

5

A-L1-G-BA (II)

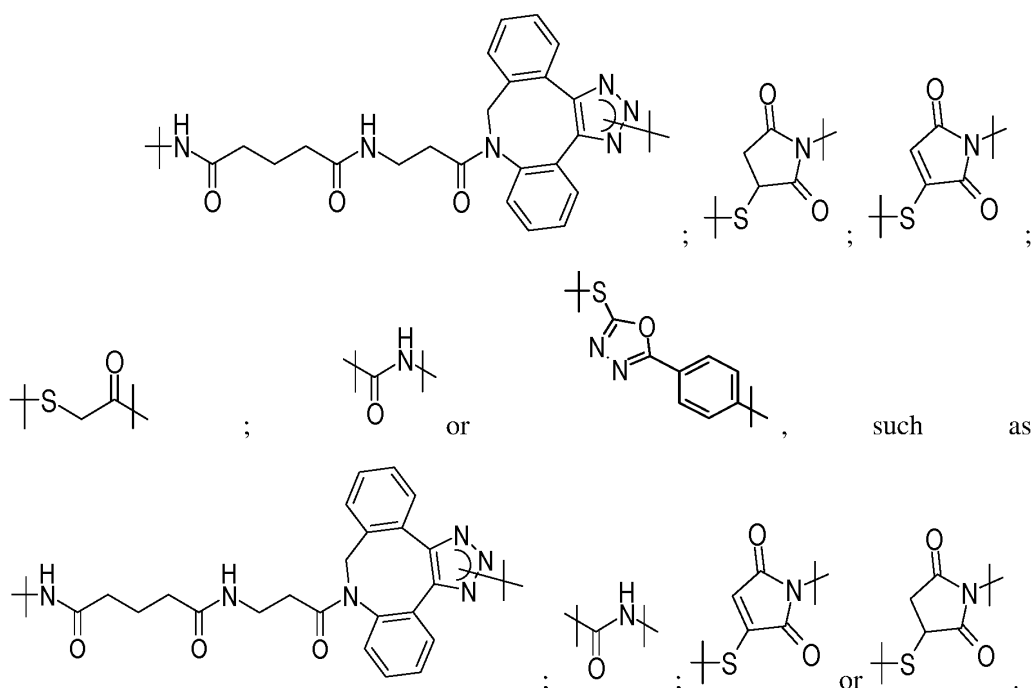
wherein:

A, and **L1** are as defined in the present disclosure;

BA represents a binding agent for instance a protein, a polypeptide, a ligand, a peptide, an oligonucleotide or an oligosaccharide, for instance **BA** is a targeting agent for instance a

10

cell-binding agent such as an antibody, such as a monoclonal antibody or a VHH antibody; **G** represents the product of reaction between RCG1 as defined in the present disclosure and RCG2, a reactive group present on the binding agent, for instance G is selected from the group consisting of :

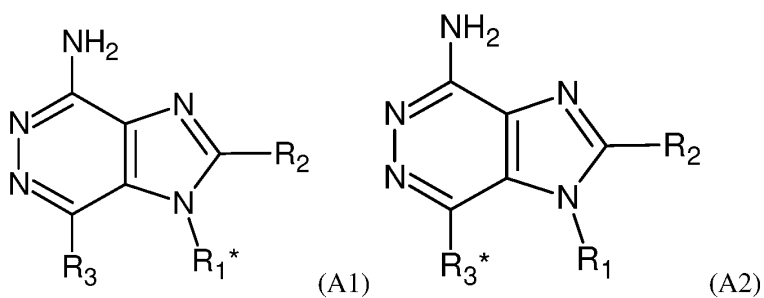


- 5 According to a particular embodiment, **L1** represents a cleavable linker or a non - cleavable linker.

According to one particular embodiment, L1 is a cleavable linker which is a peptidic linker. According to another particular embodiment, L1 is a non-cleavable linker which is a PEGylated linker.

10

Among the compounds of formula (II) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2)



- 15 in which:

R₁, R₂ and R₃ are as defined in the present disclosure;

the single asterisk (*) indicates the site of covalent attachment to -L1-G-BA;

R₁* represents:

5 - either a single bond, which means that -L1-G-BA is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from **R₁** as defined in the present disclosure, which means that -L1-G-BA is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* **R₁**;

R₃* represents:

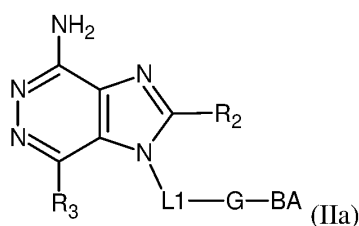
10 - either a single bond, which means that -L1-G-BA is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from **R₃** as defined in the present disclosure, which means that -L1-G-BA is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* **R₃**.

15 According to one particular embodiment, **A** is of formula (A1).

According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when **R₁*** represents a single bond, the compounds of formula (II) or a pharmaceutically acceptable salt thereof in accordance with
20 the present disclosure have the following formula (IIa):

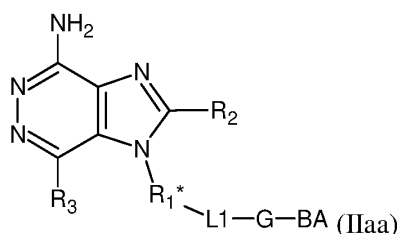


wherein **R₂, R₃, L1, G and BA** are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₁** is a hydrogen atom.

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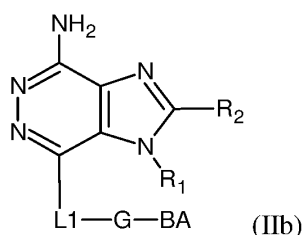
According to another particular embodiment, when **R₁*** represents a divalent radical formed from **R₁** as defined in the present disclosure, the compounds of formula (II) or a

pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIaa):



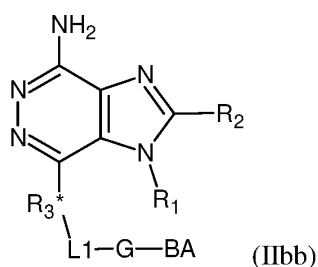
- 5 wherein **R₂**, **R₃**, **L1**, **G** and **BA** are as defined in the present disclosure and **R₁^{*}** is a divalent **R₁** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₁** is not a hydrogen atom.

- 10 According to another particular embodiment, when **R₃^{*}** represents a single bond, the compounds of formula (II) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIb):



- wherein **R₁**, **R₂**, **L1**, **G** and **BA** are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

- 15 According to another particular embodiment, when **R₃^{*}** represents a divalent radical formed from **R₃** as defined in the present disclosure, the compounds of formula (II) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIbb):



wherein **R₁**, **R₂**, **L1**, **G** and **BA** are as defined in the present disclosure and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is not a hydrogen atom.

- 5 According to a particular embodiment, the binding agent **BA** represents an antibody **Ab**, for instance a monoclonal antibody or a VHH antibody.

Among the compounds of formula (II) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

- 10 **A** is of formula (A) in which:

R₁ represents:

- a (C₁-C₆)alkyl- group, in particular a methyl group;
- a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or
- 15 substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:
 - a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted) or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:
 - 20 - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group, for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in
 - 25 particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or
 - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at

least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

R2 represents :

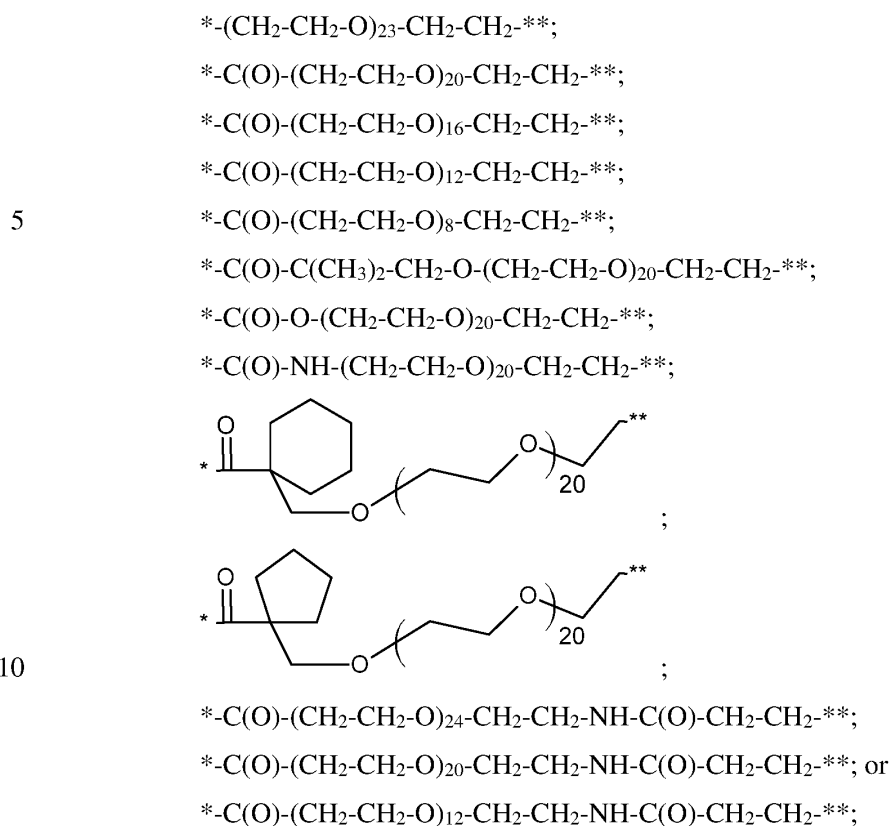
- a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;
- 5 - a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

R3 represents :

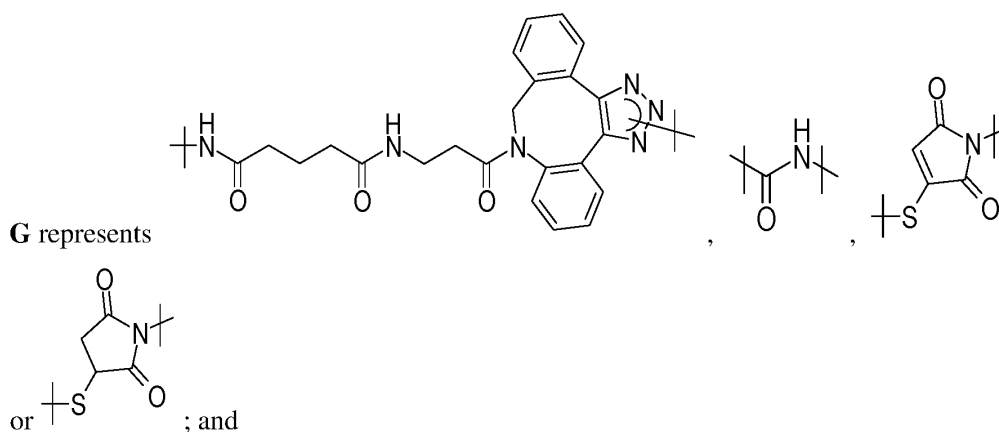
- a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that is to say a -O-CH(CH₃)₂ group);
- 10 - a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperazinyl group; or
- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected
- 15 from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group;

L1 represents:

- *-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-
- 20 CH₂-O)₃-(CH₂)₂-** ;
- *-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-
- CH₂-O)₃-(CH₂)₂-**;
- *-Cit-Val-C(O)-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- *-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;
- 25 *-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;
- *-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-
- CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-** ;
- *-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-
- CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- 30 *-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- *-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-**;

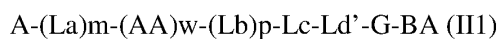


the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group;



BA represents an antibody, such as a monoclonal antibody or a VHH antibody.

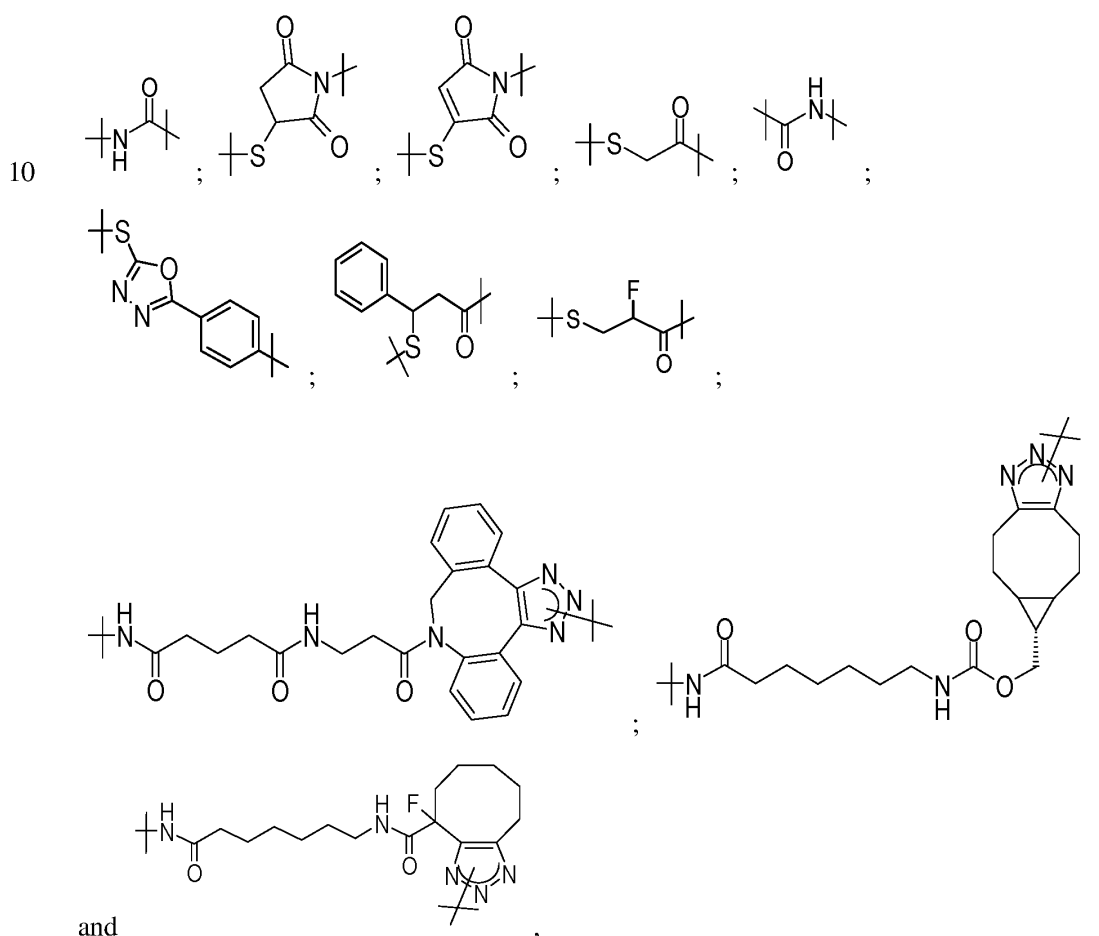
According to a particular embodiment, the compounds of formula (II) or a pharmaceutically acceptable salt thereof are chosen among compounds of formula (III) or of formula (II2):



5 wherein:

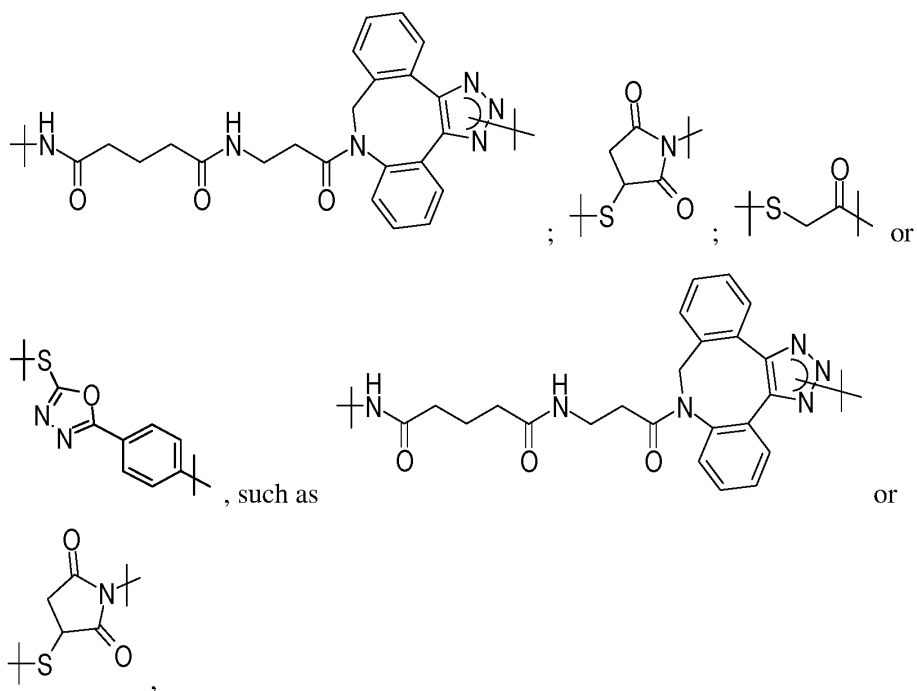
A, La, m, (AA)_w, AA, w, Lb, p, Lc, Ld' and **BA** are as defined in the present disclosure;

G represents the product of reaction between RCG1 as defined in the present disclosure and RCG2, a reactive group present on the binding agent, for instance G is selected from the group consisting of :



for instance, the left side of the G groups being linked to BA and the right side of the G

15 groups being linked to Ld', for instance G represents the following groups:



5

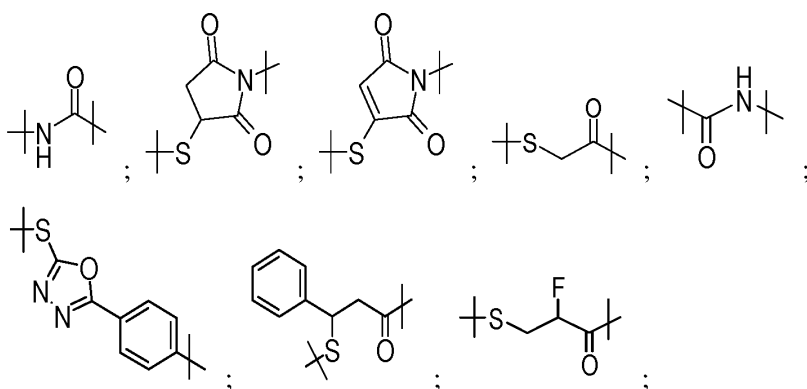
A-(P)o-L-Ld-G-BA (II2)

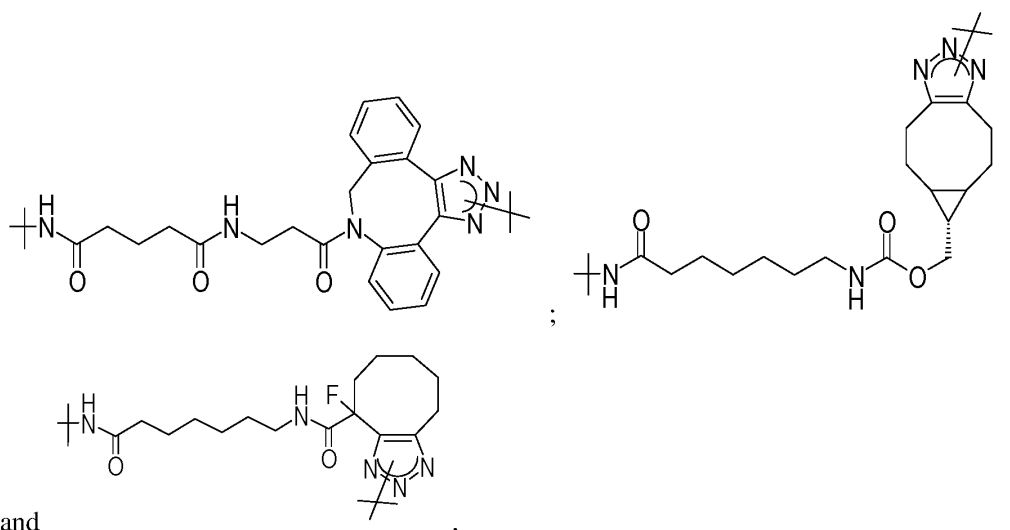
wherein:

A, P, o, L, Ld and **BA** are as defined in the present disclosure;

G represents the product of reaction between RCG1 as defined in the present disclosure and

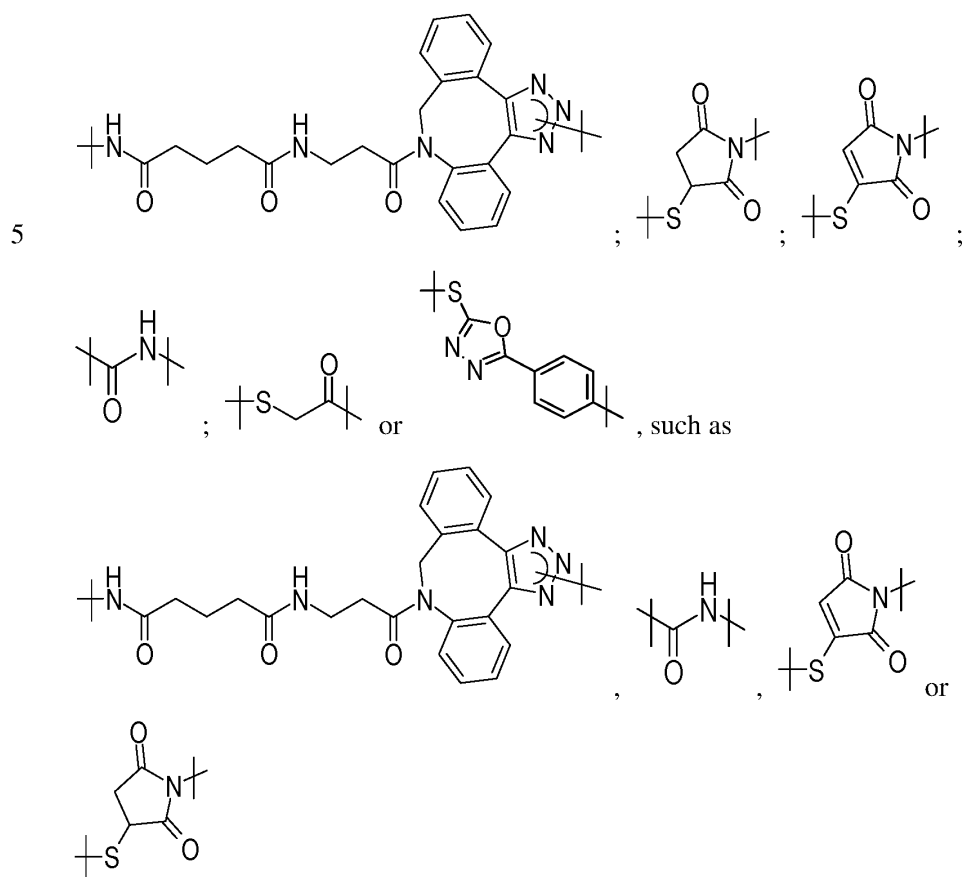
10 **RCG2**, a reactive group present on the binding agent instance **G** is selected from the group consisting of:





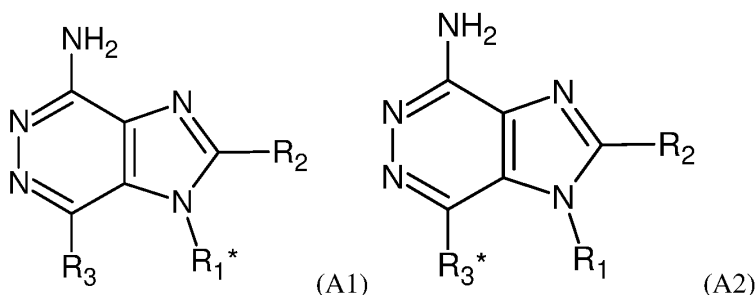
and

for instance, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld, for instance G represents the following groups:



According to a particular embodiment, the binding agent **BA** is an antibody Ab such as a monoclonal antibody or a VHH antibody.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of
5 compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2)



in which:

R₁, **R₂** and **R₃** are as defined in the present disclosure;

10 the single asterisk (*) indicates the site of covalent attachment to -(La)m-(AA)_w-(Lb)_p-Lc-Ld'-G-BA;

R₁^{*} represents:

- either a single bond, which means that -(La)m-(AA)_w-(Lb)_p-Lc-Ld'-G-BA is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,
- 15 - or a divalent radical formed from R₁ as defined in the present disclosure, which means that -(La)m-(AA)_w-(Lb)_p-Lc-Ld'-G-BA is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* R₁;

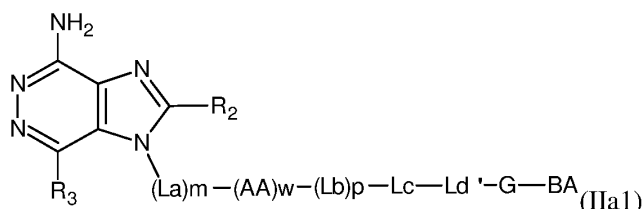
R₃^{*} represents:

- either a single bond, which means that -(La)m-(AA)_w-(Lb)_p-Lc-Ld'-G-BA is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,
- 20 - or a divalent radical formed from R₃ as defined in the present disclosure, which means that -(La)m-(AA)_w-(Lb)_p-Lc-Ld'-G-BA is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* R₃.

According to one particular embodiment, **A** is of formula (A1).

25 According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when R_1^* represents a single bond, the compounds of formula (II1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIa1):

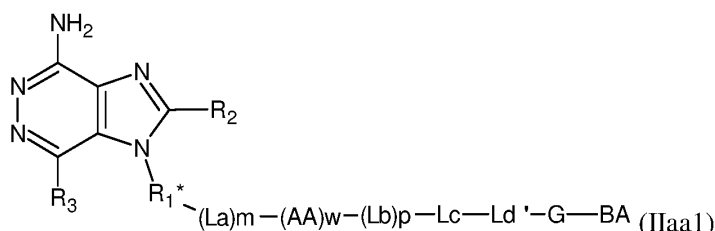


5

wherein R_2 , R_3 , La , m , AA , w , Lb , p , Lc , Ld' , G and BA are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, R_1 is a hydrogen atom.

- 10 According to another particular embodiment, when R_1^* represents a divalent radical formed from R_1 as defined in the present disclosure, the compounds of formula (II1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIaa1):

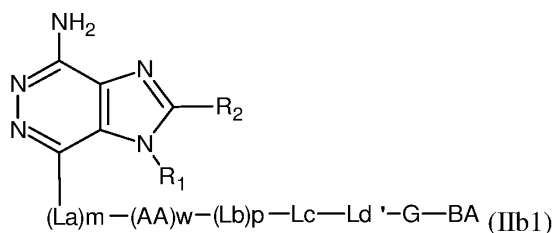
15



wherein R_2 , R_3 , La , m , AA , w , Lb , p , Lc , Ld' , G and BA are as defined in the present disclosure and R_1^* is a divalent R_1 as defined in the present disclosure. This means that in this particular embodiment, in the drug A, R_1 is not a hydrogen atom.

20

According to another particular embodiment, when R_3^* represents a single bond, the compounds of formula (II1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIb1):

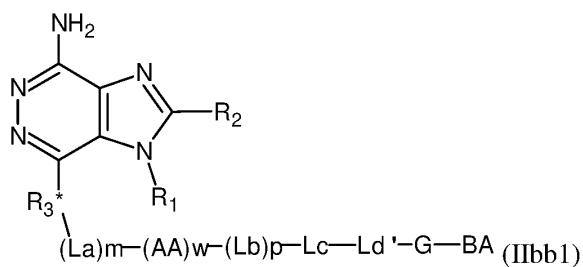


wherein **R₁**, **R₂**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'**, **G** and **BA** are as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

5

According to another particular embodiment, when **R₃*** represents a divalent radical formed from **R₃** as defined in the present disclosure, the compounds of formula (II1) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIbb1):

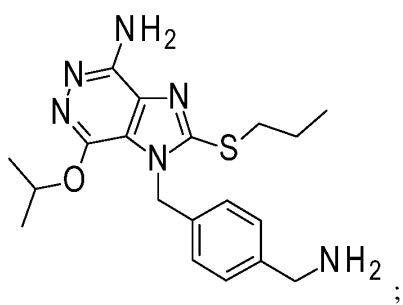
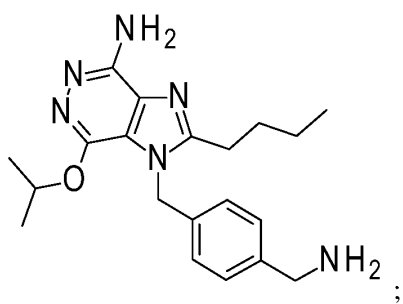
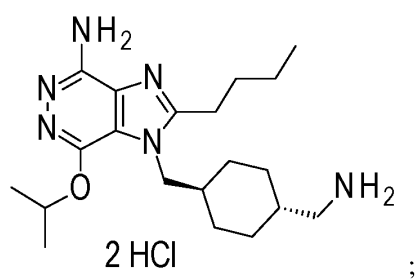
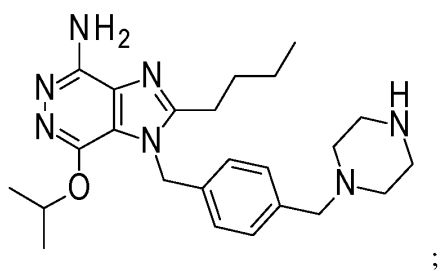
10

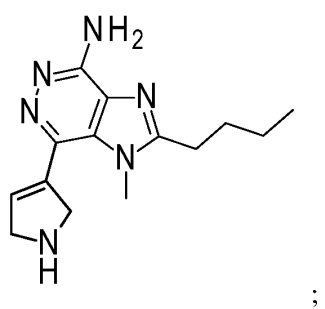
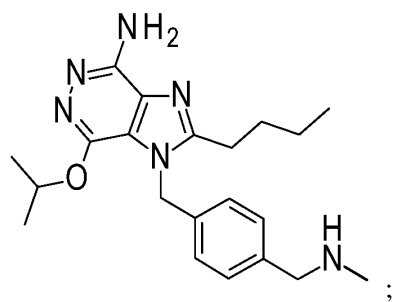
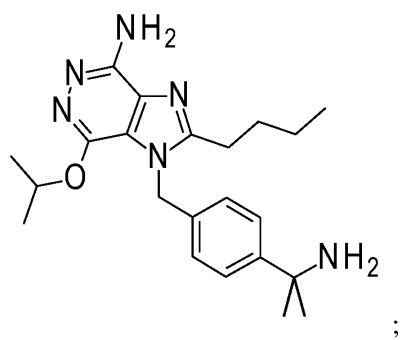
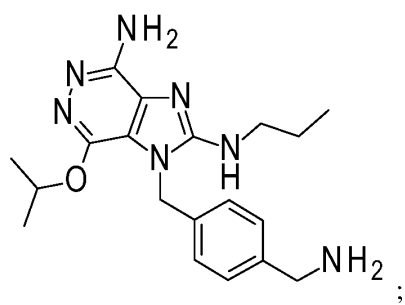


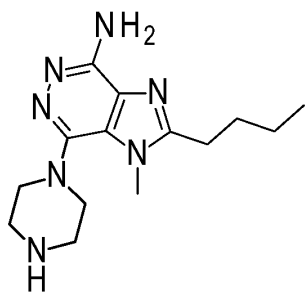
wherein **R₁**, **R₂**, **La**, **m**, **AA**, **w**, **Lb**, **p**, **Lc**, **Ld'**, **G** and **BA** are as defined in the present disclosure and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is not a hydrogen atom.

15

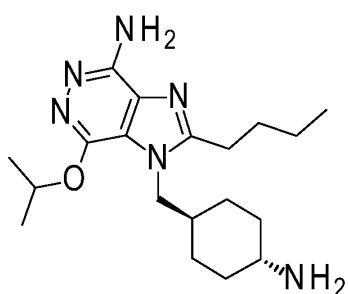
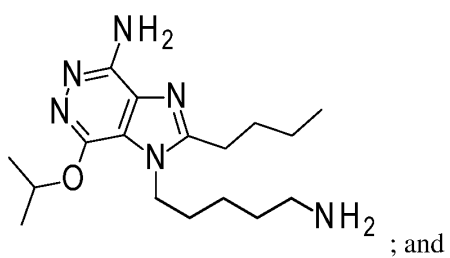
Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:



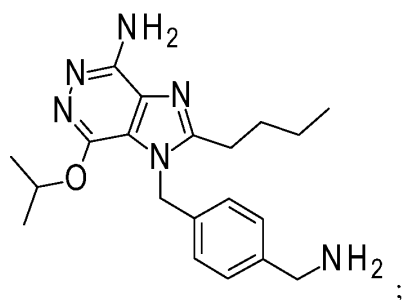


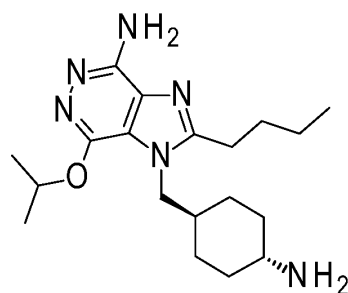
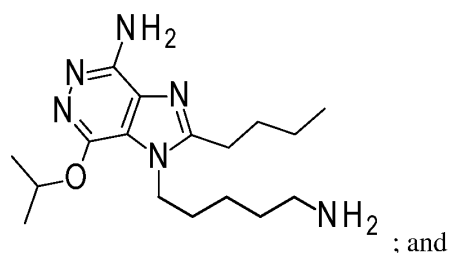


;



- 5 Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:





Among the compounds of formula (III) that are subject matter of the disclosure, a group of
 5 compounds is composed of the compounds for which **La** is a self-immolative linker. Self
 immolative linkers are well known for the person of the art. **La** is a covalent assembly
 tailored to correlate the cleavage of two chemical bonds after activation of a protective part
 in a precursor. Thus, its list has not to be limited or restricted.

For instance, **La** represents a *-C(O)-O-(CR₁₄R₁₅)-aryl-NR₁₆-** group, *-C(O)-O-
 10 (CR₁₄R₁₅)-heteroaryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-**, a *-C(O)-NR₁₇-
 (C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-aryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-
 NR₁₈-C(O)-O-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, a *-CR₁₅R₁₄-aryl-NR₁₆-** group or a *-
 CR₁₅R₁₄-heteroaryl-NR₁₆-** group, aryl being a (C₆-C₁₀) membered aryl group and
 15 heteroaryl being a (C₅-C₁₀) membered heteroaryl group comprising one to four heteroatoms
 selected from oxygen, nitrogen and sulfur, the single asterisk (*) indicating the site of
 covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site
 of covalent attachment to the (AA)_w radical, R₁₄, R₁₅, R₁₆, R₁₇ and R₁₈ being a hydrogen
 atom or a (C₁-C₆)alkyl group.

20 Among the compounds of formula (III) that are subject matter of the disclosure, a group of
 compounds is composed of the compounds for which **La** represents a *-C(O)-O-CH₂-
 phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the

A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)w radical.

5 Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which in (AA)w, w represents an integer from 1 to 6, and AA is chosen from valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu).

10 Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which in (AA)w, w represents an integer 2 or 3, and AA is chosen from valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu).

For instance, (AA)w comprises a Citrulline (Cit).

For instance, (AA)w is chosen among Glu-Val-Cit; Glu-Val-Ala; Val-Cit or Val-Ala.

15 For instance, when the amino acid sequence (AA)w is Glu-Val-Cit, the citrulline (Cit) is linked to La or to A (if La is absent, that is to say if m is 0) and the glutamic acid (Glu) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

20 For instance, when the amino acid sequence (AA)w is Glu-Val-Ala, the alanine (Ala) is linked to La or to A (if La is absent, that is to say if m is 0) and the glutamic acid (Glu) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

For instance, when the amino acid sequence (AA)w is Val-Cit, the citrulline (Cit) is linked to La or to A (if La is absent, that is to say if m is 0) and the valine (Val) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

25 For instance, when the amino acid sequence (AA)w is Val-Ala, the alanine (Ala) is linked to La or to A (if La is absent, that is to say if m is 0) and the valine (Val) is linked to Lb or to Lc (if Lb is absent, that is to say if p is 0).

For instance, (AA)w is Val-Cit.

30 Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which Lb represents a *-C(O)-(C₁-C₆)alkyl-C(O)-NH-** group or a *-C(O)-** group, the single asterisk (*) indicating the

site of covalent attachment to the (AA)_w radical and the double asterisk (**) indicating the site of covalent attachment to the L_c radical.

Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_b** represents a *-C(O)-(CH₂)₃-C(O)-NH-**- group or a *-C(O)-**- group, the single asterisk (*) indicating the site of covalent attachment to the (AA)_w radical and the double asterisk (**) indicating the site of covalent attachment to the L_c radical.

Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-**- group in which n represents an integer from 2 to 12, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-**- group in which n represents an integer from 3 to 7, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_c** represents a *-(CH₂-CH₂-O)_n-**- group in which n represents an integer 3 or 4, the single asterisk (*) indicating the site of covalent attachment to the L_b radical if present (that is to say if p is 1) or directly to the (AA)_w radical if L_b is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the L_d' radical.

Among the compounds of formula (II1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L_d'** represents a *-(C₂-C₆)alkyl-**- group, a *-(C₂-C₆)alkyl-NH-C(O)-(C₂-C₆)alkyl-**- group or a *-(C₂-C₆)alkyl-C(O)-NH-(C₂-C₆)alkyl-**- group, the single asterisk (*) indicating the site of

covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical.

Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **Ld'** represents a
 5 $^{*}-(\text{CH}_2)_2-^{**}$ group, a $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^{*}-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical.

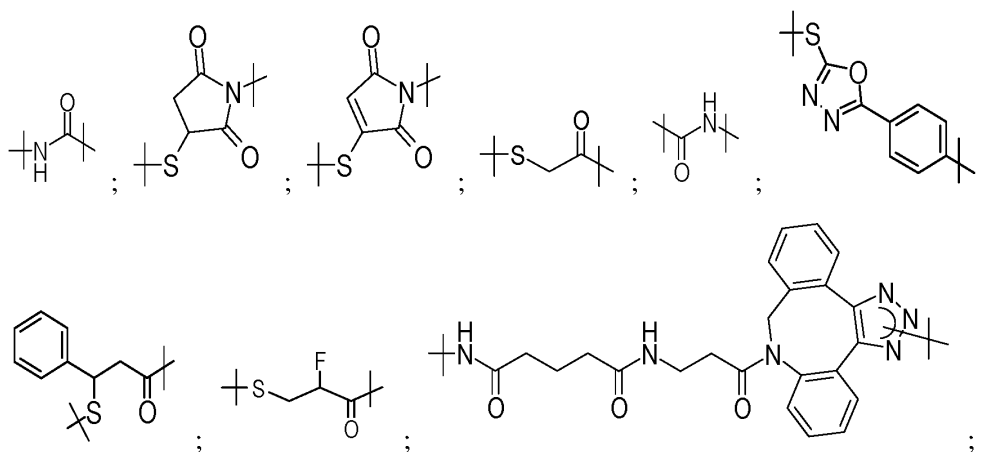
Among the compounds of formula (III1) that are subject matter of the disclosure,
 10 a group of compounds is composed of the compounds for which **m** represents an integer 1.

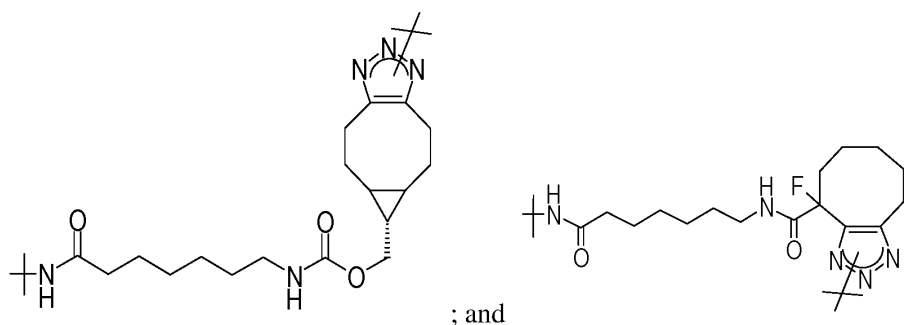
Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **m** represents 0.

Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **p** represents an integer 1.

15 Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **p** represents 0.

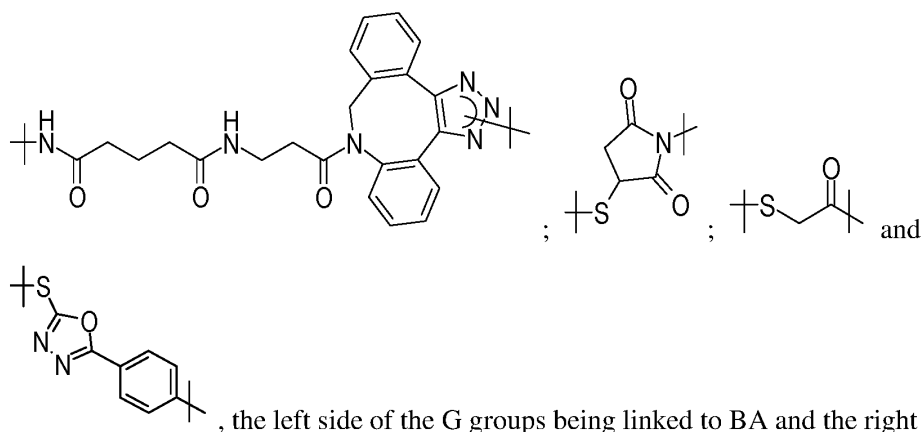
Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting
 20 of:





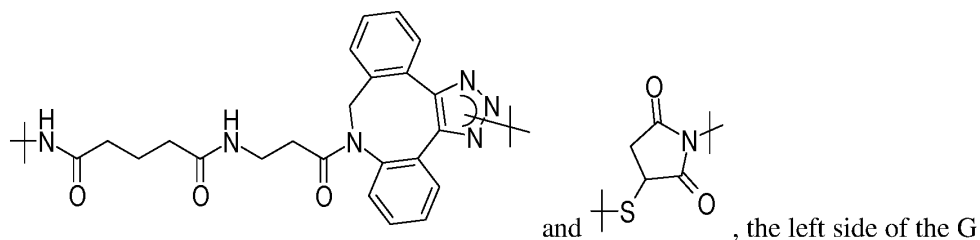
the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld'.

- 5 Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting of:



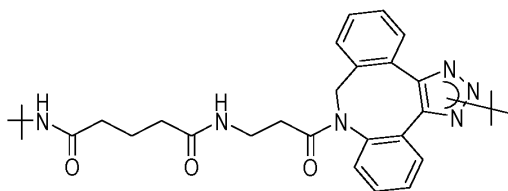
- 10 the G groups being linked to Ld'.

Among the compounds of formula (III1) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting of:



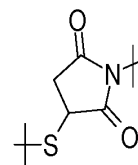
- 15 groups being linked to BA and the right side of the G groups being linked to Ld'.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical, then **G** is



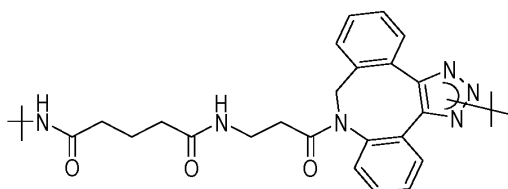
, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld'.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(C_2-C_6)alkyl-NH-C(O)-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical, then **G** is



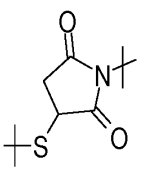
, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld'.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical, then **G** is



, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld'.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld'** represents a $^{*}-(CH_2)_2-NH-C(O)-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent

- 5 attachment to the G group belonging to the -G-BA radical, then **G** is , the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld'.

- 10 Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which BA is an antibody Ab.

Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which Ab is a monoclonal antibody, for instance tusamitamab, and avelumab variants.

- 15 Among the compounds of formula (III) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which Ab is a VHH antibody.

All these sub-groups taken alone or in combination are part of the present disclosure.

- 20 According to a particular embodiment, the disclosure relates to a compound/conjugate of formula (III) or a pharmaceutically acceptable salt thereof wherein **A**, **G** and **BA** are as defined in the present disclosure; and

L_a is $^{*}-C(O)-O-CH_2$ -para-phenyl-NH ** ;

m is 1;

AA is selected from Ala, Val, Cit and Glu;

- 25 w is 2 or 3;

L_b is $^{*}-C(O)-(CH_2)_3-C(O)-NH-^{**}$;

p is 1;

L_c is $^{*}-(CH_2-CH_2-O)_3-^{**}$; and

L_{d'} is $^{*}-(CH_2)_2-^{**}$ or $^{*}-(CH_2)_2-NH-C(O)-(CH_2)_2-^{**}$.

According to another particular embodiment, the disclosure relates to a compound/conjugate of formula (II1) or a pharmaceutically acceptable salt thereof wherein:

A, G and BA are as defined in the present disclosure;

5 m is 0;

AA is selected from Ala, Val and Cit;

w is 2;

Lb is $^*\text{-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-}^{**}$ or $^*\text{-C(O)-}^*$;

p is 1;

10 Lc is $^*\text{-(CH}_2\text{-CH}_2\text{-O)}_3\text{-}^{**}$; and

Ld' is $^*\text{-(CH}_2\text{)}_2\text{-}^{**}$ or $^*\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$.

According to another particular embodiment, the disclosure relates to a compound/conjugate of formula (II1) or a pharmaceutically acceptable salt thereof wherein

15 the $\text{-(La)}_m\text{-(AA)}_w\text{-(Lb)}_p\text{-Lc-Ld'}$ part is selected from the group consisting of:

$^*\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;

$^*\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;

20 $^*\text{-Cit-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;

$^*\text{-Ala-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-}^{**}$;

$^*\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;

25 $^*\text{-C(O)-O-CH}_2\text{-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;

$^*\text{-Cit-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;

$^*\text{-Ala-Val-C(O)-(CH}_2\text{)}_3\text{-C(O)-NH-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$;

and

$^*\text{-Cit-Val-C(O)-(CH}_2\text{-CH}_2\text{-O)}_3\text{-(CH}_2\text{)}_2\text{-NH-C(O)-(CH}_2\text{)}_2\text{-}^{**}$,

30 the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group.

Among the compounds of formula (III1) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

A is of formula (A) in which:

R₁ represents:

- 5 - a (C₁-C₆)alkyl- group, in particular a methyl group;
- a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:
- 10 - a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted) or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:
- a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group,
- 15 for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or
- 20 - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

R₂ represents :

- 25 - a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;
- a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

R₃ represents :

- a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that is to say a -O-CH(CH₃)₂ group);
- 30 - a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms

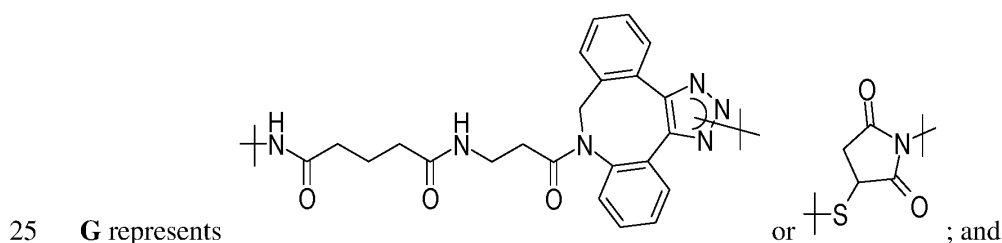
selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for instance a piperazinyl group; or

- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group;

*-(La)m-(AA)w-(Lb)p-Lc-Ld'-** represents:

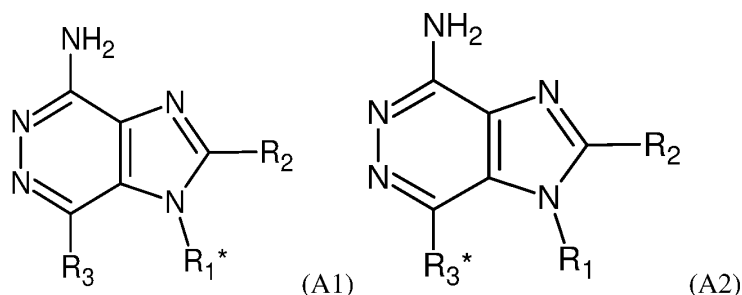
- *-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-** ;
- 10 *-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;
- *-Cit-Val-C(O)-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- *-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;
- *-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;
- 15 *-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-** ;
- *-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- *-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;
- 20 or
- *-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**,

the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group;



BA represents an antibody, such as a monoclonal antibody or a VHH antibody.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is of formula (A1) or of formula (A2)



5 in which:

R₁, R₂ and R₃ are as defined in the present disclosure;

the single asterisk (*) indicates the site of covalent attachment to $-(P)o-L-Ld-G-BA$;

R₁^{*} represents:

10 - either a single bond, which means that $-(P)o-L-Ld-G-BA$ is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R₁ as defined in the present disclosure, which means that $-(P)o-L-Ld-G-BA$ is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring *via* R₁;

R₃^{*} represents:

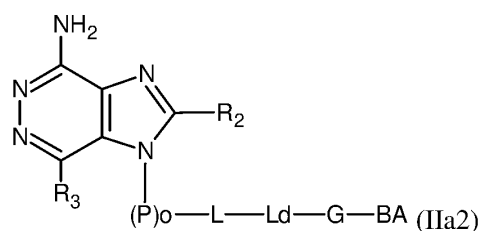
15 - either a single bond, which means that $-(P)o-L-Ld-G-BA$ is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R₃ as defined in the present disclosure, which means that $-(P)o-L-Ld-G-BA$ is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* R₃.

20 According to one particular embodiment, **A** is of formula (A1).

According to another embodiment, **A** is of formula (A2).

Thus, according to a particular embodiment, when **R₁^{*}** represents a single bond, the compounds of formula (II2) or a pharmaceutically acceptable salt thereof in accordance with
25 the present disclosure have the following formula (IIa2):



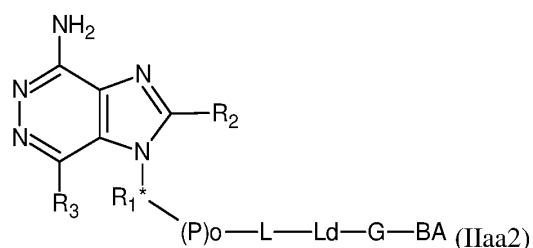
wherein **R₂**, **R₃**, **P**, **o**, **L**, **Ld**, **G** and **BA** are as defined in the present disclosure.

This means that in this particular embodiment, in the drug A, **R₁** is a hydrogen atom.

5

According to another particular embodiment, when **R₁*** represents a divalent radical formed from **R₁** as defined in the present disclosure, the compounds of formula (II2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIaa2):

10

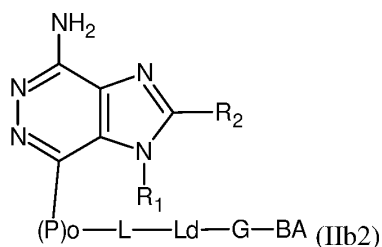


wherein **R₂**, **R₃**, **P**, **o**, **L**, **Ld**, **G** and **BA** are as defined in the present disclosure and **R₁*** is a divalent **R₁** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₁** is not a hydrogen atom.

15

According to another particular embodiment, when **R₃*** represents a single bond, the compounds of formula (II2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIb2):

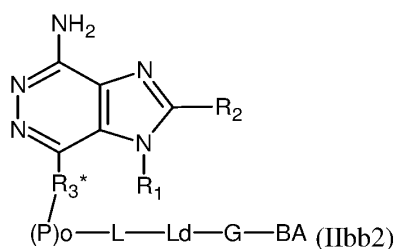
20



wherein **R₁**, **R₂**, **P**, **o**, **L**, **L_d**, **G** and **BA** are as defined in the present disclosure.

This means that in this particular embodiment, in the drug A, **R₃** is a hydrogen atom.

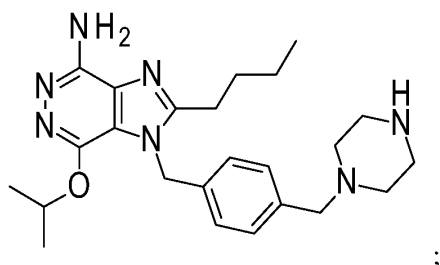
- 5 According to another particular embodiment, when **R₃*** represents a divalent radical formed from **R₃** as defined in the present disclosure, the compounds of formula (II2) or a pharmaceutically acceptable salt thereof in accordance with the present disclosure have the following formula (IIbb2):

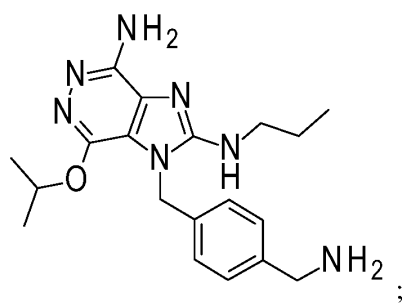
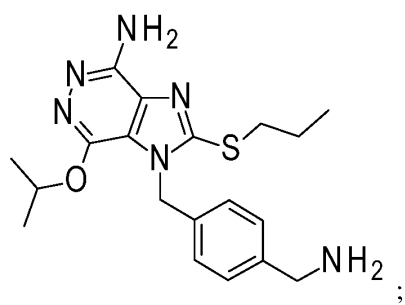
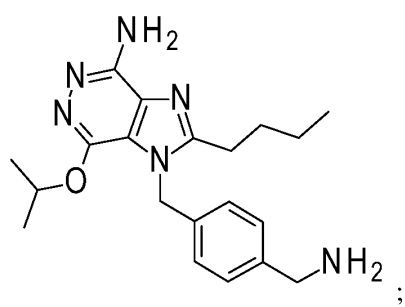
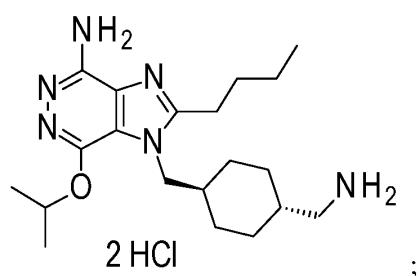


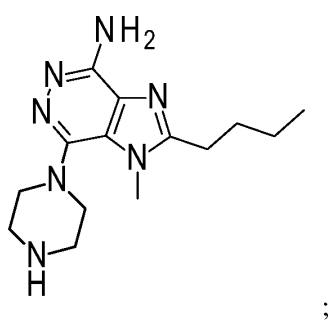
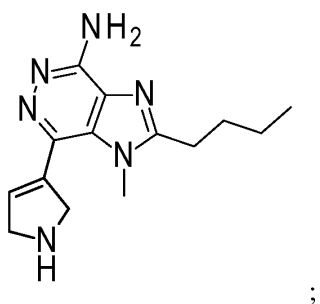
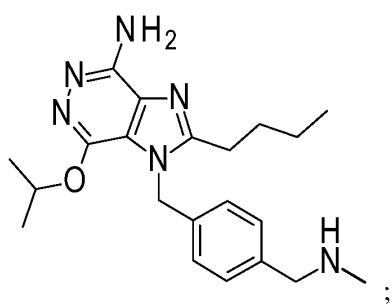
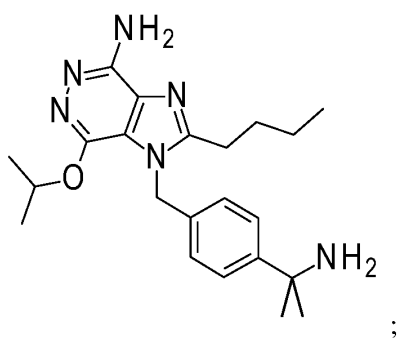
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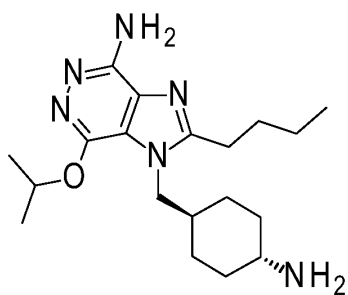
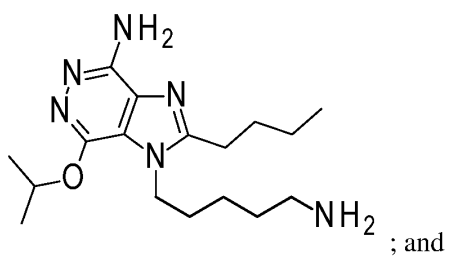
wherein **R₁**, **R₂**, **P**, **o**, **L**, **L_d**, **G** and **BA** are as defined in the present disclosure and **R₃*** is a divalent **R₃** as defined in the present disclosure. This means that in this particular embodiment, in the drug A, **R₃** is not a hydrogen atom.

- 15 Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:

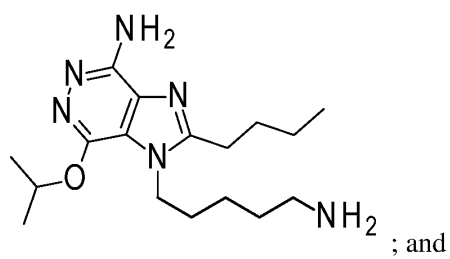
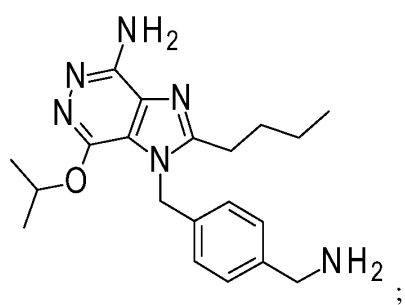


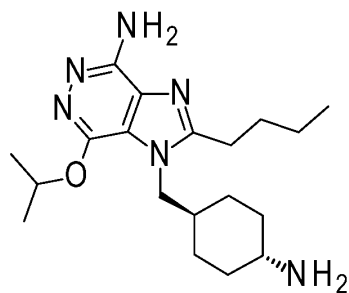






- Among the compounds of formula (II2) that are subject matter of the disclosure,
- 5 a group of compounds is composed of the compounds for which **A** is selected from the group consisting of:





Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)- group.

5 Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-O- group.

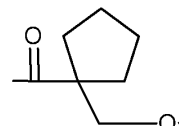
Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -S(O₂)- group.

10 Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-C(CH₃)₂-CH₂-
O- group.

Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which P is a -C(O)-NH- group.

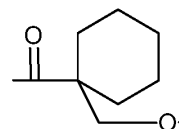
Among the compounds of formula (II2) that are subject matter of the disclosure,

15 a group of compounds is composed of the compounds for which P is a a
group.



Among the compounds of formula (II2) that are subject matter of the disclosure,

a group of compounds is composed of the compounds for which P is a
group.



20 Among the compounds of formula (II2) that are subject matter of the disclosure,
a group of compounds is composed of the compounds for which o is 0.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which o is 1.

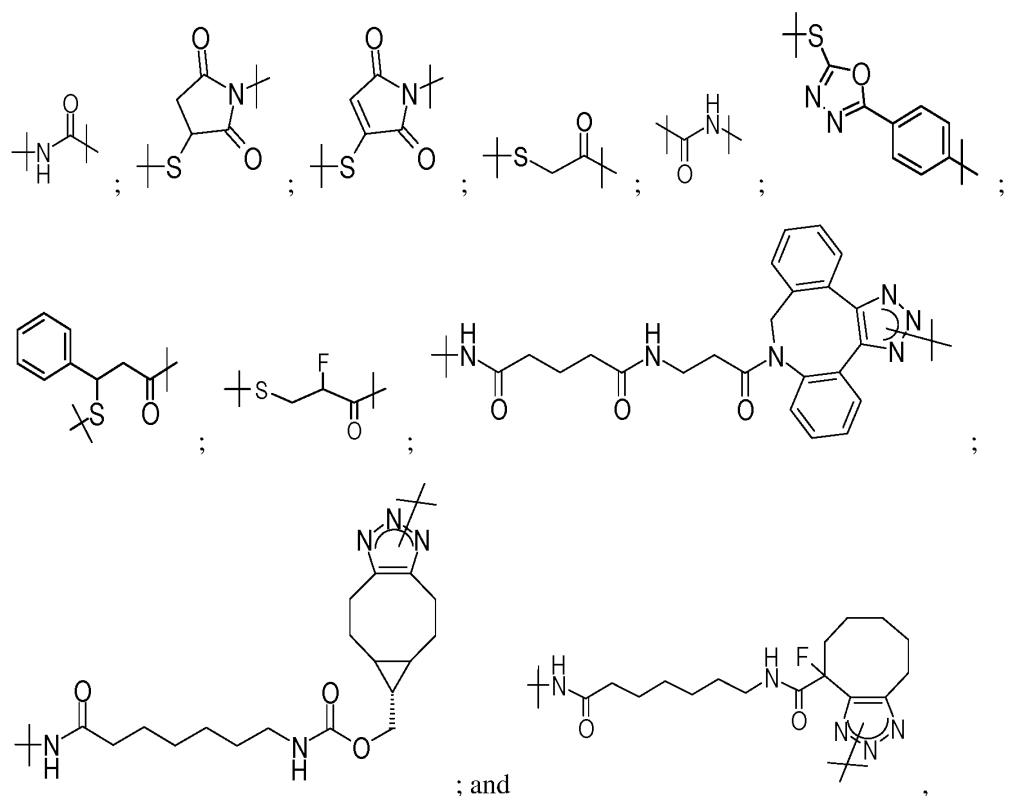
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents an integer from 8 to 25, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents 8, 12, 16, 20, 23 or 24, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical.

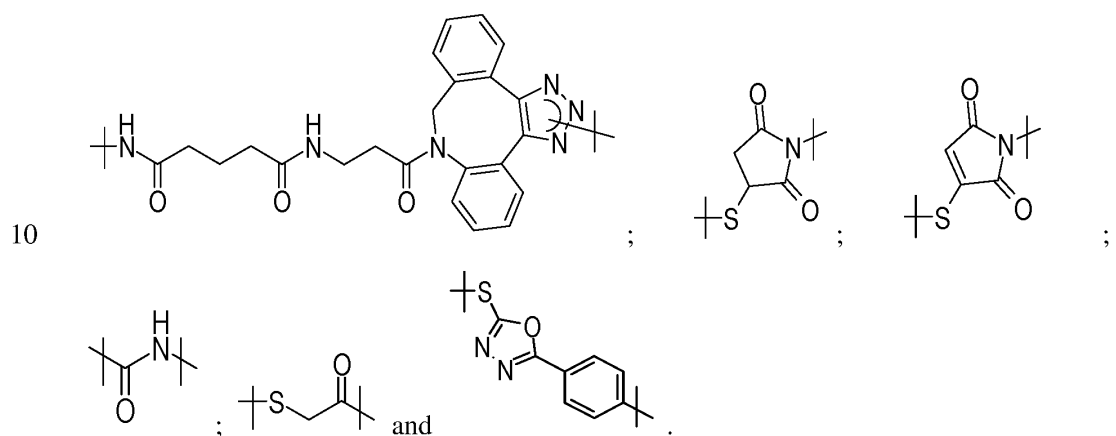
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **Ld** represents a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{NH}-\text{C}(\text{O})-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group or a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which **Ld** represents a $^{*}-(\text{CH}_2)_2-^{**}$ group, a $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^{*}-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical.

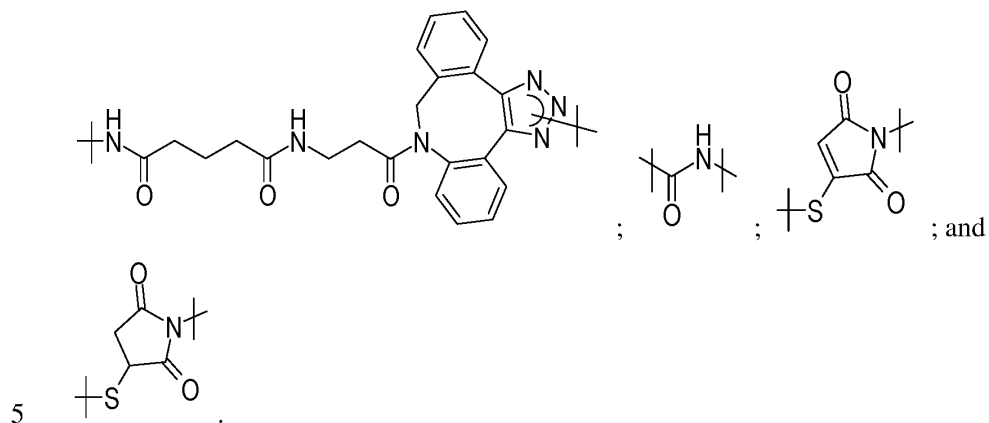
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting of:



Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting of:

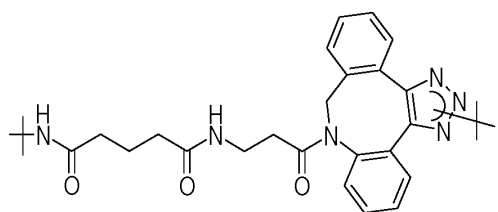


Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which G is selected from the group consisting of:



Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G group belonging to the -G-BA radical, then **G** is

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, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld.

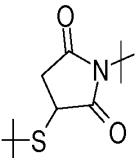
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)alkyl-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G

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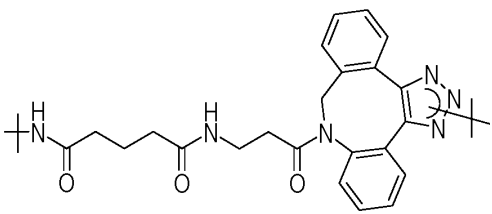
group belonging to the -G-BA radical, then **G** is

, the right side of the G groups being linked to BA and the left side of the G groups being linked to Ld.

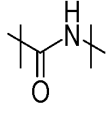
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(C_2-C_6)\text{alkyl}-NH-C(O)-(C_2-C_6)\text{alkyl}-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of

covalent attachment to the G group belonging to the -G-BA radical, then **G** is , the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld.

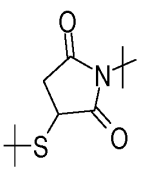
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G group

belonging to the -G-BA radical, then **G** is , the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(CH_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the G group

belonging to the -G-BA radical, then **G** is , the right side of the G groups being linked to BA and the left side of the G groups being linked to Ld.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which when **Ld** represents a $^{*}-(\text{CH}_2)_2\text{-NH-C(O)-}(\text{CH}_2)_2\text{-}^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent

- 5 attachment to the G group belonging to the -G-BA radical, then **G** is , the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which BA is an antibody Ab.

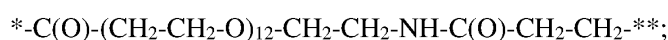
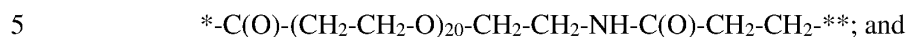
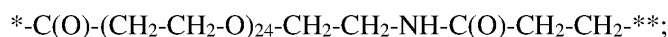
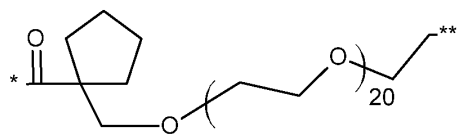
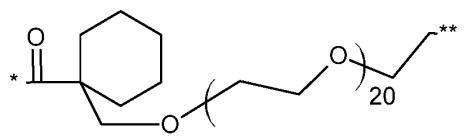
Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which BA is a monoclonal antibody, for instance tusamitamab (CAS [2349294-95-5]) and avelumab variants.

Among the compounds of formula (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which BA is a VHH antibody.

All these sub-groups taken alone or in combination are part of the present disclosure.

According to a particular embodiment, the disclosure relates to a compound/conjugate of formula (II2) or a pharmaceutically acceptable salt thereof wherein the -(P)o-L-Ld- part is selected from the group consisting of:

- 25 $^{*}\text{-C(O)-}(\text{CH}_2\text{-CH}_2\text{-O})_{24}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-(CH}_2\text{-CH}_2\text{-O)}_{23}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-}(\text{CH}_2\text{-CH}_2\text{-O})_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-}(\text{CH}_2\text{-CH}_2\text{-O})_{16}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-}(\text{CH}_2\text{-CH}_2\text{-O})_{12}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-}(\text{CH}_2\text{-CH}_2\text{-O})_8\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-C(CH}_3)_2\text{-CH}_2\text{-O-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;
 $^{*}\text{-C(O)-O-(CH}_2\text{-CH}_2\text{-O)}_{20}\text{-CH}_2\text{-CH}_2\text{-}^{**}$;



the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group.

- 10 Among the compounds of formula (II2) that are subject-matter of the present disclosure, a group of compounds is composed of the compounds wherein:

A is of formula (A) in which:

R₁ represents:

- a (C₁-C₆)alkyl- group, in particular a methyl group;
- 15 - a NH₂-(C₁-C₆)alkyl- group, in particular a NH₂-(CH₂)₅- group;
- a phenyl(C₁-C₆)alkyl- group, in particular a phenyl-CH₂- group, being unsubstituted or substituted by at least one substituent, in particular substituted on the phenyl ring by at least one substituent, for instance by one substituent, selected from:
 - a (C₁-C₆)alkyl- group, in particular a methyl group (or a -CH₂- group if substituted)
 - 20 or an isopropyl group (or a -CH(CH₃)₂- group if substituted), being unsubstituted or substituted by at least one substituent, in particular by one substituent, selected from:
 - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from: a hydrogen atom and a (C₁-C₁₆)alkyl- group, in particular a methyl group, for instance -NR₄R₅ is -NH₂ or -NH(CH₃); or R₄ and R₅ form together with the nitrogen
 - 25 atom to which they are attached an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular an unsubstituted (C₃-C₁₀)membered heterocycloalkyl- group comprising two nitrogen atoms, for instance a piperazinyl group; or

- a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group, in particular a cyclohexyl-CH₂- group, being unsubstituted or substituted, in particular substituted on the (C₃-C₁₀)cycloalkyl- group, by at least one substituent, in particular by one substituent, selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group such as a NH₂-CH₂- group;

5 **R₂** represents :

- a (C₁-C₆)alkyl- group, in particular a -(CH₂)₃-CH₃ group;
- a (C₁-C₆)alkylthio- group, in particular a -S-(CH₂)₂-CH₃ group; or
- a (C₁-C₆)alkyl-NH- group, in particular a -NH-(CH₂)₂-CH₃ group; and

R₃ represents :

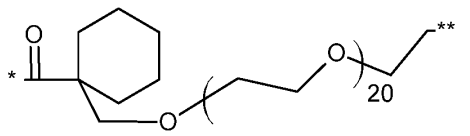
- 10 - a -OR₆ group wherein R₆ is a (C₁-C₆)alkyl- group, in particular a -O-isopropyl group (that is to say a -O-CH(CH₃)₂ group);
- a -NR₇R₈ group wherein R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur, in particular comprising two nitrogen atoms, for
- 15 instance a piperazinyl group; or
- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, in particular one nitrogen atom or two nitrogen atoms, for instance a dihydropyrrolyl group such as a 2,5-dihydro-1H-pyrrolyl group, or a piperazinyl group;

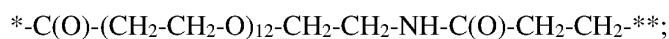
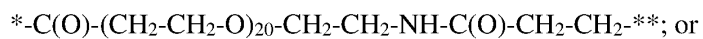
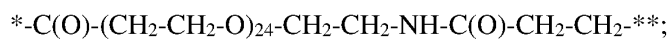
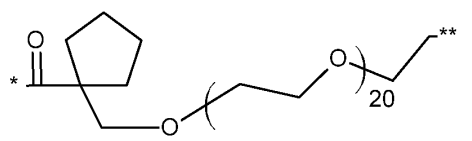
20 ***(P)o-L-Ld-*** represents:

- *-C(O)-(CH₂-CH₂-O)₂₄-CH₂-CH₂-**;
- *-(CH₂-CH₂-O)₂₃-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-(CH₂-CH₂-O)₁₆-CH₂-CH₂-**;

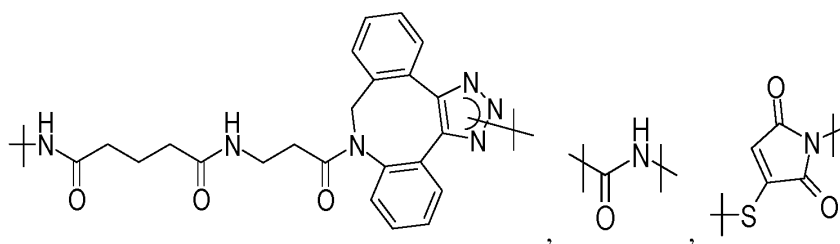
25 *-C(O)-(CH₂-CH₂-O)₁₂-CH₂-CH₂-**;

- *-C(O)-(CH₂-CH₂-O)₈-CH₂-CH₂-**;
- *-C(O)-C(CH₃)₂-CH₂-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-O-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;
- *-C(O)-NH-(CH₂-CH₂-O)₂₀-CH₂-CH₂-**;

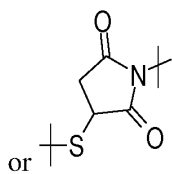




- 5 the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group;



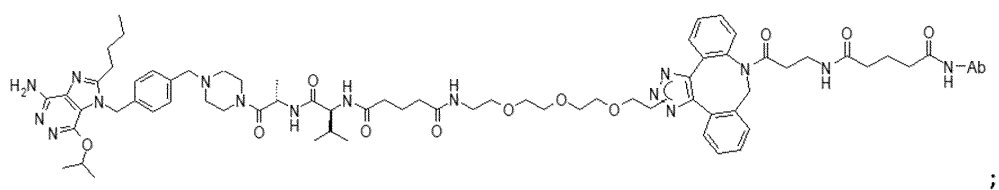
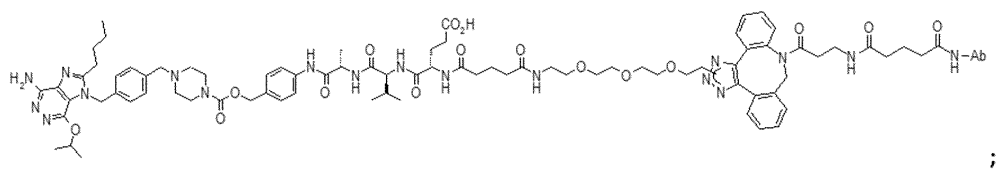
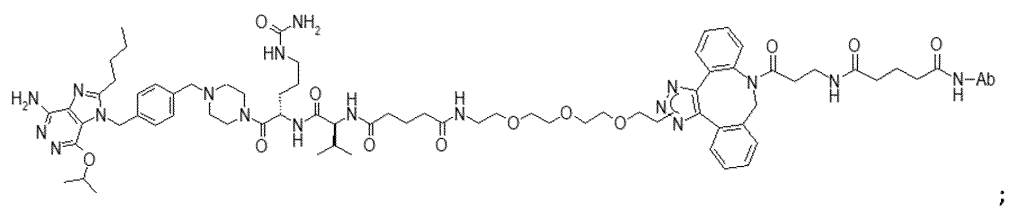
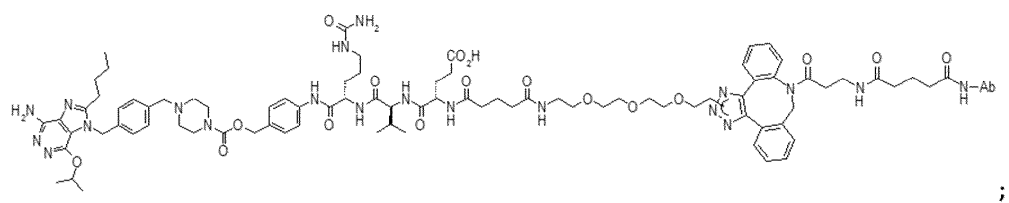
G represents

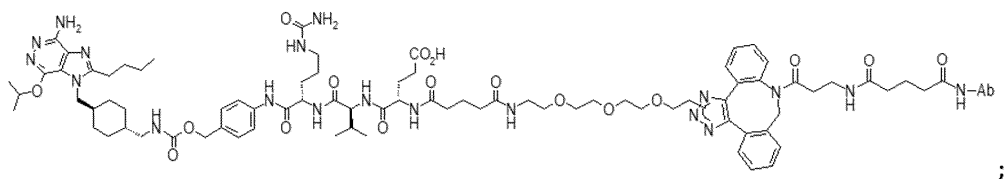


or ; and

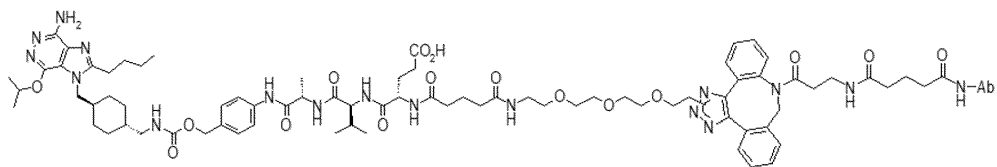
- 10 **BA** represents an antibody, such as a monoclonal antibody or a VHH antibody.

Among the compounds/conjugates of formulae (II), (II1) or (II2) that are subject matter of the present disclosure, mention may be made for instance to the following compounds:

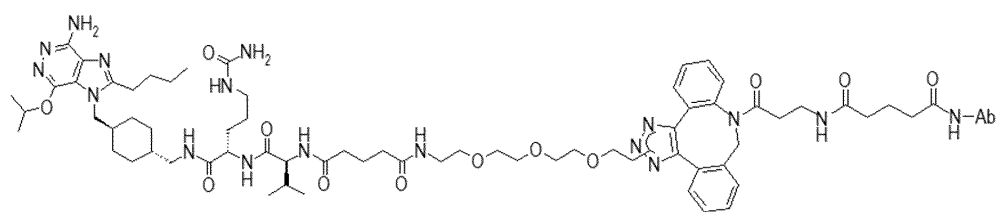




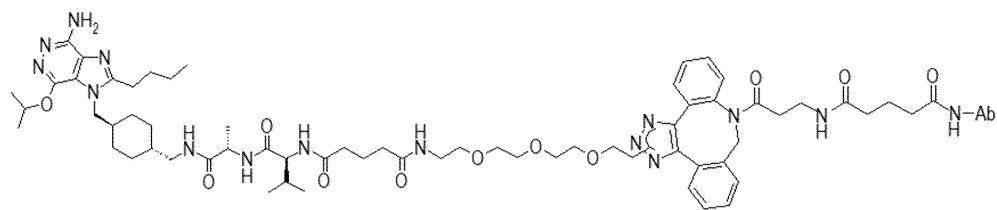
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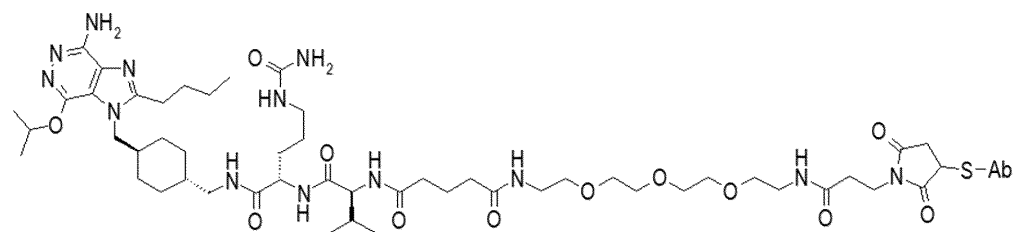
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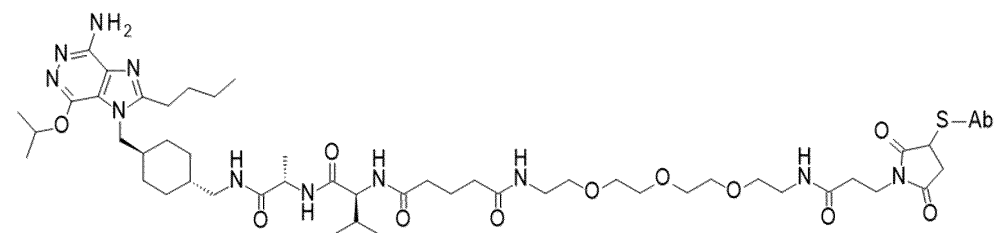
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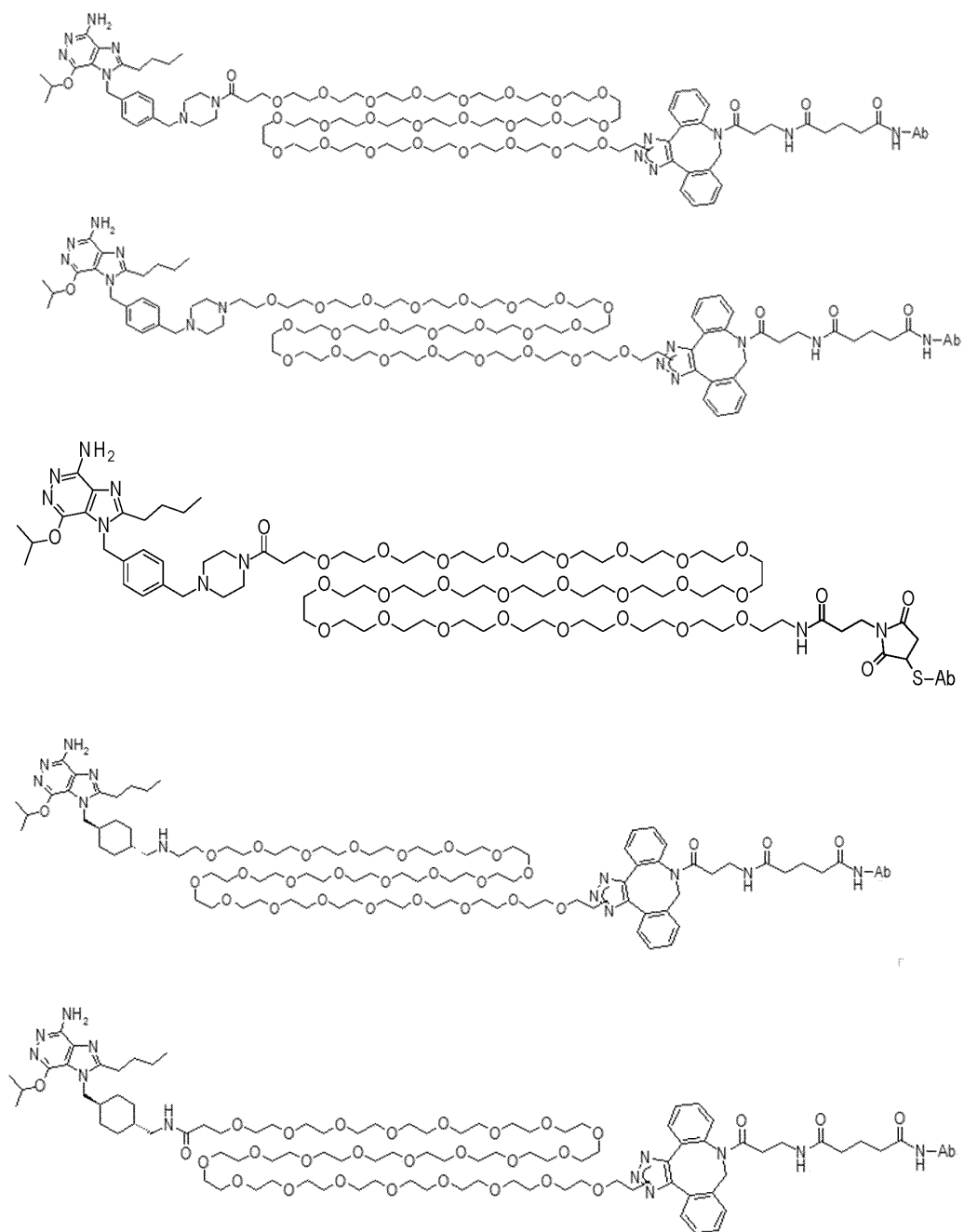
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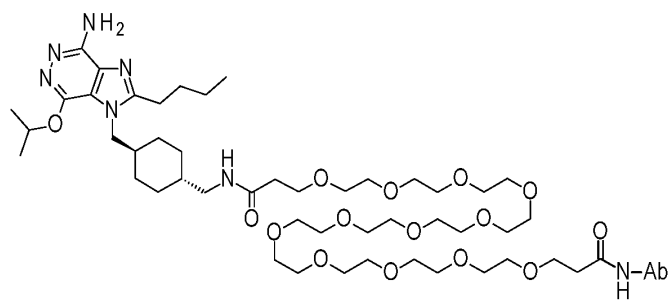
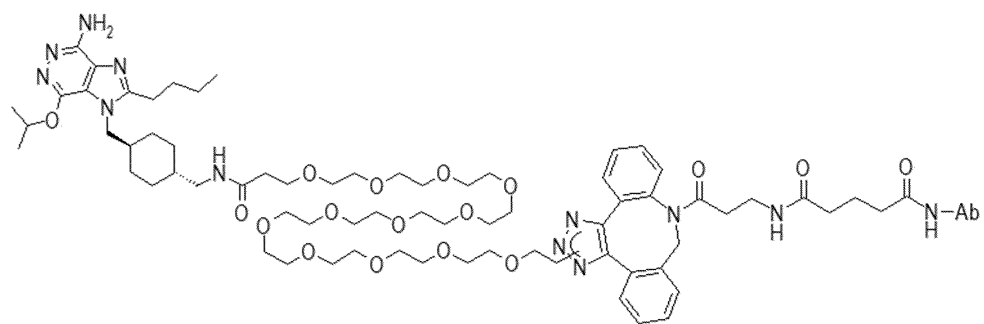
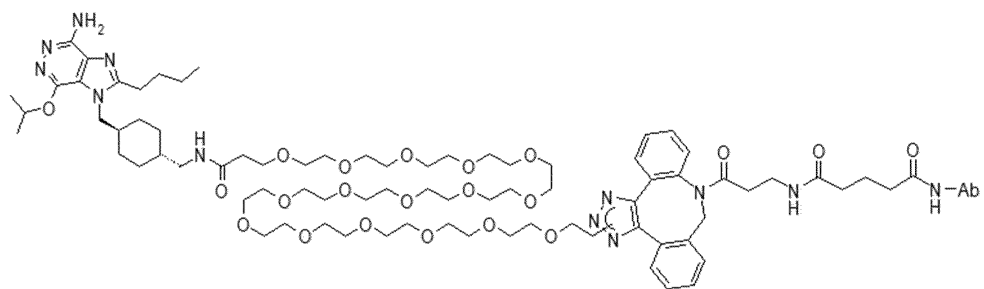
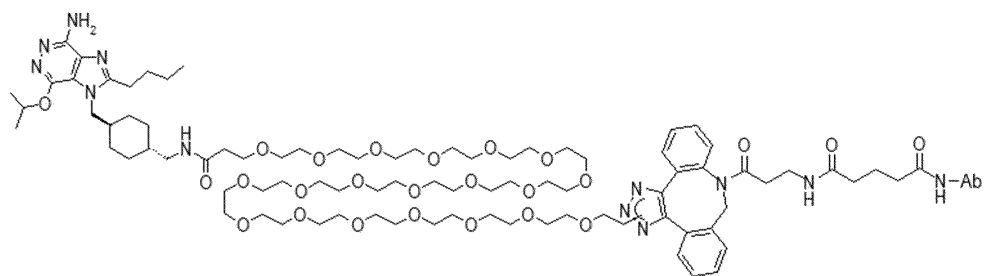


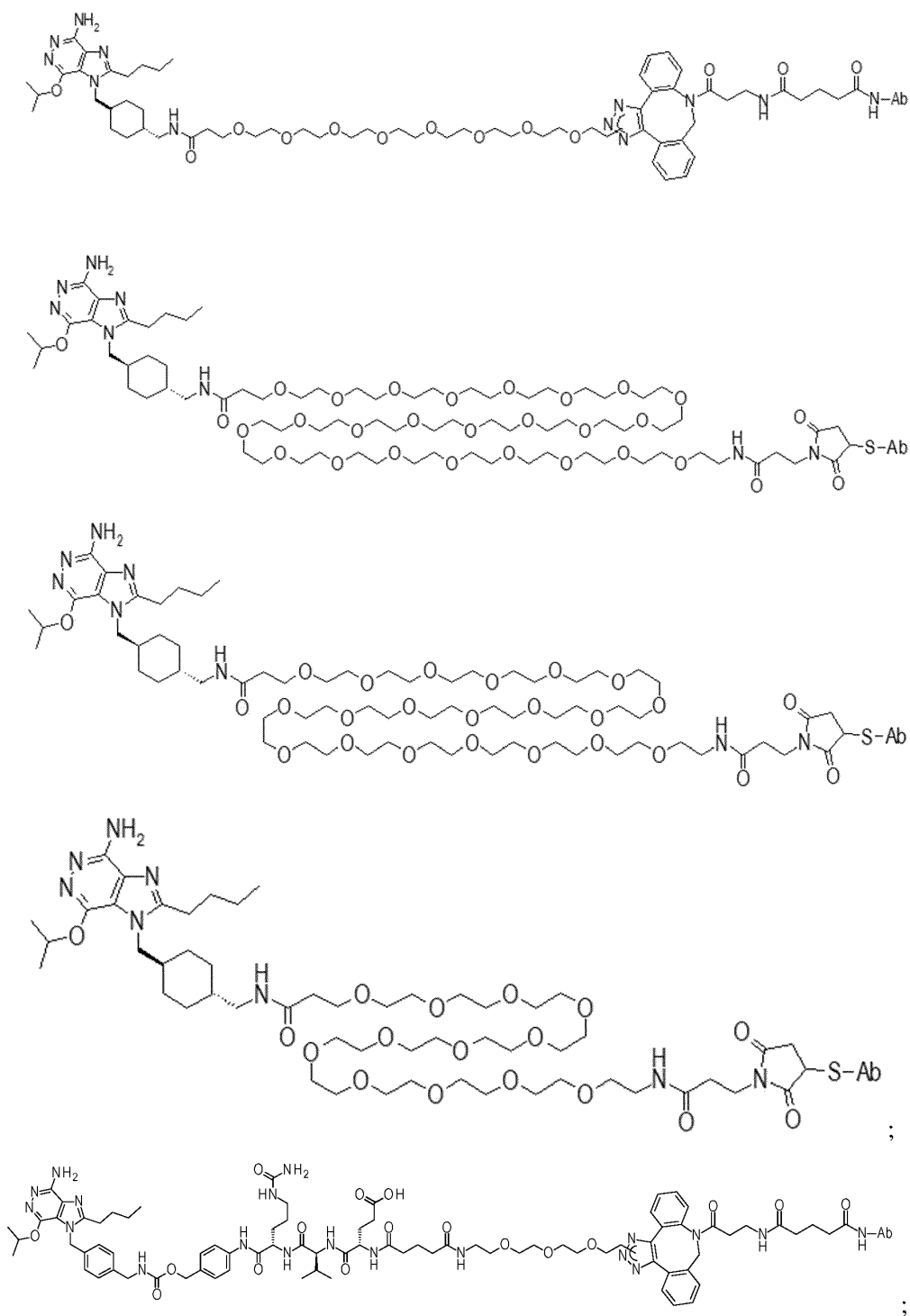
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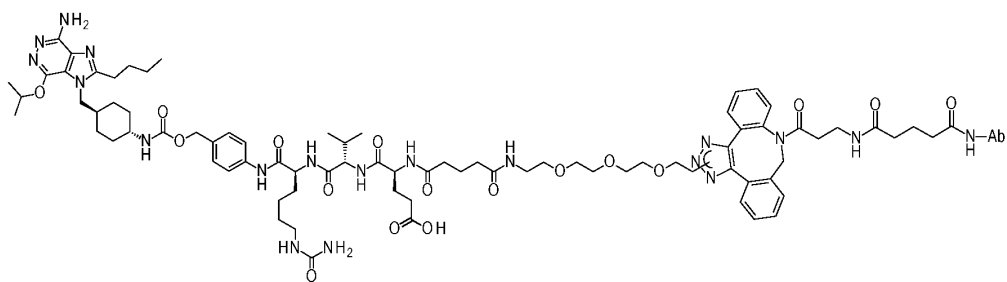


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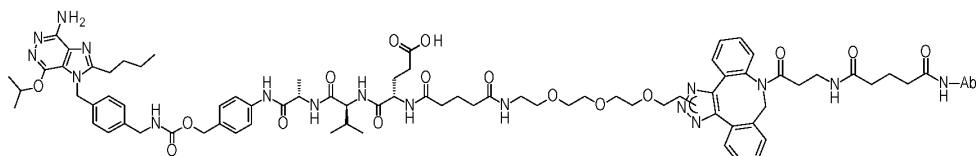




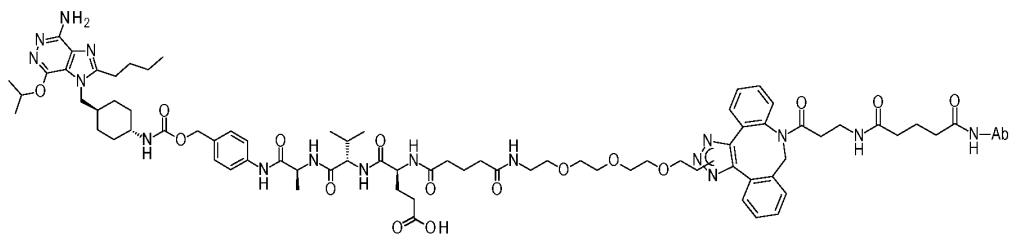




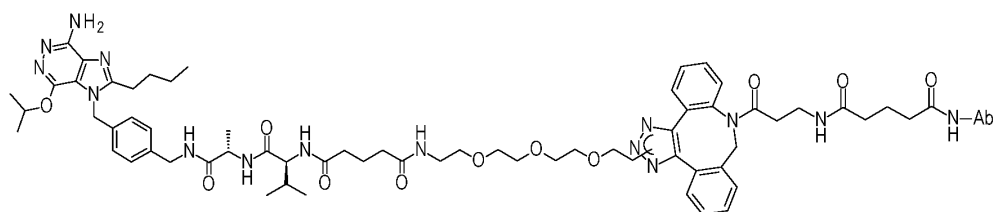
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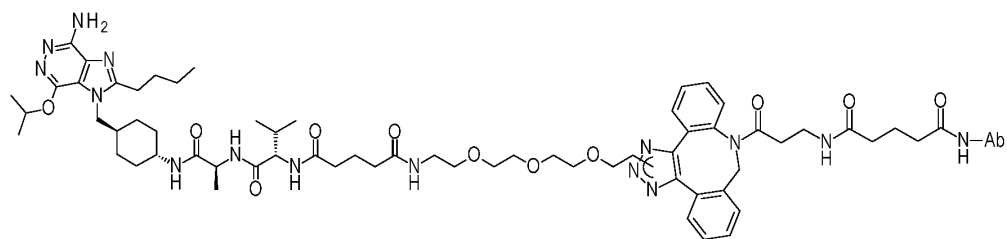
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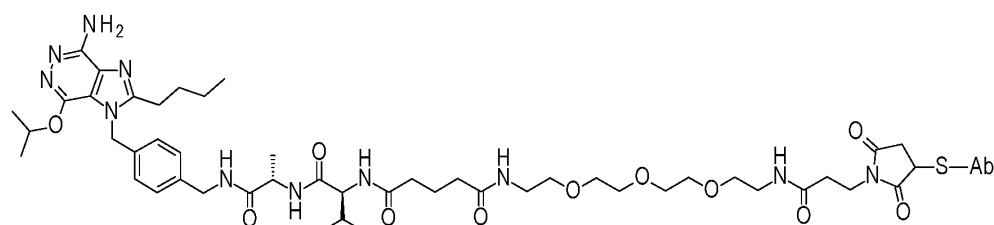


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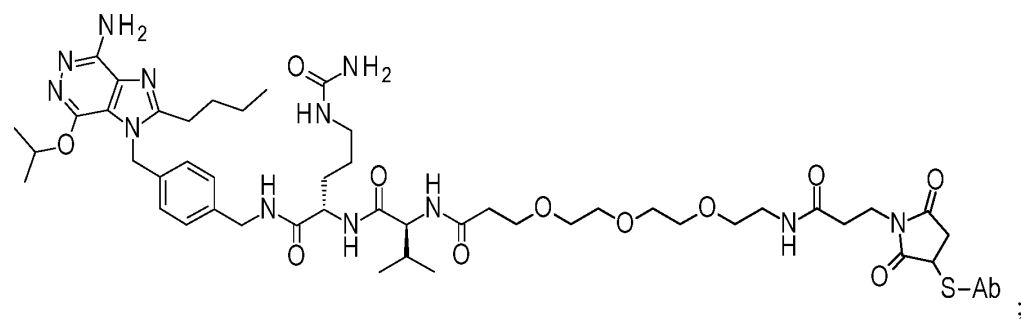
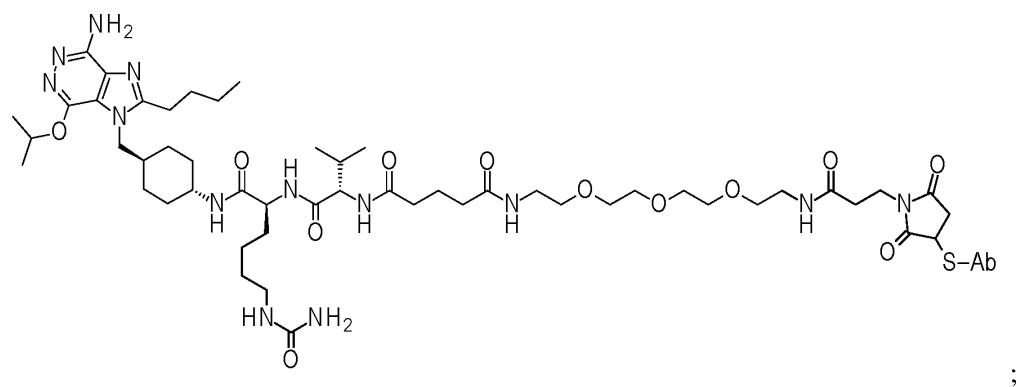
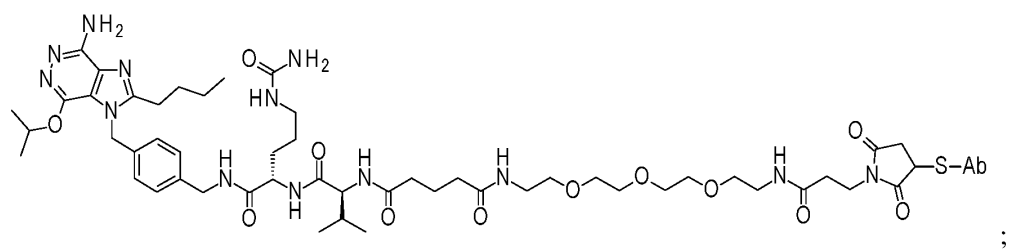
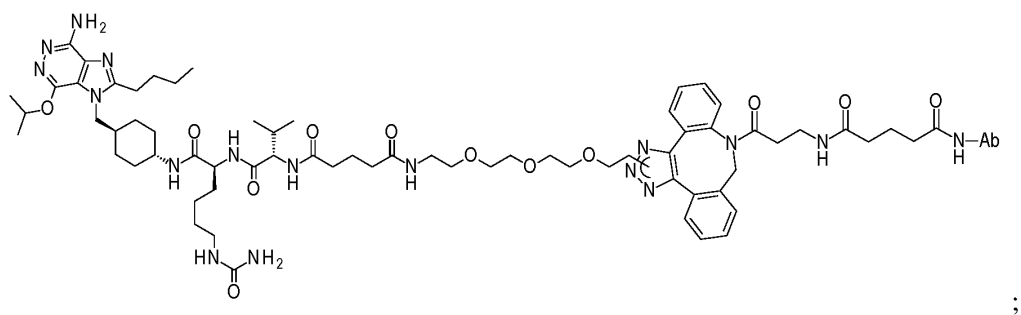
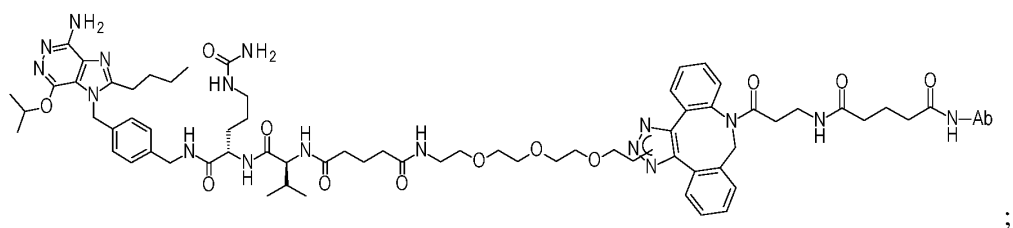


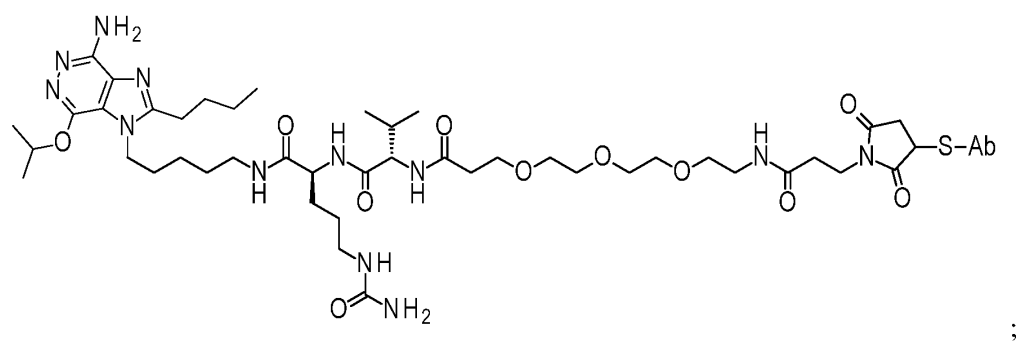
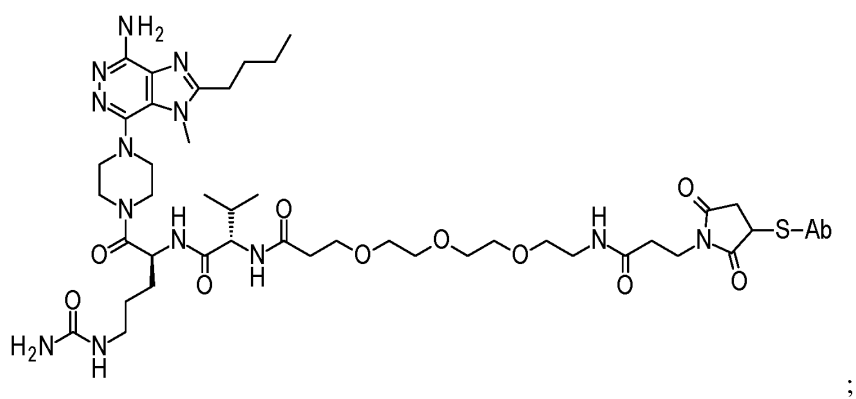
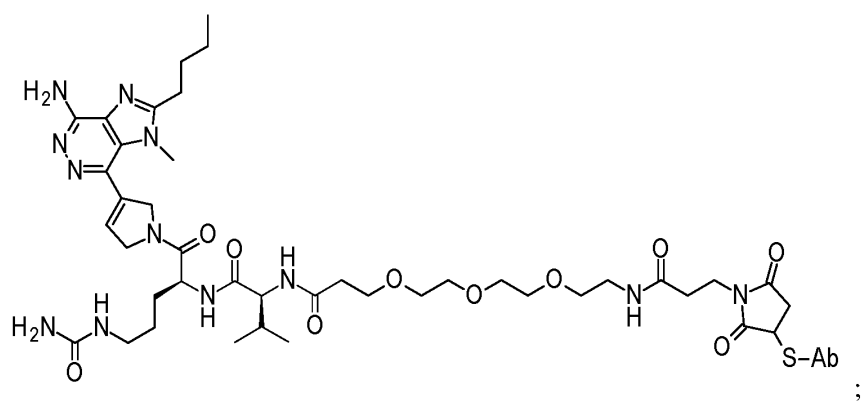
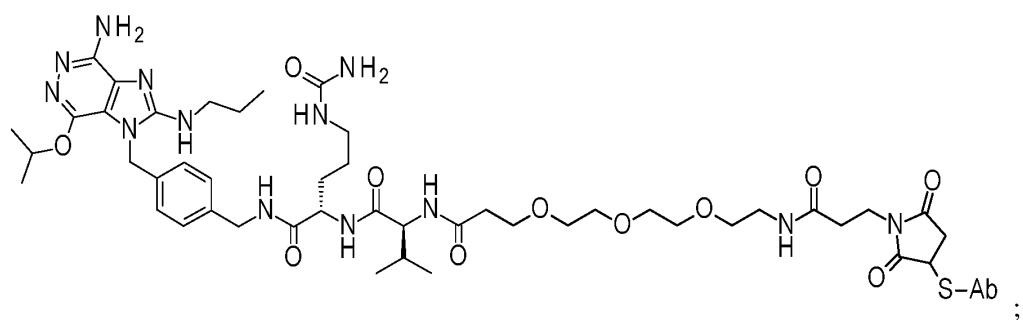
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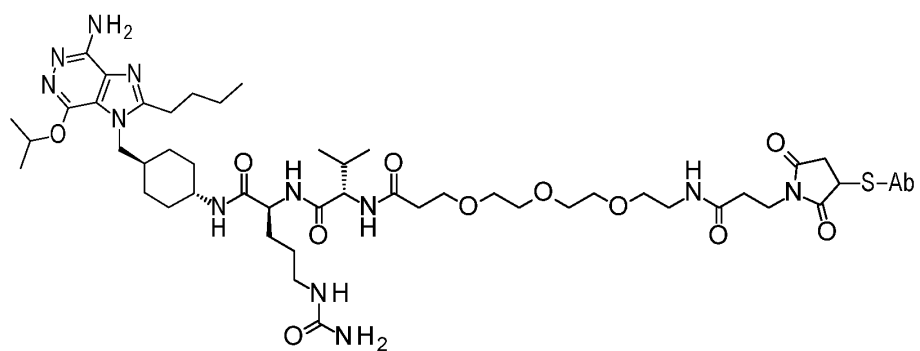
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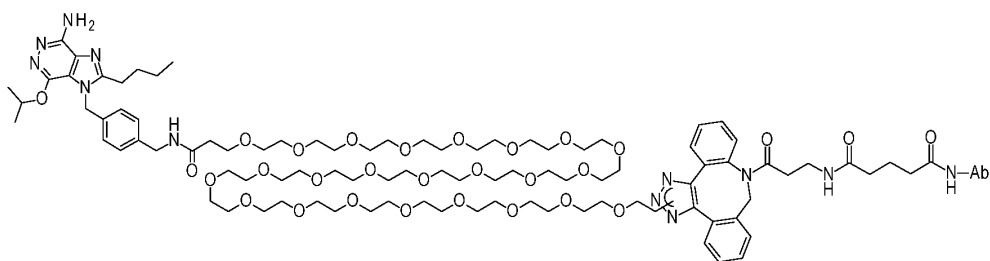
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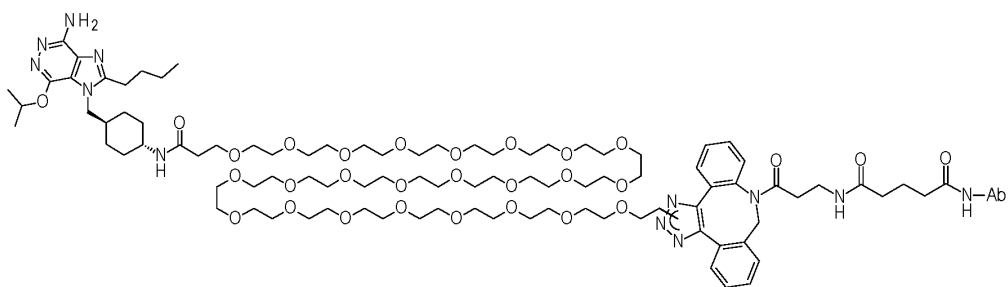




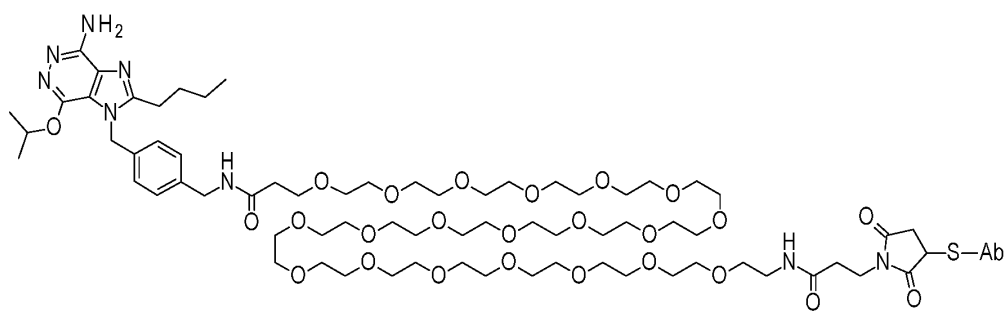
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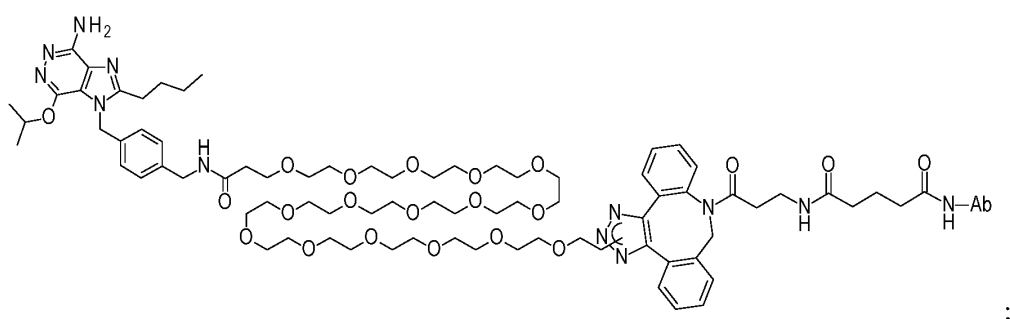
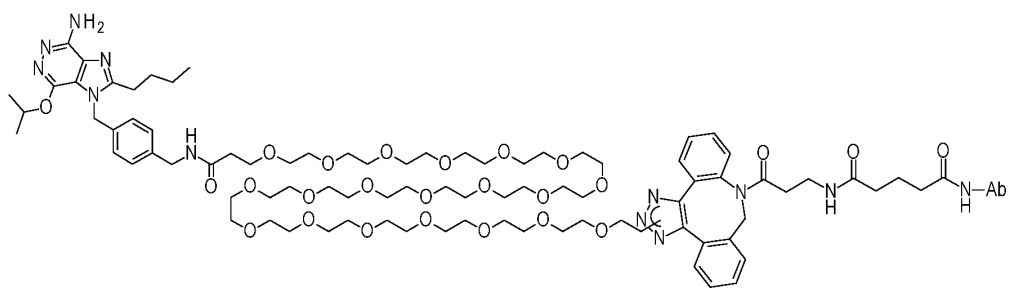
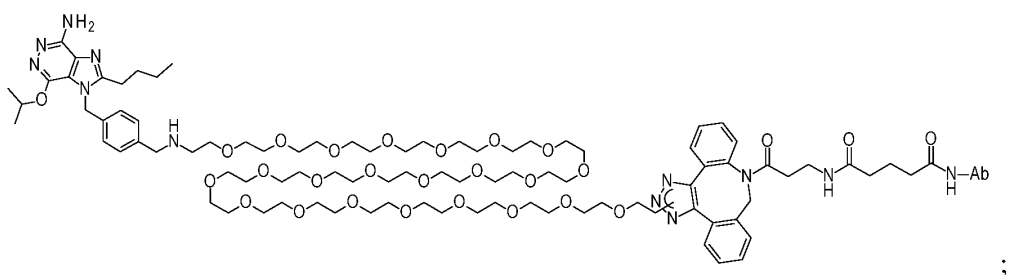
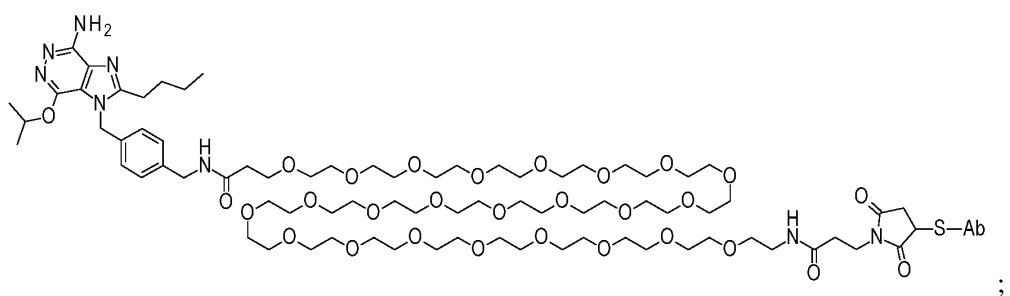
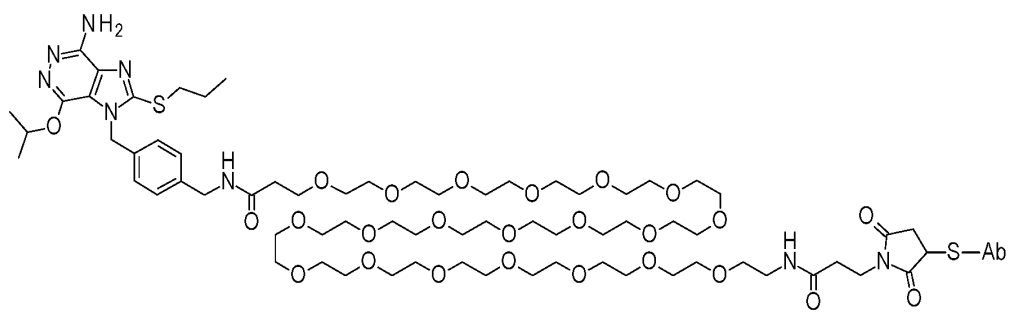
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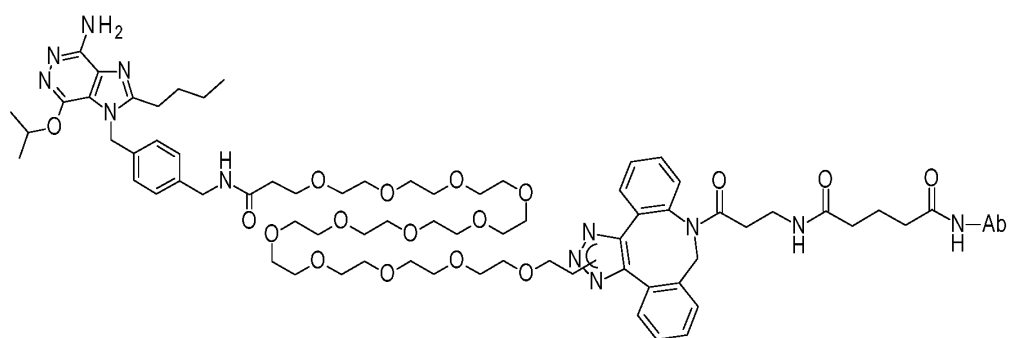


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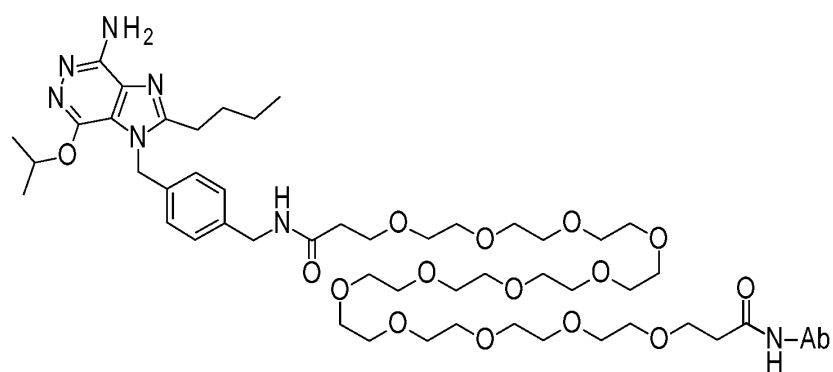


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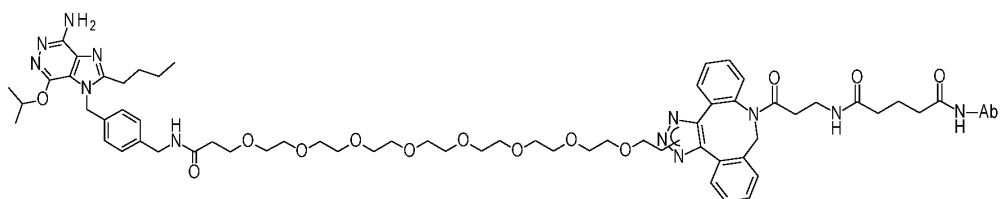




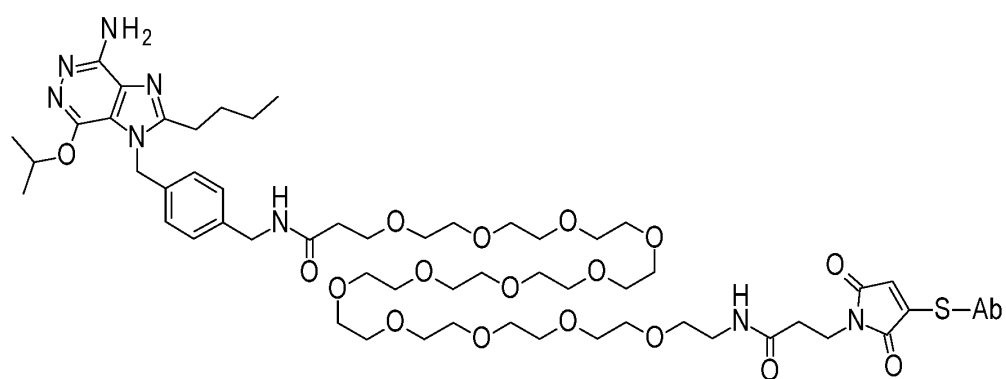
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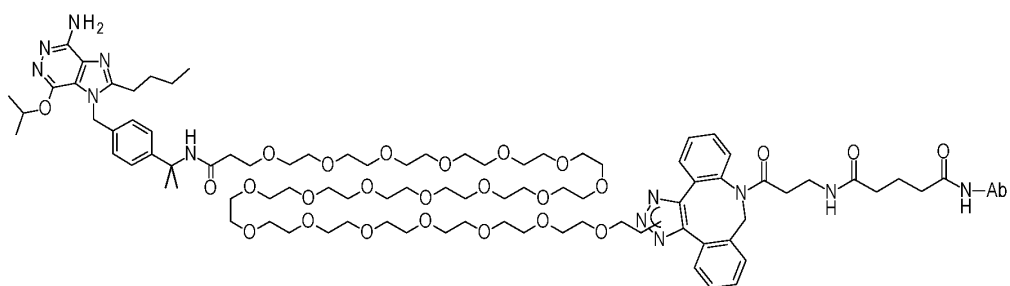
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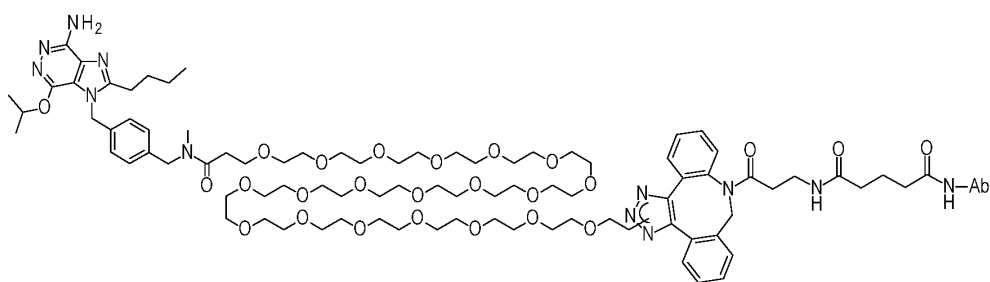
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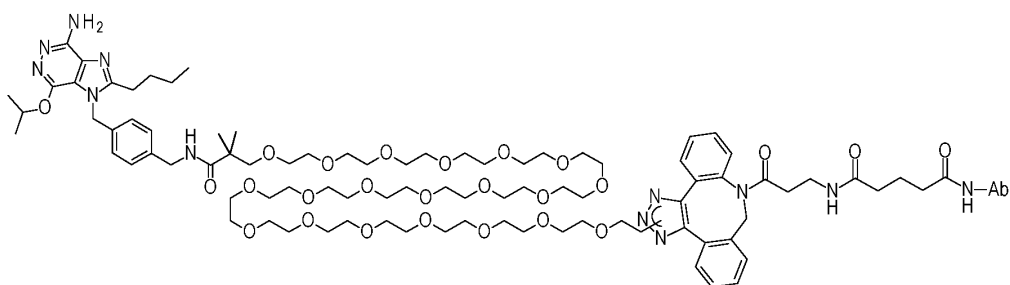
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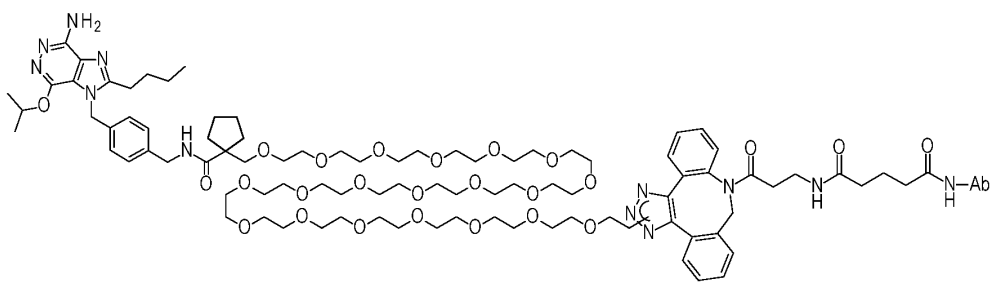
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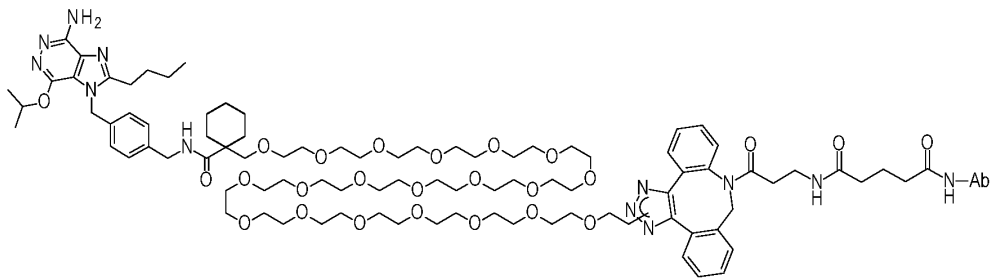
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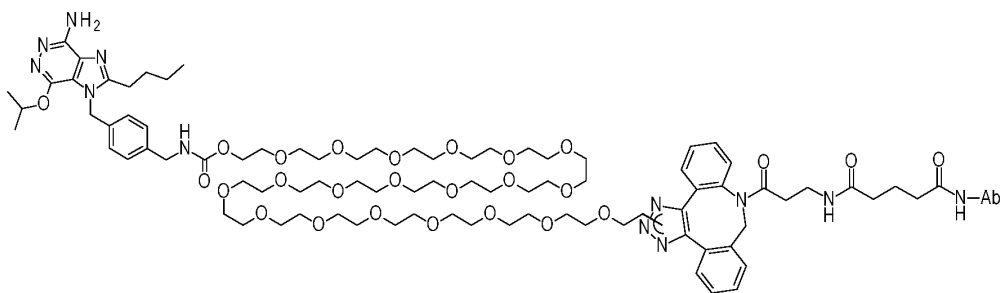
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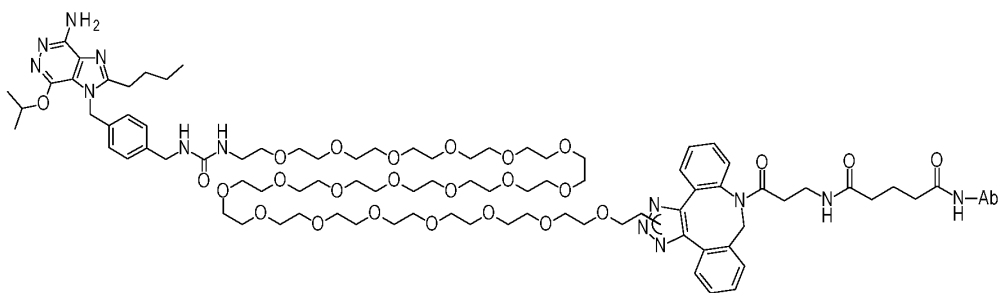
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and



wherein Ab represents an antibody, for instance a monoclonal antibody or a VHH antibody.

5

Among the compounds of formulae (II), (II1) and (II2) that are subject matter of the disclosure, a group of compounds is composed of the compounds for which BA is a monoclonal antibody, for instance:

Tusamitamab (CAS [2349294-95-5]),

10 Avelumab_K76R-K100R,

Avelumab_K360C,

Avelumab_LALA-K360C,

769_VarB_VH7/VL2,

p01_CEA_1_07,

15 p01_CEA_1_07_A118C,

p01_CEA_1_07_E152C,

p01_CEA_1_07_K360C,

p01_CEA_1_07_K414C,

p07_CEA_3_50_VH1_A118C,

20 p07_CEA_3_50_VH1_K360C,

p07_CEA_3_50_VH1_K414C,

- p01_CEA_1_07_A118C_K360C,
p01_CEA_1_07_E152C_K360C,
p01_CEA_1_07_K274C_K414C,
p07_CEA_3_50_VH1_A118C_K360C,
5 p07_CEA_3_50_VH1_K274C_K414C,

In particular, among the compounds/conjugates of formulae (II), (III) or (II2) or a pharmaceutically acceptable salt thereof that are subject matter of the present disclosure, mention may be made for instance to the following compounds:

- 10 (c1) CEACAM5_Tusamitamab-P5 ADC (Example 13)
(c2) PDL1_Avelumab_K76R-K100R-P5 ADC (Example 14)
(c3) CEACAM5_Tusamitamab-P8 ADC (Example 15)
(c4) PDL1_Avelumab_K76R-K100R-P8 ADC (Example 16)
(c5) CEACAM5_Tusamitamab-P3 ADC (Example 17)
15 (c6) PDL1_Avelumab_K76R_K100R-P3 ADC (Example 18)
(c7) CEACAM5_Tusamitamab-Example 3 ADC (Example 19)
(c8) PDL1_Avelumab_K76R_K100R-Example 3 ADC (Example 20)
(c9) CEACAM5_Tusamitamab-Example 5 ADC (Example 21)
(c10) PDL1_Avelumab_K76R_K100R-Example 5 ADC (Example 22)
20 (c11) PDL1_Avelumab_K360C-Example 6 ADC (Example 23)
(c12) PDL1_Avelumab_LALA_K360C-Example 6 ADC (Example 24)
(c13) CEACAM5_Tusamitamab-Example 2 ADC (Example 25)
(c14) PDL1_Avelumab_K76R_K100R-Exemple 2 ADC (Example 26)
(c15) CEACAM5_Tusamitamab-Example 7 ADC (Example 27)
25 (c16) PDL1_Avelumab_K76R_K100R-Example 7 ADC (Example 28)
(c17) CEACAM5_Tusamitamab-Example 11b ADC (Example 29)
(c18) PDL1_Avelumab_K76R_K100R- Example 11b ADC (Example 30)
(c19) CEACAM5_Tusamitamab-Example 8 ADC (Example 31)
(c20) PDL1_Avelumab_K76R_K100R-Example 8 ADC (Example 32)
30 (c21) PDL1_Avelumab_K360C-P16 ADC (Example 33)
(c22) PDL1_Avelumab_K360C-Example 10 ADC (Example 34)
(c23) PDL1_Avelumab_LALA_K360C-Example 10 ADC (Example 35)

- (c24) PDL1_Avelumab_K360C-P14 ADC (Example 36)
- (c25) PDL1_Avelumab_LALA_K360C-P14 ADC (Example 37)
- (c26) PDL1_Avelumab_K76R_K100R-Example 12 ADC (Example 38)
- (c27) CEACAM5_Tusamitamab-Example 11a ADC (Example 39)
- 5 (c28) PDL1_Avelumab_K76R_K100R-Example 11a ADC (Example 40)
- (c29) CEACAM5_Tusamitamab-Example 43 ADC (Example 62)
- (c30) PDL1_Avelumab_K76R_K100R-Example 43 ADC (Example 63)
- (c31) CEACAM5_Tusamitamab-Example 45 ADC (Example 64)
- (c32) CEACAM5_769_VarB_VH7/VL2-Example 45 ADC (Example 65)
- 10 (c33) CEACAM5_p01_CEA_1_07-Example 45 ADC (Example 66)
- (c34) PDL1_Avelumab_K76R_K100R-Example 45 ADC (Example 67)
- (c35) CEACAM5_p01_CEA_1_07-Example 46 ADC (DAR4) (Example 68)
- (c36) CEACAM5_p01_CEA_1_07-Example 46 ADC (DAR8) (Example 69)
- (c37) CEACAM5_p01_CEA_1_07_A118C-Example 46 ADC (Example 70)
- 15 (c38) CEACAM5_p01_CEA_1_07_E152C-Example 46 ADC (Example 71)
- (c39) CEACAM5_p01_CEA_1_07_K360C-Example 46 ADC (Example 72)
- (c40) CEACAM5_p01_CEA_1_07_K414C-Example 46 ADC (Example 73)
- (c41) CEACAM5_p07_CEA_3_50_VH1_A118C-Example 46 ADC (Example 74)
- (c42) CEACAM5_p07_CEA_3_50_VH1_K360C-Example 46 ADC (Example 75)
- 20 (c43) CEACAM5_p07_CEA_3_50_VH1_K414C-Example 46 ADC (Example 76)
- (c44) CEACAM5_p01_CEA_1_07_A118C_K360C-Example 46 ADC (Example 77)
- (c45) CEACAM5_p01_CEA_1_07_E152C_K360C-Example 46 ADC (Example 78)
- (c46) CEACAM5_p01_CEA_1_07_K274C_K414C-Example 46 ADC (Example 79)
- (c47) CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-Example 46 ADC (Example 80)
- 25 (c48) CEACAM5_p07_CEA_3_50_VH1_K274C_K414C-Example 46 ADC (Example 81)
- (c49) PDL1_Avelumab_K360C-Example 46 ADC (Example 82)
- (c50) PDL1_Avelumab_LALA_K360C-Example 46 ADC (Example 83)
- (c51) CEACAM5_p01_CEA_1_07_K360C-Example 47 ADC (Example 84)
- (c52) CEACAM5_p01_CEA_1_07_K360C-P36 ADC (Example 85)
- 30 (c53) PDL1_Avelumab_K360C-P36 ADC (Example 86)
- (c54) CEACAM5_p01_CEA_1_07_K360C-Example 48 ADC (Example 87)
- (c55) PDL1_Avelumab_K360C-Example 48 ADC (Example 88)

- (c56) CEACAM5_p01_CEA_1_07_K360C-Example 49 ADC (Example 89)
- (c57) PDL1_Avelumab_K360C-Example 49 ADC (Example 90)
- (c58) CEACAM5_Tusamitamab-Example 42 ADC (Example 91)
- (c59) PDL1_Avelumab_K76R_K100R-Example 42 ADC (Example 92)
- 5 (c60) CEACAM5_Tusamitamab-Example 41 ADC (Example 93)
- (c61) PDL1_Avelumab_K76R_K100R-Example 41 ADC (Example 94)
- (c62) CEACAM5_Tusamitamab-P29 ADC (Example 95)
- (c63) PDL1_Avelumab_K76R_K100R-P29 ADC (Example 96)
- (c64) CEACAM5_Tusamitamab-P32 ADC (Example 97)
- 10 (c65) PDL1_Avelumab_K76R_K100R-P32 ADC (Example 98)
- (c66) CEACAM5_Tusamitamab-P27 ADC (Example 99)
- (c67) PDL1_Avelumab_K76R_K100R-P27 ADC (Example 100)
- (c68) CEACAM5_Tusamitamab-P25 ADC (Example 101)
- (c69) PDL1_Avelumab_K76R_K100R-P25ADC (Example 102)
- 15 (c70) CEACAM5_p07_CEA_3_50_VH1_K360C-P39 ADC (Example 103)
- (c71) CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-P39 ADC (Example 104)
- (c72) CEACAM5_p07_CEA_3_50_VH1_K360C-P40 ADC (Example 105)
- (c73) CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-P40 ADC (Example 106)
- (c74) CEACAM5_Tusamitamab-Example 50 ADC (Example 107)
- 20 (c75) PDL1_Avelumab_K76R_K100R-Example 50 ADC (Example 108)
- (c76) PDL1_Avelumab_K360C-P45 ADC (Example 109)
- (c77) CEACAM5_769_VarB_VH7/VL2-P47 ADC (Example 110)
- (c78) CEACAM5_p01_CEA_1_07-P47 ADC (Example 111)
- (c79) CEACAM5_p01_CEA_1_07-Example 51 ADC (DAR4) (Example 112)
- 25 (c80) CEACAM5_p01_CEA_1_07-Example 51 ADC (DAR8) (Example 113)
- (c81) PDL1_Avelumab_K360C-Example 51 ADC (Example 114)
- (c82) PDL1_Avelumab_LALA_K360C-Example 51 ADC (Example 115)
- (c83) CEACAM5_p01_CEA_1_07_A118C-Example 51 ADC (Example 116)
- (c84) CEACAM5_p01_CEA_1_07_E152C-Example 51 ADC (Example 117)
- 30 (c85) CEACAM5_p01_CEA_1_07_K360C-Example 51 ADC (Example 118)
- (c86) CEACAM5_p01_CEA_1_07_K414C-Example 51 ADC (Example 119)
- (c87) CEACAM5_p01_CEA_1_07_A118C_K360C-Example 51 ADC (Example 120)

- (c88) CEACAM5_p01_CEA_1_07_E152C_K360C-Example 51 ADC (Example 121)
- (c89) CEACAM5_p01_CEA_1_07_K274C_K414C-Example 51 ADC (Example 122)
- (c90) CEACAM5_Tusamitamab-Example 57 ADC (Example 123)
- (c91) CEACAM5_Tusamitamab-Example 58 ADC (Example 124)
- 5 (c92) CEACAM5_Tusamitamab-Example 59 ADC (Example 125)
- (c93) CEACAM5_Tusamitamab-Example 60 ADC (Example 126)
- (c94) CEACAM5_Tusamitamab-Example 61 ADC (Example 127)
- (c95) PDL1_Avelumab_K360C-P52 ADC (Example 128)
- (c96) PDL1_Avelumab_LALA_K360C-P52 ADC (Example 129)
- 10 (c97) PDL1_Avelumab_K76R_K100R-Example 54 ADC (Example 130)
- (c98) CEACAM5_Tusamitamab-Example 53 ADC (Example 131)
- (c99) PDL1_Avelumab_K76R_K100R-Example 53 ADC (Example 132)
- (c100) CEACAM5_Tusamitamab-Example 55 ADC (Example 133)
- (c101) CEACAM5_Tusamitamab-Example 56 ADC (Example 134)
- 15 (c102) PDL1_Avelumab_K360C-Example 52 ADC (Example 135)
- (c103) CEACAM5_Tusamitamab-P42 ADC (Example 136)
- (c104) PDL1_Avelumab_K76R_K100R- P42 ADC (Example 137)

20 The compounds/conjugates of formulae (II), (II1) and (II2) or a pharmaceutically acceptable salt thereof according to the present disclosure can be prepared according to any process known by the skilled person, and for instance by the following processes. Processes are also detailed in the experimental part of the present disclosure.

- The compound/conjugate of formulae (II), (II1) and (II2) may be prepared by
- 25 (i) placing in contact and leaving to react:
 - an, optionally buffered, aqueous solution of a binding agent as defined in the present disclosure for instance of an antibody Ab, comprising a reactive RCG2 group, optionally modified by means of a modifying agent,
 - and
 - 30 - a solution of a compound/payload of formula (I), (I1) or (I2) as defined in the present disclosure, comprising a reactive RCG1 group;

RCG1 groups of the compounds/payloads of formula (I), (II) or (II2) being reactive towards RCG2 groups of the binding agent as defined in the present disclosure for instance of the antibody to form the G group by a covalent bond and forming compounds/conjugates of formula (II), (II1) or (II2);

- 5 (ii) and then optionally separating the compound of formula (II), (II1) or (II2) formed in step (i) from the unreacted compound of formula (I), (II) or (II2) and/or from the unreacted antibody and/or from any aggregates that may have been formed.

10 Examples of RCG2, present on the binding agent as defined in the present disclosure for instance of the antibody, that may be mentioned include (Garnett M.C., *et al.*, *Advanced Drug Delivery Reviews* **2001**, 53, 171-216):

- (i) a ϵ -amino group (ϵ -NH₂ group) borne by the side chain of a lysine residue that is present at the surface of the binding agent as defined in the present disclosure for instance of the antibody;
- 15 (ii) an α -amino group (α -NH₂ group) of an N-terminal amino acid of a heavy chain or a light chain of the binding agent as defined in the present disclosure for instance of the antibody;
- (iii) the saccharide groups of the hinge region;
- (iv) a thiol (a -SH group) of a cysteine residue generated by reducing an intra-chain disulfide bond of the binding agent as defined in the present disclosure for instance of the antibody or
- 20 a -SH group of an engineered cysteine residue of the binding agent as defined in the present disclosure for instance of the antibody;
- (v) an amide group (a -C(O)NH₂ group) borne by the side chains of a glutamine residue that is present at the surface of the binding agent as defined in the present disclosure for instance of the antibody;
- 25 (vi) an aldehyde group (a -C(O)H group) introduced using formylglycine generating enzyme; and
- (vii) **RCG2** group, optionally introduced by means of a modifying agent.

30 According to a particular embodiment, in the compounds of formula (II), (II1) or (II2) as defined in the present disclosure, **RCG2** is a reactive group of a cysteine engineered amino acid residue of an antibody.

For instance, in the compounds of formula (II), (II1) or (II2) as defined in the present

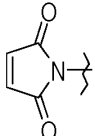
disclosure, the cysteine engineered amino acid residue is at least one position according to the numbering of the EU numbering index, selected from an interchain cysteine, 118, 152, 360, 274, 414 of the heavy chain constant (C_H) domain, in particular the cysteine engineered amino acid residue is at position 360 of the heavy chain constant (C_H) domain according to the numbering of the EU numbering index.

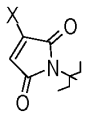
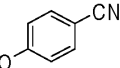
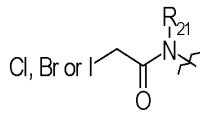
More recently, other site-specific conjugation approaches have been considered, for instance the introduction of cysteines by mutation (Junutula J.R., *et al.*, *Nature Biotechnology* **2008**, 26, 925-932), the introduction of unnatural amino acids allowing other types of chemistry (Axup J.Y., *et al.*, *PNAS* **2012**, 109, 40, 16101-16106) or the conjugation on antibody glycans (Zhou Q., *et al.*, *Bioconjugate Chem.* **2014**, 25, 510-520). Use of cysteine bridging dibromomaleimides (Behrens C.R., *et al.*, *Mol. Pharmaceutics* **2015**, 3986-3998) and bis-sulfone reagents (Bryant P., *et al.*, *Mol. Pharmaceutics* **2015**, 1872-1879) in order to cross-link antibodies have also been described and could be applied to the present disclosure.

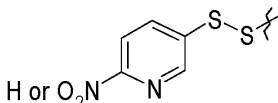
Another approach for site-specific modifications of antibodies is based on enzymatic conjugation using for example bacterial transglutaminase (Jeger S., *et al.*, *Angew. Chem. Int. Ed.* **2010**, 49, 9995-9997; Strop P., *et al.*, *Chem. Biol.* **2013**, 20, 161-167) or formylglycine generating enzyme (Hudak J.E., *et al.*, *Angew. Chem. Int. Ed.* **2012**, 51, 4161-4165). For a review of site-specific conjugation strategies, see Agarwal P. and Bertozzi C.R., *Bioconjugate Chem* **2015**, 26, 176-192. These conjugation technologies may also be applied to compounds/payloads of formula (I), (I1) or (I2) described in the present disclosure.

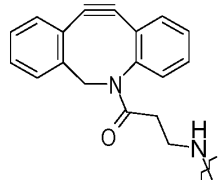
It is also possible to chemically modify the binding agent as defined in the present disclosure for instance the antibody so as to introduce novel RCG2 reactive groups. Thus, it is well known to those skilled in the art how to modify a binding agent as defined in the present disclosure for instance an antibody with the aid of a modifying agent introducing for example activated disulfide, thiol, maleimido, haloacetamido, azido, alkyne or cycloalkyne groups (see especially WO2005/077090 page 14 and WO2011/001052). The modification makes it possible to improve the conjugation reaction and to use a wider variety of RCG1 groups, present on compound/payload of formula (I), (I1) or (I2).

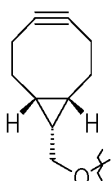
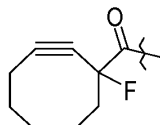
For instance, in the case where RCG1 is of the type (ii) above defined, that is to say one of

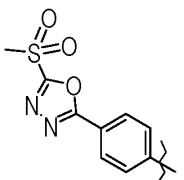
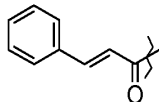
the following reactive groups: a maleimido  group; a substituted maleimido group

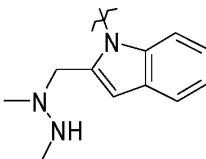
such as  with X = Br, O- CN; a haloacetamido  group with R₂₁ representing a hydrogen atom or a (C₁-C₆)alkyl group, such as a methyl group; Cl-; N₃-; HO-

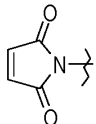
5 ; HS-; an activated disulfide such as ; H₂N-; HC≡C- or an activated

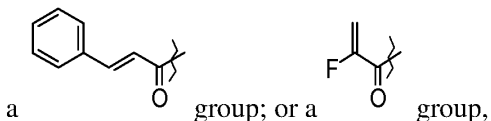
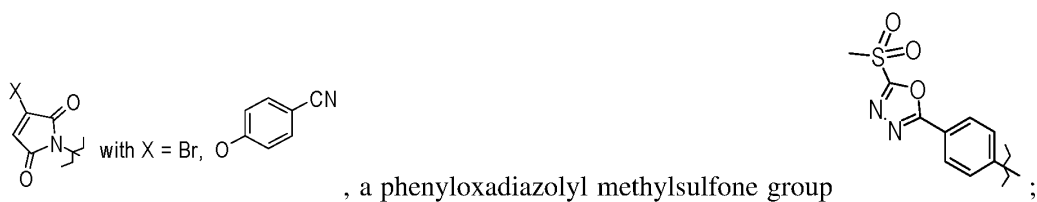
C≡C such as a cyclooctyne moiety for instance a DBCO-amine  or BCN

 or MFCO ; a phenyloxadiazolyl methylsulfone group (PODS)

such as ; a  group; a  group; an O-alkyl

hydroxylamine or a Pictet-Spengler reaction substrate such as , for

10 instance RCG₁ is N₃-, a maleimido , a substituted maleimido group



it is possible to chemically modify the antibody using an adequate modifying agent or to introduce one or more unnatural amino acids so as to introduce the adequate RCG2 groups.

5 For example:

- when RCG1 represents a N-hydroxysuccinimidyl ester, RCG2 represents a -NH₂ group;
- when RCG1 represents a maleimido function, a haloacetamido function, a chlorine atom or an activated disulfide function, RCG2 may be a -SH group;
- when RCG1 represents a -N₃ group, RCG2 may be a -C=CH function or an activated C=C

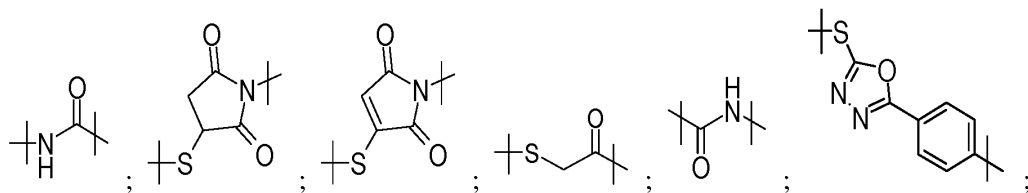
10 function such as a cyclooctyne moiety;

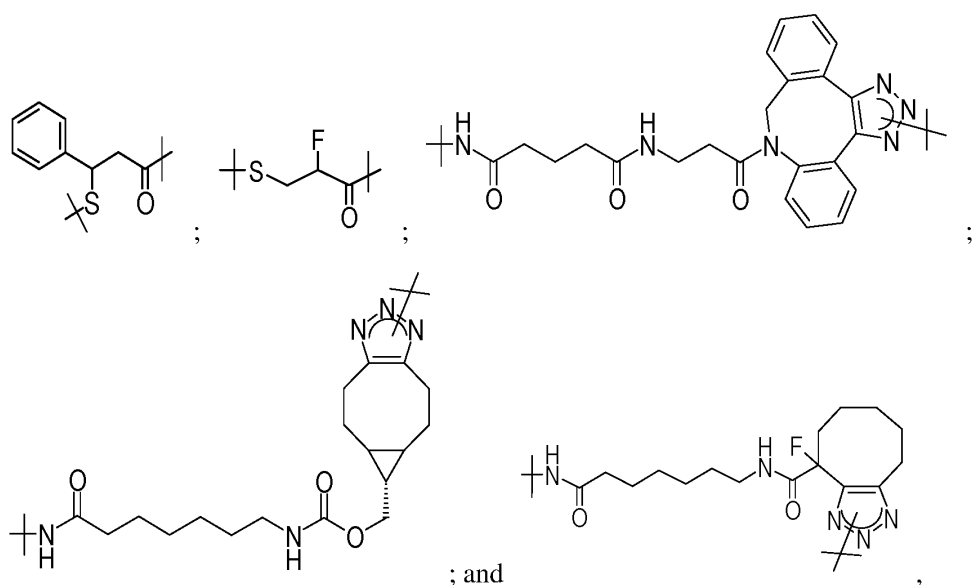
- when RCG1 represents a -OH group or -NH₂ group, RCG2 may be a carboxylic acid or amide function;
- when RCG1 represents a -SH group, RCG2 may be a maleimido function, a haloacetamido function or an activated disulfide function;

15 - when RCG1 represents a -C=CH function or an activated -C=C function, RCG2 may be a -N₃ group;

- when RCG1 represents a O-alkyl hydroxylamine function or a Pictet-Spengler reaction substrate, RCG2 may be an aldehyde or a ketone function.

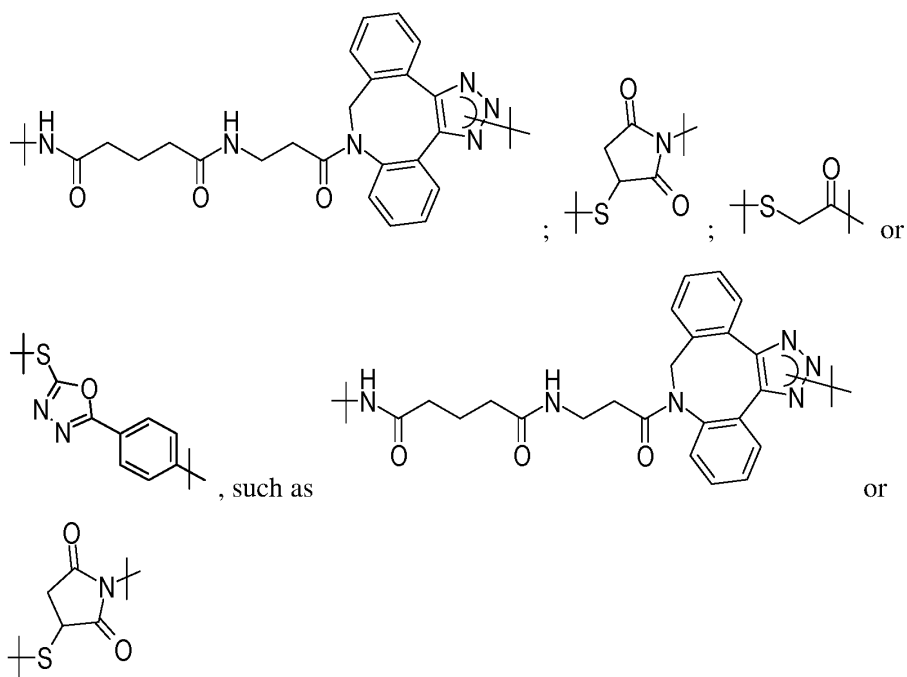
20 As indicated above, examples of **G** that may be mentioned include

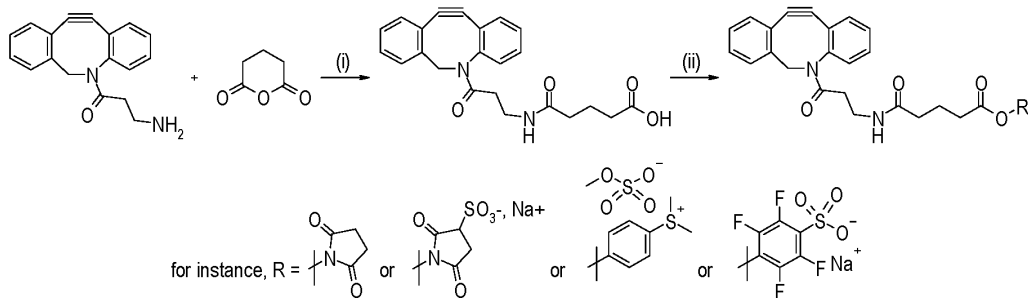




for instance, the left side of the G groups being linked to BA and the right side of the G groups being linked to L1.

- 5 For instance, G represents the following groups:



**Scheme 5**

Step (i): coupling of dibenzocyclooctyne-amine to glutaric anhydride;

Step (ii): activation of the carboxylic acid as an activated ester by treatment

- 5
 - with DSC in the presence of a base such as, for example, DIEA; or
 - with N-hydroxysulfosuccinimide sodium salt in the presence of a coupling reagent such as, for example, DCC; or
 - with 4-hydroxyphenyldimethylsulfonium methyl sulfate in the presence of a coupling reagent such as, for example, DIC; or
- 10
 - with 2,3,5,6-tetrafluoro-4-hydroxybenzenesulfonic acid sodium salt in the presence of a coupling reagent such as, for example, DIC.

Scheme 5 depicted the synthesis starting with dibenzocyclooctyne-amine (CAS number [1255942-06-3]) but may also apply to other cyclooctyne-amines which are commercially available. It depicted the synthesis using glutaric anhydride but may also apply to succinic anhydride or alkyl diacids which are commercially available for n ranging from 3 to 10.

Preparation of the compounds/conjugates of formula (II), (II1) and (II2):

The compound/conjugates of formula (II), (II1) or (II2) or a pharmaceutically acceptable salt thereof of the present disclosure can be obtained *via* the process comprising at least the steps of:

(i) placing in contact and leaving to react:

- an, optionally buffered, aqueous solution of a binding agent as defined in the present disclosure for instance of an antibody Ab, comprising a reactive RCG2 group, optionally modified by means of a modifying agent,

25 and

- a solution of a compound/payload of formula (I), (II) or (I2) as defined in the present

disclosure, comprising a reactive RCG1 group,
RCG1 groups of compound/payload of formula (I), (I1) or (I2) being reactive towards RCG2
groups of the binding agent as defined in the present disclosure for instance of the antibody
to form the G groups by a covalent bond and forming compounds/conjugates of formula (II),
5 (II1) or (II2).

According to one variant for example, in step (ii) the compound/conjugate of formula (II),
(II1) or (II2) from step (i) is separated from the unreacted compound/payload of formula (I),
(I1) or (I2) from any aggregates formed, and/or any unreacted binding agent as defined in
10 the present disclosure for instance any unreacted antibody.

The function of the placing in contact is to react RCG1 groups and RCG2 groups in order to
ensure attachment of the compound/payload of formula (I), (I1) or (I2) to the binding agent
as defined in the present disclosure for instance to the antibody by formation of a covalent
15 bond; such as:

- when RCG1 represents a chlorine atom or a maleimido or haloacetamido group, the
binding agent as defined in the present disclosure for instance the antibody may comprise
thiol groups;
- when RCG1 represents an azido group, the binding agent as defined in the present
20 disclosure for instance the antibody may comprise a $-C=CH$ moiety or an activated $C\equiv C$
such as a cyclooctyne group;
- when RCG1 represents a $-NH_2$ group, the reaction may take place on amide function of
the antibody using an enzymatic catalysis, such as the amide groups borne by the side
chains of glutamine (Gln) residues of a binding agent as defined in the present disclosure
25 for instance an antibody.
- when RCG1 represents a $HC\equiv C-$ or an activated $C\equiv C$ group such as a cyclooctyne
moiety, the binding agent as defined in the present disclosure for instance the antibody
may comprise azido groups.

30 The term “aggregates” means associations that may form between two or more binding
agents as defined in the present disclosure for instance two or more antibodies, the antibodies
possibly having been modified by conjugation. Aggregates are liable to form under the

influence of a wide variety of parameters such as a high concentration of binding agent as defined in the present disclosure for instance antibody in the solution, the pH of the solution, high shear forces, the number of grafted drugs and their hydrophobic nature, the temperature (see the references cited in the introduction of *J. Membrane Sci.* **2008**, 318, 311–316), the
5 influence of some of them, however, having not been clearly elucidated. In the case of proteins or antibodies, reference may be made to AAPS Journal, “Protein Aggregation and Bioprocessing” 2006, 8(3), E572-E579. The aggregate content may be determined via known techniques such as SEC (see in this respect *Analytical Biochemistry* **1993**, 212 (2), 469-480).

10

The aqueous solution of the binding agent as defined in the present disclosure for instance the antibody may be buffered with buffers for example, potassium phosphate or HEPES or a mixture of buffers such as buffers A, B, C, D and E described later. The buffer depends on the nature of the binding agent as defined in the present disclosure for instance the antibody.

15 The compound/payload of formula (I), (I1) or (I2) is dissolved in a polar organic solvent such as DMSO or DMA.

The reaction takes place at a temperature generally ranging from 20°C to 40°C. The reaction time may be ranging from 1 to 24 hours. The reaction between the binding agent as defined
20 in the present disclosure for instance the antibody and the compound/payload of formula (I), (I1) or (I2) may be monitored by SEC with a refractometric and/or ultraviolet detector and/or HRMS in order to determine its degree of progress. If the degree of substitution is insufficient, the reaction can be left for longer and/or compound/payload of formula (I), (I1) or (I2) can be added. Reference may be made to the example section for further details
25 regarding particular conditions. Particular embodiments are described in Examples 9 to 40.

A person skilled in the art has at his disposal various chromatographic techniques for the separation of step (ii): the compound/conjugate of formula (II), (II1) or (II2) may be purified, for example, by steric exclusion chromatography (SEC), by adsorption chromatography (for
30 instance ion exchange, IEC), by hydrophobic interaction chromatography (HIC), by affinity chromatography, by chromatography on mixed supports such as ceramic hydroxyapatite, or by HPLC. Purification by dialysis or diafiltration may also be used.

After step (i) or (ii), the solution of the compound/conjugate of formula (II), (III) or (II2) may undergo an ultrafiltration and/or diafiltration step (iii). After these steps, the compound/conjugate of formula (II), (III) or (II2) in aqueous solution is thus obtained.

5

The present disclosure also relates to compounds of formula (II), (III) or (II2) that may be obtained *via* the process(es) in accordance with the present disclosure.

Binding agent

- 10 As mentioned above, a “**binding agent**” represents a molecule with affinity for a biological target, such as a ligand, a protein, a polypeptide, an antibody such as a monoclonal antibody or a VHH antibody, a peptide, an oligonucleotide, an oligosaccharide for instance a targeting agent for instance a cell-binding agent. The function of the binding agent is to direct the biologically active compound, such as a TLR7 and/or 8 agonist compound towards the
- 15 biological target.

Cell-binding agent

- As mentioned above, cell binding agents may be of any kind presently known, or that become known and includes peptides and non-peptides. The cell binding agent may be any
- 20 compound that can bind a cell, either in a specific or non-specific manner. Generally, these can be antibodies (especially monoclonal antibodies), lymphokines, hormones, growth factors, vitamins, nutrient-transport molecules (such as transferrin), or any other cell binding molecule or substance.

More specific examples of cell binding agents that can be used include:

- 25 (a) polyclonal antibodies;
- (b) monoclonal antibodies;
- (c) fragments of antibodies such as Fab, Fab1, and F(ab')₂, Fv (Parham, J. Immunol. 131:2895-2902 (1983); Spring et al. J. Immunol. 113:470-478 (1974); Nisonoff et al. Arch. Biochem. Biophys. 89:230-244 (1960));
- 30 (d) interferons (e.g. alpha, beta, gamma);

- (e) lymphokines such as IL-2, IL-3, IL-4, IL-6;
- (f) hormones such as insulin, TRH (thyrotropin releasing hormone), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens;
- (g) growth factors and colony-stimulating factors such as EGF, TGF- α , FGF, VEGF, G-CSF, M-CSF and GM-CSF (Burgess, Immunology Today 5:155-158 (1984)); or
- (h) transferrin (O'Keefe et al. J. Biol. Chem. 260:932-937 (1985)); and (i) vitamins, such as folate.

According to a particular embodiment, the binding agent (also named BA in the present text) can be an antibody (also named Ab in the present text), for instance a monoclonal antibody or a VHH antibody.

Antibody

The antibody can be a monoclonal antibody selected from the group consisting of a murine, chimeric, a humanized and a human antibody.

In one embodiment, the antibody is a monospecific antibody, i.e., an antibody specifically binding to one single target. Alternatively, it might be a multispecific antibody.

In one embodiment, the antibody is an IgG antibody, for instance an IgG1, an IgG2, an IgG3 or an IgG4 antibody.

The antibody according to the disclosure specifically binds to a target, thereby directing the compound/conjugate of formula (II), (II1) or (II2) towards said target. As used herein, "specifically binds" or "binds specifically to" or "binds to" or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiological conditions. Specific binding can be characterized by an equilibrium dissociation constant (K_D) of at least about 1×10^{-8} M or less (e.g., a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been characterized, for example, by their specific binding to target and/or target antigen using surface plasmon resonance, e.g., BIACORE™.

The target typically corresponds to a protein expressed at the cell surface, e.g. a protein

expressed at the surface of tumor cells.

In one embodiment, the target is the CEACAM5 receptor. The CEACAM5 receptor is a member of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) receptor family and is also referred to as “CEA cell adhesion molecule 5”, or “CD66e”. The antibody specifically binding to the CEACAM5 receptor might for instance correspond to one of the antibodies described in WO2014/079886 (US 9617345B2).

In one embodiment, the target is the Programmed Cell Death Ligand 1 (PDL1). The Programmed Cell Death Ligand 1 is a trans-membrane protein that is considered to be a co-inhibitory factor of the immune response. The antibody specifically binding to PDL1 might for instance correspond to one of the antibodies described in WO2013/079174A1.

The antibody may optionally be modified with a modifying agent so as to promote the attachment of the compound/payload of formula (I), (II) or (I2) as previously described. The antibody may especially be monoclonal, polyclonal or multispecific. It may also be an antibody fragment. It may also be a murine, human, humanized or chimeric antibody. The antibodies used in the examples of the present disclosure are:

- Tusamitamab (CAS [2349294-95-5]), an antibody against CEACAM5 receptor. The sequence of Tusamitamab (CAS [2349294-95-5]) is represented by SEQ ID NO: 88 (light chain of antibody Tusamitamab (CAS [2349294-95-5])) and by SEQ ID NO: 87 (heavy chain of antibody Tusamitamab (CAS [2349294-95-5])) following the procedure described in WO2014/079886 (US9617345B2). For the amino acid sequence of tusamitamab, it may also be referred to SEQ ID NO. 1 (light chain) and SEQ ID NO. 2 (heavy chain) disclosed herein after,
- Avelumab_K76R-K100R, an antibody against PDL1. It corresponds to the antibody Avelumab (CAS [1537032-82-8]) in which Lys 76 and Lys 100 have been replaced by Arg. For the amino acid sequence of Avelumab_K76R-K100R, it may be referred to SEQ ID NO. 7 (light chain) and SEQ ID NO. 8 (heavy chain) disclosed herein after,
- Avelumab_K360C, an antibody against PDL1. It corresponds to the antibody Avelumab (CAS [1537032-82-8]) in which Lys 360 has been replaced by Cys. For the amino acid sequence of Avelumab_K360C, it may be referred to SEQ ID NO. 5 (light chain) and SEQ ID NO. 6 (heavy chain) disclosed herein after,

- Avelumab_LALA-K360C, an antibody against PDL1. It corresponds to the antibody Avelumab (CAS [1537032-82-8]) in which Lys 360 has been replaced by Cys and Leu 234 and Leu 235 have been replaced by Ala. For the amino acid sequence of Avelumab_LALA-K360C, it may be referred to SEQ ID NO. 3 (light chain) and SEQ ID NO. 4 (heavy chain) disclosed herein after,
5
- Anti-CEACAM5_hyb_769graft_VHg2-7_VLg2-2 also named anti-CEACAM5_769_VarB_VH7/VL2, an antibody against CEACAM5 receptor. For the amino acid sequence of anti-CEACAM5_hyb_769graft_VHg2-7_VLg2-2, it may be referred to SEQ ID NO. 9 (light chain) and SEQ ID NO. 10 (heavy chain) disclosed herein after,
10
- Anti-CEACAM5_p01_CEA-1_07_IgG1-dK, an antibody against CEACAM5 receptor. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1-dK, it may be referred to SEQ ID NO. 11 (light chain) and SEQ ID NO. 12 (heavy chain) disclosed herein after,
15
- Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Ala 118 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_dK, it may be referred to SEQ ID NO. 13 (light chain) and SEQ ID NO. 14 (heavy chain) disclosed herein after,
20
- Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Glu 152 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_dK, it may be referred to SEQ ID NO. 15 (light chain) and SEQ ID NO. 16 (heavy chain) disclosed herein after,
25
- Anti-CEACAM5_p01_CEA-1_07_IgG1_K360C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Lys 360 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_K360C_dK, it may be referred to SEQ ID NO. 17 (light chain) and SEQ ID NO. 18 (heavy chain) disclosed herein after,
30

- Anti-CEACAM5_p01_CEA-1_07_IgG1_K414C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Lys 414 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_K414C_dK, it may be referred to SEQ ID NO. 19 (light chain) and SEQ ID NO. 20 (heavy chain) disclosed herein after,
5
- Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A1118C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_dK in which Ala 118 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_dK, it may be referred to SEQ ID NO. 21 (light chain) and SEQ ID NO. 22 (heavy chain) disclosed herein after,
10
- Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K360C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_dK in which Lys 360 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K360C_dK, it may be referred to SEQ ID NO. 23 (light chain) and SEQ ID NO. 24 (heavy chain) disclosed herein after,
15
- Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K414C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_dK in which Lys 414 has been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K414C_dK, it may be referred to SEQ ID NO. 25 (light chain) and SEQ ID NO. 26 (heavy chain) disclosed herein after,
20
- Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_K360C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Ala 118 and Lys 360 have been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_K360C_dK, it may be referred to SEQ ID NO. 27 (light chain) and SEQ ID NO. 28 (heavy chain) disclosed herein after,
25
30
- Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_K360C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-

1_07_IgG1-dK in which Glu 152 and Lys 360 have been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_K360C_dK, it may be referred to SEQ ID NO. 29 (light chain) and SEQ ID NO. 30 (heavy chain) disclosed herein after,

- 5 • Anti-CEACAM5_p01_CEA-1_07_IgG1_K274C_K414C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p01_CEA-1_07_IgG1-dK in which Lys 274 and Lys 414 have been replaced by Cys. For the amino acid sequence of Anti-CEACAM5_p01_CEA-1_07_IgG1_K274C_K414C_dK, it may be referred to SEQ ID NO. 31 (light chain) and SEQ ID NO. 32 (heavy chain) disclosed herein after,
- 10 • Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_K360C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_dK in which Ala 118 and Lys 360 have been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_K360C_dK, it may be referred to SEQ ID NO. 33 (light chain) and SEQ ID NO. 34 (heavy chain) disclosed herein after,
- 15 • Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K274C_K414C_dK, an antibody against CEACAM5 receptor. It corresponds to the antibody anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_dK in which Lys 274 and Lys 414 have been replaced by Cys. For the amino acid sequence of anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K274C_K414C_dK, it may be referred to SEQ ID NO. 35 (light chain) and SEQ ID NO. 36 (heavy chain) disclosed herein after.
- 20

Compound/conjugate of formula (II), (II1) or (II2) (Conjugate):

- 25 A compound/conjugate of formula (II), (II1) or (II2) generally comprises from about 1 to 10 compound/payload of formula (I), (I1) or (I2) covalently attached to the binding agent as defined in the present disclosure for instance the antibody (this is the degree of grafting or “drug-to-antibody ratio” or “DAR”). This number varies as a function of the nature of the binding agent as defined in the present disclosure for instance the antibody and of the
- 30 compound/payload of formula (I), (I1) or (I2) and also of the operating conditions used in the conjugation process (for example the number of equivalents of compound/payload of formula (I), (I1) or (I2) relative to the binding agent as defined in the present disclosure for

instance the antibody, the reaction time, the nature of the solvent and of any cosolvent). Placing of the binding agent as defined in the present disclosure for instance the antibody and the compound/payload of formula (I), (II) or (I2) in contact leads to a mixture comprising several compounds/conjugates of formula (II), (II1) or (II2) that are individually distinguished from each other by different DARs; optionally the unreacted binding agent as defined in the present disclosure for instance the unreacted antibody; optionally aggregates. The DAR that is determined on the final solution thus corresponds to an average DAR. The DAR may be calculated from the deconvolution of the SEC-HRMS spectrum of the compound/conjugate of formula (II), (II1) or (II2). The DAR (HRMS) is for example greater than 0.5, for instance ranging from 1 to 10, such as ranging from 2 to 5.

The compound/conjugate of formula (II), (II1) or (II2) may be used as an anticancer agent. Owing to the presence of the binding agent as defined in the present disclosure for instance the antibody, the compound/conjugate of formula (II), (II1) or (II2) is made highly selective towards tumor cells rather than healthy cells. This makes it possible to direct the compound/conjugate of formula (II), (II1) or (II2) in an environment similar thereto or directly therein. It is possible to treat solid or liquid cancers. The compound/conjugate of formula (II), (II1) or (II2) may be used alone or in combination with at least one other anticancer agent.

The compound/conjugate of formula (II), (II1) or (II2) is formulated in the form of a buffered aqueous solution at a concentration generally ranging from 1 to 10 mg/mL. This solution may be injected in perfusion form per se or may be re-diluted to form a perfusion solution.

The examples which follow describe the preparation of some compounds of formulae (I), (II) or (I2) and (II), (II1) or (II2) in accordance with the disclosure. The numbers of the compounds exemplified below match those given above. All reactions are performed under inert atmosphere, unless otherwise stated.

In the following examples, when the source of the starting products is not specified, it should be understood that said products are known compounds.

EXAMPLES

The examples which follow describe the preparation of certain compounds in accordance
5 with the disclosure. These examples are not limitative, and merely illustrate the present disclosure.

Materials and methods

The progress of the synthetic reactions is monitored by TLC. The plates are made of glass
10 and are coated with Merck 60 F254 silica gel. After elution, the plates are observed under ultraviolet light at 254 nm.

The microwave reactions were performed using a Biotage Initiator 8 EXP microwave machine. The products were purified, when necessary, on a Biotage Isolera chromatograph or a Spot 2 chromatograph from Merck. The columns used are Merck 15-40 µm silica
15 columns (2.5 g to 800 g).

Analytical methods used

High Pressure Liquid Chromatography – Mass Spectrometry (LCMS)

Method M: the spectra were acquired on an Agilent 1200 & 6110B; ionization: electrospray
20 in positive mode (ES+).

- Kinetex C18 50 x 2.1 mm, 5 µm
- Solvents: A: H₂O+0.037 % (v/v) TFA B: ACN+0.018 % (v/v) TFA
- Column temperature: 40°C
- Flow rate: 1 mL/min
- 25 • Gradient (5 min): from 5 to 95% B in 3 min; 1 min: 95% B; 1.50 min: 5% B

Method M1: the spectra were acquired on a Waters UPLC-SQD; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 – 1.7 µm – 2.1 x 50 mm
- 30 • Solvents: A: H₂O (0.1 % formic acid) B: CH₃CN (0.1 % formic acid)
- Column temperature: 50°C
- Flow rate: 0.90 mL/min

- Gradient (2.5 min): from 5 to 100% B in 1.8 min; 2.4 min: 100% B; 2.45 min: 5% B

Method M2: the spectra were acquired on a Waters XEVO Q TOF; ionization: electrospray in positive mode (ES+).

- Column ACQUITY CSH C18 – 1.7 μ m – 2.1 x 100 mm
- Solvents: A: H₂O (0.1 % formic acid) B: CH₃CN (0.1 % formic acid)
- Column temperature: 45°C
- Flow rate: 0.50 mL/min
- Gradient (3 min): 0 to 0.3 min, 5% of B - 0.3 to 4 min, 5 to 100% of B - 4 to 4.6 min, 100% of B - 4.6 to 5.3 min, 5% of B

Method N: the spectra were acquired on a Waters UPLC-SQD2; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 - 1.7 μ m - 2.1 x 50 mm
- Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
- Column temperature: 60°C
- Flow rate: 1 mL/min
- Gradient (2.5 min): from 3 to 100% B in 2.1 min; 2.45 min: 100% B; 2.50 min: 3% B

Method N1: the spectra were acquired on a Waters UPLC-SQD2; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 - 1.7 μ m - 2.1 x 50 mm
- Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
- Column temperature: 60°C
- Flow rate: 1 mL/min
- Gradient (3 min): 0.1 min: 3% B; from 3 to 97% B in 2 min; 2.45 min: 97% B; 2.50 min: 3% B; 3 min: 3% B

Method N2: the spectra were acquired on a Waters UPLC-SQD2; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 - 1.7 μ m - 2.1 x 30 mm
- Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)

- Column temperature: 50°C
- Flow rate: 0.7 mL/min
- Gradient (6 min): 0.3 min: 3% B; from 3 to 97% B in 4.7 min; 5 min: 100% B; 6 min: 3% B

5

Method N3: the spectra were acquired on a Waters UPLC-SQD2; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 - 1.7 μm - 2.1 x 30 mm
 - Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
 - Column temperature: 50°C
 - Flow rate: 0.8 mL/min
- Gradient (5 min): 0.2 min: 5% B; from 5 to 98% B in 3.4 min; 3.7 min: 98% B; 4.10 min: 5% B; 5 min: 5% B

10

15 Method N4: the spectra were acquired on a Waters UPLC-SQD; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CORTECS C18+ - 1.6 μm - 2.1 x 50 mm
 - Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
 - Column temperature: 50°C
 - Flow rate: 1 mL/min
- Gradient (2 min): 0 to 1.5 min 5 to 99% of B - 1.5 to 1.9 min 99% of B - 1.9 to 1.95 min 99 to 5% of B - 1.95 to 2.15

20

25 Method N5: the spectra were acquired on a Waters UPLC-SQD2; ionization: electrospray in positive and/or negative mode (ES+/-).

- Column ACQUITY CSH C18 - 1.7 μm - 2.1 x 30 mm
 - Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
 - Column temperature: 50°C
 - Flow rate: 0.7 mL/min
- Gradient (4 min): 0.18 min: 5% B; from 5 to 99% B in 3.02 min; 3.4 min: 99% B; 4 min: 5% B

30

Method O: the spectra were acquired on a WATERS QUATTRO PREMIER; ionization: electrospray in positive and/or negative mode (ES+/-)

- Column ACQUITY MSS T3 - 1.8 μ m - 2.1 x 50 mm
- Solvents: A: H₂O (0.1% formic acid) B: CH₃CN (0.1% formic acid)
- 5 • Column temperature: 55°C
- Flow rate: 0.9 mL/min
- Gradient (3.7 min): de 5 to 100% B in 3 min; 3.1 min: 5% B

¹H Nuclear Magnetic Resonance (NMR)

- 10 The ¹H NMR spectra were recorded on a Brüker Avance and/or Varian G spectrometer (300 MHz, 400 MHz, 500 MHz, or 600 MHz) in deuterated DMSO. The chemical shifts are expressed in units (ppm) using tetramethyl silane (TMS) as internal reference. For the interpretation of the spectra, the following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, dd = doubled doublet, ddd = doublet of
- 15 doubled doublets, m = multiplet, ax. = axial, equat. = equatorial.

Reverse phase chromatography – high resolution mass spectrometry (RP-HRMS)

- The analyses were performed on an UPLC-XEVO-G2-XS-QTOF (Waters), an UPLC-XEVO-G2-S-QTOF (Waters) or an UPLC-XEVO-G3-QTOF (Waters). The reverse phase
- 20 chromatographic analysis was performed using an Agilent BioHPLC PLR 5 μ m column (2.1 x 50 mm) at 80°C with a flow rate of 0.5 mL/min and the following elution gradient: 5% B at 1 min, 50% B at 4 min, 50% B at 5 min, 5% B at 6 min, 5% B at 7 min (solvents: A: H₂O (0.1% formic acid); B: CH₃CN (0.1% formic acid)). The mass spectrometry was performed with electrospray ionization in positive mode (ES+). The mass spectra were deconvoluted
- 25 with the Waters MaxEnt1 software. The observed molecular masses correspond respectively to the mass of the naked antibody, if present, and to the conjugation of 1 (D1), 2 (D2),... n (Dn) drugs on the antibody.

- When needed, preliminary ADC reduction was performed using the following conditions: ADC was treated with 1/10 (in volume) of a 0.5M TCEP solution in water and left for 30 min
- 30 at RT before RP-HRMS analysis. In this case, the observed molecular masses correspond respectively to the mass of the naked light and heavy chains (LC and HC), if present, and to the conjugation of 1 (LC1, HC1), 2 (LC2, HC2)... n (LCn, HCn) drugs on the antibody.

Analytical Size Exclusion Chromatography (SEC)

The analysis was performed on a Hitachi Labchrom or a Thermo Vanquish LC system equipped with a photodiode array detector and a Tosoh Bioscience TSK-GEL SuperSW 4 μm (4.6×150 mm) column with a flow rate of 0.2 mL/min or a Tosoh Bioscience TSKgel G3000 SWXL 5 μm (7.8×300 mm) column with a flow rate of 0.5 mL/min and an isocratic elution of 15 minutes with a pH 7 buffer containing 0.2 M of KCl, 0.052 M of KH_2PO_4 , 0.107 M of K_2HPO_4 and 20 % by volume of isopropanol.

10 Buffers

- DPBS: Na_2HPO_4 (8.10 mM), KH_2PO_4 (1.47 mM), NaCl (136.9 mM), KCl (2.67 mM)
- Buffer A (pH 5.5): sodium acetate (20 mM), 10% (w/v) sucrose
- Buffer B (pH 5.5): sodium acetate (20 mM), 5% (w/v) sucrose, 0.01% PS80
- Buffer C (pH 7.4): NaPi (10 mM), NaCl (140 mM)
- 15 • Buffer D (pH 5.5): histidine (10 mM), 8% (w/v) sucrose
- Buffer E (pH 6.5): histidine (10 mM), 10% (w/v) sucrose

General methods used for the preparation of compounds/conjugates of formula (II), (II1) or (II2) (M1 to M4)

- 20 • **General method used for the preparation of mAb-DBCO using NHS ester (M1)**

M1: To a solution of antibody in an aqueous buffer eventually diluted with DPBS was added 1 N HEPES (composition varying from 94:6 to 100:0, in other terms from 0% to 6% HEPES (v/v)). The solution was treated with an excess (4 to 10 equivalents) of a solution at 2 to 20 mM of NHS ester DBCO linker in DMA or DMSO such that the final antibody concentration is 1-11 mg/mL and the percentage of DMA or DMSO in the aqueous buffer is 1-20%. After stirring at RT for 45 min to 3 hours, the mixture was analyzed by SEC HPLC and/or HRMS so as to determine the linker-to-antibody ratio (LAR) on the population of monomeric antibodies. If the LAR was found insufficient, the mixture was treated with a further excess of linker solution in DMA or DMSO for up to 2 additional hours at RT under stirring.

30

- **General method used for the preparation of compounds/conjugates of formula (II), (II1) or (II2) (M2 & M3)**

M2: A solution of mAb-DBCO in an aqueous buffer containing 1-20% DMA or DMSO (eventually diluted with DPBS) was treated with an excess (6 to 12 equivalents) of a solution at 10 to 20 mM of azido payload in DMA or DMSO such that the final antibody concentration is 1-4 mg/mL and the percentage of DMA or DMSO in the aqueous buffer is 2-20%. After overnight stirring at RT, the reaction mixture was analyzed by RP-HRMS to determine the DAR on the population of monomeric antibodies. The mixture was eventually concentrated by ultrafiltration with ultrafiltration spin column (Vivaspin®, PES membrane 50K, Sartorius) with DPBS buffer containing 20% of DMA. The mixture was then purified by gel filtration using a Superdex™ 200 pg matrix (HiLoad® 16/60 or 26/60 desalting column, GE Healthcare) pre-equilibrated in DPBS buffer containing up to 10-20 % of DMA. The fractions containing the monomeric conjugated antibody were pooled and eventually concentrated by ultrafiltration on spin columns (Vivaspin®: PES membrane 50K, Sartorius or Amicon® Ultra: Ultracel membrane, 50K, Millipore) to reach a concentration between 2 and 5 mg/mL. In some cases, the conjugate was formulated by gel filtration using a Sephadex™ G25 matrix (NAP® or Hitrap™ desalting columns, GE Healthcare) pre-equilibrated with the final aqueous buffer A, buffer D or buffer E. The conjugate was finally sterile filtered (Millex®-SV 0.22µm, PVDF, Durapore®, Millipore). then assayed by UV spectrometry or SEC HPLC so as to measure the conjugate concentration, by SEC HPLC so as to determine the monomeric purity and by RP-HRMS so as to determine the DAR from the deconvolution of the mass spectrum of the conjugate.

M3: A solution of mAb-DBCO in an aqueous buffer containing 1-20% DMA or DMSO (eventually diluted with DPBS) was treated with an excess (6 to 12 equivalents) of a solution at 10 to 20 mM of azido payload in DMA or DMSO such that the final antibody concentration is 1-4 mg/mL and the percentage of DMA or DMSO in the aqueous buffer is 2-20%. After overnight stirring at RT, the mixture was analyzed by RP-HRMS so as to determine the DAR on the population of monomeric antibodies. The mixture was purified by diafiltration with ultrafiltration spin columns (Vivaspin®: PES membrane 50K, Sartorius or Amicon® Ultra: Ultracel membrane, 50K, Millipore) then formulated using Sephadex™ G25 matrix (Hitrap™ desalting GE Healthcare) pre-equilibrated with the final aqueous buffer A, buffer D or buffer E. Depending on the residual free drug content as assessed by HRMS, additional purification by diafiltration with final buffer was performed. The conjugate was finally sterile filtered (Millex®-SV 0.22 µm (PVDF, Durapore, Millipore)).

The final conjugate was assayed by UV spectrometry or SEC HPLC to measure the conjugate concentration, by SEC-HPLC so as to determine the monomeric purity and by RP-HRMS to determine the DAR from the deconvolution of the mass spectrum of the conjugate.

5 • **General method used for the preparation of compounds/conjugates of formula (II), (II1) or (II2) using thiomAbs (M4)**

M4a: To a solution of thiomAb at 1-11 mg/mL in DPBS was added DTT (64 equiv of a 100-300 mM solution in H₂O or DPBS). The mixture was stirred at 37°C during 45 min to 1 h and then purified by gel filtration using a SephadexTM G25 matrix (NAP® or HiprepTM 26/10 desalting columns, GE Healthcare) pre-equilibrated with DPBS buffer. To the solution of reduced thiomAb was added DHAA (14-15 equiv of a 25-100 mM solution in DPBS) and the reaction mixture was stirred at RT for 3 hours.

M4b: after addition of DMA (10-20% v/v), the mixture was treated with an excess of maleimido compound (1.5 to 2 equiv per uncapped thiols of a 10 to 20 mM solution in DMA) such that the final antibody concentration is 1-4 mg/mL and the percentage of DMA in the aqueous buffer is ~10-20%. After overnight stirring at RT, the mixture was analyzed by RP-HRMS so as to determine the DAR on the population of monomeric antibodies. The mixture was concentrated by diafiltration with ultrafiltration spin columns (Vivaspin®: PES membrane 50K, Sartorius or Amicon® Ultra: Ultracel membrane, 50K, Millipore) before or after purification by gel filtration using a Superdex 200 pg matrix (HiLoad® 16/60 or 26/60 desalting column, GE Healthcare) pre-equilibrated in aqueous buffer such as DPBS containing 5 to 20 % of an organic solvent such as DMA or EtOH and then formulated by gel filtration using a SephadexTM G25 matrix (NAP® or HiprepTM 26/10 desalting columns, GE Healthcare) pre-equilibrated with the final aqueous buffer A, buffer D or buffer E. Depending on the residual free drug content as assessed by HRMS, additional purification by diafiltration with final buffer was performed. The conjugate was finally sterile filtered (Millex®-SV 0.22 µm, PVDF, Durapore, Millipore). The conjugate was assayed by UV spectrometry or SEC HPLC so as to measure the conjugate concentration, by SEC-HPLC so as to determine the monomeric purity and by RP-HRMS so as to determine the DAR from the deconvolution of the mass spectrum of the conjugate.

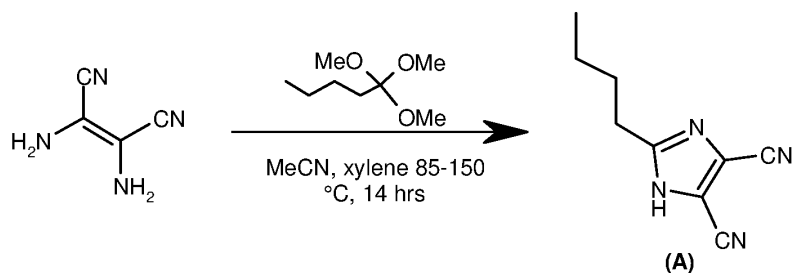
Preparation

A) Preparation of TLR agonists

All the following compounds were synthesized according to the protocols described below.

Synthesis of intermediates

5 **Preparation 1: Intermediate (A)** 2-butyl-1H-imidazole-4,5-dicarbonitrile

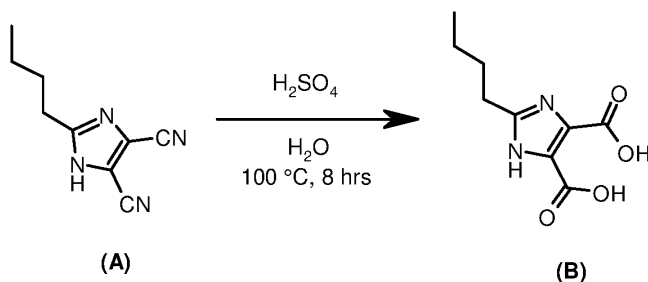


A suspension of (Z)-2,3-diaminobut-2-enenitrile (45.0 g, 416 mmol) and 1,1,1-trimethoxypentane (67.5 g, 416 mmol) in MeCN (90 mL) was stirred in an oil bath and it was kept at 85°C for 6 h. The reaction mixture was then concentrated by evaporation under reduced pressure. The whole of this compound was dissolved in xylene (90 mL), and the resulting solution was stirred in an oil bath kept at 150°C for 8 h. The mixture was concentrated to give a residue, which was filtered, and washed with methylbenzene. Then the filter cake was dried to give the expected product. **Intermediate (A)** (63.0 g, 87% yield) was used in the next step without any purification.

¹H NMR (400 MHz, δ in ppm, CDCl₃): 2.82 (t, J=7.6 Hz, 15.2 Hz, 2H); 1.72-1.80 (m, 2H); 1.36-1.45 (m, 2H); 0.93-0.97 (m, 3H)

MS Method M: t_R =1.019 min, m/z 175.1 (M+H)⁺

Preparation 2: Intermediate (B) 2-butyl-1H-imidazole-4,5-dicarboxylic acid



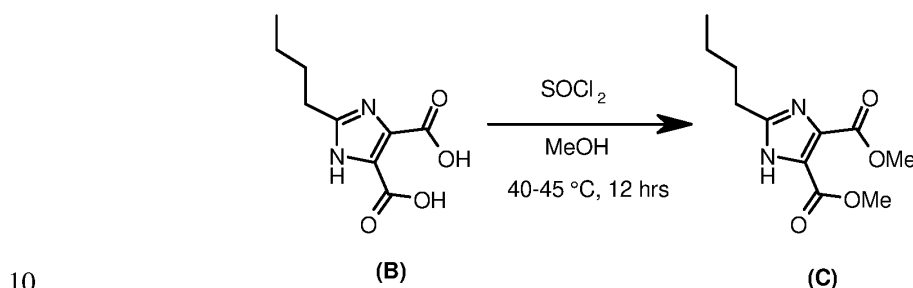
A solution of **intermediate (A)** (63.0 g, 362 mmol) in H₂SO₄ (284 mL) and H₂O (126 mL) was stirred at 100°C for 8 h. By TLC one major new spot with lower polarity was observed.

The pH of the solution was adjusted to 9-10 using 10% sodium hydroxide solution. The mixture was filtered, and the filter cake was concentrated to give the expected product which was used into the next step without further purification. **Intermediate (B)** (76.5 g, 99.7% yield) was obtained as a black brown solid.

- 5 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 2.59 (t, J=7.6 Hz, 2H); 1.57-1.63 (m, 2H); 1.19-1.28 (m, 2H); 1.27-1.33 (m, 2H); 0.86 (t, J=7.2 Hz, 14.8 Hz, 2H)

MS Method M: t_R =1.163 min, m/z 213.1 (M+H)⁺.

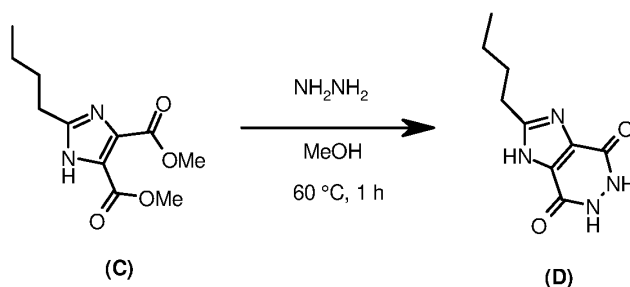
Preparation 3: Intermediate (C) dimethyl 2-butyl-1H-imidazole-4,5-dicarboxylate



- To a mixture of **intermediate (B)** (76.5 g, 361 mmol) in MeOH (230 mL) was added SOCl₂ (214 g, 1.80 mol, 131 mL) slowly and degassed and purged with N₂ for 3 times, and then the mixture was stirred at 40-45°C for 12 h under N₂ atmosphere. The mixture was cooled and then poured slowly to chilled water (500 mL). It was neutralized by the addition of 10 %
- 15 sodium hydroxide solution. Then the mixture was extracted with EtOAc (3 x 500 mL). The combined organic layers were washed with brine (1.00 L), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without further purification. **Intermediate (C)** (81.0 g, 93.5% yield) was obtained as a black brown solid.

- 20 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 3.87 (s, 6H); 2.77 (t, J=8.0 Hz, 2H); 1.68-1.76 (m, 2H); 1.30-1.40 (m, 2H); 0.90 (t, J=7.6 Hz, 3H)

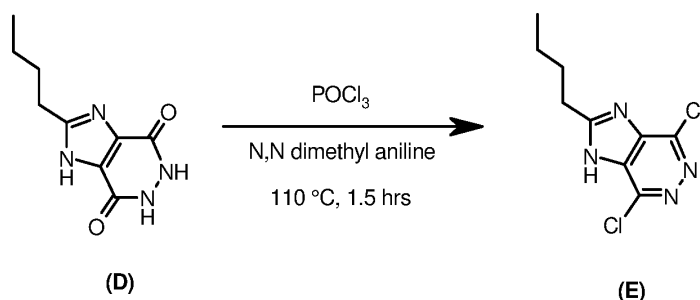
Preparation 4: Intermediate (D) 2-butyl-5,6-dihydro-1H-imidazo[4,5-d]pyridazine-4,7-dione



A mixture of **intermediate (C)** (81.0 g, 337 mmol), $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (51.7 g, 1.01 mol, 50.2 mL, 98.0 % purity) in MeOH (243 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 60°C for 1 h under N_2 atmosphere. The reaction mixture
 5 was filtered and washed with methanol. This material was suspended in water (1.00 L), heated to 85°C, and then rendered acidic by addition of conc. HCl (100 mL). After stirring for 12 h at 85°C, the mixture was cooled and the white product filtered with suction and washed with water. The crude product was used into the next step without further purification. **Intermediate (D)** (49.5 g, 70.5% yield) was obtained as an off-white solid.

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Preparation 5: Intermediate (E) 2-butyl-4,7-dichloro-1H-imidazo[4,5-d]pyridazine

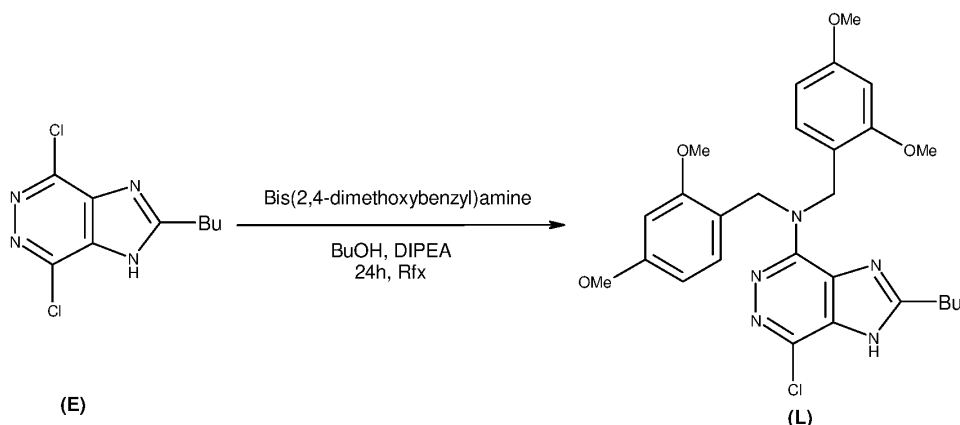


To a cooled solution of **intermediate (D)** (49.5 g, 238 mmol) in POCl_3 (248 mL) was added N,N-dimethyl aniline (50 mL) and the reaction mixture was stirred at 110°C for 90 min. The
 15 reaction mixture was concentrated under reduced pressure to give a residue. The residue was poured into cold water (2.00 L) and adjusted pH to 7 with solid sodium bicarbonate and extracted with EtOAc (2 x 1 L). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue in MTBE (200 mL) was stirred for 2 h. The suspension was filtered,
 20 and the filter cake was collected. **Intermediate (E)** (54.5 g, 92% yield) was obtained as a yellow solid.

^1H NMR (400 MHz, δ in ppm, CDCl_3): 3.18 (t, $J=7.6$ Hz, 2H); 1.90-1.97 (m, 2H); 1.40-1.49 (m, 1H); 0.92 (t, $J=7.2$ Hz, 3H)

MS Method M: $t_R = 2.161$ min, m/z 245.0 ($M+H$) $^+$.

5 **Preparation 6: Intermediate (L)** 2-butyl-7-chloro-N,N-bis(2,4-dimethoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine

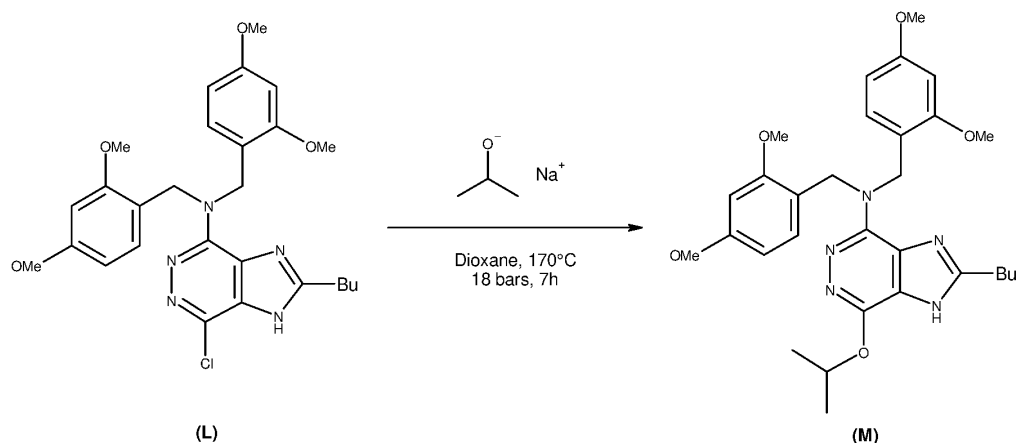


To a solution of **intermediate (E)** (15 g, 61.20 mmol) in n-butanol (150 mL) was added DIPEA (107 mL, 612.63 mmol) and bis(2,4-dimethoxybenzyl)amine (7 g, 22.1 mmol). The reaction mixture was refluxed during 1 h. A further 5 g (15.8 mmol) of bis(2,4-dimethoxybenzyl)amine were added and the heating was continued for 1 h. This operation was repeated twice again (2 x 5 g, 31.5 mmol). The resulting mixture was heated overnight. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to remove most of n-butanol. The residue was diluted with DCM (500 mL), then washed with H_2O (250 mL), brine (200 mL), dried over anhydrous MgSO_4 , filtered and the filtrate was concentrated under reduced pressure to give 38.8 g of crude product. The residue was purified by chromatography on a Merck cartridge (800 g of 15-40 μm silica) using as eluent DCM/acetone 96/4 to 90/10 to give **intermediate (L)** (12 g, 37% yield) as a pale-yellow solid.

20 ^1H NMR (400MHz, δ in ppm, $\text{DMSO}-d_6$): 0.85 (t, $J=7.4$ Hz, 3 H); 1.28 (m, 2 H); 1.68 (m, 2 H); 2.82 (t, $J=7.6$ Hz, 2 H); 3.72 (s, 12 H); 5.07 (s broad, 4 H); 6.39 (dd, $J=2.4$ & 8.4 Hz, 2 H); 6.54 (d, $J=2.4$ Hz, 2 H); 6.95 (d, $J=8.4$ Hz, 2 H); 13.35 (s broad, 1 H)

MS method N: t_R (min): 1.59; $[M+H]^+$ 526

Preparation 7: Intermediate (M) 2-butyl-N,N-bis(2,4-dimethoxybenzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine.

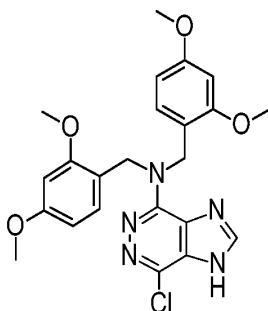


To a suspension of **intermediate (L)** (4 g, 7.60 mmol) in dioxane (30 mL) was added sodium isopropoxide (3.74 g, 45.62 mmol) and 2-propanol (30 mL, 1.05 mol). The mixture was heated at 170°C under 18 bars for 7 h. The reaction mixture was cooled and filtered, and the cake was washed with EtOAc (100 mL). The filtrate was concentrated under reduced pressure. The crude product was dissolved with EtOAc (150 mL) then washed with H₂O (2 x 75 mL), brine (75 mL), dried over anhydrous MgSO₄, filtered and the filtrate was concentrated under reduced pressure to give 4.37 g of product. The residue was purified by chromatography on a Merck cartridge (200 g of 15-40 µm silica) with an EtOAc/Heptane (55/45) elution. **Intermediate (M)** (2.11 g, 50% yield) was obtained as a white solid.

¹H NMR (400MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.4 Hz, 3 H); 1.26 (m, 2 H); 1.37 (d, J=6.2 Hz, 6 H); 1.66 (m, 2 H); 2.77 (t, J=7.6 Hz, 2 H); 3.70 (s, 6 H); 3.71 (s, 6 H); 5.00 (s broad, 4 H); 5.41 (sept, J=6.2 Hz, 1 H); 6.38 (dd, J=2.4 & 8.4 Hz, 2 H); 6.52 (d, J=2.4 Hz, 2 H); 6.94 (d, J=8.4 Hz, 2 H); 13.01 (s broad, 1 H)

MS method N: t_R (min): 1.58; [M+H]⁺ 550

Preparation 8: Intermediate (N) 7-chloro-N,N-bis(2,4-dimethoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine

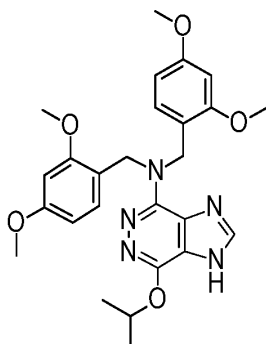


To a 25 mL round bottom flask equipped with a magnetic stir and a reflux condenser, were added commercially available 4,7-dichloro-1H-imidazo[4,5-d]pyridazine (3 g, 15.87 mmol), n-butanol (30 mL) and DIPEA (27.79 mL, 158.73 mmol), resulting in a homogeneous solution. Bis(2,4-dimethoxybenzyl)amine (1.45 g, 4.48 mmol) was then added and the brown reaction mixture was heated at reflux for 1 h. 3 other portions of bis(2,4-dimethoxybenzyl)amine were added every hour (t₀ + 2 h: 1.5 g, 4.63 mmol; t₀ + 3 h: 1.5 g, 4.63 mmol and t₀ + 4 h: 1.09 g, 3.37 mmol). The mixture was heated at reflux for 92 h. It was cooled to rt and evaporated under vacuum to dryness. The remaining oil was dissolved in EtOAc (400 mL), transferred to a separating funnel and washed with water (3 x 100 mL). The aqueous phase was extracted with 100 mL EtOAc. The combined organic layer was washed with brine, dried over MgSO₄, filtered and evaporated to dryness, to give 9.06 g of crude product. The crude material was purified by chromatography on a Merck cartridge (600 g of 15-40 μm silica) with CHCl₃/MeOH (97/3) elution, to afford 1.79 g (24% yield) of expected **intermediate (N)** as a yellow solid.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 3.72 (s, 12 H); 5.07 (br s, 4 H); 6.40 (dd, J=8, 3 Hz, 2 H); 6.54 (d, J=3 Hz, 2 H); 6.95 (d, J=8 Hz, 2 H); 8.40 (s, 1 H); 13.74 (br s, 1 H)

MS method N: t_R (min): 1.37, [M+H]⁺ 470

Preparation 9: Intermediate (R) N,N-bis(2,4-dimethoxybenzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine

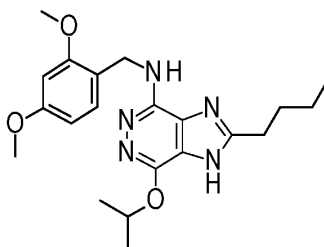


To a round bottom flask (50 mL), were added 7-chloro-N,N-bis(2,4-dimethoxybenzyl)-1H-imidazo[4,5-d]pyridazin-4-amine, **intermediate (N)** (1.44 g, 3.06 mmol), dioxane (16 mL) and isopropanol (16 mL). The resulting solution was transferred equally into 2 microwave tubes of 10-20 mL capacity, to each was added sodium isopropoxide (755 mg, 9.2 mmol). Both tubes were heated at 170°C for 8 h. The reaction mixtures were combined and diluted in EtOAc (100 mL). The organic solution was washed with water (50 mL), the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layer was washed with brine, dried over MgSO₄, filtered and evaporated to dryness, to give 1.38 g of crude product, which was purified by chromatography on a Merck cartridge (150 g of 15-40 µm silica) with DCM/MeOH (99/1 to 98/2) elution, to afford 0.763 g (50% yield) of expected **intermediate (R)** as a white foam.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.38 (d, J=6.2 Hz, 6 H); 3.71 (s, 12 H); 5.02 (br s, 4 H); 5.41 (m, 1 H); 6.40 (dd, J=8.4, 2.4 Hz, 2 H); 6.53 (d, J=2.4 Hz, 2 H); 6.95 (d, J=8.4 Hz, 2 H); 8.21 (br s, 1 H); 13.30-13.60 (br s, 1H)

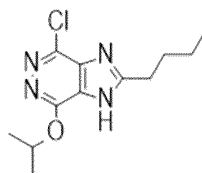
MS method N: t_R (min): 1.29, [M+H]⁺ 494

Preparation 10: Intermediate (S) 2-butyl-N-[(2,4-dimethoxyphenyl)methyl]-4-isopropoxy-3H-imidazo[4,5-d]pyridazin-7-amine



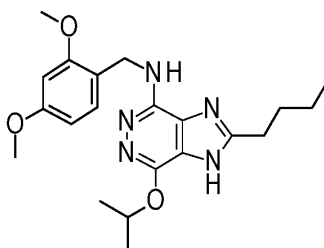
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Step 1: 2-butyl-4-chloro-7-isopropoxy-1H-imidazo[4,5-d]pyridazine



- NaH (12.3 g, 307.3 mmol) was added at 0-5 °C to a mixture of i-PrOH (300 mL) under N₂. The mixture was stirred at 0-5 °C for 30 min, then heated to 95-100 °C. To the reaction mixture was added dropwise a solution of 2-butyl-4,7-dichloro-1H-imidazo[4,5-d]pyridazine, **Intermediate (E)** (15 g, 61.5 mmol) in dioxane (150 mL) and the reaction was heated at 95-100 °C for a period of 12 h under N₂. The residue was poured into cold water (2 V) and extracted with EtOAc (2 V x 3). The combined organic layers were washed with brine (2 V), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give 16 g (97 % yield) of the expected compound.
- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.91 (t, J=7 Hz, 3 H); 1.35 (m, 8 H); 1.71 (m, 2 H); 2.72 (t, J=7.6 Hz, 2 H); 3.78 (m, 1 H); 5.49 (m, 1 H)

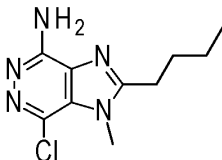
Step 2: 2-butyl-N-[(2,4-dimethoxyphenyl)methyl]-4-isopropoxy-3H-imidazo[4,5-d]pyridazin-7-amine



- To a solution of 2-butyl-4-chloro-7-isopropoxy-1H-imidazo[4,5-d]pyridazine from **Step 1** (12 g, 44.75 mmol) and (2,4-dimethoxyphenyl)methanamine (7.48 g, 44.75 mmol) in THF (240 mL) were added NaOtBu (10.75 g, 111.89 mmol) and tBuXPhos Pd G-3 (1 g, 1.34 mmol). The reaction mixture was stirred at 60-65 °C for 3 h then poured into cold water (2 V) and extracted with EtOAc (2 V x 3). The combined organic layers were washed with brine (2 V), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give 11.1 g (62 % yield) of **Intermediate (S)**.
- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.89 (t, J=7.2 Hz, 3 H); 1.32 (m, 8 H); 1.72 (m, 2 H); 2.82 (t, J=7.6 Hz, 2 H); 3.72 (s, 3 H); 3.78 (s, 3 H); 4.53 (d, J=4.8 Hz, 2H); 5.38 (m,

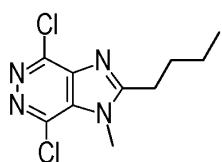
1 H), 6.43 (d, J=7.6, 1 H); 6.53 (s, 1H)); 7.14 (bs, 1 H)

Preparation 11: Intermediate (T) 2-butyl-4-chloro-3-methyl-imidazo[4,5-d]pyridazin-7-amine



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Step1: 2-butyl-4,7-dichloro-3-methyl-imidazo[4,5-d]pyridazine

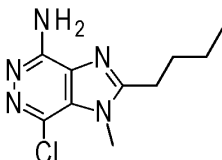


To a solution of 2-butyl-4,7-dichloro-1H-imidazo[4,5-d]pyridazine, **Intermediate (E)** (5 g, 20.4 mmol) in acetone (65 mL) was added potassium carbonate (8.5 g, 61.20 mmol). After 30 min of stirring at rt methyl iodide (1.9 mL, 30.60 mmol) was added dropwise and the reaction mixture was stirred for 4 h. The solids were removed by filtration over a glass frit and the filtrate was concentrated to dryness. The residue was taken up in DCM (50 mL) and washed with water (2 x 40 mL) and brine (40 mL). The organic phase was collected, dried over MgSO₄, filtered and concentrated to dryness to give a dark orange solid that was then triturated in isopropyl ether, collected by filtration and air dried. The expected product was obtained as a light pink solid (4.5 g, 85 % yield).

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MS method N1: t_R (min): 1.38; $[M+H]^+$ 258.9

Step2: 2-butyl-4-chloro-3-methyl-imidazo[4,5-d]pyridazin-7-amine



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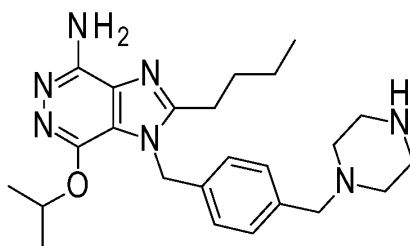
A solution of 2-butyl-4,7-dichloro-3-methyl-imidazo[4,5-d]pyridazine from **Step 1** (1.55 g, 5.98 mmol) was reacted with a solution of 35 % ammonia (30 mL) at 160 °C (36 bars of pressure) for 3 h. The resulting suspension was concentrated to dryness, taken up in water

(20 mL), stirred for 2 h, then further triturated in isopropylalcohol, filtered and air dried to give 533 mg (37 % yield) of **Intermediate (T)** as a beige solid.

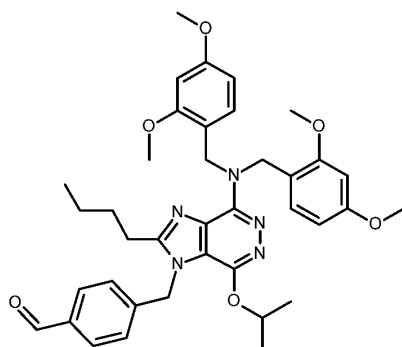
MS method N1: t_R (min): 0.85; $[M+H]^+$ 239.9

5 Synthesis of TLR agonists

Example (TLR1): Preparation of 2-butyl-7-isopropoxy-1-(4-(piperazin-1-ylmethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine



Step 1: 4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzaldehyde



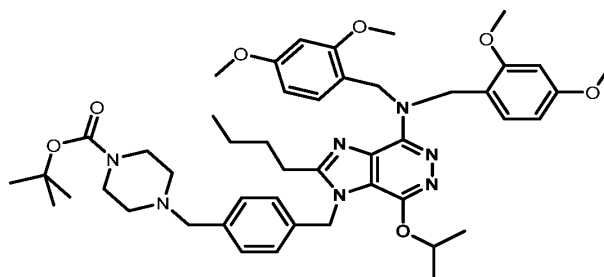
To a suspension of **intermediate (M)** (2.2 g, 3.12 mmol) in Me-THF (21 mL) was added 4-(bromomethyl)benzaldehyde (847 mg, 4.26 mmol) and Cs_2CO_3 (1.32 g, 4.11 mmol). The mixture was stirred at rt during 24 h. The mixture was poured into Me-THF (15 mL) then washed with H_2O (15 mL), dried over anhydrous MgSO_4 , filtered and the filtrate was concentrated under reduced pressure to give 3 g of crude product, which was purified by chromatography on a Merck cartridge (150 g of 15-40 μm silica) with EtOAc/heptane (38/62) elution. Expected product (1.5 g, 71% yield) was obtained as a white solid.

^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.76 (t, $J=7.4$ Hz, 3 H); 1.13 (d, $J=6.2$ Hz, 6 H); 1.24 (m, 2 H); 1.56 (m, 2 H); 2.77 (t, $J=7.5$ Hz, 2 H); 3.71 (s, 12 H); 5.02 (s, 4 H); 5.26 (sept,

J=6.2 Hz, 1 H); 5.69 (s, 2 H); 6.40 (dd, J=2.5 & 8.4 Hz, 2 H); 6.53 (d, J=2.5 Hz, 2 H); 6.99 (d, J=8.4 Hz, 2 H); 7.25 (d, J=8.3 Hz, 2 H); 7.89 (d, J=8.3 Hz, 2 H); 9.97 (s, 1 H)

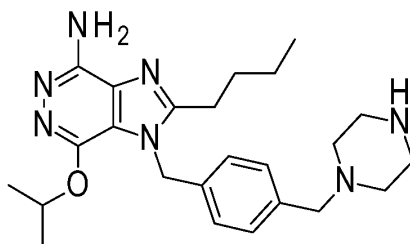
MS method N: t_R (min): 1.66; $[M+H]^+$ 668; ES^- $[2M-H+HCO_2H]^-$: m/z 1379

- 5 **Step 2:** *tert*-butyl 4-(4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)piperazine-1-carboxylate



- To a solution of 4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzaldehyde of **Step 1** (200 mg, 0.30 mmol) in methanol (2.5 mL) was added 1-Boc-piperazine (85.38 mg, 0.45 mmol), acetic acid (34.29 μ L, 0.60 mmol). The mixture was stirring at rt during 10 min and then sodium cyanoborohydride (28.23 mg, 0.45 mmol) was added. The stirring was continued at rt during 4.5 h. The reaction was poured into water and pH adjusted to 9 by addition of ammonia solution (35%). Then the mixture was extracted by EtOAc. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to give 0.45 g of oil. The expected crude product (289 mg) as white gum was used directly in the next step.

Step 3: Example (TLR1) Preparation of 2-butyl-7-isopropoxy-1-(4-(piperazin-1-ylmethyl)benzyl)-1H-imidazo[4,5-d]pyridazin-4-amine



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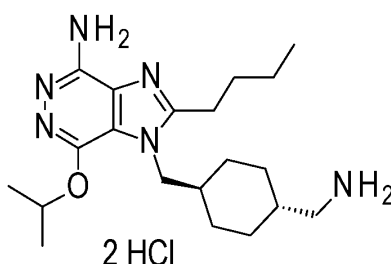
To a solution of *tert*-butyl 4-(4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)piperazine-1-carboxylate of

Step 2 (250 mg, 0.3 mmol) in DCM (6.3 mL) was added 2,2,2-trifluoroacetic acid (919.29 μ L, 11.93 mmol). The mixture was stirred at rt for 24 h. The reaction was poured into DCM and washed with sodium hydroxide 1M. The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated under vacuum to give 145 mg of crude product, which was purified by chromatography on a Merck cartridge (20 g of 15-40 μ m silica) with $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (90/10/0.2) elution, to afford **Example (TLR1)** (45 mg, 35% yield) as a white solid.

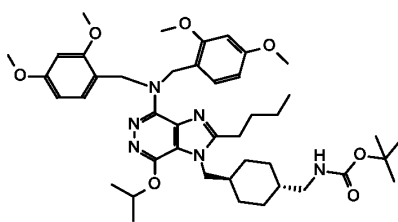
^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.81 (t, $J=7$ Hz, 3 H); 1.17 (d, $J=6$ Hz, 6 H); 1.29 (dq, $J=7$ & 15 Hz, 2 H); 1.59 (quin, $J=8$ Hz, 2 H); 2.22 (s broad, 4 H); 2.63 (t, $J=5$ Hz, 4 H); 2.81 (t, $J=7$ Hz, 2 H); 3.36 (s, 2 H); 5.30 (spt, $J=6$ Hz, 1 H); 5.55 (s, 2 H); 5.94 (s, 2 H); 7.00 (d, $J=8$ Hz, 2 H); 7.24 (d, $J=8$ Hz, 2 H)

MS method N: t_R (min): 0.80; $[\text{M}+\text{H}]^+$ 438

Example (TLR2): Preparation of 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine dihydrochloride



Step 1: *tert*-butyl (4-(*trans*-4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)cyclohexyl)methyl)carbamate



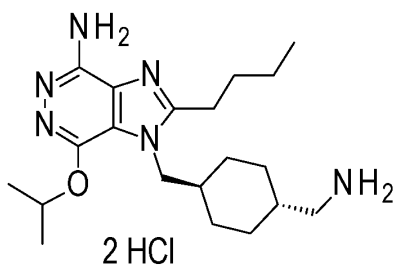
To a suspension of 2-butyl-N,N-bis(2,4-dimethoxybenzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, **intermediate (M)** (1 g, 1.82 mmol) in Me-THF (20 mL) was added Cs_2CO_3 (1.78 g, 5.46 mmol). The mixture was stirred at rt for 30 min. Then *tert*-butyl n-([(1R,4R)-4(bromomethyl)cyclohexyl]methyl)carbamate (879 mg, 2.73 mmol) in Me-THF

(12 mL) was added. The mixture was stirred for 5 min at rt then refluxing for 24 h. The reaction mixture was then diluted with EtOAc (150 mL), washed with water and brine, concentrated under reduced pressure to give 1.73 g of crude product, which was purified by chromatography on a Merck cartridge (70 g of 15-40 μ m silica) with a 0/100 to 50/50 EtOAc/heptane elution. Expected product (1.03 g, 73% yield) was obtained as a white solid.

^1H NMR (400 MHz, δ in ppm, DMSO- d_6): 0.75 (m, 2 H); 0.83 (t, $J=7$ Hz, 3 H); 1.08 (m, 2 H); 1.24 to 1.40 (m, 18 H); 1.48 (d, $J=12$ Hz, 2 H); 1.58 to 1.81 (m, 5 H); 2.73 (t, $J=6$ Hz, 2 H); 2.77 (t, $J=7$ Hz, 2 H); 3.66 to 3.76 (m, 12 H); 4.10 (d, $J=6$ Hz, 2 H); 5.00 (broad s, 4 H); 5.37 (spt, $J=6$ Hz, 1 H); 6.39 (dd, $J=2$ & 8 Hz, 2 H); 6.52 (d, $J=2$ Hz, 2 H); 6.78 (t, $J=6$ Hz, 1 H); 6.96 (d, $J=8$ Hz, 2 H)

MS method N: t_R (min): 1.87; $[\text{M}+\text{H}]^+$ 775

Step 2: Example (TLR2) Preparation of 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine dihydrochloride



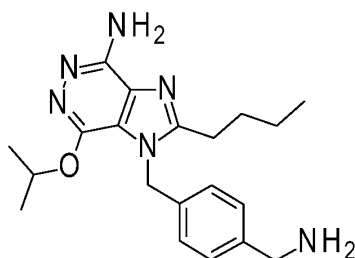
To a solution of *tert*-butyl 4-(*trans*-4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methylcyclohexyl)methylcarbamate of **Step 1** (1.03 g, 1.33 mmol) in DCM (6 mL) was added 2,2,2-trifluoroacetic acid (6 mL, 77.9 mmol) and 1,3-dimethoxybenzene (522 μ L, 3.99 mmol). The mixture was stirred for 24 h at rt. The reaction was diluted with DCM (50 mL) and H₂O (10 mL). The pH of the mixture was adjusted to pH 10-12 with 30% NaOH under stirring in an ice bath. The product was extracted with DCM (4 x 50 mL), the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure to give 3.1 g of crude product. The residue was purified by chromatography on a Macherey Nagel cartridge (40 g of 15-40 μ m diol silica) with 100//0 to 50//50 DCM-DCM/MeOH/H₂O(80/10/1) to give an oil. The oil was treated with iPr₂O (10 mL), a white

solid was filtered and washed with $i\text{Pr}_2\text{O}$ (3 x 5 mL) dried in vacuo to give 617 mg of powder. 500 mg of this product was purified on a C18 Waters Xbridge column (19 x 150 mm 5 μm), eluted with ammonium bicarbonate 10 mM (pH 10) / acetonitrile to afford 188 mg of material, which was dissolved in HCl in MeOH (1.25N) and the mixture was evaporated under reduced pressure. The residue was then dissolved in water, filtrated over a 0.22 μm membrane, the filtrate was freeze - dried to give 190 mg (39.5%, yield) of **Example (TLR2)**, under hydrochloric acid form (2 HCl).

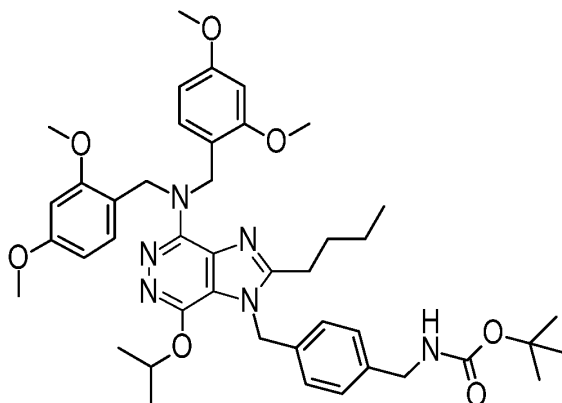
^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.87 (q, $J=13$ Hz, 2 H); 0.94 (t, $J=7$ Hz, 3 H); 1.12 (q, $J=13$ Hz, 2 H); 1.33 to 1.48 (m, 8 H); 1.51 to 1.63 (m, 3 H); 1.66 to 1.92 (m, 5 H); 2.63 (t, $J=6$ Hz, 2 H); 2.93 (t, $J=8$ Hz, 2 H); 4.21 (d, $J=7$ Hz, 2 H); 5.24 (quin, $J=6$ Hz, 1 H); 7.90 (s broad, 3 H); 8.57 (s broad, 2 H); 13.89 (s, 1 H)

MS method N: t_R (min): 0.83; $[\text{M}+\text{H}]^+$ 375

Example (TLR3): Preparation of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine



Step 1: *tert*-butyl (4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate

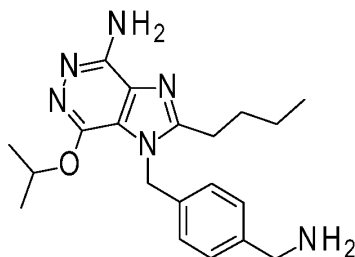


To a suspension of 2-butyl-N,N-bis(2,4-dimethoxybenzyl)-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, **intermediate (M)** (1 g, 1.82 mmol) in Me-THF (30 mL) was added Cs₂CO₃ (1.78 g, 5.46 mmol). The mixture was stirred 30 min at rt, *tert*-butyl 4-(bromomethyl)benzylcarbamate (819 mg, 2.73 mmol) was then added. The mixture was stirred at rt for 16 h. The reaction mixture was diluted with 150 mL of Me-THF and washed with water and brine, dried over anhydrous MgSO₄, filtered and the filtrate was concentrated under reduced pressure to give 1.73 g of crude product. The residue was purified by chromatography on a Merck cartridge (50 g of 15-40 µm silica) with 100/0 to 50/50 DCM-DCM/MeOH (90/10) elution. Expected product (1.34 g, 96 % yield) was obtained as a white foam.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.76 (t, J=7 Hz, 3 H); 1.21 (m, 8 H); 1.37 (s, 9 H); 1.55 (quin, J=7 Hz, 2 H); 2.74 (t, J=7 Hz, 2 H); 3.71 (s, 12 H); 4.07 (d, J=6 Hz, 2 H); 5.01 (s, 4 H); 5.31 (quin, J=6 Hz, 1 H); 5.55 (s, 2 H); 6.39 (dd, J=2 et 8 Hz, 2 H); 6.52 (d, J=2 Hz, 2 H); 6.97 (d, J=8 Hz, 2 H); 7.03 (d, J=8 Hz, 2 H); 7.19 (d, J=8 Hz, 2 H); 7.34 (t, J=6 Hz, 1 H)

MS method N: t_R (min): 1.87; [M+H]⁺ 769

Step 2: Example (TLR3) Preparation of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine



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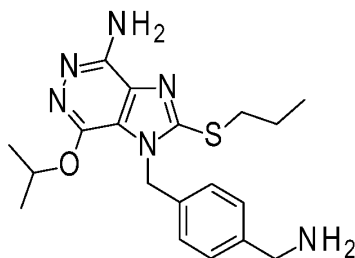
To a solution of *tert*-butyl 4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate of **Step 1** (684 mg, 0.889 mmol) in DCM (10 mL) cooled in an ice bath were added 1,3-dimethoxybenzene (349 µL, 2.67 mmol) and 2,2,2-trifluoroacetic acid (10 mL, 129.8 mmol). After 15 min, the ice bath was removed, and the mixture was stirred 16 h at rt. The reaction mixture was diluted with DCM (100 mL) and H₂O (50 mL), the aqueous phase was washed by DCM (2 x 50 mL). The pH of the aqueous layer was adjusted to pH 11 with 30% NaOH under stirring

25

in an ice bath. The product was extracted with DCM (4 x 50 mL), the combined organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure to give 306 mg of crude product, which was purified by chromatography on a Macherey Nagel cartridge (26 g of 15-40 µm diol silica) with 100/0 to 0/100 DCM-DCM/MeOH (90/10) elution, to give 91 mg of purified compound, which was further purified on a C18 Waters Xbridge column (19 x 150 mm 5 µm), eluted with ammonium bicarbonate 10 mM (pH10) / MeCN elution to afford 64 mg (20% yield) of **Example (TLR3)**.

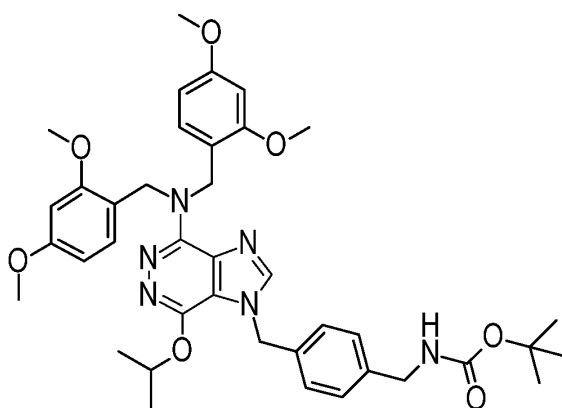
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7 Hz, 3 H); 1.21 (s, 3 H); 1.23 (s, 3 H); 1.33 (m, 2 H); 1.63 (m, 2 H); 1.97 (m, 2 H); 2.79 (d, J=15 Hz, 2 H); 3.66 (s, 2 H); 5.33 (spt, J=6 Hz, 1 H); 5.54 (s, 2 H); 5.91 (s, 2 H); 7.01 (d, J=8 Hz, 2 H); 7.28 (d, J=8 Hz, 2 H)
MS method N: t_R (min): 0.76; [M+H]⁺ 369

Example (TLR4): Preparation of 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-7-amine



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Step 1: *tert*-butyl N-[[4-[[7-bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]carbamate



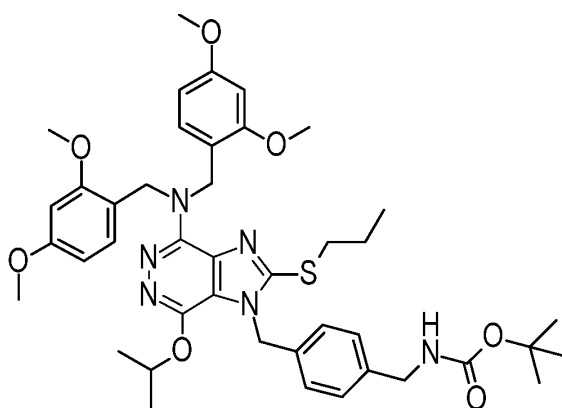
To a round-bottom flask were added N,N-bis[(2,4-dimethoxyphenyl)methyl]-4-isopropoxy-

3H-imidazo[4,5-d]pyridazin-7-amine, **Intermediate (R)** (450 mg, 0.91 mmol), MeTHF (6 mL), *tert*-butyl 4-(bromomethyl)benzylcarbamate (310 mg, 1.00 mmol) and Cs₂CO₃ (891 mg, 2.74 mmol). The resulting mixture was stirred at rt for 2 h. The reaction was then diluted with EtOAc and washed with brine. The organic layer was separated, dried over MgSO₄, filtered and evaporated to give a white foam. The crude material was purified by a silica gel column (80 g) using as solvent heptane/EtOAc (60/40) to give 0.52 g (80% yield) of a white solid.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.28 (d, J=6.1 Hz, 6 H); 1.37 (s, 9 H); 3.70 (m, 12 H); 4.05 (m, 2 H); 4.98 (s, 4 H); 5.34 (sept, J=6.2 Hz, 1 H); 5.54 (s, 2 H); 6.39 (dd, J=8.3, 2.4 Hz, 2 H); 6.52 (d, J=2.4 Hz, 2 H); 6.94 (d, J=8.3 Hz, 2 H); 7.21 (m, 4 H); 7.33 (m, 1 H); 8.46 (s, 1 H)

MS method N: t_R (min): 1.57, [M+H]⁺ 713

Step 2: *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]carbamate



To a Keller tricol equipped with magnetic stir, thermometer probe, under argon, were added *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]carbamate of **Step 1** (50 mg, 0.07 mmol), and anhydrous THF (1 mL). The homogenous solution was cooled to -30°C, to which was added *n*-BuLi (50 μL, 1.6 M in heptane, 0.077 mmol) and stirred at -30°C for 20 min, followed by addition of propyl disulfide (13 μL, 0.084 mmol). The reaction mixture was allowed to reach slowly rt and stirred for 2 h. It was then cooled again to -30°C, *n*-BuLi (100 μL, 1.6 M in heptane, 0.154 mmol) was added, the reaction mixture turned to red with some slight

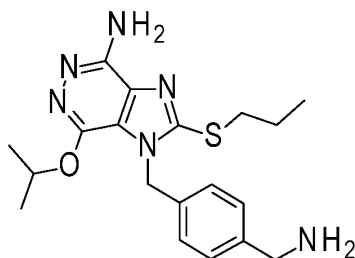
precipitation. It was stirred for 30 min and followed by addition of propyl disulfide (26 μ L, 0.168 mmol). The reaction mixture was allowed to reach slowly rt and stirred overnight. 20 mL of water and 30 mL of EtOAc were then added and the crude media was transferred to a separating funnel. After separation of organic phase, the aqueous phase was extracted with EtOAc (2 x 30 mL). The combined organic phase was washed with brine, dried over MgSO_4 , filtered and evaporated to dryness to give 55 mg of crude product. The crude material was purified by a silica gel column (5 g, Merck) using as heptane/EtOAc (70/30) elution to give 0.015 g (28% yield) of a white gum.

^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.76 (t, $J=7.3$ Hz, 3 H); 1.11 - 1.31 (m, 6 H); 1.37 (s, 9 H); 1.52 (sxt, $J=7.3$ Hz, 2 H); 3.06 (t, $J=7.2$ Hz, 2 H); 3.67 - 3.78 (m, 12 H); 4.07 (br d, $J=6.1$ Hz, 2 H); 4.97 (s, 4 H); 5.33 (m, 1 H); 5.41 (s, 2 H); 6.40 (dd, $J=8.5, 2.2$ Hz, 2 H); 6.53 (d, $J=2.2$ Hz, 2 H); 6.98 (d, $J=8.5$ Hz, 2 H); 7.12 (d, $J=8.3$ Hz, 2 H); 7.19 (d, $J=8.3$ Hz, 2 H); 7.33 (t, $J=6.2$ Hz, 1 H)

MS method N: t_R (min): 1.75, $[\text{M}+\text{H}]^+$ 787

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Step 3: Example (TLR4) Preparation of 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-7-amine

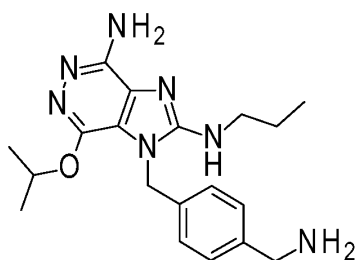


To a solution of *tert*-butyl N-[[4-[[7-bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl] carbamate of **Step 2** (147 mg, 0.187 mmol) in DCM (1.5 mL) was added dropwise TFA (0.50 mL, 5.55 mmol). The mixture became pink and was stirred at rt for 5.5 h, then evaporated to dryness. The crude product was dissolved in MeOH with some purple precipitates which were filtered. The filtrate was loaded into a SCX cartridge (20 g, pre-washed with 10 mL of MeOH), the cartridge was eluted with MeOH (60 mL) and 2N MeOH-NH₃. The ammoniacal methanolic solution was evaporated to dryness to give 56 mg of foam which was purified by a silica gel column (Merck, 10 g) using as eluent a mixture

DCM/NH₃-MeOH 7N (97/3) to give 22 mg (31% yield) of **Example (TLR4)** as a white solid.

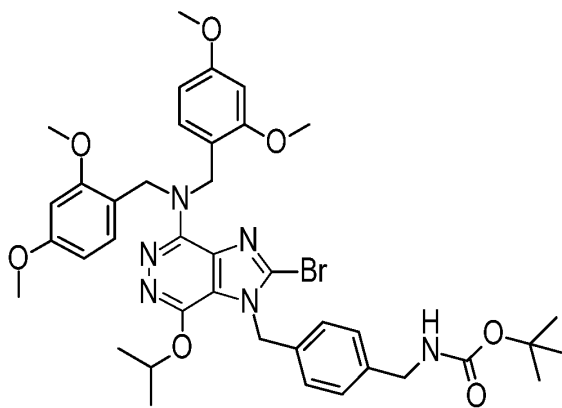
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.96 (t, J=7 Hz, 3 H); 1.25 (d, J=6 Hz, 6 H); 1.72 (sxt, J=7 Hz, 2 H); 2.06 - 2.30 (m, 2 H); 3.30 - 3.33 (m, 2 H); 3.67 (s, 2 H); 5.34 (quin, J=6 Hz, 1 H); 5.43 (s, 2 H); 5.91 (s, 2 H); 7.11 (d, J=1 Hz, 2 H); 7.29 (d, J=1 Hz, 2 H)
MS method N: t_R (min): 0.90, [M+H]⁺ 387

Example (TLR5): Preparation of 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-N2-propyl-imidazo[4,5-d]pyridazine-2,7-diamine



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Step 1: *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-bromo-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]carbamate



To a -30°C solution of *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]carbamate of **Step 2** in **Example (TLR4)** (103 mg, 0.144 mmol) in dry THF (2 mL), under argon, was added a 2.5 M solution of *n*-BuLi in hexane (0.35 mL, 0.86 mmol). The mixture was kept 30 min at the same temperature then a solution of NBS (67.42 mg, 0.36 mmol) in dry THF (2 mL) was added. After the addition was completed, the mixture was left at rt. The reaction color turned

15

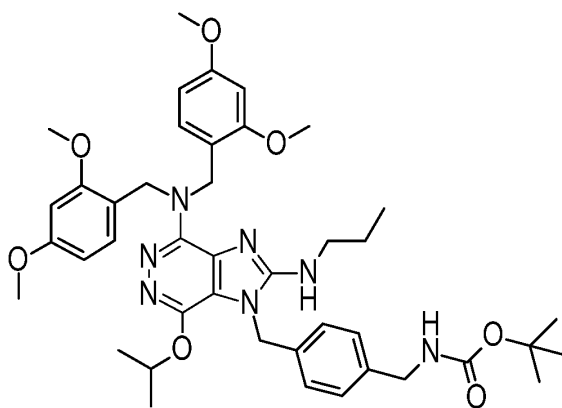
to yellow/orange. The reaction was stopped after 2 h at rt. The reaction mixture was cooled at -10°C, added saturated solution of NH₄Cl, and diluted with EtOAc. The organic solution was separated, washed with brine, dried over MgSO₄, filtered and evaporated to give a yellow oil. The crude material was purified by a silica gel column using as eluent a mixture

5 CHCl₃/acetone (96/4) to give 24.6 mg (21% yield) of the expected product, as a yellow solid.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.24 (d, J=6.0 Hz, 6 H); 1.37 (s, 9 H); 3.67 - 3.74 (m, 12 H); 4.08 (d, J=6.3 Hz, 2 H); 4.94 (s, 4 H); 5.33 (sept, J=6.1 Hz, 1 H); 5.56 (s, 2 H); 6.41 (dd, J=8.4, 2.4 Hz, 2 H); 6.53 (d, J=2.4 Hz, 2 H); 6.97 (d, J=8.4 Hz, 2 H); 7.12 (m, J=8.3 Hz, 2 H); 7.21 (m, J=8.3 Hz, 2 H); 7.31 (t, J=5.8 Hz, 1 H)

10 MS method N: t_R (min): 1.67, [M+H]⁺ 791

Step 2: *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-2-(propylamino)imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]carbamate



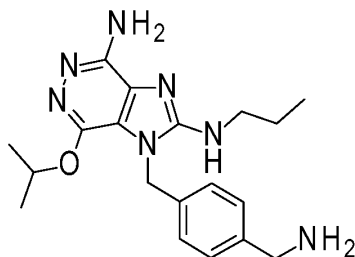
15 A mixture of *tert*-butyl N-[[4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-bromo-4-isopropoxy imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]carbamate of **Step 1** (85.2 mg, 0.108 mmol), *n*-propylamine (1.44 mL) and DMF (0.02 mL) was heated at 100°C in the microwave for 2.5 h. The reaction mixture was diluted with EtOAc and washed with H₂O. The organic layer was separated, dried over MgSO₄, filtered and evaporated to give a white transparent oil. The crude material was purified by a silica gel column using as eluent a mixture DCM/*i*PrOH (98/2 to 96/4) to give 67 mg of the expected product.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.77 (t, J=7.3 Hz, 3 H); 1.19 (d, J=6.1 Hz, 6 H); 1.36 (s, 9 H); 1.41 - 1.55 (m, 2 H); 3.16 - 3.24 (m, 2 H); 3.71 (s, 12 H); 4.06 (d, J=5.9 Hz, 2 H); 4.94 (s, 4 H); 5.25 (m, 1 H); 5.33 (s, 2 H); 6.39 (dd, J=8.3, 2.3 Hz, 2 H); 6.51 (d,

J=2.3 Hz, 2 H); 6.95 - 7.04 (m, 3 H); 7.06 - 7.12 (m, 2 H); 7.13 - 7.20 (m, 2 H); 7.28 (t, J=5.8 Hz, 1 H)

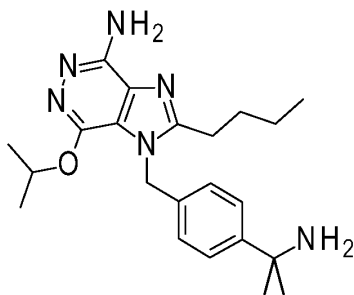
MS method N: t_R (min): 1.70, $[M+H]^+$ 772

- 5 **Step 3: Example (TLR5)** Preparation of 3-[[4-(aminomethyl)phenyl]methyl]-4-isopropoxy-N2-propyl-imidazo[4,5-d]pyridazine-2,7-diamine

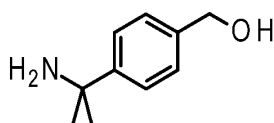


- To a solution of *tert*-butyl N-[[4-[[7-bis[(2,4-dimethoxyphenyl)methyl]amino]-4-isopropoxy-2-(propylamino)imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl] carbamate of **Step 2** (63 mg, 0.0818 mmol) in DCM (0.5 mL) was added TFA (0.19 mL, 2.11 mmol). The mixture was stirred at rt for 17 h. Then it was diluted with EtOAc and evaporated (twice). The resulting crude material was diluted with MeOH and it was loaded into a SCX column (10 g), firstly it was eluted with MeOH then with a 50/50 MeOH/2N NH₃ in MeOH and finally with pure 2N NH₃ in MeOH. The fraction containing the product was evaporated to give 33 mg of an oil, which was crystalized by adding Et₂O. The solid was filtered and dried to give 24.1 mg (80% yield) of **Example (TLR5)** as a white solid.
- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.87 (t, J=7 Hz, 3 H); 1.21 (d, J=6 Hz, 6 H); 1.60 (sxt, J=7 Hz, 2 H); 3.31 - 3.81 (m, 6 H); 5.27 (dt, J=12, 6 Hz, 1 H); 5.34 (s, 2 H); 5.39 (br s, 2 H); 7.00 (br t, J=5 Hz, 1 H); 7.10 (d, J=8 Hz, 2 H); 7.27 (d, J=8 Hz, 2 H)
- 20 MS method N: t_R (min) : 0.74 $[M+H]^+$ 370

Example (TLR6): Preparation of 3-[[4-(1-amino-1-methyl-ethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine



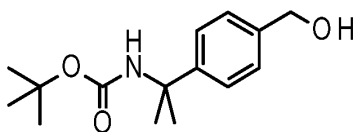
Step 1: 4-(1-amino-1-methyl-ethyl)phenyl]methanol



To a solution of lithium triethylborohydride 1N in THF (6.5 mL) was slowly added a solution of methyl 4-(2-aminopropan-2-yl)benzoate hydrochloride (200 mg, 0.870 mmol) in dry THF (10 mL). The reaction mixture was stirred at rt for 5 h then cooled with an ice bath. HCl 1 N was added to reach pH 1, the solution was washed with Et₂O (20 mL); a saturated aqueous solution of NaHCO₃ was added to reach pH 10 before extraction with DCM (4 x 30 mL). The combined organic solutions were then dried over MgSO₄, filtered and concentrated to dryness. The crude material was purified by silica gel column chromatography using as eluent a mixture of CH₂Cl₂/MeOH/NH₄OH (12:1.5:0.25) to give 72 mg (50% yield) of the expected product, as a white solid.

MS method N2: t_R (min): 0.27; [M+H]⁺ 166.0

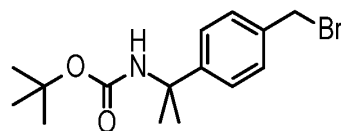
Step 2: *tert*-butyl N-[1-[4-(hydroxymethyl)phenyl]-1-methyl-ethyl]carbamate



To a solution of 4-(1-amino-1-methyl-ethyl)phenyl]methanol (70 mg, 0.423 mmol) from **Step 1** in THF (5 mL) was added DIPEA (89 μ L, 0.511 mmol) and di-*tert*-butyl dicarbonate (110 μ L, 0.480 mmol). The reaction mixture was stirred for 3.5 h at rt then concentrated to dryness. The crude material was purified by silica gel column chromatography using as eluent CH₂Cl₂/EtOAc (100:0 to 90:10) to give 90 mg (80% yield) of the expected product, as a white solid.

MS method N1: t_R (min): 1.44; $[M+H]^+$ 266.2

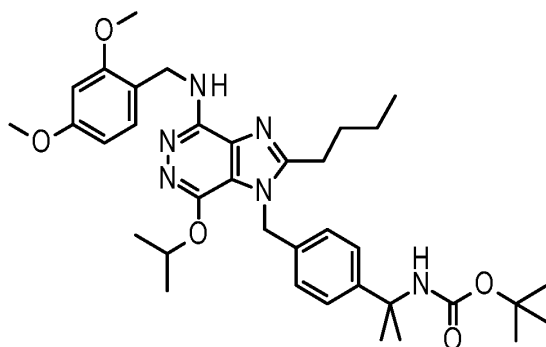
Step 3: *tert*-butyl N-[1-[4-(bromomethyl)phenyl]-1-methyl-ethyl]carbamate



- 5 To a solution of *tert*-butyl N-[1-[4-(hydroxymethyl)phenyl]-1-methyl-ethyl]carbamate (90 mg, 0.339 mmol) from **Step 2** in DCM (3 mL) cooled at -10°C were successively added DIPEA (177 μL , 1.02 mmol) and methanesulfonyl bromide (56 μL , 0.687 mmol). The reaction mixture was stirred for 4 h at -10°C then overnight at rt. DCM (10 mL) was added then the solution was successively washed with a saturated aqueous solution of NaHCO_3
- 10 (15 mL) and water (15 mL), dried over MgSO_4 , filtered and concentrated to dryness. The crude material was purified by silica gel column chromatography using as eluent CH_2Cl_2 to give 55 mg (49% yield) of the expected product, as a white solid.

MS method N1: t_R (min): 1.87; $[M+H]^+$ 328.0

- 15 **Step 4:** *tert*-butyl N-[1-[4-[[2-butyl-7-[(2,4-dimethoxyphenyl)methylamino]-4-isopropoxyimidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]-1-methyl-ethyl]carbamate



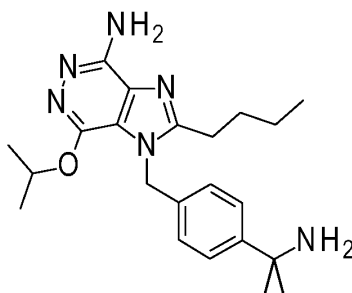
- To a solution of 2-butyl-N-[(2,4-dimethoxyphenyl)methyl]-4-isopropoxy-3H-imidazo[4,5-d]pyridazin-7-amine, **intermediate (S)** (164 mg, 0.410 mmol) in DMF (4 mL) were added
- 20 potassium carbonate (190 mg, 1.374 mmol) and a solution of *tert*-butyl N-[1-[4-(bromomethyl)phenyl]-1-methyl-ethyl]carbamate (180 mg, 0.548 mmol) from **Step 3** in DMF (4 mL). The mixture was reacted at 60°C for 20 h then cooled to rt, DCM (60 mL) was added, and the solution was washed with water (80 mL), dried over MgSO_4 , filtered and

concentrated to dryness. The crude material was purified by silica gel column chromatography using as eluent CH₂Cl₂/EtOAc (100:0 to 60:40) to give 73 mg (27% yield) of the expected product, as a colorless glassy solid.

MS method N1: *t_R* (min): 1.59; [M+H]⁺ 647.3

5

Step 5: Example (TLR6) Preparation of 3-[[4-(1-amino-1-methyl-ethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine

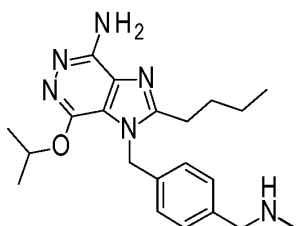


To a solution of *tert*-butyl N-[1-[4-[[2-butyl-7-[(2,4-dimethoxyphenyl)methylamino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]-1-methyl-ethyl]carbamate (70 mg, 0.108 mmol) from **Step 4** in DCM (5 mL) was added TFA (420 µL, 5.45 mmol). The reaction mixture was stirred for 20 h at rt then diluted with DCM (10 mL) and successively washed with a saturated aqueous solution of NaHCO₃ (15 mL) and water (15 mL), dried over MgSO₄, filtered and concentrated to dryness. The crude material was purified by silica gel column chromatography using as eluent a mixture of CH₂Cl₂/MeOH/H₂O (10:1.5:0.1) to give 22 mg (51% yield) of **Example (TLR6)**, as a colorless glassy solid.

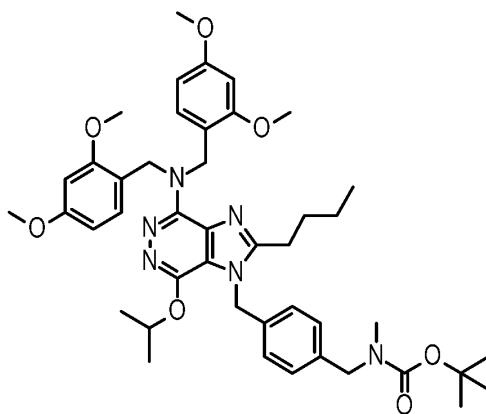
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.83 (t, *J*=7.3 Hz, 3 H); 1.21 (d, *J*=6.1 Hz, 6 H); 1.25 - 1.37 (m, 2 H); 1.31 (s, 6 H); 1.55 - 1.67 (m, 2 H); 1.68 - 2.00 (m, 2 H); 2.77 - 2.85 (m, 2 H); 5.27 - 5.38 (m, 1 H); 5.53 (s, 2 H); 5.90 (s, 2 H); 7.00 (d, *J*=8.3 Hz, 2 H); 7.47 (d, *J*=8.3 Hz, 2 H)

MS method N1: *t_R* (min): 0.82; [M+H]⁺ 397.3

Example (TLR7): Preparation of 2-butyl-4-isopropoxy-3-[[4-(methylaminomethyl)phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine



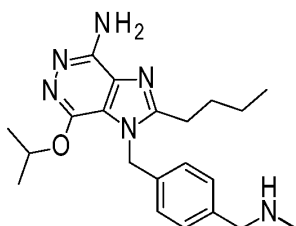
Step 1: *tert*-butyl N-[[4-[[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]-N-methyl-carbamate



- 5 To a solution of *tert*-butyl (4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)carbamate of **Step 1** in **Example (TLR3)** (210 mg, 0.273 mmol) in dry DMF (3 mL), were added sodium hydride (16 mg, 0.400 mmol) and methyl iodide (25 μ L, 0.399 mmol) a few minutes later. The reaction mixture was stirred for 45 min at rt then poured into 75 mL of ice-cold water. The precipitated solid was collected
- 10 by filtration, washed with water and dried at 30°C under vacuum to give 189 mg (88%) of the expected product as a white solid.

MS method M: t_R (min): 3.3; $[M+H]^+$ 783.6

- Step 2: Example (TLR7)** Preparation of 2-butyl-4-isopropoxy-3-[[4-(methylaminomethyl)phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine
- 15



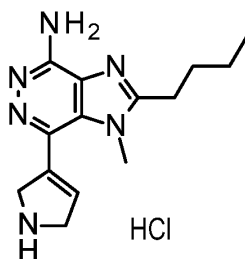
To a solution of *tert*-butyl N-[[4-[[[7-bis[(2,4-dimethoxyphenyl)methyl]amino]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]-N-methyl-carbamate (184 mg, 0.240 mmol) from **Step 1** in DCM (1 mL) was added TFA (1 mL, 12.93 mmol).

- 5 The reaction mixture was stirred overnight at rt then diluted with DCM (100 mL) and extracted with water (3 x 50 mL). NaOH 5N was added to the aqueous phase to reach pH 12 then the solution was extracted with EtOAc (3 x 50 mL) and the combined organic phases were washed with brine, dried over MgSO₄, filtered and concentrated to dryness. The crude product was dissolved in MeOH and loaded into a SCX cartridge (10 g, pre-washed with
- 10 10 mL of MeOH), the cartridge was eluted with MeOH and 2N MeOH-NH₃. The ammoniacal methanolic solution was evaporated to dryness to give a gum that crystallized in iPr₂O. 37 mg (42% yield) of **Example (TLR7)** were finally obtained after filtration and drying overnight at 27°C under vacuum.

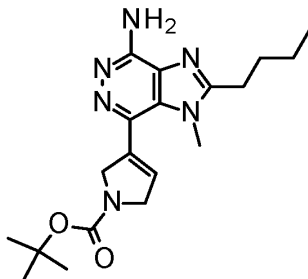
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.83 (t, J=7.3 Hz, 3 H); 1.19 (d, J=6.1 Hz, 6 H);
 15 1.25 - 1.38 (m, 2 H); 1.56 - 1.69 (m, 2 H); 2.23 (s, 3 H); 2.77 - 2.86 (m, 2 H); 3.63 (s, 2 H);
 5.24 - 5.38 (m, 1 H); 5.56 (s, 2 H); 5.92 (br s, 2 H); 7.02 (d, J=8.1 Hz, 2 H); 7.28 (d, J=8.1 Hz,
 2 H)

MS method M: t_R (min): 0.62 & 1.39; [M+H]⁺ 383.3

- 20 **Example (TLR8):** Preparation of 2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)-1-methyl-imidazo[4,5-d]pyridazin-4-amine; hydrochloride

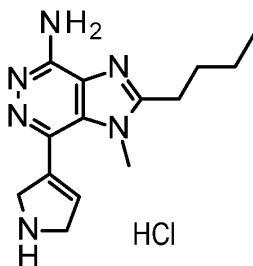


Step 1: *tert*-butyl 3-(4-amino-2-butyl-1-methyl-imidazo[4,5-d]pyridazin-7-yl)-2,5-dihydropyrrole-1-carboxylate



Under argon, 1,1'-bis(diphenylphosphino)ferrocene-palladium(ii)dichloride
 5 dichloromethane complex (170 mg, 0.208 mmol), Cs₂CO₃ (1.36 g, 4.17 mmol) and *tert*-butyl
 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,5-dihydro-1H-pyrrole-1-carboxylate
 (678 mg, 2.29 mmol) were added to a suspension of 2-butyl-4-chloro-3-methyl-imidazo[4,5-
 d]pyridazin-7-amine, **intermediate (T)** (500 mg, 2.08 mmol) in degassed 1,4-dioxane
 (7.5 mL) and water (2.5 mL). The mixture was reacted at 160°C under microwaves for
 10 30 min. The reaction mixture was then diluted with DCM (50 mL), washed with water and
 brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting
 solid was washed with iPr₂O (30 mL) then purified by silica gel column chromatography
 using as eluent a mixture of CH₂Cl₂/MeOH-NH₃ 7N (95:5) to give 243 mg (31% yield) of
 the expected product, as a light brown solid.
 15 MS method N1: t_R (min): 1.11; [M+H]⁺ 373.2

Step 2: Example (TLR8) Preparation of 2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)-1-methyl-imidazo[4,5-d]pyridazin-4-amine;hydrochloride



20 To a suspension of *tert*-butyl 3-(4-amino-2-butyl-1-methyl-imidazo[4,5-d]pyridazin-7-yl)-
 2,5-dihydropyrrole-1-carboxylate (243 mg, 0.652 mmol) from **Step 1** in 1,4-dioxane (5 mL)

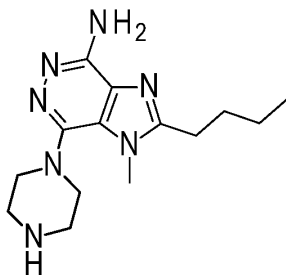
was added dropwise a solution of HCl 4M in dioxane (1.7 mL). The mixture was reacted overnight at rt then the solid product was collected by filtration, successively washed with dioxane (2 x 5 mL) and iPr₂O (5 mL) then dried at 30°C under vacuum to give 162 mg (80% yield) of **Example (TLR8)** as a light beige solid.

- 5 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.95 (t, J=7.5 Hz, 3 H); 1.45 (sxt, J=7.5 Hz, 2 H); 1.79 (quin, J=7.5 Hz, 2 H); 3.01 (t, J=7.5 Hz, 2 H); 3.85 (s, 3 H); 4.22 - 4.33 (m, 2 H); 4.32 - 4.47 (m, 2 H); 6.49 (br s, 1 H); 8.65 - 9.20 (m, 2 H); 9.97 (br s, 2 H); 14.59 - 15.10 (m, 1 H)

MS method N1: t_R (min): 0.15; [M+H]⁺ 273.1

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Example (TLR9): Preparation of 2-butyl-3-methyl-4-piperazin-1-yl-imidazo[4,5-d]pyridazin-7-amine



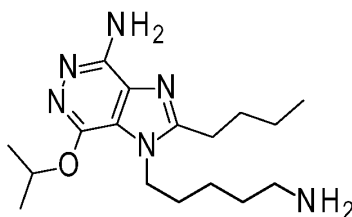
- To a suspension of 2-butyl-4-chloro-3-methyl-imidazo[4,5-d]pyridazin-7-amine, 15 **intermediate (T)** (500 mg, 2.08 mmol) in 1-butanol (10 mL) were added piperazine (898 mg, 10.4 mmol) and 3-chloropyridine hydrochloride (1.03 g, 6.88 mmol). The mixture was reacted at 180°C under microwaves for 7 h. The reaction mixture was then diluted with DCM (100 mL) and the solids were filtered off and rinsed with DCM. The filtrate was then concentrated under reduced pressure. The resulting solid was washed with EtOAc then 20 purified by silica gel column chromatography using as eluent a mixture of CH₂Cl₂/MeOH-NH₃ 7N (90:10). The compound containing fractions were combined and concentrated then further purified by preparative HPLC using a Phenomenex Lux Cellulose-4 column (250 x 30 mm, 5μm), with EtOH/MeOH/TEA (1:1:0.1) as mobile phase, under UV (265 nm) detection. The compound containing fractions were combined and concentrated under 25 vacuum to give **Example (TLR9)** (94 mg, 16% yield), as a white solid.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.94 (t, *J*=7.3 Hz, 3 H); 1.35 - 1.48 (m, 2 H); 1.70 - 1.81 (m, 2 H); 2.10 - 2.31 (m, 1 H); 2.80 - 2.93 (m, 6 H); 2.96 - 3.04 (m, 4 H); 3.96 (s, 3 H); 5.94 (s, 2 H)

MS method N1: *t*_R (min): 0.17; [M+H]⁺ 290.2

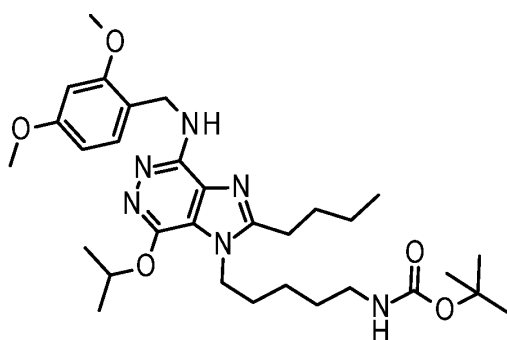
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Example (TLR10): Preparation of 3-(5-aminopentyl)-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine



Step 1: *tert*-butyl N-[5-[2-butyl-7-[(2,4-dimethoxyphenyl)methylamino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]pentyl]carbamate

10



To a solution of 2-butyl-N-[(2,4-dimethoxyphenyl)methyl]-4-isopropoxy-3H-imidazo[4,5-d]pyridazin-7-amine, **intermediate (O)** (2 g, 5.01 mmol) in 2-methyltetrahydrofuran (60 mL) were added *tert*-butyl N-(5-bromopentyl)carbamate (1.73 g, 6.51 mmol) and cesium carbonate (4 g, 12.52 mmol). The reaction mixture was stirred overnight at rt then 6 h at 60°C. Additional *tert*-butyl N-(5-bromopentyl)carbamate (132 mg, 0.508 mmol) was added and the reaction was pursued for 24 h to go to completion. Water was then added, and the reaction mixture was extracted twice with EtOAc. The combined organic solutions were washed with water and brine, dried over MgSO₄, filtered and concentrated to dryness. The crude product was then purified by silica gel column chromatography using as eluent a

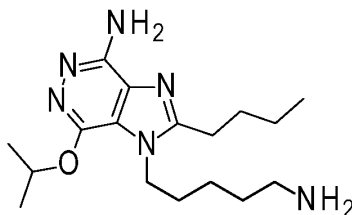
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mixture of heptane/(EtOAc/EtOH 3:1) (100:0 to 30:70) to give 1.57 g (53% yield) of the expected product, as a light yellow gum.

MS method N4: t_R (min): 0.90; $[M+H]^+$ 585

- 5 **Step 2: Example (TLR10)** Preparation of 3-(5-aminopentyl)-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine

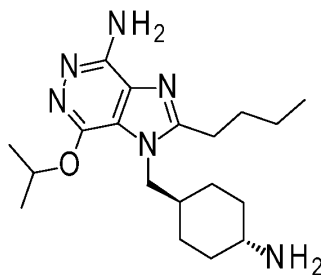


- To a solution of *tert*-butyl N-[5-[2-butyl-7-[(2,4-dimethoxyphenyl)methylamino]-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]pentyl]carbamate from **Step 1** (1.570 g, 2.685 mmol) in dichloromethane (10 mL), was added TFA (13 mL). The solution was stirred overnight at rt then concentrated to dryness. The crude product was dissolved in MeOH with some purple precipitates which were filtered off. The filtrate was loaded into a SCX cartridge (25 g, pre-washed with MeOH), the cartridge was eluted with MeOH (4 x 25 mL) and 7N MeOH-NH₃. The ammoniacal methanolic solution was evaporated to dryness to give 950 mg (100% yield) of **Example (TLR10)** as a light brown oil that crystallize on standing.

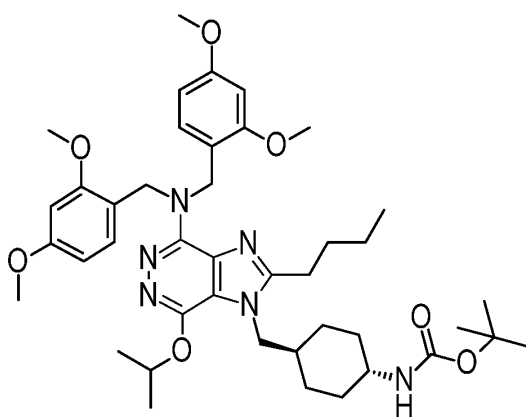
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.93 (t, J = 7.4 Hz, 3 H); 1.29 - 1.46 (m, 12 H); 1.65 - 1.80 (m, 4 H); 2.57 (t, J = 6.9 Hz, 2 H); 2.85 (m, 2 H); 3.47 (m broad, 2 H); 4.23 (m, 2 H); 5.39 (sept, J = 6.2 Hz, 1 H); 5.85 (s, 2 H)

- 20 MS method N: t_R (min): 0.86; $[M+H]^+$ 335

Example (TLR11): Preparation of *Trans* 1-(4-aminocyclohexyl)methyl-2-butyl-7-isopropoxy-1H-imidazo [4,5-d]pyridazin-4-amine



Step 1: *tert*-butyl 4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)cyclohexyl)carbamate

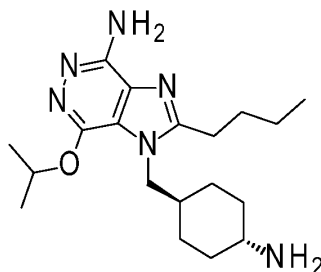


- 5 To a suspension of **intermediate (M)** (400 mg, 0.73 mmol) in Me-THF (15 mL), was added Cs_2CO_3 (720 mg, 2.19 mmol). The mixture was stirred for 30 min at rt. Then, *tert*-butyl 4-(bromomethyl)cyclohexyl)carbamate (320 mg, 1.10 mmol) was added. The mixture was stirred at rt for 24 h, then heated at 90°C under microwave for 18 h. The reaction mixture was then diluted with Me-THF (250 mL), washed with water and brine, dried over MgSO_4 , filtered and concentrated under reduced pressure to give 692 mg of crude product, which was purified by chromatography on a Merck cartridge (40 g of 15-40 μm silica) with a 10/90 to 100/00 EtOAc/heptane elution. Expected product (357 mg, 64% yield) was obtained as a white solid.

^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.83 (t, $J=7.5$ Hz, 3 H); 1.04 (m, 2 H); 1.14 (m, 2 H); 1.09 (m, 2 H); 1.36 (s, 9 H); 1.37 (d, $J=6.2$ Hz, 6 H); 1.47 (m, 2 H); 1.60 to 1.79 (m, 5 H); 2.77 (t, $J=7.4$ Hz, 2 H); 3.17 (m, 1 H); 3.70 (s, 6 H); 3.71 (s, 6 H); 4.10 (d, $J=7.2$ Hz, 2 H); 5.00 (s, 4 H); 5.38 (sept, $J=6.2$ Hz, 1 H); 6.39 (dd, $J=2.5$ & 8.4 Hz, 2 H); 6.52 (d, $J=2.5$ Hz, 2 H); 6.68 (d, $J=7.9$ Hz, 1 H); 6.96 (d, $J=8.4$ Hz, 2 H)

MS method N: t_R (min): 1.79; $[\text{M}+\text{H}]^+$ 761

Step 2: Example (TLR11) Preparation of *Trans* 1-(4-aminocyclohexyl)methyl)-2-butyl-7-isopropoxy-1H-imidazo [4,5-d]pyridazin-4-amine



- 5 To a solution of *tert*-butyl 4-((4-(bis(2,4-dimethoxybenzyl)amino)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)cyclohexyl)carbamate of **Step 1** (345 mg, 0.45 mmol) in DCM (2 mL) was added TFA (2 mL, 26.66 mmol). The mixture was stirred 24 h at rt. The reaction mixture was diluted with DCM (100 mL) and extracted with H₂O (2 x 80 mL). The aqueous phase was neutralized with NaOH (5M) up to pH 10,
- 10 extracted with EtOAc (3 x 75 mL), the organic phase was washed with water and brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. **Example (TLR11)** (142 mg, 87% yield) was obtained as a white solid.

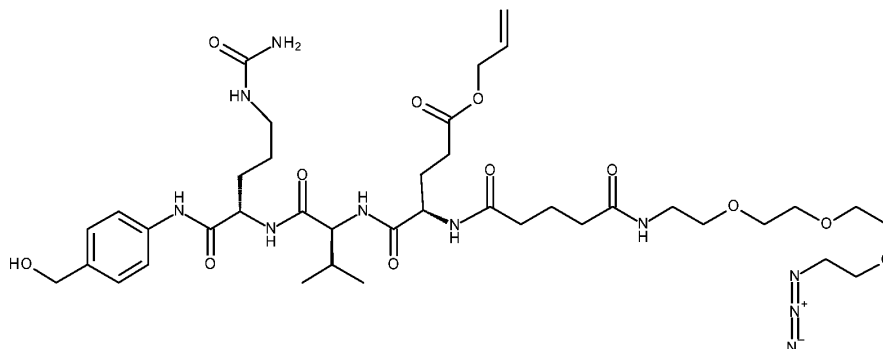
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.92 (t, J=7.3 Hz, 3 H); 0.94 (m, 2 H); 1.12 (m, 2 H); 1.34 to 1.48 (m, 5 H); 1.36 (d, J=6.2 Hz, 6 H); 1.65 to 1.81 (m, 5 H); 2.82 (m, 2 H);

15 2.95 (m broad, 2 H); 4.10 (d, J=7.5 Hz, 2 H); 5.39 (sept, J=6.2 Hz, 1 H); 5.85 (s, 2 H)

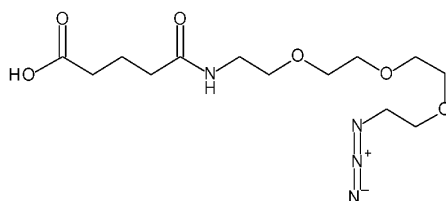
MS method N: t_R (min): 0.78; [M+H]⁺ 361; ES+ [M+2H]²⁺ 181

B) Preparation of linkers

- 20 **Linker (L1):** Preparation of allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbonyl]-4-ureido-butyl]carbonyl]-2-methyl-propyl]amino]-5-oxo-pentanoate



Step 1: 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid



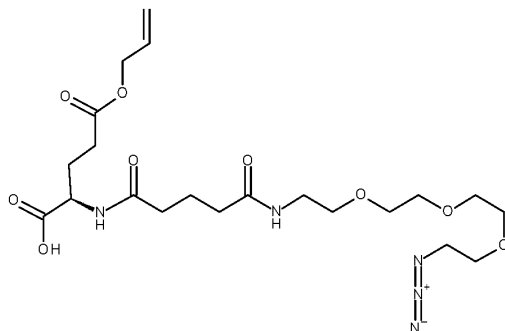
Under argon, to a round-bottom flask containing 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (200 mg, 0.916 mmol) in anhydrous THF (10 mL) were added TEA (127 μ L, 0.916 mmol) and glutaric anhydride (104.5 mg, 0.916 mmol), the reaction mixture was stirred at rt for 20 h. It was then concentrated under vacuum to give a colorless oil. The crude material was purified by chromatography on silica gel (Merck, 10 g, 15-40 μ m), with eluent DCM//DCM/MeOH/H₂O (8/2/0.1), from 1//0 to 0//1 to afford 223 mg (73% yield) of the expected compound as a pale-yellow oil.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.69 (quin, J=7 Hz, 2 H); 2.09 (t, J=7 Hz, 2 H); 2.19 (t, J=7 Hz, 2 H); 3.18 (q, J=6 Hz, 2 H); 3.36 - 3.42 (m partially hidden, 4 H); 3.46 - 3.57 (m, 8 H); 3.58 - 3.63 (m, 2 H); 7.87 (br t, J=5 Hz, 1 H)

MS method N: t_R (min): 0.99; [M+H]⁺ 333

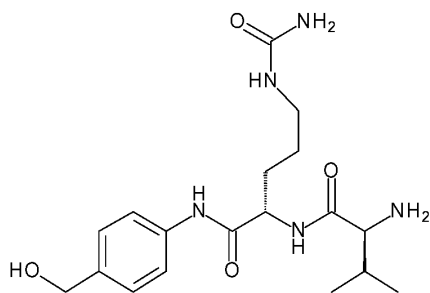
15

Step 2: (2S)-5-allyloxy-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoic acid



Under argon, to a round-bottom flask containing 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid of **Step 1** (139 mg, 0.418 mmol) and anhydrous DMF (6 mL), were added N,N'-Disuccinimidyl carbonate (117 mg, 0.460 mmol) and DIPEA (72 μ L, 0.418 mmol), the reaction mixture was stirred at rt for 3 h. It was then added L-Glutamic acid 5-allyl ester (86 mg, 0.460 mmol) and DIPEA (219 μ L, 1.25 mmol). The reaction mixture was stirred at rt for 20 h and concentrated under vacuum. The crude material was purified by chromatography on silica gel (Merck, 15 g, 15-40 μ m), with eluent DCM//DCM/MeOH/H₂O (8/2/0.1), from 1//0 to 0//1 to afford 180 mg (86% yield) of the expected compound as a colorless oil.

Step 3: (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureidopentanamide



Under argon, to a three neck round-bottom flask containing (S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanoic acid (400 mg, 0.805 mmol), anhydrous DCM (8 mL) and MeOH (4 mL), were added p-amino-benzyl alcohol (204.4 mg, 1.66 mmol) and N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (396.4 mg, 1.60 mmol). The reaction mixture was protected from light and stirred overnight at rt. It was then concentrated under vacuum to give a white pasty residue which

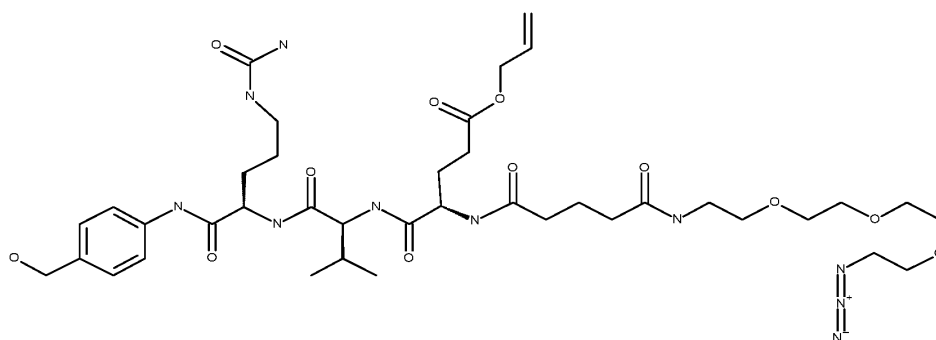
was triturated in diisopropyl ether (3 mL) and filtrated. The white cake was washed with diisopropyl ether and dried under vacuum to give a yellow slide (495 mg). It was then dissolved, in a round bottom flask, in anhydrous DMF (8 mL), followed by addition of piperidine (398.8 μ L, 4.03 mmol) and triethyl silane (195 μ L, 1.21 mmol), the reaction mixture stirred at rt for 30 min and evaporated to dryness. The crude material was purified by chromatography on silica gel (Merck, 5 g, 15-40 μ m), with eluent EtOAc//EtOAc/MeOH/H₂O (7/2/1), from 1//0 to 0//1 to afford 163 mg (53% yield) of the expected compound as a colorless oil.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.78 (d, J=7 Hz, 3 H); 0.88 (d, J=7 Hz, 3 H); 1.28 - 1.48 (m, 2 H); 1.51 - 1.63 (m, 1 H); 1.64 - 1.73 (m, 1 H); 1.84 - 2.00 (m, 1 H); 2.85 - 3.03 (m, 2 H); 3.01 (d, J=5 Hz, 1 H); 4.41 (s, 2 H); 4.42 - 4.52 (m, 1 H); 4.98 - 5.27 (m, 1 H); 5.44 (s, 2 H); 6.06 (br t, J=6 Hz, 1 H); 7.24 (d, J=8 Hz, 2 H); 7.54 (d, J=8 Hz, 2 H); 8.16 (br d, J=8 Hz, 1 H); 10.10 (s, 1 H)

MS method N: t_R (min): 1.34; [M+H]⁺ 380.2

15

Step 4: Preparation of (L1) allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate



20

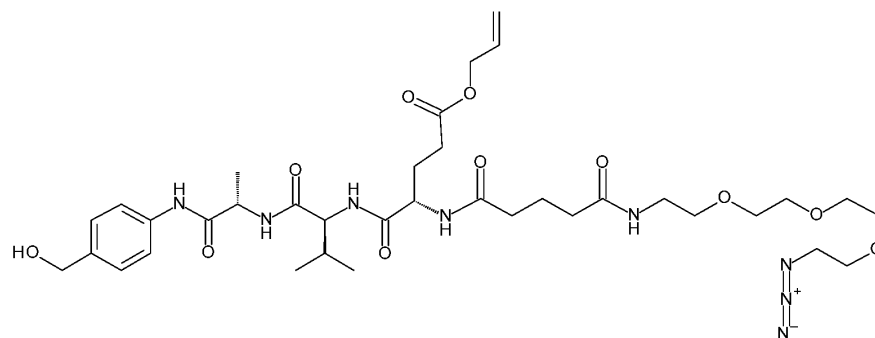
Under argon, to a round-bottom flask containing (2S)-5-allyloxy-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoic acid of **Step 2** (255 mg, 0.509 mmol), (S)-2-((S)-2-amino-3-methylbutanamido)-N-(4-(hydroxymethyl)phenyl)-5-ureido-pentanamide of **Step 3** (185 mg, 0.463 mmol) and anhydrous DMF (10 mL), were added HATU (193 mg, 0.509 mmol), HOBt (62 mg,

25

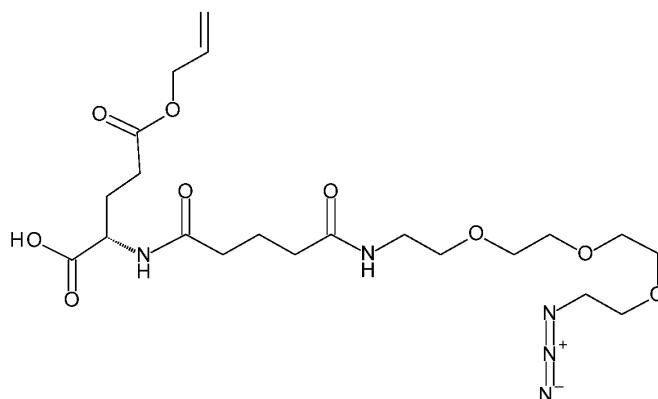
0.463 mmol) and drop wisely DIPEA (80 μ L, 0.463 mmol). The reaction mixture was protected from light and stirred at rt for 20 h. The resulting solution was poured into 300 mL of Et₂O and the solid that precipitate was collected by filtration then purified by chromatography on silica gel (Merck, 20 g, 15-40 μ m), with eluent DCM//DCM
 5 /MeOH/H₂O (8/2/0.1), from 1//0 to 1//1 to afford 122 mg (29% yield) of the **Linker L1** as a colorless resin.

¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.82 (d, J=6.9 Hz, 3 H), 0.86 (d, J=6.6 Hz, 3 H), 1.30 - 1.49 (m, 2 H), 1.54 - 1.63 (m, 1 H), 1.64 - 1.84 (m, 4 H), 1.87 - 2.03 (m, 2 H), 2.06 (t, J=7.4 Hz, 2 H), 2.11 (td, J=7.4, 2.5 Hz, 2 H), 2.34 - 2.39 (m, 2 H), 2.88 - 2.98 (m, 1 H), 2.98
 10 - 3.08 (m, 1 H), 3.18 (q, J=5.7 Hz, 2 H), 3.36 - 3.42 (m, 4 H), 3.47 - 3.57 (m, 8 H), 3.58 - 3.62 (m, 2 H), 4.19 (dd, J=8.4, 6.8 Hz, 1 H), 4.29 - 4.35 (m, 1 H), 4.35 - 4.41 (m, 1 H), 4.42 (d, J=5.7 Hz, 2 H), 4.53 (d, J=5.4 Hz, 2 H), 5.07 (t, J=5.7 Hz, 1 H), 5.20 (br dd, J=10.4, 1.6 Hz, 1 H), 5.28 (br dd, J=17.3, 1.6 Hz, 1 H), 5.40 (br s, 2 H), 5.85 - 5.94 (m, 1 H), 5.96 (br t, J=5.7 Hz, 1 H), 7.22 (d, J=8.5 Hz, 2 H), 7.53 (d, J=8.5 Hz, 2 H), 7.72 (d, J=8.5 Hz,
 15 1 H), 7.82 (t, J=5.5 Hz, 1 H), 8.02 (d, J=7.9 Hz, 1 H), 8.13 (d, J=7.6 Hz, 1 H), 9.91 (s, 1 H)
 MS method N: t_R (min): 1.06; [M+H]⁺ 863.7

Linker (L2): Preparation of allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate
 20



Step 1: (2S)-5-allyloxy-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoic acid

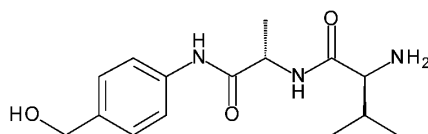


Under argon, to a round-bottom flask containing 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid of **Step 1** of **Linker 1** (139 mg, 0.418 mmol) and anhydrous DMF (6 mL), were added N,N'-Disuccinimidyl carbonate (117.8 mg, 0.46 mmol) and DIPEA (73 μ L, 0.418 mmol), the reaction mixture was stirred at rt for 3 h. It was then added (2S)-5-allyloxy-2-amino-5-oxo-pentanoic acid (86.1 mg, 0.46 mmol) and DIPEA (219 μ L, 1.26 mmol). The reaction mixture was stirred at rt for 16 h and concentrated under vacuum. The crude material was purified by chromatography on silica gel (Merck, 10 g, 15-40 μ m), with eluent DCM//DCM/MeOH/H₂O (8/2/0.1), from 1//0 to 0//1 to afford 180 mg (86% yield) of the expected compound as a colorless oil.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.69 (quin, J=7 Hz, 2 H); 1.75 - 1.84 (m, 1 H); 1.94 - 2.04 (m, 1 H); 2.03 - 2.13 (m, 4 H); 2.28 - 2.42 (m, 2 H); 3.11 - 3.25 (m, 2 H); 3.37 - 3.44 (m, 4 H); 3.48 - 3.57 (m, 8 H); 3.58 - 3.61 (m, 2 H); 4.01 - 4.15 (m, 1 H); 4.53 (d, J=5 Hz, 2 H); 5.20 (dd, J=10, 1 Hz, 1 H); 5.29 (br dd, J=17, 2 Hz, 1 H); 5.81 - 6.09 (m, 1 H); 7.65 - 7.80 (m, 1 H); 7.89 - 8.08 (m, 1 H)

MS method N: *t*_R (min): 1.21; [M+H]⁺ 502.2

Step 2: ((2S)-2-amino-N-[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide



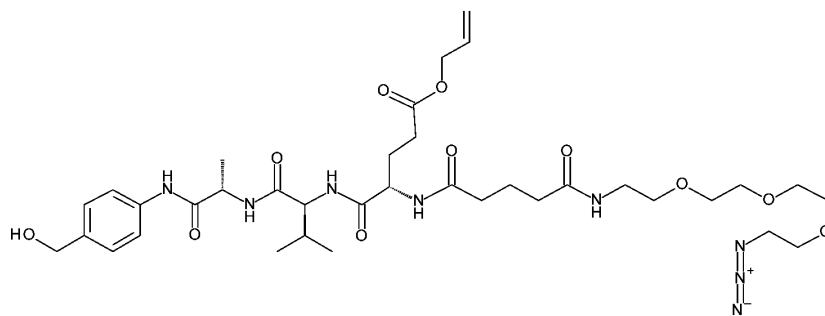
Under argon, to a three-neck round-bottom flask containing Fmoc-Val-Ala-OH (150 mg, 0.365 mmol) and anhydrous DCM (3 mL) and MeOH (1.5 mL), were added p-amino-benzyl

alcohol (90.0 mg, 0.73 mmol) and N-Ethoxycarbonyl-2-2-ethoxy-1,2-dihydroquinoline (EEDQ) (180.7 mg, 0.73 mmol). The reaction mixture was protected from light and stirred for 16 h. It was then concentrated under vacuum to give a white solid residue which was triturated in diisopropyl ether (3 mL) and filtrated. The white cake was washed with diisopropyl ether and dried under vacuum to give a white solid (165 mg). It was then dissolved, in a round bottom flask, in anhydrous DMF (3 mL), followed by addition of piperidine (180 μ L, 1.87 mmol) and triethylsilane (87.5 μ L, 0.55 mmol), the reaction mixture was stirred at rt for 30 min and evaporated to dryness. The crude material was purified by chromatography on silica gel (Merck, 5 g, 15-40 μ m), with eluent EtOAc//EtOAc/MeOH/H₂O (7/2/1), from 1//0 to 0//1 to afford 71 mg (66% yield) of the expected compound.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.78 (d, J=7 Hz, 3 H); 0.88 (d, J=7 Hz, 3 H); 1.29 (d, J=7 Hz, 3 H); 1.86 - 1.98 (m, 1 H); 1.90 - 2.60 (large m, 2H); 3.00 (d, J=5 Hz, 1 H); 4.40 - 4.52 (m, 3 H); 5.09 (t, J=6 Hz, 1 H); 7.24 (d, J=8 Hz, 2 H); 7.53 (d, J=8 Hz, 2 H); 8.09-8.22 (m, 1 H); 9.97 (br s, 1 H)

MS method M: t_R (min): 0.62; [M+H]⁺ 294.2

Step 3: Preparation of (L2) allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate



Under argon, to a round-bottom flask containing (2S)-5-allyloxy-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoic acid of **Step 1** (136.8 mg, 0.273 mmol), ((2S)-2-amino-N-[[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]-3-methyl-butanamide of **Step 2** (71 mg, 0.237 mmol) and anhydrous

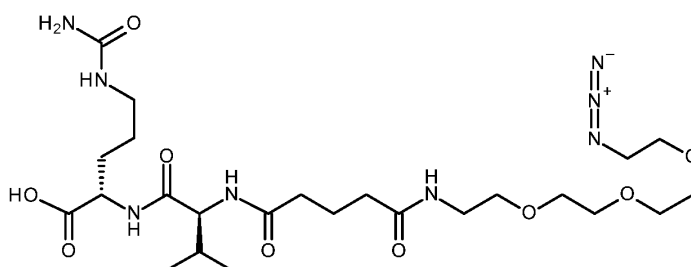
Under argon, to a round-bottom flask containing 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid of **Step 1** of **Linker (L1)** (188 mg, 0.566 mmol) and anhydrous DMF (6 mL), were added N,N'-Disuccinimidyl carbonate (168 mg, 0.622 mmol) and DIPEA (73 μ L, 0.565 mmol), the mixture was stirred at rt for 3 h. It was then added L-valyl-L-alanine (117.1 mg, 0.622 mmol) in DMF (2.5 mL) and DIPEA (296 μ L, 1.70 mmol). The reaction mixture was stirred at rt for 3.5 h and concentrated under vacuum to give 427 mg of crude product, which was purified by chromatography on silica gel (Merck, 10 g, 15-

40 μ m), with eluent DCM//DCM/MeOH/H₂O (8/2/0.1), from 1//0 to 0//1 to afford 158 mg (55% yield) of **Linker (L3)** as a colorless oil.

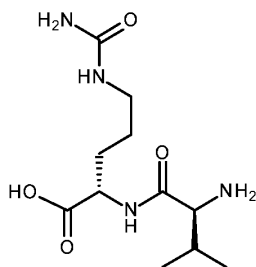
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.83 (d, J=7 Hz, 1 H); 0.85 (d, J=7 Hz, 1 H); 1.19 (d, J=7 Hz, 3 H); 1.62 - 1.78 (m, 2 H); 1.98 - 2.22 (m, 6 H); 3.12 - 3.22 (m, 2 H); 3.37-
5 3.44 (m partially hidden, 4 H); 3.47 - 3.57 (m, 8 H); 3.57 - 3.62 (m, 2 H); 3.77 - 3.92 (m, 1 H); 4.09 (dd, J=9, 6 Hz, 1 H); 7.62 - 7.82 (m, 1 H); 7.88 (br d, J=9 Hz, 1 H); 8.16 - 8.38 (m, 1 H)

MS method D: *t*_R (min): 2.07; [M+H]⁺ 503.4

- 10 **Linker (L4):** Preparation of (2S)-2-[[[(2S)-2-[[5-[2-[2-[2-(2- azidoethoxy)ethoxy]ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl] amino]-3-methyl-butanoyl]amino]- 5-ureido-pentanoic acid



Step 1: (2S)-2-[[[(2S)-2-amino-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid

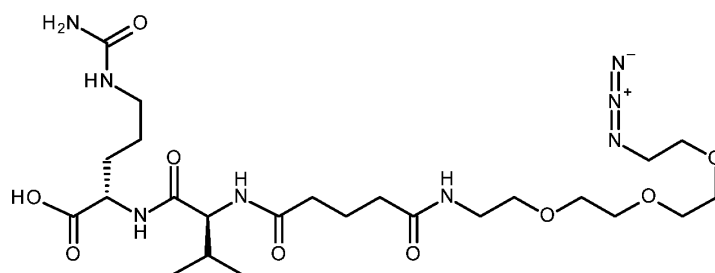


- 15 Under argon, to a round-bottom flask containing (2S)-2-[[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid (5.23 g, 10 mmol) and anhydrous DMF (50 mL), was added DMAP (1.22 g, 10 mmol), the reaction mixture was stirred at rt overnight to give a thick white gel. Then were added EtOAc (500 mL) and water (250 mL). After decantation, the aqueous phase was washed with EtOAc
20 (125 mL), and taken into a round-bottom flask, frozen and free-dried to give expected compound, 3.17 g (80% yield) of white lyophilizate, with the presence of 0.3 equivalents of DMAP.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.82 (d, J=7 Hz, 3 H); 0.90 (d, J=7 Hz, 3 H); 1.37 (quin, J=7 Hz, 2 H); 1.48 - 1.62 (m, 1 H); 1.64 - 1.79 (m, 1 H); 1.97 (m, 1 H), 2.84 - 3.01 (m, 2 H); 2.95 (s, 6 H); 3.18 (br d, J=5 Hz, 1 H); 4.05 - 4.13 (m, 1 H); 5.37 (s, 2 H); 5.90 - 6.11 (m, 1 H); 6.50 - 6.72 (m, 2 H); 7.98 - 8.32 (m, 3 H)

5 MS method N: t_R (min):0.15 ; [M+H]⁺ 275.0

Step 2: Preparation of **linker (L4)** (2S)-2-[[[(2S)-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy] ethylamino]-5-oxo-pentanoyl] amino]-3-methyl-butanoyl]amino]- 5-ureido-pentanoic acid

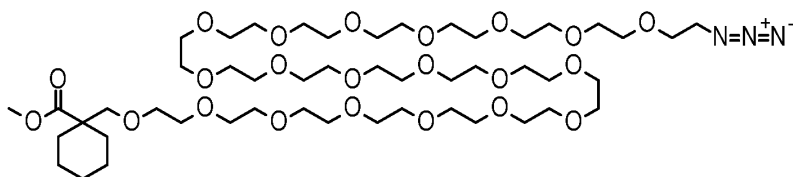


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Under argon, to a round-bottom flask containing 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid of **Step 1** of **Linker (L1)** (1.40 g, 4.21 mmol) and anhydrous DMF (30 mL), were added N,N'-Disuccinimidyl carbonate (983 mg, 4.63 mmol) and DIPEA (603 μ L, 4.21mmol), the reaction mixture was stirred at rt for 3 h. It was then added (2S)-2-[[[(2S)-2-amino-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid of **Step 1** (1.2 g, 4.63 mmol) in DMF (30 mL) and DIPEA (1500 μ L, 12.64 mmol). The reaction mixture was stirred at rt for 3.5 h and concentrated under vacuum. The crude material was purified by chromatography on silica gel (Merck,15-40 μ m), with eluent DCM/MeOH (8/2) to give 1.36 g (55 % yied) of **Linker (L4)** as a white powder.

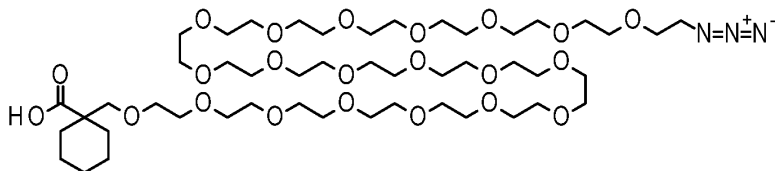
20 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.83 (d, J=7 Hz, 3 H); 0.86 (d, J=7 Hz, 3 H); 1.30 - 1.45 (m, 2 H); 1.50 - 1.61 (m, 1 H); 1.64 - 1.75 (m, 3 H); 1.97 (dq, J=13, 7 Hz, 1 H); 2.06 (t, J=7 Hz, 2 H); 2.09 - 2.23 (m, 2 H); 2.94 (q, J=7 Hz, 2 H); 3.18 (q, J=6 Hz, 2 H); 3.37-3.43 (m, 4 H); 3.47 - 3.57 (m, 8 H); 3.58 - 3.65 (m, 2 H); 4.05 - 4.13 (m, 1 H); 4.19 (dd, J=9, 7 Hz, 1 H); 5.36 (s, 2 H); 5.93 (br t, J=5 Hz, 1 H); 7.78 (d, J=9 Hz, 1 H); 7.90 (br t, J=5 Hz, 1 H); 8.06 (d, J=7 Hz, 1 H); 10.88 - 13.71 (m, 1 H)

25 MS method N: t_R (min): 1.03; [M+H]⁺ 589.3



To a cooled solution of methyl 1-(hydroxymethyl)cyclohexanecarboxylate (178.2 mg, 0.983 mmol) in anhydrous DMF (500 µL) was added sodium hydride (39 mg, 0.985 mmol). The solution was stirred for 5 min then a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-
5 [2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]
ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl
methanesulfonate from **Step 1** (195 mg, 0.194 mmol) in anhydrous DMF (2.25 mL) was
added. The reaction mixture was reacted overnight at rt then cooled to 0°C before a new
addition of sodium hydride (39 mg, 0.985 mmol). The mixture was reacted for 30 min at
10 0°C and 30 min at rt. Na₂SO₄.10H₂O (6.4 g) and DCM (10 mL) were then added, vigorously
stirred then filtrated and concentrated to dryness. The crude product was then purified by
reverse phase column chromatography using as eluent a mixture of H₂O + 0.1% TFA/
CH₃CN (95:5 to 10:90). The compound containing fractions were pooled and concentrated
to dryness. The residue was taken up in DCM then dried over MgSO₄, filtrated and
15 concentrated to dryness to give 84 mg of the expected product (40% yield) as a light yellow
oil.

MS method N1: t_R (min): 1.78; $[M+H]^+$ 1078.3; $[M+2H]^{2+}$ 540.0

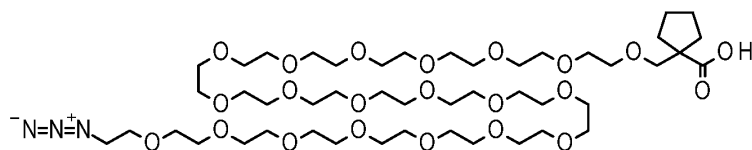
[illegible]

To a solution of methyl 1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-
azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]et
hoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl)cyclohexane carb

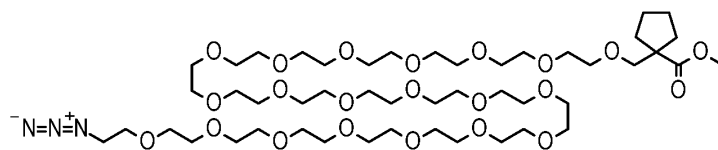
oxylate from **Step 2** (78 mg, 0.0725 mmol) in THF (2.6 mL) were added H₂O (870 µL) and a 1M aqueous solution of LiOH (365 µL, 0.365 mmol). The reaction was stirred for 5.5 h at rt then, under LCMS monitoring, new additions of 2M aqueous LiOH (a total of 433 µL, 0.866 mmol) were needed to go to reaction completion. HCl 5M (196 µL) was added and the mixture was evaporated to dryness. The residue was taken up in DCM, dried over MgSO₄, filtered and concentrated. The crude product was then purified by silica gel column chromatography using as eluent a mixture of DCM/(DCM/MeOH/H₂O, 40:5:0.5) (100:0 to 0:100) to give 57 mg (74% yield) of the **Linker (L5)**, as a light yellow oil.

MS method N1: t_R (min): 1.63; [M+H]⁺ 1064.2

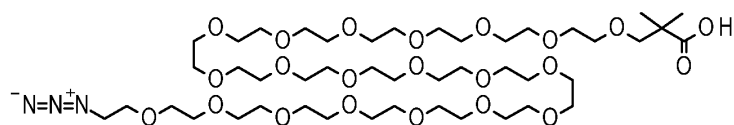
Linker (L6): Preparation of 1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclopentanecarboxylic acid



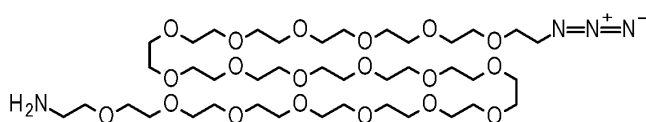
Step 1: methyl 1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclopentanecarboxylate



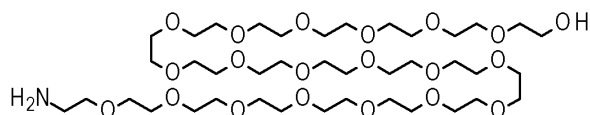
To a cooled solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclopentanecarboxylate, **Linker (L5)** from **Step 1** (98 mg, 0.0982 mmol) in anhydrous DMF (1 mL) was added methyl 1-(hydroxymethyl)cyclopentanecarboxylate (63 mg, 0.3783 mmol) and sodium hydride (12 mg, 0.313 mmol). The mixture was reacted for 30 min at 0°C and 5 h at rt then, under LCMS monitoring, new additions of methyl 1-(hydroxymethyl)cyclopentanecarboxylate (2



To *tert*-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]-2,2-dimethyl-propanoate from **Step 1** (73 mg, 0.068 mmol) was added a HCl solution 4M in dioxane (3.5 mL, 14 mmol). The resulting limpid solution was stirred 3.5 h then concentrated under vacuum to afford 76 mg (87% yield) of the expected compound **Linker (L7)** as a light yellow wax.

MS method N1: t_R (min): 1.49; $[M-H]^-$ 1022.3[illegible]

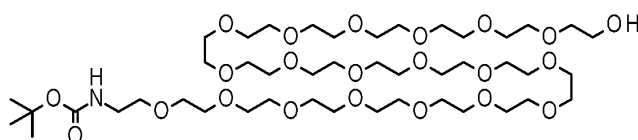
Step 1: 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]
15 ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]
 [ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol



To a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol (225 mg, 0.243 mmol) in THF (6 mL) and H₂O (1 mL) was added triphenylphosphine (320 mg, 1.22 mmol). The reaction mixture was stirred for 48 h then concentrated to dryness. The residue was diluted in MeOH and purified by size exclusion chromatography (LH20 resin, 70 g), with 100% MeOH then further purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/NH₄OH, 12:1.5:0.25 to give 175 mg (80% yield) of the expected product, as a white solid.

MS method N5: t_R (min): 0.94; $[M+H]^+$ 898.7; $[M+2H]^{2+}$ 450.0

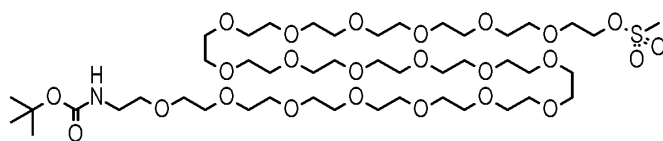
Step 2: *tert*-butyl N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate



To a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol from **Step 1** (170 mg, 0.189 mmol) in THF (5 mL) were added DIPEA (40 μ l, 0.230 mmol) and di-*tert*-butyl dicarbonate (46 mg, 0.210 mmol). The reaction mixture was stirred at rt for 1 h then concentrated to dryness. The residue was purified by size exclusion chromatography (LH20 resin, 70 g), with 100% DCM then further purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O, 40:5:0.5 to give 143 mg (75% yield) of the expected product, as a white solid.

MS method N5: t_R (min): 1.52; $[M+H]^+$ 998.8; $[M+2H]^{2+}$ 500

Step 3: 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(*tert*-butoxycarbonyl amino)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl methanesulfonate



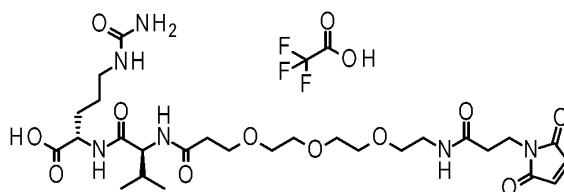
To a solution of *tert*-butyl N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate (140 mg, 0.140 mmol) from **Step 2** in DCM (4 mL) cooled with an ice bath were successively added TEA (78 μ L, 0.560 mmol) and methanesulfonyl chloride (22 μ L, 0.284 mmol). The reaction mixture was stirred for 30 min at 0°C then poured onto a cooled mixture of DCM (10 mL) /

MS method N5: t_R (min): 1.66; [M+H]⁺ 1076.6[illegible]

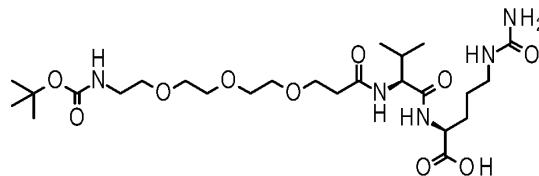
hoxyl]ethoxyl]ethoxyl]ethoxyl]ethoxyl]ethoxyl]ethoxyl]ethyl]carbamate from **Step 4** (70 mg, 0.068 mmol) in dioxane (3 mL) was added a HCl solution 4M in dioxane (1.7 mL, 6.8 mmol). The resulting solution was stirred 1 h at rt then concentrated under vacuum and purified by silica gel column chromatography using as eluent a mixture of
 5 DCM/MeOH/H₂O, 40: 5:0.5 to give 37 mg (56% yield) of **Linker (L8)**, as a whitish glassy solid.

MS method N1: t_R (min): 1.11; [M+H]⁺ 923.2

Linker (L9): Preparation of (2S)-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid; 2,2,2-trifluoroacetic acid
 10



Step 1: (2S)-2-[[[(2S)-2-[3-[2-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid

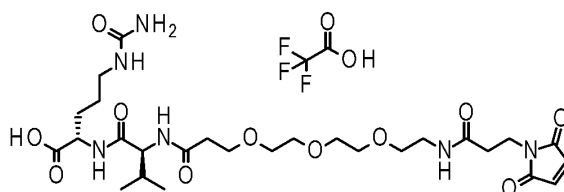


15

To a solution of (2S)-2-[[[(2S)-2-(*tert*-butoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid prepared as described in *Bioorg. Med. Chem.*, **2020**, 28 (24), 115808 (810 mg, 2.164 mmol) in DCM (10 mL) was added a HCl solution 4M in dioxane (5 mL, 20 mmol). The resulting suspension was stirred for 1 h at rt and concentrated under vacuum.
 20 The residue was solubilized in DMF (5 mL) and reacted with DIPEA (760 μL, 4.4 mmol) and a solution of (2,5-dioxopyrrolidin-1-yl) 3-[2-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy]ethoxy]propanoate (924 mg, 2.164 mmol) in DMF (5 mL). The reaction mixture was stirred overnight, concentrated to dryness and purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH, 70:30 to give 980 mg (78% yield) of the expected
 25 product, as a white foamy solid.

MS method N1: t_R (min): 1.19; $[M+H]^+$ 578.2

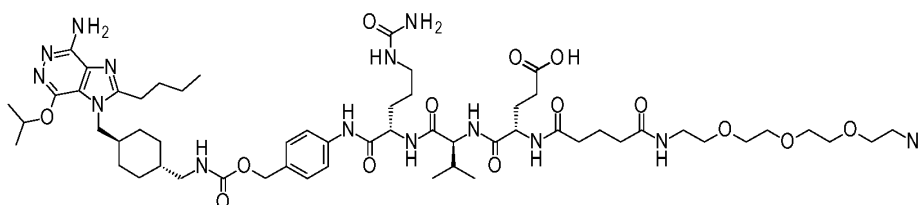
Step 2: Preparation of **Linker (L9)** (2S)-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid; 2,2,2-trifluoroacetic acid



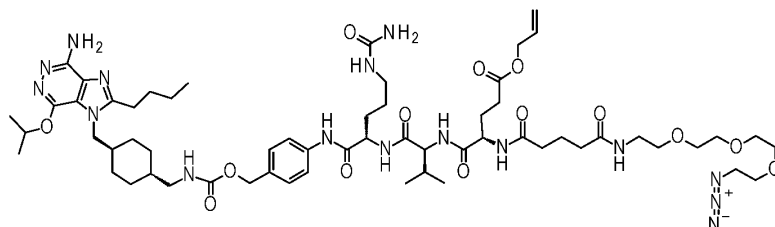
To a solution of (2S)-2-[[[(2S)-2-[3-[2-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid (980 mg, 1.698 mmol) from **Step 1** in DCM (15 mL) was added TFA (5 mL). The solution was reacted at rt for 1 h then concentrated to dryness. The residue was solubilized in a mixture of DCM (2.5 mL) and DMF (2 mL) and reacted with DIPEA (1.45 mL, 8.38 mmol) and a solution of N-succinimidyl 3-maleimidopropionate (464 mg, 1.729 mmol) in DCM (2 mL) and DMF (1 mL). The reaction mixture was stirred at rt for 1 h, concentrated to dryness and purified by reverse phase column chromatography using as eluent a mixture of H₂O + 0.1% TFA/CH₃CN (95:5 to 5:95). The compound containing fractions were pooled, concentrated under vacuum then freeze dried to give 838 mg (66% yield) of **Linker (L9)** as a white foamy solid. MS method N1: t_R (min): 0.94; $[M+H]^+$ 629.1

C) Preparation of payloads

Example 1: Preparation of (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylcarbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid (Payload P2)



Step 1: Allyl (4S)-5-[[[(1S)-1-[[[(1S)-1-[[4-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]carbamoyloxy]methyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate

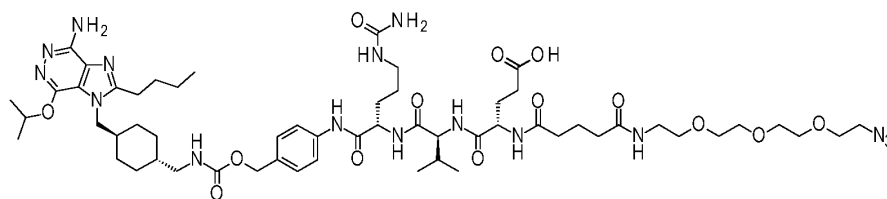


5

To a solution of allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate **Linker (L1)** (150 mg, 0.174 mmol) in anhydrous DMF (3. mL), were added bis(4-nitrophenyl) carbonate (46 mg, 0.348 mmol) and DIPEA (60 μ L, 0.348 mmol), the yellow mixture was stirred under argon 15 min at 0°C, 2h30 at 50°C then overnight at rt. To the mixture were added a solution of 3-[[*trans*-4-(aminomethyl)cyclohexyl)methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine (**Example (TLR2)**) (65 mg, 0.174 mmol) in anhydrous DMF (1.5 mL), followed by addition of DIPEA (45 μ L, 0.26 mmol). The reaction mixture was stirred 3h30 at rt then poured into Et₂O to give a yellow solid that is collected by filtration then purified by silica gel flash chromatography (Merck, 15 g, silica gel 15-40 μ m), elution with DCM//DCM/MeOH/H₂O (8/2/0.1), from 1//0 to 1//1 to afford the expected compound (169 mg, 77% yield) as a yellow glassy solid.

Step 2: Preparation of **Example 1** (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]carbamoyloxy]methyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid

20



To a solution of allyl (4S)-5-[[[(1S)-1-[[[(1S)-1-[[4-[[trans-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylcarbamoyloxymethyl]phenyl] carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azido ethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate of **Step 1** (168 mg, 0.133 mmol) in a degassed mixture of dioxane (20 mL) and H₂O (7 mL), were added at 0°C, 1,3-dimethylbarbituric acid (50 mg, 0.319 mmol) and tetrakis(triphenylphosphine)palladium(0) (61 mg, 0.053 mmol). The reaction mixture was stirred 15 min at 0°C and 2 h at RT then poured into Et₂O to give a yellow solid that is collected by filtration then purified by HPLC using a C18 Waters Xbridge OBD column (19 x 150mm, 5 µm), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under ELSD detection. The compound containing fractions were combined, concentrated under vacuum (31°C, 100 mb to 80 mb), and freeze-dried to give **Example 1** (38 mg, 23% yield) as a white lyophilisate.

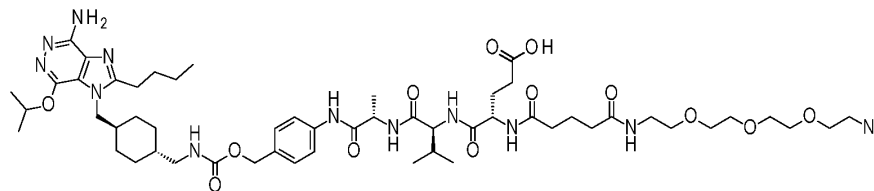
¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.72 - 0.80 (m, 2 H), 0.82 (d, J=7 Hz, 3 H), 0.85 (d, J=7 Hz, 3 H), 0.92 (t, J=7 Hz, 3 H), 1.00-1.12 (m, 2 H), 1.35 (d, J=6 Hz, 6 H), 1.37-1.51 (m, 6 H), 1.60-1.91 (m, 12 H), 1.95-2.22 (m, 7 H), 2.77-2.86 (m, 4 H), 2.94-3.00 (m, 2 H), 3.18 (q, J=6 Hz, 2 H), 3.36 - 3.42 (m, 4 H), 3.47-3.57 (m, 8 H), 3.57-3.62 (m, 2 H), 4.10 (d, J=7 Hz, 2 H), 4.21 (dd, J=9, 6 Hz, 1 H), 4.24-4.34 (m, 2 H), 4.91 (s, 2 H), 5.38 (spt, J=6 Hz, 1 H), 5.56-5.79 (m, 2 H), 5.87 (br s, 2 H), 7.20 (br t, J=6 Hz, 1 H), 7.25 (d, J=9 Hz, 2 H), 7.58 (d, J=9 Hz, 2 H), 7.77-7.98 (m, 2 H), 8.05-8.25 (m, 1 H), 8.43-8.86 (m, 1 H), 10.01 (s, 1 H)

MS method N: t_R (min): 1.31; [M+H]⁺ 1223.0

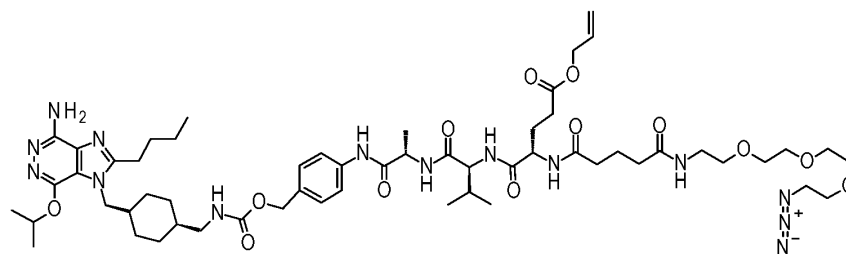
Compound **P1** was prepared by analogy according to this example.

Example 2: Preparation of (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[trans-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylcarbamoyloxy methyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-

[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid (Payload P4)



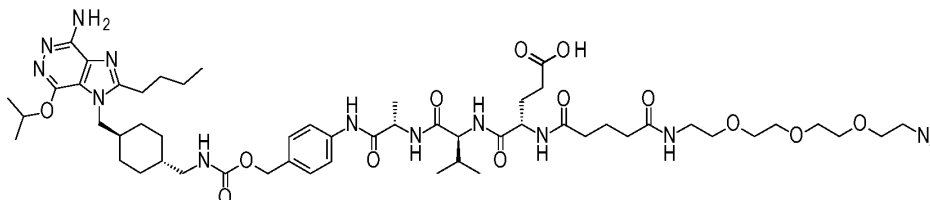
Step 1: Preparation of allyl (4S)-5-[[[(1S)-1-[[[(1S)-2-[4-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylcarbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate



To a solution of allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-1-[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate **Linker (L2)** (114 mg, 0.146 mmol) in anhydrous DMF (4 mL), were added at 0°C bis(4-nitrophenyl) carbonate (67 mg, 0.220 mmol) and DIPEA (130 µL, 0.746 mmol), the yellow mixture was stirred 24 h at rt. To the mixture were added a solution of 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine (**Example (TLR2)**) (55 mg, 0.146 mmol) in anhydrous DMF (1 mL). The reaction mixture was stirred 24 h at rt then poured into cold water and extracted with EtOAc (3 x 100 mL) and CH₂Cl₂ (2 x 100 mL). The combined organic solutions were concentrated to dryness then purified by silica gel flash chromatography (Merck, 10 g, silica gel 15-40 µm), with eluent EtOAc // EtOAc/EtOH/H₂O (85/15/1), from 40//60 to 30//70 to afford the expected compound (93 mg, 54% yield).

Step 2: Preparation of **Example 2** (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylcarbamoyloxy

methyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid



- 5 To a solution of allyl (4S)-5-[[[(1S)-1-[[[(1S)-2-[4-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylcarbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate of **Step 1** (65 mg, 0.052 mmol) in a degassed mixture of dioxane (9 mL) and H₂O (3 mL), was added at 0°C,
- 10 tetrakis(triphenylphosphine)palladium(0) (11 mg, 0.029 mmol) in a mixture of dioxane (1.5 mL) and H₂O (0.5 mL) and 1,3-dimethylbarbituric acid (27 mg, 0.177 mmol) in a mixture of dioxane (1.5 mL) and H₂O (0.5 mL). The reaction mixture was stirred 10 min at 0°C and 1 h at RT then concentrated to dryness and taken up in EtOAc. The resulting solid, collected by filtration was then purified by preparative SFC using a Princeton 2-EP column
- 15 (4.6 x 250mm, 5μm), with mobile phase CO₂/MeOH/TEA (75/25/0.1), under UV (230 nm) detection. The compound containing fractions were combined, concentrated under vacuum (30°C), taken up in water and freeze-dried to give **Example 2** (34 mg, 41% yield) as a white lyophilisate.

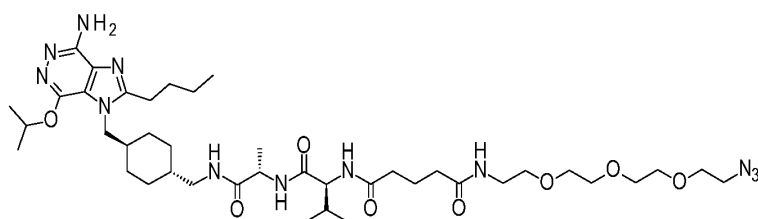
¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.69 - 0.82 (m, 2 H), 0.82 (d, J=7 Hz, 3 H), 0.86 (d, J=7 Hz, 3 H), 0.92 (t, J=7 Hz, 3 H), 1.07 (br q, J=12 Hz, 2 H), 1.30 (d, J=7 Hz, 3 H), 1.35 (d, J=6 Hz, 6 H), 1.37-1.44 (m, 2 H), 1.47 (br d, J=12 Hz, 2 H), 1.60-1.80 (m, 9 H), 1.88 (m, 1 H), 1.98 (m, 1 H), 2.06 (t, J=7 Hz, 2 H), 2.08-2.14 (m, 2 H), 2.18-2.26 (m, 2 H), 2.78-2.89 (m, 4 H), 3.18 (q, J=6 Hz, 2 H), 3.36-3.64 (m, 14 H), 4.10 (br d, J=7 Hz, 2 H), 4.19 (m, 1 H), 4.28 (m, 1 H), 4.37 (quin, J=7 Hz, 1 H), 4.91 (s, 2 H), 5.37 (m, 1 H), 5.94 (br s, 2 H),

20 7.20 (t, J=6 Hz, 1 H), 7.26 (d, J=8 Hz, 2 H), 7.56 (d, J=8 Hz, 2 H), 7.69 (d, J=9 Hz, 1 H), 7.85 (t, J=5 Hz, 1 H), 8.06 (br d, J=7 Hz, 1 H), 8.24 (d, J=7 Hz, 1 H), 9.98 (s, 1 H), 11.59 - 13.21 (m, 1 H)

MS method N: t_R (min): 1.35; [M+H]⁺ 1137.6

Compound **P3** was prepared by analogy according to this example.

Example 3: Preparation of N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl] pentanediamide (Payload P6)



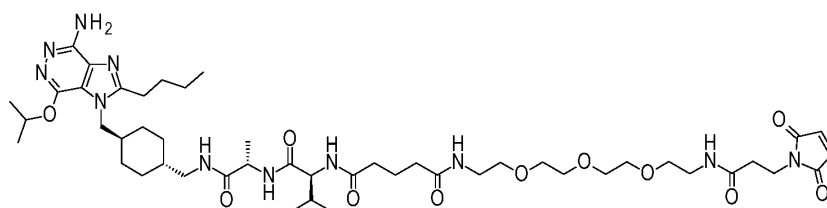
To a solution of 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine (**Example (TLR2)**) (57 mg, 0.153 mmol) and (1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oyl)-L-valyl-L-alanine **Linker (L3)** (70 mg, 0.139 mmol) in anhydrous DMF (2 mL), were added HATU (169 mg, 0.445 mmol), and 1-hydroxy-7-azabenzotriazole (55 mg, 0.404 mmol), followed by dropwise addition of DIPEA (97 μ L, 0.557 mmol). The resulting solution was stirred at rt under argon for 24 h. Then, the reaction mixture was concentrated to dryness and purified by silica gel flash chromatography (Merck, 20 g, silica gel 15-40 μ m), with eluent CH₂Cl₂/MeOH, 95/5. The compound containing fractions were combined, concentrated and further purified by preparative SFC using a DCPACK B column (4.6 x 250 mm, 5 μ m), with mobile phase CO₂/MeOH/TEA (80/20/0.1), under UV (230nm) detection. The compound containing fractions were combined, concentrated under vacuum (40°C), taken up in water and freeze-dried to give **Example 3** (15 mg, 12% yield) as a white lyophilisate.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.76-0.86 (m, 8 H), 0.93 (t, J=7.4 Hz, 3 H), 1.05 (q, J=12.1 Hz, 2 H), 1.17 (d, J=7.0 Hz, 3 H), 1.32-1.53 (m, 5 H), 1.36 (d, J=6.0 Hz, 6 H), 1.64-1.81 (m, 7 H), 1.89-2.00 (m, 1 H), 2.05 (t, J=7.5 Hz, 2 H), 2.10-2.19 (m, 2 H), 2.79-2.94 (m, 4 H), 3.18 (q, J=5.9 Hz, 2 H), 3.35-3.43 (m, 4 H), 3.47-3.61 (m, 10 H), 4.06-4.16 (m, 3 H), 4.22 (quin, J=7.2 Hz, 1 H), 5.38 (m, 1 H), 5.80 (s, 2 H), 7.71 (t, J=5.8 Hz, 1 H), 7.75-7.82 (m, 2 H), 7.90 (d, J=7.5 Hz, 1 H)

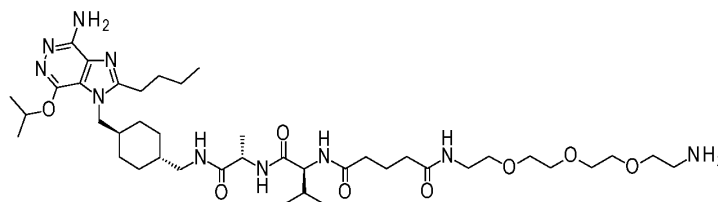
MS method N: t_R (min): 1.05; [M+H]⁺ 859.9

Compound **P5** was prepared by analogy according to this example.

Example 4: Preparation of N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoyl amino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide (Payload P7)

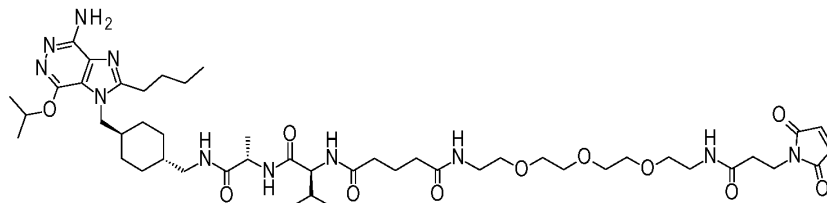


Step 1: Preparation of N'-[(1S)-1-[[[(1S)-2-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]pentanediamide



To an round bottom flask were added Pd/C (10%) (3.5 mg, 3.3 μ mol), N'-[(1S)-1-[[[(1S)-2-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide of **compound (P6)** (40 mg, 46.6 μ mol), ammonium formate (29 mg, 0.465 mmol) and MeOH (3 mL). The reaction mixture was heated under reflux for 1 h then filtrated over whatman filter paper and evaporated to dryness (30°C, 15 mb) to give 43 mg of crude product, which was purified by chromatography on silica gel (Interchim, 4 g, silica gel 30 μ m), with eluent DCM/MeOH/ammonia (35%) 12/3/0.5 to afford the expected compound (20 mg, 52% yield).

Step 2: Preparation of **Example 4** N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoyl amino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide



5

To a round-bottom flask were added N'-[(1S)-1-[[[(1S)-2-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]pentanediamide from **Step 1** (18.0 mg, 21.6 μ mol), resin supported DIEA (11 mg, 43 μ mol),
 10 N-succinimidyl 3-maleimidopropionate (5.2 mg, 19.5 μ mol) and DCM (4 mL). The resulting cloudy white solution was stirred at rt for 0.5 h then filtrated over whatman filter paper. EtOAc (5 mL) containing 10% formic acid was added and the reaction mixture was evaporated to dryness under vacuum. The crude product was purified by reverse phase chromatography (RP18 Merck, 5 g, 25-40 μ m), elution with H₂O + 0.5% form/CH₃CN +
 15 0.5% formic acid, from 80/20 to 0/100 to afford **Example 4** (6.8 mg, 32%) as a colorless glassy solid.

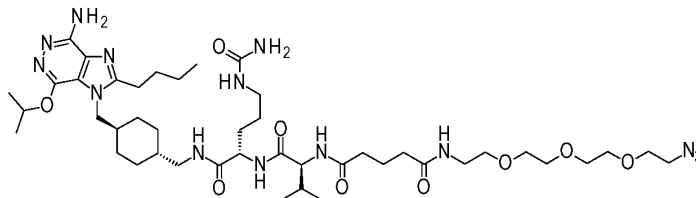
¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.73-0.85 (m, 8 H), 0.92 (t, J=7.4 Hz, 3 H), 0.99-1.12 (m, 2 H), 1.17 (d, J=7.3 Hz, 3 H), 1.33-1.49 (m, 6 H), 1.35 (d, J=6.0 Hz, 6 H), 1.64-1.79 (m, 6 H), 1.90-1.98 (m, 1 H), 2.05 (t, J=7.6 Hz, 2 H), 2.10-2.18 (m, 2 H), 2.27-2.35 (m,
 20 2 H), 2.82 (t, J=7.7 Hz, 2 H), 2.84-2.93 (m, 2 H), 3.08-3.64 (m partially hidden, 18 H), 4.05-4.17 (m, 3 H), 4.22 (m, 1 H), 5.38 (m, 1 H), 5.85 (s, 2 H), 7.00 (s, 2 H), 7.72 (t, J=6.4 Hz, 1 H), 7.78 (d, J=8.2 Hz, 1 H), 7.83 (t, J=5.5 Hz, 1 H), 7.92 (d, J=7.9 Hz, 1 H), 7.97-8.11 (m, 1 H)

MS method N: t_R (min): 1.16; [M+H]⁺ 984.5

25

Example 5: Preparation of N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-5-(carbamoylamino)

-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide (Payload P9)

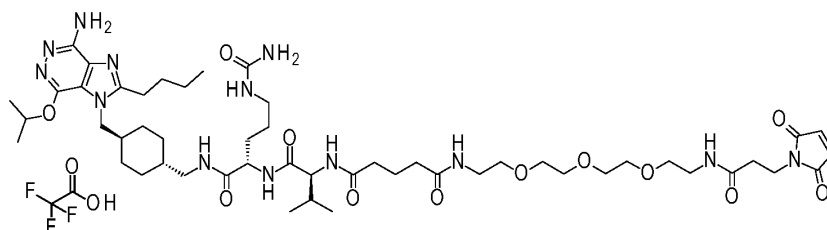


- Under argon, to a three-neck round-bottom flask were added (2S)-2-[[[(2S)-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid **Linker (L4)** (55.0 mg, 0.094 mmol), anhydrous THF (3.5 mL), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) (54 mg, 0.28 mmol), HOBt (18.9 mg, 0.14 mmol) and DIPEA (32.5 μ L, 0.187 mmol). The mixture was stirred for 5 min, then were added 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine (**Example (TLR2)**) (35 mg, 0.094 mmol) and DIPEA (32.5 μ L, 0.187 mmol). The resulting slightly cloudy white solution was stirred at rt overnight. It was then evaporated under vacuum (40 $^{\circ}$ C, 15 mb) to dryness to give 184 mg of crude product. The crude material was purified by chromatography on silica gel (Interchim, 25 g, silica gel 30 μ m), with DCM/MeOH from 85/15 to 80/20 to afford 46 mg of expected compound as a 'white laquer', which was further purified by HPLC using a C18 XSelect Peptide column (30 x 250 mm 5 μ m), with mobile phase A: H₂O + 0.1% formic acid and mobile phase B: MeCN + 0.1% formic acid, under UV detection (210 nm). The compound containing fractions were combined, concentrated under vacuum, and freeze-dried to give **Example 5** (27.9 mg, 32%) as a yellow solid.
- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.67-0.86 (m, 2 H); 0.81 (m, 6 H); 0.92 (t, J=7 Hz, 3 H); 1.06 (q, J=12 Hz, 2 H); 1.20-1.53 (m, 9 H); 1.36 (d, J=6 Hz, 6 H); 1.53-1.84 (m, 7 H); 1.94 (m, 1 H); 2.05 (t, J=8 Hz, 2 H); 2.10-2.21 (m, 2 H); 2.77 - 2.96 (m, 6 H); 3.18 (q, J=6 Hz, 2 H); 3.36 - 3.67 (m, 14 H); 4.05 - 4.15 (m, 3 H); 4.19 (m, 1 H); 5.33 (s, 2 H); 5.38 (spt, J=6 Hz, 1 H); 5.82 (s, 2 H); 5.89 (t, J=6 Hz, 1 H); 7.63 - 7.91 (m, 4 H)
- MS method N: t_R (min): 1.18; [M+H]⁺ 945.5

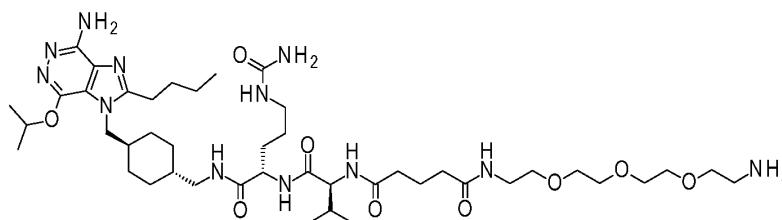
Compound **P8** was prepared by analogy according to this example.

Example 6: Preparation of N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide, 2,2,2-trifluoroacetate

5 (Payload P10)



Step 1: N'-[(1S)-1-[[[(1S)-1-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylcarbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]pentanediamide



10

To an autoclave were added Pd/C (10%) (16 mg, 0.015 mmol), N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide (**Example 5**) (159.5 mg, 0.169 mmol) and MeOH (75 mL). The autoclave was submitted to hydrogenation (4 b), at 20°C for 2.5 h. The reaction mixture was filtrated over glass microfiber filter (Wahntman) and evaporated to dryness (40°C, 15 mb) to give 150 mg of crude product, which was purified by chromatography on silica gel (Interchim, 12 g, silica gel 30 µm), with eluent DCM/MeOH/ammonia (35%) 12/3/0.5 to afford expected compound (91.5 mg, 59%) as a white solid.

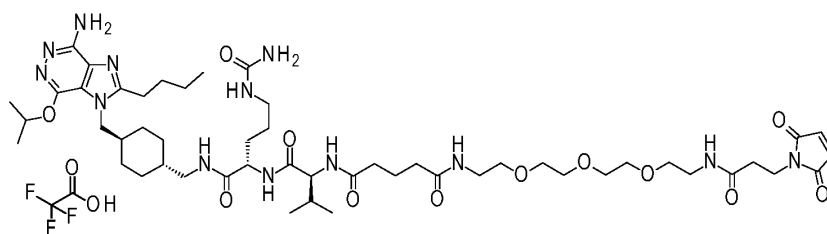
20

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.67-0.86 (m, 8 H); 0.92 (t, J=7 Hz, 3 H); 0.98-1.13 (m, 2 H); 1.18-1.51 (m, 15 H); 1.51-1.63 (m, 1 H); 1.64-1.82 (m, 8 H); 1.87-2.00 (m, 1 H); 2.05 (t, J=7 Hz, 2 H); 2.14 (m, 2 H); 2.64 (t, J=6 Hz, 2 H); 2.75-2.97 (m, 6 H); 3.18 (q, J=6 Hz, 2 H); 3.33-3.43 (m partially hidden, 4 H); 3.44-3.58 (m, 8 H); 4.01-4.24 (m, 4 H);

5.27-5.44 (m, 3 H); 5.84 (br s, 2 H); 5.90 (br t, J=6 Hz, 1 H); 7.68-7.83 (m, 2 H); 7.83-7.94 (m, 2 H)

MS method N: t_R (min): 0.93; $[M+H]^+$ 919.5

- 5 **Step 2:** Preparation of **Example 6** N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide,2,2,2-trifluoroacetate



10

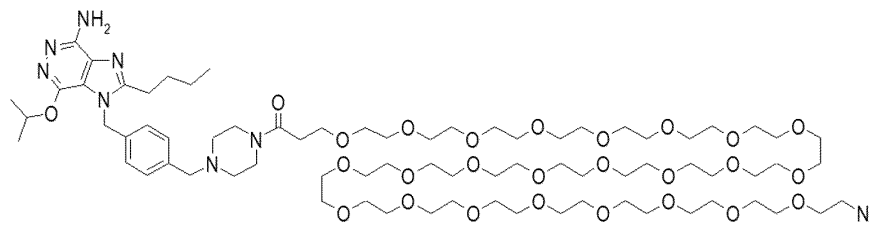
Under argon, to a three-neck round-bottom flask were added N'-[(1S)-1-[[[(1S)-1-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]pentanediamide of **Step 1** (89.0 mg, 0.097 mmol) and anhydrous DMF (4 mL), followed by addition of (2,5-dioxopyrrolidin-1-yl) 3-(2,5-dioxopyrrol-1-yl)propanoate (26.3 mg, 0.097 mmol) and DIPEA (50.6 μ L, 0.291 mmol). The reaction mixture was stirred at rt for 1.5 h, giving a pale pink yellow solution. To this solution, was added EtOAc (15 mL) containing 10% of TFA and followed by evaporation under vacuum (30°C, 15 mb) to give pale yellow oil, which was diluted with H₂O. After free drying, 179 mg of crude product was obtained, which was purified by HPLC using a C18 Waters Xbridge column (19 x 150 mm 5 μ m), with mobile phase A: H₂O + 10 mM TFA, and mobile phase B: MeCN + 10% TFA, under UV detection (210 nm). The compound containing fractions were combined, concentrated under vacuum (30°C, 80 mb), and freeze-dried to give **Example 6** (92.5 mg, 80.5% yield) as a white lyophilisate.

- 25 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.69-0.86 (m, 2 H); 0.81 (t, J=6 Hz, 6 H); 0.93 (t, J=7 Hz, 3 H); 1.01-1.14 (m, 2 H); 1.20-1.63 (m, 10 H); 1.39 (d, J=6 Hz, 6 H); 1.64-1.73 (m, 4 H); 1.74-1.83 (m, 2 H); 1.94 (m, 1 H); 2.05 (t, J=8 Hz, 2 H); 2.11-2.19 (m, 2 H); 2.33 (t, J=7 Hz, 2 H); 2.84-2.99 (m, 6 H); 3.12-3.53 (m partially hidden, 16 H); 3.59 (t, J=7 Hz,

2 H); 4.03-4.32 (m, 4 H); 4.77-5.46 (m, 2 H); 5.27 (m, 1 H); 5.34 (s, 2 H); 5.90 (t, J=6 Hz, 1 H); 7.00 (s, 2 H); 7.72-7.81 (m, 2 H); 7.82-7.90 (m, 2 H); 8.00 t, J=5 Hz, 1 H); 11.62-12.70 (m, 1 H)

MS method N: t_R (min): 1.12; $[M+H]^+$ 1070.3

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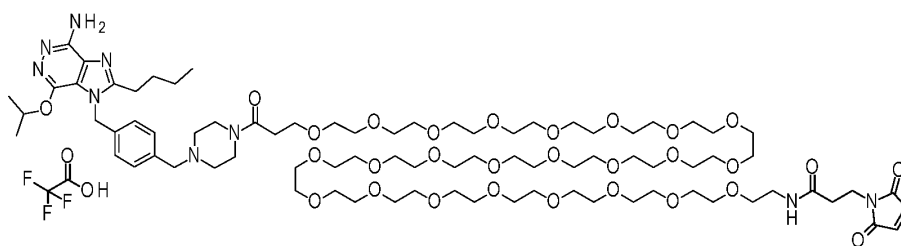
[illegible]

To a solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (**Example (TLR1)**) (300 mg, 0.686 mmol) in anhydrous DCM (120 mL), under argon, was added commercial available 2,5-dioxopyrrolidin-1-yl 1-azido-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72-tetracosaoxapentaheptacontan-75-oate (BP-23542 from BroadPharm, CAS [2375600-46-5], 870 mg, 0.686 mmol). The mixture was stirred overnight at rt. It was then concentrated under vacuum till ~20 mL of solution, which were loaded to a Sephadex LH20 column (3.1 cm diam, 48 g of Sephadex LH-20m resin, washed previously with DCM). The column was then eluted with DCM (5 mL/min), the compound containing fractions were combined, and evaporated to dryness to give **Example 7** (1.09 g, 100% yield) as a colorless glassy solid.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.82 (t, J=7 Hz, 3 H); 1.17 (d, J=6 Hz, 6 H); 1.26-1.38 (m, 2 H); 1.53-1.66 (m, 2 H); 2.18-2.37 (m, 4 H); 2.52-2.54 (m partially hidden, 2 H); 2.74-2.86 (m, 2 H); 3.36-3.42 (m, 4 H); 3.43-3.71 (m, 100 H); 5.31 (quin, J=6 Hz, 1 H); 5.56 (s, 2 H); 5.89 (s, 2 H); 7.02 (d, J=8 Hz, 2 H); 7.26 (d, J=8 Hz, 2 H)

MS method N: t_R (min): 1.14; [M+H]⁺ 1592.4

Example 9: Preparation of N-[2-[3-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]piperazin-1-yl]-3-oxopropoxy]ethyl]-3-(2,5-dioxopyrrol-1-yl)propanamide, 2,2,2-trifluoroacetate (Payload P13)

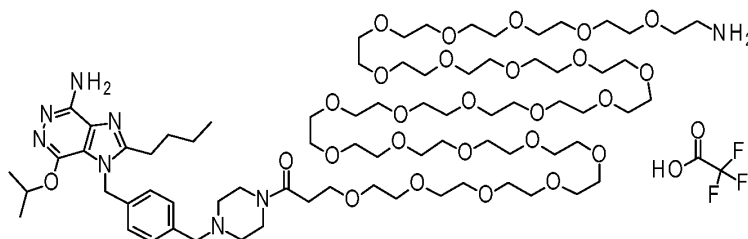


Step **1:** 1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-

10 yl)methyl]phenyl)methyl]piperazin-1-yl]-3-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]

ethoxy]

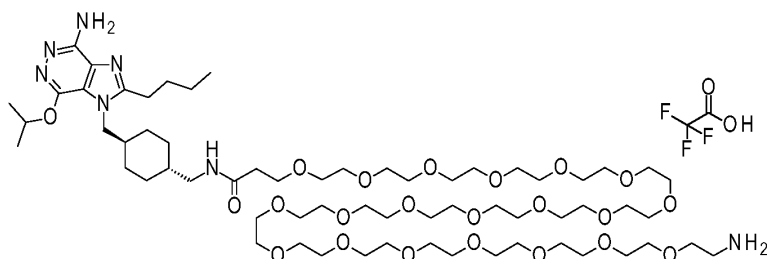
[ethoxy]ethoxy]ethoxy]ethoxy]propan-1-one,2,2,2-trifluoroacetate



To a hydrogenation tube, was added a solution of 1-[4-[[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazin-1-yl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propan-1-one (**Example 7**) (122 mg, 0.077 mmol), in MeOH (45 mL) and Pd/C (12 mg, 0.011 mmol). The mixture was stirred under hydrogen (4 b) at 25°C for 2 h 30. The reaction mixture was then filtered over a Wattman filter (0.2 μm), the filtrate was evaporated, and the residue was taken with 3 x 3 mL of MeCN containing 10 mmol of TFA/mL. It was then evaporated to dryness to give

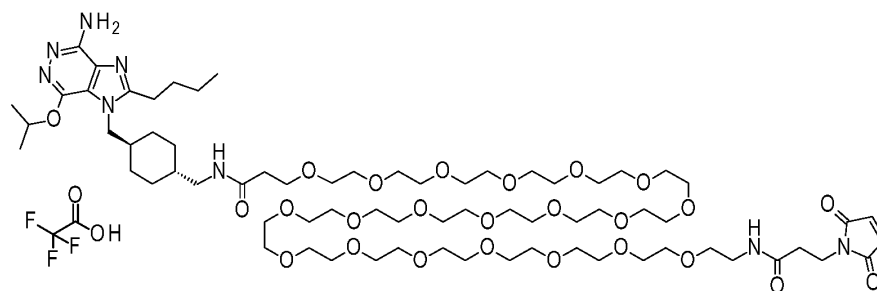
To a vial equipped with magnetic stir, was added a solution of 1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazin-1-yl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propan-1-one

of **Step 1** (73 mg, 0.044 mmol) in DMF (3 mL), followed by addition of (2,5-dioxopyrrolidin-1-yl) 3-(2,5-dioxopyrrol-1-yl)propanoate (11.6 mg, 0.044 mmol) and DIPEA (23 µL, 0.13 mmol). The vial was capped and stirred at rt for 2 h 30. The reaction mixture was directly purified by HPLC using a C18 Waters XBridge column (19.0 x 150 mm



To an autoclave was added Pd/C (10%) (4 mg, 0.037 mmol), N-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanamide (**Example 8**) (40 mg, 29.5 μmol) and MeOH (6 mL). The autoclave was submitted to hydrogenation (2 b), at 25°C for 2 h. The reaction mixture was filtrated over Dicalite 4158 and evaporated to dryness (25°C, 2.5 mb) to give 35 mg of crude product, which was purified by HPLC using a C18 Waters XBridge column (19.0 x 150 mm 5μm), with mobile phase A: water containing 10 mM TFA and mobile phase B: MeCN containing 10 mM TFA, under UV (222 nm) and ELSD detection. The compound containing fractions were combined, concentrated under vacuum (30°C, 15 mb), and freeze-dried to give the expected product (21.5 mg, 59% yield).

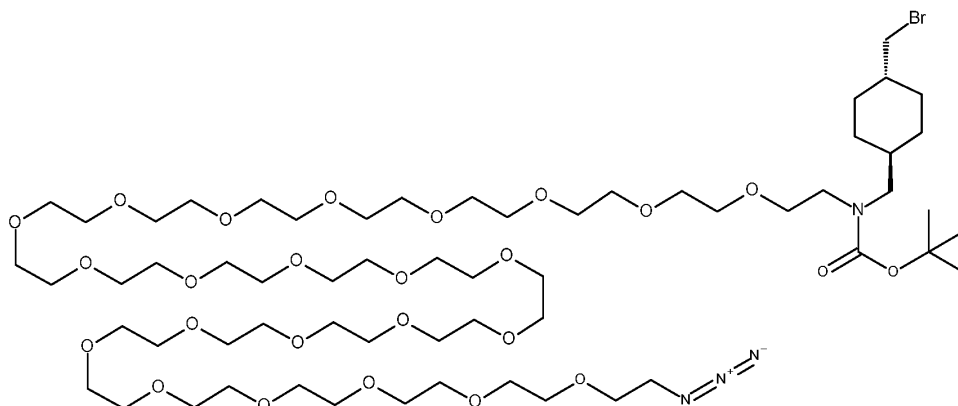
Step 2: Preparation of **Example 10** N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl] cyclohexyl)methylamino]-3-oxopropoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-3-(2,5-dioxopyrrol-1-yl)propanamide, 2,2,2-trifluoroacetate



To a round-bottom flask were added N-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanamide-trifluoroacetate from **Step 1** (16 mg, 11.1 μmol), DIEA (5.8 μL, 33.3 μmol), N-succinimidyl 3-maleimidopropionate (3 mg, 11 μmol) and DMF (0.7 mL). The resulting solution was stirred

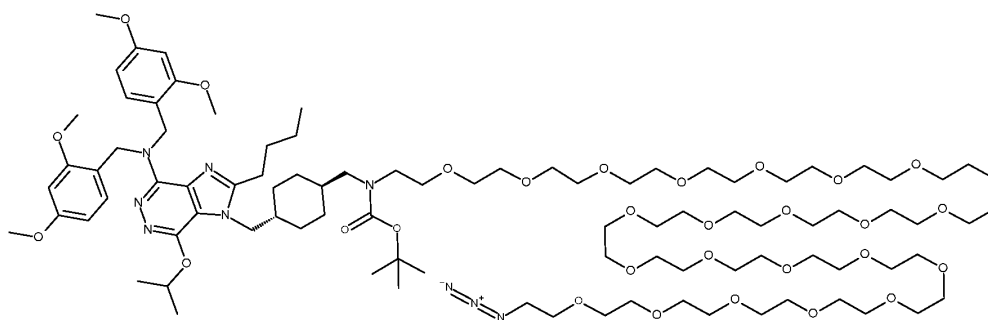
Step 1: Preparation of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]-N-[[*trans*-4-(bromomethyl)cyclohexyl]methyl]ethanamine

Step 2: Preparation of *tert*-butyl N-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-N-[[*trans*-4-(bromomethyl)cyclohexyl]methyl]carbamate



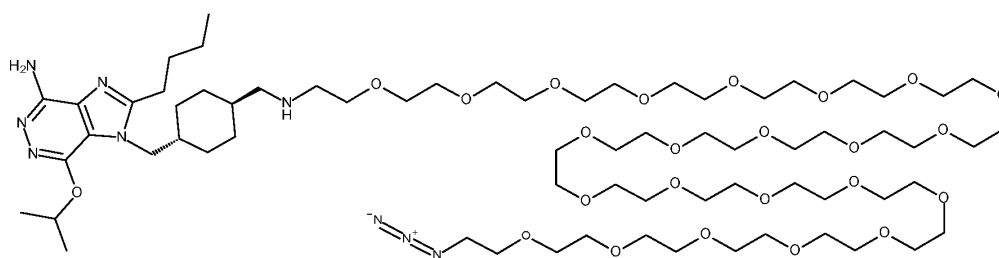
To a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]-N-[[*trans*-4-(bromomethyl)cyclohexyl]methyl]ethanamine of **Step 1** (252 mg, 0.195 mmol) in DCM (11 mL) were added di-*tert*-butyl dicarbonate (215 mg, 0.977 mmol) and pyridine (16 µL, 0.196 mmol). The solution was stirred for 20 h at RT then concentrated to dryness and the crude residue was purified by flash chromatography (Merck, 5 g, 15 -40 µm silica gel), elution with DCM//DCM/MeOH/H₂O (8/2/0.1) 100//0 to 75//25 to afford the expected compound (188 mg, 69%) as a colorless oil.

Step 3: preparation of *tert*-butyl N-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-N-[[*trans*-4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl]methyl]cyclohexyl]methyl]carbamate



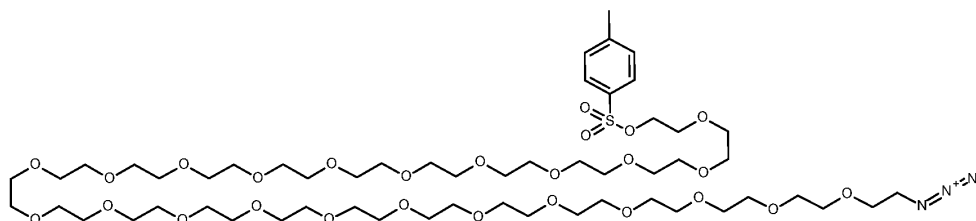
To a solution of **Intermediate M** in Me-THF (3 mL) was added Cs₂CO₃ (139 mg, 0.428 mmol). The suspension was stirred for 1 h at rt then a solution of *tert*-butyl N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-N-[[*trans*-4-(bromo methyl)cyclohexyl]methyl]carbamate from **Step 2** (188 mg, 0.135 mmol) in Me-THF (1 mL) was added. The reaction mixture was heated at 80°C overnight then cooled to rt, diluted with EtOAc (50 mL) and washed with water (3 x 25mL) and brine. The organic phase was dried over MgSO₄, filtered and concentrated to dryness. The crude product was purified by flash chromatography (Merck, 15 g, 15-40 µm silica gel), elution with DCM//DCM/MeOH/H₂O (8/1/0.1) 100//0 to 30//70. The product containing fractions were combined, concentrated and further purified by preparative HPLC using a CSH column (250 x 50mm, 5 µm) with mobile phase A: H₂O + 0.1% formic acid and mobile phase B: MeCN + 0.1% formic acid, under UV detection (222 nm). The compound containing fractions were combined, concentrated under reduced pressure then freeze dried to afford the expected compound (48 mg, 21%).

Step 4: preparation of **Example 11a** 3-[[*trans*-4-[[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-N-[[*trans*-4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-butyl]-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine



To a solution of *tert*-butyl N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-N-[[*trans*-4-[[7-[bis[(2,4-dimethoxyphenyl)methyl]amino]-2-butyl]-4-

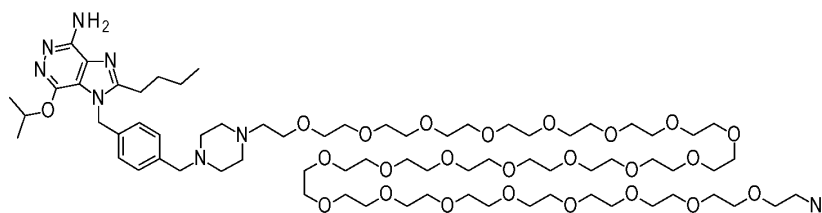
25 **Step 1:** preparation of 71-azido-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,
51,54,57,60,63,66,69-tricosaoxahenheptacontyl 4-methylbenzenesulfonate



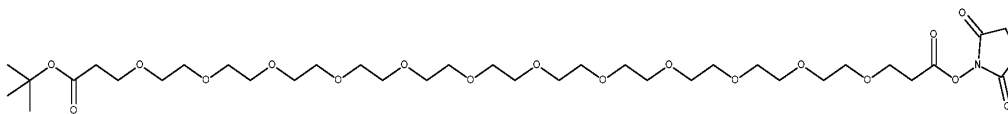
To a solution of 71-azido-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60, 63,66,69-tricosaoxahenptacontan-1-ol (70 mg, 0.064 mmol) in anhydrous DCM (2 mL), at 0°C (ice bath), under argon, was added TEA (71.5 µL, 0.51 mmol), DMAP (3.93 mg, 0.032 mmol) and a solution of p-toluenesulfonyl chloride (85.8 mg, 0.445 mmol) in DCM (2.2 mL). The ice bath was removed after 15 min, and the limpid solution was stirred overnight. To the reaction mixture was added water (5 mL), followed by, 5 min later, DCM (30 mL). After decantation, the organic layer was separated, washed with H₂O (2 x 10 mL) and brine (3 x 10 mL). The organic phase was dried with anhydrous MgSO₄, filtered and concentrated under vacuum to give 91 mg of colorless lacquer, which was purified by silica gel chromatography (Merck, 10 g, silica gel 15-40 µm), with eluent DCM//DCM/MeOH (9/1), from 1//0 to 0//1 to afford the expected compound (71 mg, 87% yield) as colorless oil. ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 2.42 (s, 3 H); 3.36 - 3.41 (m, 2 H); 3.42 - 3.71 (m, 92 H); 4.11 (dd, J=5, 4 Hz, 2 H); 7.48 (d, J=8 Hz, 1 H); 7.78 (d, J=8 Hz, 1 H)

15

Step 2: Preparation of 3-[[[4-[[(4-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl)piperazin-1-yl)methyl]phenyl)methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine

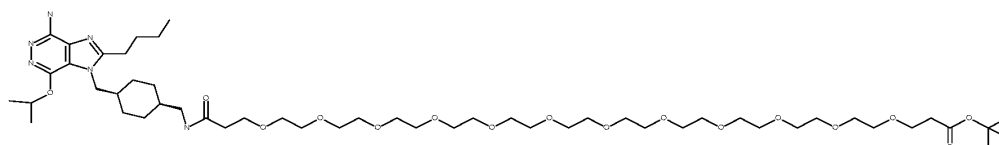


To a solution of 2-butyl-4-isopropoxy-3-[[4-(piperazin-1-ylmethyl)phenyl]methyl]imidazo[4,5-d]pyridazin-7-amine (**Example (TLR1)**) (150 mg, 0.342 mmol) in anhydrous THF (12 mL) was added a solution of 71-azido-



To a solution of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(3-tert-butoxy-3-oxo-propoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid (280 mg, 0.398 mmol) in DCM (5 mL) was added DSC (102 mg, 0.398 mmol) and DIPEA (68 μL, 0.398 mmol). The reaction was stirred overnight at RT then concentrated to dryness and purified by silica gel chromatography (Interchim, 12 g, silica gel 15μm), with DCM/MeOH (100:0 to 90:10) to afford the expected compound (288 mg, 90% yield) as colorless oil.

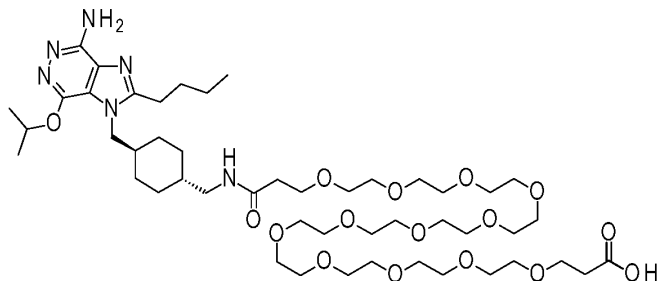
Step 2: preparation of *tert*-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate



To a solution of *tert*-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-(2,5-dioxopyrrolidin-1-yl)oxy-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate from **Step 1** (75 mg, 0.093 mmol) in DCM (5 mL) was added 3-[[*trans*-4-(aminomethyl)cyclohexyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine (**Example (TLR2)**) (35 mg, 0.093 mmol). The resulting solution was stirred overnight at RT then concentrated to dryness and purified by silica gel chromatography (Interchim, 4 g, silica gel 15µm), with eluent DCM //DCM/MeOH/NH₄OH (40/5/0.5) 100//0 to 0//100 to afford the expected compound (86 mg, 78% yield) as a colorless oil.

25 **Step 3:** preparation of **Example 12** 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]

methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]
ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid

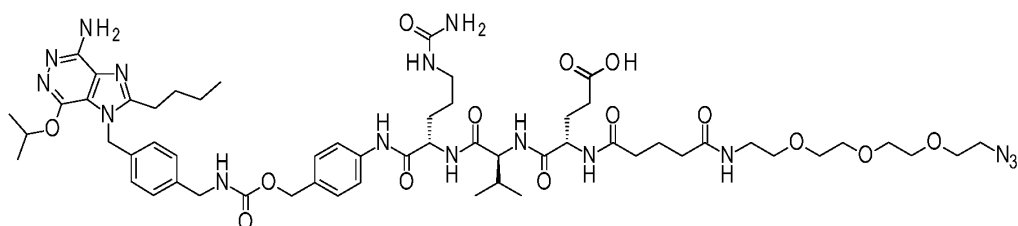


To a solution of 4M HCl in 1,4-dioxane (1 mL, 4 mmol) was added *tert*-butyl 3-[2-[2-[2-
[2-[2-[2-[2-[2-[2-[3-[[*trans*-4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-
3-yl)methyl]cyclohexyl]methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]
ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate from **Step 2** (61 mg,
0.057 mmol). The reaction mixture was stirred for 30 min at RT then concentrated to dryness
and purified by reverse phase chromatography (Merck, 5 g, RP-C18),
H₂O+0.1%TFA/CH₃CN (100/0 to 0/100). The compound containing fractions were
combined, concentrated and further purified by silica gel chromatography (Interchim, 4 g,
silica gel 15µm), with DCM //DCM/MeOH/NH₄OH (12/3/0.5) 100//0 to 0//100 to afford
Example 12 (43 mg, 74% yield) as a colorless oil.

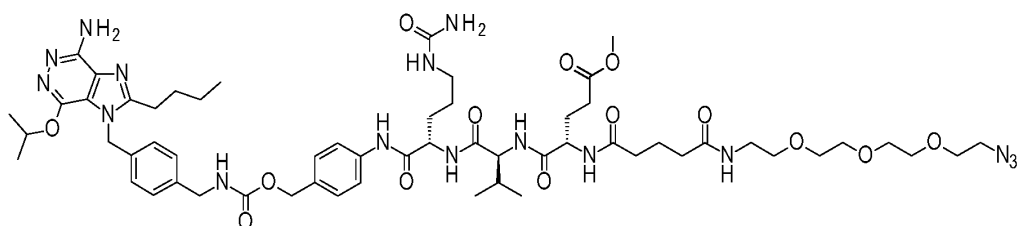
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.71-0.85 (m, 2 H); 0.93 (t, J=7.4 Hz, 3 H); 1.00-1.14 (m, 2 H); 1.28-1.54 (m, 5 H); 1.36 (d, J=6.1 Hz, 6 H); 1.64-1.85 (m, 5 H); 2.28 (t, J=6.4 Hz, 2 H); 2.43 (t, J=6.4 Hz, 2 H); 2.78-2.94 (m, 4 H); 3.41-3.72 (m, 48 H); 4.11 (br d, J=7.3 Hz, 2 H); 5.31-5.47 (m, 1 H); 5.92 (s, 2 H); 7.74 (t, J=5.7 Hz, 1 H)

MS method M2: t_R (min): 2.13; $[M+H]^+$ 1003.6

20 **Example 41:** Preparation of ((4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid (Payload 24)



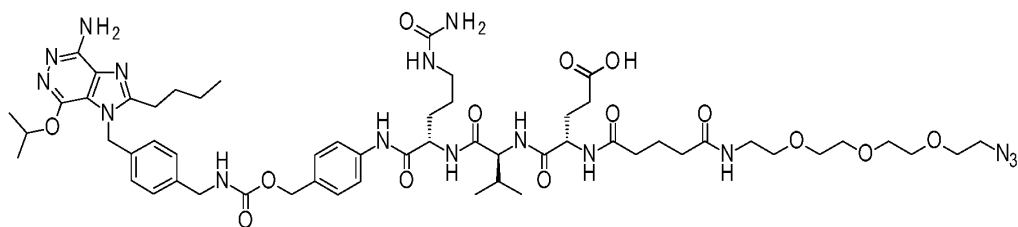
Step 1: Methyl ((4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoate



To a solution of methyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-1-[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate **Linker (L1)** (147 mg, 0.176 mmol) in anhydrous DMF (3.5 mL), were added DIPEA (61 μ L, 0.351 mmol) and 4-nitrophenyl chloroformate (70.8 mg, 0.351 mmol), the yellow mixture was stirred under argon overnight, 1.5 equivalents of DIPEA and of 4-nitrophenyl chloroformate were added in 3 times during 48 h. To the mixture were added a solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, (**Example (TLR3)**) (70.7 mg, 0.19 mmol) in anhydrous DMF (1 mL), followed by addition of DIPEA (33 μ L, 0.19 mmol). The reaction mixture was stirred overnight and then purified by HPLC using a C18 Waters Xbridge OBD column (19 x 150 mm 5 μ m), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (222 nm). The compound containing fractions were combined, concentrated under vacuum (25°C, 100 mb to 30 mb), and freeze-dried to give expected compound (34 mg, 15% yield), which was used directly in the next step.

Step 2: Preparation of **Example 41** ((4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]

anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid

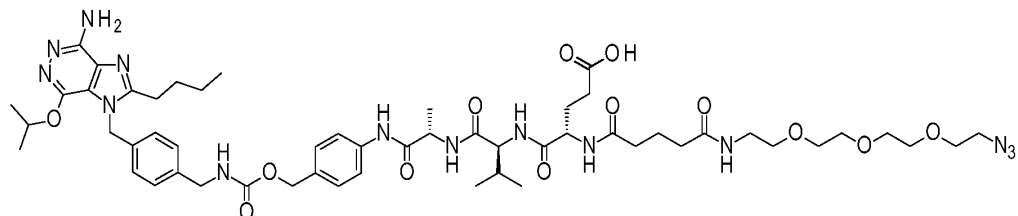


- 5 To a solution of methyl ((4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[[(7-amino-2-butyl-4-propan-2-yl)oxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoate of **Step 1** (63 mg, 0.051 mmol) in a mixture of THF (1.2 mL) and MeOH (0.3 mL), was added at 0 °C, a solution of LiOH (1.47 mg, 0.061 mmol) in H₂O (0.3 mL). The reaction mixture was stirred at 0°C for 4 h and neutralized with IR 120 H⁺ Dowex resin. After filtration, the aqueous layer was purified by HPLC using a C18 Waters Xbridge OBD column (19 x 150 mm, 5µm), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (222 nm). The compound containing fractions were combined, concentrated under vacuum (25°Cc, 100 mb to 30 mb), and freeze-dried to give **Example 41** (15 mg, 24% yield).

- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.80 - 0.87 (m, 9 H); 1.19 (d, J=6 Hz, 6 H); 1.27 - 1.53 (m, 4 H); 1.59 - 1.76 (m, 7 H); 1.84 - 1.91 (m, 1 H); 1.95 - 2.04 (m, 1 H); 2.06 (t, J=7 Hz, 2 H); 2.11 (td, J=7, 3 Hz, 2 H); 2.22 (br s, 2 H); 2.79 (t, J=8 Hz, 2 H); 2.91 - 3.05 (m, 2 H); 3.14 - 3.23 (m, 2 H); 3.36 - 3.64 (m, 14 H); 4.14 (d, J=6 Hz, 2 H); 4.20 (m, 1 H); 4.29 (m, 1 H); 4.35 (q, J=7 Hz, 1 H); 4.94 (s, 2 H); 5.30 (m, 1 H); 5.47 (br s, 2 H); 5.55 (s, 2 H); 6.00 (br s, 2 H); 6.04 - 6.23 (m, 1 H); 7.02 (d, J=8 Hz, 2 H); 7.21 (d, J=8 Hz, 2 H); 7.26 (d, J=8 Hz, 2 H); 7.57 (d, J=8 Hz, 2 H); 7.73 (br t, J=6 Hz, 2 H); 7.84 (t, J=6 Hz, 1 H); 8.04 (d, J=8 Hz, 1 H); 8.18 - 8.38 (m, 1 H); 10.00 (s, 1 H); 11.55 - 13.21 (m, 1 H)
- 25 MS method N: t_R (min): 1.26; [M+H]⁺ 1217.5

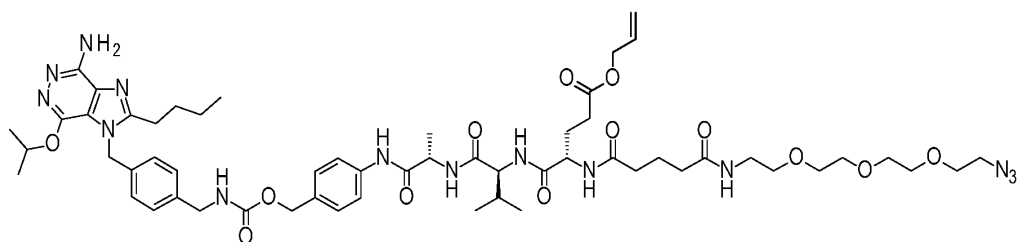
Compound **P25** was prepared by analogy according to this example.

Example 42: Preparation of 4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid (Payload P26)



5

Step 1: Allyl (4S)-5-[[[(1S)-1-[[[(1S)-2-[4-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate



10

To a solution of allyl (4S)-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-[[[(1S)-1-[[[(1S)-2-[4-(hydroxymethyl)anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-5-oxo-pentanoate **Linker (L2)** (84 mg, 0.108 mmol) in anhydrous DMF (3.0 mL), at 0 °C, were added pyridine (17.5 µL, 0.216 mmol) and 4-nitrophenyl chloroformate (22.9 mg, 0.114 mmol), after 15 min, the ice bath was removed and the reaction mixture was stirred at rt for 5 h. Then, to the mixture was added a solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, (**Example (TLR3)**) (39.8 mg, 0.108 mmol) in anhydrous DMF (1 mL), followed by addition of pyridine (8.8 µL, 0.108 mmol). The reaction mixture was stirred overnight and an additional solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (19.9 mg, 0.054 mmol) was added and followed by addition of DIPEA (19 µL, 0.108 mmol). The resulting mixture was further stirred for 3 h and directly purified by HPLC using a C18 Waters Xbridge OBD column (19 x 150 mm 5µm), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (197

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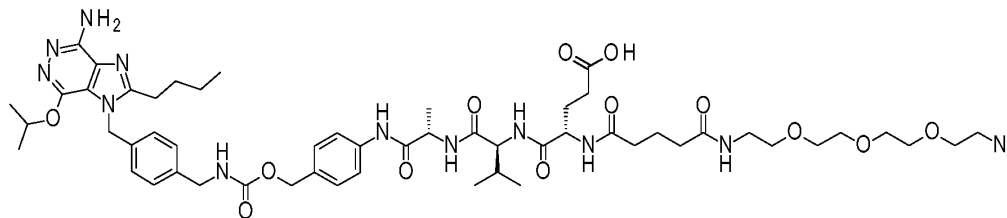
20

nm). The compound containing fractions were combined, concentrated under vacuum, and freeze-dried to give the expected compound (26 mg, 20% yield).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.78 - 0.89 (m, 9 H); 1.20 (d, J=6 Hz, 6 H); 1.26 - 1.48 (m, 2 H); 1.30 (d, J=7 Hz, 3 H); 1.57 - 1.82 (m, 5 H); 1.86 - 2.02 (m, 2 H); 2.02 - 2.15 (m, 4 H); 2.31 - 2.40 (m, 2 H); 2.75 - 2.82 (m, 2 H); 3.10 - 3.22 (m partially hidden, 2 H); 3.34 - 3.42 (m partially hidden, 4 H); 3.46 - 3.56 (m partially hidden, 8 H); 3.57 - 3.63 (m partially hidden, 2 H); 4.14 (d, J=6 Hz, 2 H); 4.16 - 4.20 (m, 1 H); 4.28 - 4.35 (m, 1 H); 4.38 (t, J=7 Hz, 1 H); 4.52 (dt, J=5, 1 Hz, 2 H); 4.95 (s, 2 H); 5.15 - 5.21 (m, 1 H); 5.22 - 5.36 (m, 2 H); 5.55 (s, 2 H); 5.83 - 5.96 (m, 3 H); 7.02 (d, J=8 Hz, 2 H); 7.21 (d, J=8 Hz, 2 H); 7.26 (br d, J=8 Hz, 2 H); 7.55 (br d, J=8 Hz, 2 H); 7.67 - 7.75 (m, 2 H); 7.80 (t, J=6 Hz, 1 H); 8.02 (d, J=8 Hz, 1 H); 8.21 (d, J=7 Hz, 1 H); 9.94 (br s, 1 H)

MS method N: *t*_R (min): 1.44; [M+H]⁺ 1171.4

Step 2: Preparation of **Example 42** (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid



To a solution of allyl (4S)-5-[[[(1S)-1-[[[(1S)-2-[4-[[4-[(7-amino-2-butyl-4-isopropoxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylcarbamoyloxymethyl]anilino]-1-methyl-2-oxo-ethyl]carbamoyl]-2-methyl-propyl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-5-oxo-pentanoate of **Step 1** (25 mg, 0.021 mmol) in a mixture of EtOAc (1.5 mL) and DCM (1.5 mL), was added at 0 °C, a solution of tetrakis(triphenylphosphine)Palladium (0) (9.68 mg, 0.008 mmol) in a mixture of EtOAc (0.75 mL) and DCM (0.75 mL), followed by addition of a solution of 1,3-dimethylbarbituric acid (8.4 mg, 0.051 mmol) in a mixture of EtOAc (0.24 mL) and DCM (0.24 mL). The ice bath was removed after 15 min and the reaction mixture was stirred for 48 h. Then, the reaction mixture was evaporated to dryness and the crude material was taken

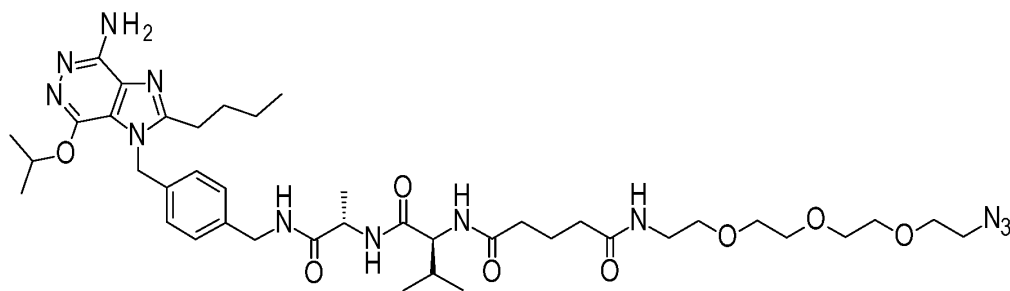
into MeOH, with after filtration over a 0.45 μ m membrane, the methanolic solution was evaporated to dryness to give a yellow lacquer (46 mg). The crude material was purified by HPLC using a C18 Waters Xbridge column (3 x 150 mm 5 μ m), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (222 nm). The compound containing fractions were combined, concentrated under vacuum, and freeze-dried to give **Example 42** (8.2 mg, 34% yield).

^1H NMR (400 MHz, δ in ppm, DMSO- d_6): 0.78 - 0.87 (m, 9 H); 1.20 (d, J=6 Hz, 6 H); 1.27 - 1.37 (m, 2 H); 1.33 (d, J=7 Hz, 3 H); 1.56 - 1.66 (m, 2 H); 1.66 - 1.74 (m, 2 H); 1.74 - 1.83 (m, 2 H); 1.96 - 2.17 (m, 7 H); 2.79 (t, J=8 Hz, 2 H); 3.14 - 3.21 (m, 2 H); 3.22 - 3.63 (m, 14 H); 4.09 - 4.18 (m, 4 H); 4.35 (quin, J=7 Hz, 1 H); 4.93 (s, 2 H); 5.31 (m, 1 H); 5.55 (s, 2 H); 5.90 (s, 2 H); 7.02 (d, J=8 Hz, 2 H); 7.21 (d, J=8 Hz, 2 H); 7.25 (d, J=8 Hz, 2 H); 7.63 (d, J=8 Hz, 2 H); 7.74 (t, J=6 Hz, 1 H); 8.02 (d, J=9 Hz, 1 H); 8.18 (t, J=5 Hz, 1 H); 8.58 (d, J=7 Hz, 1 H); 9.08 (br d, J=5 Hz, 1 H); 10.40 (s, 1 H)

MS method N: t_R (min): 1.32; $[\text{M}+\text{H}]^+$ 1131.1

Compound **P27** was prepared by analogy according to this example.

Example 43: Preparation of N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide (Payload P28)



To a solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, (**Example (TLR3)**) (50 mg, 0.136 mmol) and (1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oyl)-L-valyl-L-alanine **Linker (L4)** (75 mg, 0.149 mmol) in anhydrous DMF (2 mL), were added HATU (165 mg, 0.434 mmol), and HOAt (54.7 mg, 0.393 mmol), followed by dropwise addition of DIPEA (94.8 μ L, 0.542 mmol). The

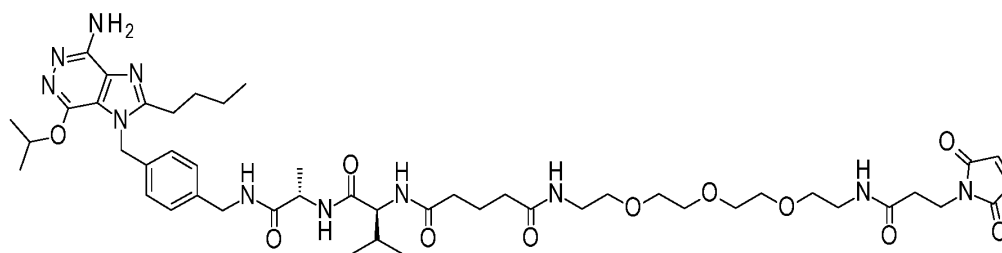
resulting yellow solution was protected from light and stirred at rt, under argon for 24 h. Then, the reaction mixture was filtered over a 0.45 µm membrane and purified by HPLC using a C18 Waters Xbridge column (19 x 150 mm 5µm), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (200 nm). The compound containing fractions were combined, concentrated under vacuum (33°C, 80 mb), and freeze-dried to give **Example 43** (45 mg, 39% yield).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.73 - 0.81 (m, 6 H); 0.84 (t, J=7.3 Hz, 3 H); 1.16 - 1.37 (m, 11 H); 1.59 - 1.71 (m, 4 H); 1.92 (m, 1 H); 2.04 (t, J=7.5 Hz, 2 H); 2.08 - 2.24 (m, 2 H); 2.71 - 2.84 (m, 2 H); 3.15 - 3.20 (m, 2 H); 3.36 - 3.67 (m, 14 H); 4.13 (dd, J=8.6, 6.6 Hz, 1 H); 4.19 - 4.30 (m, 3 H); 5.32 (quin, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.91 (s, 2 H); 7.00 (d, J=8.1 Hz, 2 H); 7.19 (d, J=8.1 Hz, 2 H); 7.79 (d, J=8.8 Hz, 1 H); 7.82 (t, J=5.6 Hz, 1 H); 8.00 (d, J=7.1 Hz, 1 H); 8.30 (t, J=5.9 Hz, 1 H)

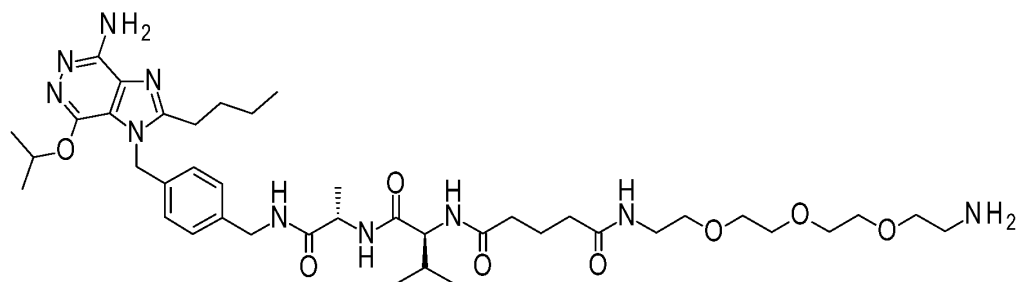
MS method N: t_R (min): 1.22; [M+H]⁺ 853.4

Compound **P29** was prepared by analogy according to this example.

Example 44: Preparation of N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide (Payload P30)

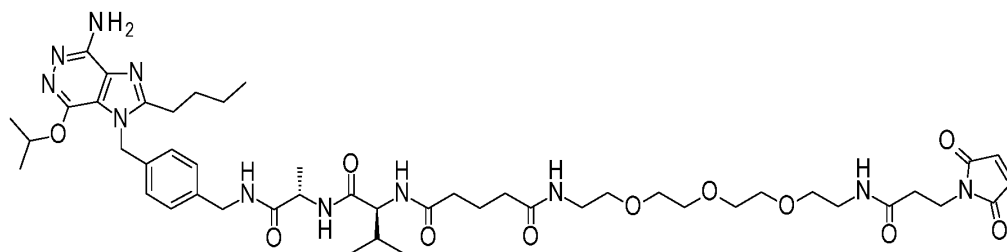


Step 1: N1-((S)-1-(((S)-1-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-N5-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)glutaramide



To an autoclave was added Pd/C (10%) (2 mg, 0.054 mmol), N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide, (**Example 43**) (29.5 mg, 34.6 μ mol) and MeOH (6 mL). The autoclave was submitted to hydrogenation (2 bars) at 25°C for 2 h. The reaction mixture was filtrated over 0.2 μ m PVDF Membrane Syringe Filter and evaporated to dryness (25°C, 2.5 mb) to give 30 mg of crude product, which was purified by chromatography on silica gel (Merck, 5 g, silica gel 15-40 μ m), with DCM/MeOH/Ammonia (35%) 12/1.5/0.25 elution to afford the expected compound (11 mg, 38% yield).

Step 2: Preparation of **Example 44** N1-((S)-1-(((S)-1-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-N5-(15-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-13-oxo-3,6,9-trioxa-12-azapentadecyl)glutaramide

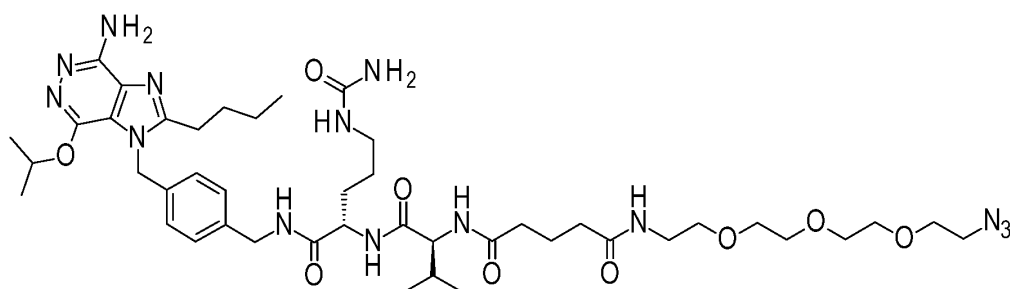


To a round-bottom flask were added N1-((S)-1-(((S)-1-((4-((4-amino-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-1-yl)methyl)benzyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-N5-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)glutaramide from **Step 1** (10.0 mg, 12.9 μ mol), DIEA (6.3 μ L, 36 μ mol), N-succinimidyl 3-maleimidopropionate (3.4 mg, 13 μ mol) and DCM (2 mL). The resulting troubling white solution was stirred at rt for 0.5 h then EtOAc (100 μ L) containing 10% AcOH was added

and the reaction mixture was evaporated to dryness under vacuum. The crude product was purified by chromatography on a Macherey Nagel cartridge (4 g, 15-40 μ m amino silica gel), with eluent $\text{CH}_2\text{Cl}_2/\text{iPrOH}$ (80/20) containing 0.5% AcOH, to afford **Example 44** (7.57 mg, 80%).

- 5 ^1H NMR (400 MHz, δ in ppm, $\text{DMSO}-d_6$): 0.71 - 0.93 (m, 9 H); 1.12 - 1.45 (m, 11 H); 1.55 - 1.77 (m, 4 H); 1.90 - 1.98 (m, 1 H); 1.98 - 2.07 (m, 2 H); 2.09 - 2.19 (m, 2 H); 2.30 - 2.37 (m, 2 H); 2.73 - 2.82 (m, 2 H); 3.17 (m, 4 H); 3.30 - 3.54 (m partially hidden, 12 H); 3.56 - 3.65 (m, 2 H); 4.13 (dd, $J=9$, 7 Hz, 1 H); 4.19 - 4.39 (m, 3 H); 5.32 (quin, $J=6$ Hz, 1 H); 5.54 (s, 2 H); 5.89 (s, 2 H); 6.94 - 7.06 (m, 4 H); 7.19 (d, $J=8$ Hz, 2 H); 7.76 (d, $J=9$ Hz, 1 H);
 10 7.80 (br t, $J=6$ Hz, 1 H); 7.96 - 8.04 (m, 2 H); 8.28 (br t, $J=6$ Hz, 1 H)
 MS method N: t_R (min): 1.12; $[\text{M}+\text{H}]^+$ 978.1

Example 45: Preparation of N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxo
 15 pentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide (Payload P31)



- Under argon, to a three-neck round-bottom flask containing EDCI (260 mg, 1.36 mmol) were added DCM (10.5 mL) and DMF (10.5 mL), the reaction mixture was stirred at rt till
 20 the end of dissolution. It was then placed in an ice bath, and to it, were added ethyl cyanoglyoxylate-2-oxime (197 mg, 1.36 mmol), and (2S)-2-[[[(2S)-2-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxo-pentanoyl]amino]-3-methyl-
 butanoyl]amino]-5-ureido-pentanoic acid **Linker (L4)** (887.4 mg, 1.36 mmol) in a mixture of DCM (10.5 mL) and DMF (10.5 mL), followed by addition of 3-[[4-
 25 (aminomethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine, (**Example (TLR3)**) (500 mg, 1.36 mmol). The reaction mixture was stirred at 0°C and allowed slowly to reach rt and stirred at rt overnight. It was then diluted in toluene (80 mL)

and evaporated under vacuum (30°C, 15 mb) to dryness to give 2.25 g of crude product. The crude material was purified by chromatography on silica gel (Interchim, 120 g, silica gel 30 µm), with EtOAc/MeOH/H₂O (7/2/1) elution, to afford 873 mg of expected compound as a pale yellow laquer which was triturated in 5 mL MeCN. The suspension was filtrated,
 5 washed with acetonitrile (2 mL), and dried (30°C, 0.2 mb) to give **Example 45** (841 mg, 66%).

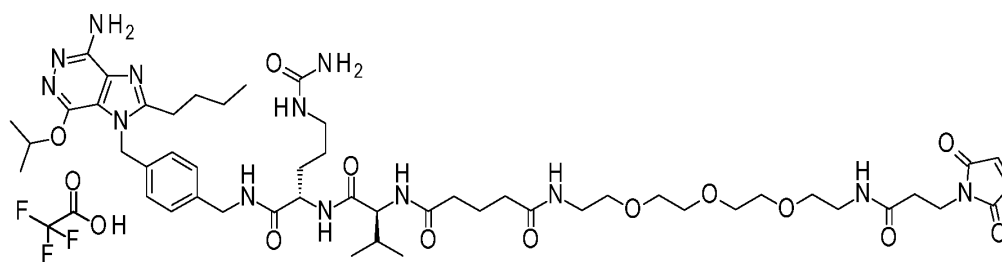
¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.78 (d, J=6 Hz, 6 H); 0.84 (t, J=7 Hz, 3 H); 1.22 (d, J=6 Hz, 6 H); 1.25 - 1.41 (m, 4 H); 1.45 - 1.56 (m, 1 H); 1.56 - 1.77 (m, 5 H); 1.93 (m, 1 H); 2.05 (t, J=7 Hz, 2 H); 2.09 - 2.21 (m, 2 H); 2.82 (t, J=8 Hz, 2 H); 2.92 (q, J=6 Hz, 2 H);
 10 3.18 (q, J=6 Hz, 2 H); 3.29 - 3.66 (m partially hidden, 14 H); 4.07 - 4.32 (m, 4 H); 5.24 (spt, J=6 Hz, 1 H); 5.35 (br s, 2 H); 5.58 (s, 2 H); 5.92 (t, J=5 Hz, 1 H); 6.85 - 7.30 (m, 2 H); 7.03 (d, J=8 Hz, 2 H); 7.19 (d, J=8 Hz, 2 H); 7.79 (d, J=9 Hz, 1 H); 7.84 (t, J=6 Hz, 1 H); 7.94 (d, J=8 Hz, 1 H); 8.36 (t, J=6 Hz, 1 H)

MS method N: t_R (min): 1.13; [M+H]⁺ 939.5

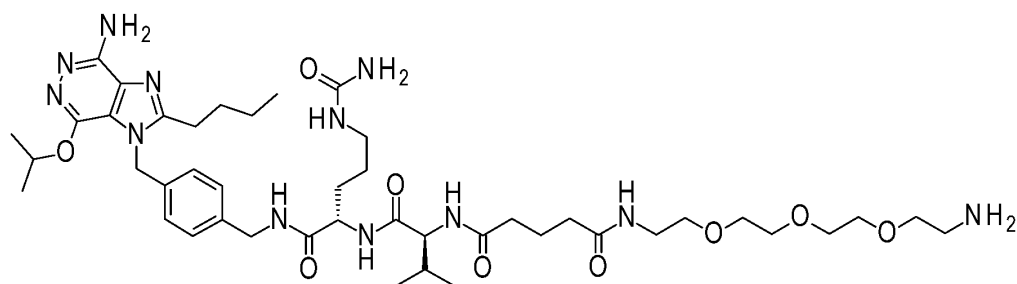
15

Compound **P32** was prepared by analogy according to this example.

Example 46: Preparation of N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-
 yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-
 20 oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-
 yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate
 (Payload P33)



Step 1: N'-[(1S)-1-[[[(1S)-1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-
 25 -yl)methyl]phenyl]methylcarbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-
 [2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl]pentanediamide

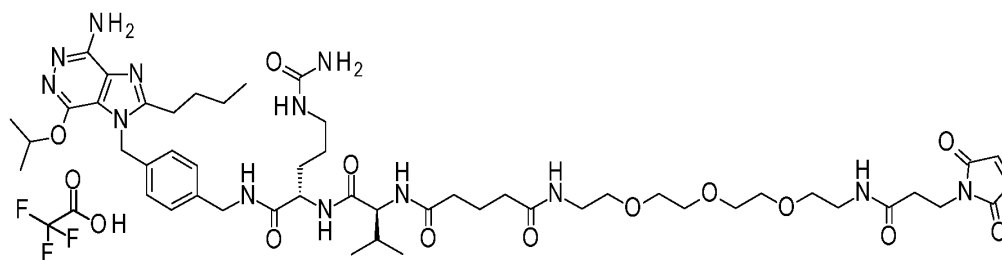


To an autoclave was added Pd/C (10%) (46 mg, 0.043 mmol), N'-[(1S)-1-[[[(1S)-1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide (**Example 45**) (456 mg, 0.485 mmol) and MeOH (220 mL). The autoclave was submitted to hydrogenation (4 bars), at 20°C for 2.5 h. The reaction mixture was filtrated over Glass Microfiber Filter (Whatman) and evaporated to dryness (40°C, 15 mb) to give 440 mg of crude product, which was purified by chromatography on silica gel (Interchim, 25g, silica gel 30 µm), with DCM/MeOH/Ammonia (35%) 12/3/0.5 elution to afford the expected compound (265 mg, 60% yield).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.78 (br d, J=7 Hz, 6 H); 0.84 (t, J=7 Hz, 3 H); 1.21 (br d, J=6 Hz, 6 H); 1.26 - 1.41 (m, 4 H); 1.42 - 1.55 (m, 1 H); 1.56 - 1.77 (m, 5 H); 1.81 - 1.98 (m, 1 H); 2.04 (t, J=7 Hz, 2 H); 2.08 - 2.25 (m, 2 H); 2.62 - 2.71 (m, 2 H); 2.73 - 2.85 (m, 2 H); 2.88 - 2.97 (m, 2 H); 3.13 - 3.21 (m, 2 H); 3.28 - 3.44 (m partially hidden, 4 H); 3.50 (br s, 8 H); 4.09 - 4.17 (m, 1 H); 4.16 - 4.38 (m, 3 H); 5.23 - 5.37 (m, 1 H); 5.35 (s, 2 H); 5.54 (s, 2 H); 5.81 - 6.05 (m, 3 H); 7.00 (d, J=8 Hz, 2 H); 7.18 (d, J=8 Hz, 2 H); 7.80 (d, J=8 Hz, 1 H); 7.87 (t, J=6 Hz, 1 H); 7.96 (d, J=8 Hz, 1 H); 8.34 (t, J=6 Hz, 1 H)
MS method N: t_R (min): 0.89; [M+H]⁺ 913.2

20

Step 2: Preparation of **Example 46** N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate



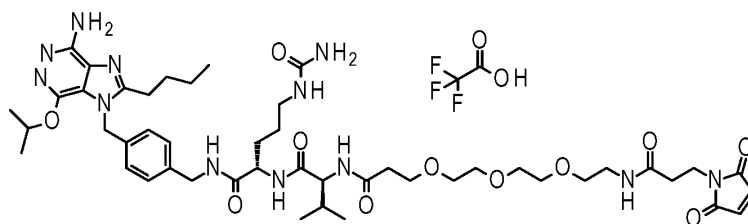
Under argon, to a three-neck round-bottom flask were added N'-[(1S)-1-[[[(1S)-1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-(2-aminoethoxy) ethoxy]ethoxy]ethyl]pentanediamide of **Step 1**, (98.5 mg, 0.108 mmol) and 1M HCl in EtOAc (5.4 mL). The resulting troubling white solution was stirred at rt for 1 h, giving a pale pink solution. The reaction mixture was then evaporated to dryness under vacuum, and dried in a stove overnight (30°C, 0.2 mb) to give 102 mg of sticky mass, which was added to a three-neck round-bottom flask, followed by addition of anhydrous DMF (4 mL), (2,5-dioxopyrrolidin-1-yl) 3-(2,5-dioxopyrrol-1-yl)propanoate (28.7 mg, 0.106 mmol) and DIPEA (56.4 µL, 0.324 mmol). The reaction mixture was stirred at rt for 1.5 h, giving a pale pink yellow solution. To this solution, was added EtOAc (30 mL) containing 10% of TFA and followed by evaporation under vacuum (30°C, 15 mb) to give pale yellow oil, which was dissolved in distilled H₂O. After free drying, 200 mg of crude product was obtained, which was purified by HPLC using a C18 Waters Xbridge column (19 x 150 mm 5µm), with mobile phase A: H₂O + 10 mM TFA, and mobile phase B: MeCN + 10 mM TFA under UV detection (210 nm). The compound containing fractions were combined, concentrated under vacuum (30°C, 80 mb), and freeze-dried to give **Example 46** (48 mg, 38% yield).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.74 - 0.80 (m, 6 H); 0.84 (t, J=7 Hz, 3 H); 1.24 (d, J=6 Hz, 6 H); 1.25 - 1.42 (m, 5 H); 1.42 - 1.55 (m, 1 H); 1.56 - 1.74 (m, 4 H); 1.88 - 1.98 (m, 1 H); 2.05 (t, J=8 Hz, 2 H); 2.09 - 2.22 (m, 2 H); 2.33 (t, J=7 Hz, 2 H); 2.85 - 2.96 (m, 4 H); 3.10 - 3.21 (m, 4 H); 3.31 - 3.52 (m partially hidden, 12 H); 3.56 - 3.62 (m, 2 H); 4.13 (dd, J=8, 7 Hz, 1 H); 4.17 - 4.34 (m, 3 H); 5.15 (spt, J=6 Hz, 1 H); 5.25 - 5.45 (m, 2 H); 5.63 (s, 2 H); 5.86 - 5.97 (m, 1 H); 7.00 (s, 2 H); 7.06 (d, J=8 Hz, 2 H); 7.21 (d, J=8 Hz, 2 H); 7.79 (d, J=9 Hz, 1 H); 7.84 (t, J=5 Hz, 1 H); 7.93 (d, J=8 Hz, 1 H); 8.01 (t, J=6 Hz, 1 H); 8.36 (t, J=6 Hz, 1 H); 8.58 (br s, 2 H); 13.69 (s, 1 H)

MS method N: t_R (min): 1.07; [M+H]⁺ 1064.5

Compound **P34** was prepared by analogy according to this example.

Example 47: Preparation of (2S)-N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide;2,2,2-trifluoroacetic acid (Payload P35)



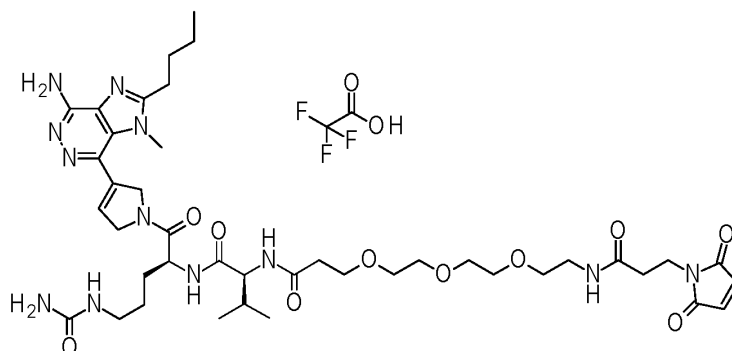
To a round-bottom flask containing EDCI (16 mg, 0.086 mmol) in DCM (1 mL) and DMF (1 mL), was added at 4°C ethyl cyanoglyoxylate-2-oxime (13 mg, 0.093 mmol), and (2S)-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid;2,2,2-trifluoroacetic acid, **Linker (L9)** (60 mg, 0.081 mmol) in DMF (1 mL), followed by addition of 3-[[4-(aminomethyl)phenyl]methyl]-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-7-amine, **Example (TLR3)** (30 mg, 0.0814 mmol). The reaction mixture was allowed to reach rt and further stirred 3 h at rt. TFA (15 µL) was added and the reaction mixture was concentrated to dryness. The crude material was purified by preparative HPLC using a C18 Waters Xselect CSH column (30 x 150 mm 5µm), with mobile phase A: H₂O + 0.1% TFA, and mobile phase B: MeCN, under UV detection. The compound containing fractions were combined, concentrated under vacuum, and freeze-dried to give **Example 47** (27 mg, 30% yield).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.73 - 0.81 (m, 6 H); 0.84 (t, J=7.4 Hz, 3 H); 1.24 (d, J=6.1 Hz, 6 H); 1.26 - 1.42 (m, 4 H); 1.43 - 1.55 (m, 1 H); 1.56 - 1.73 (m, 3 H); 1.86 - 1.99 (m, 1 H); 2.28 - 2.50 (m partially hidden, 4 H); 2.83 - 2.99 (m, 4 H); 3.14 (q, J=5.8 Hz, 2 H); 3.36 (t, J=5.8 Hz, 2 H); 3.41 - 3.52 (m, 8 H); 3.53 - 3.64 (m, 4 H); 4.12 - 4.35 (m, 4 H); 5.09 - 5.21 (m, 1 H); 5.22 - 5.52 (m, 2 H); 5.63 (s, 2 H); 5.87 - 5.97 (m, 1 H); 7.00 (s, 2 H); 7.06 (d, J=8.3 Hz, 2 H); 7.21 (d, J=8.3 Hz, 2 H); 7.84 (d, J=8.6 Hz, 1 H); 7.96 (d, J=7.9 Hz, 1 H); 8.01 (br t, J=5.5 Hz, 1 H); 8.35 (t, J=5.9 Hz, 1 H); 8.60 (br s, 2 H); 13.74 (br s, 1 H)

MS method N1: t_R (min): 1.09; $[M+H]^+$ 979.3; $[M+2H]^{2+}$ 490.4

Compounds **P36**, **P39** and **P40** were prepared by analogy according to this example.

- 5 **Example 48:** Preparation of (2S)-N-[(1S)-1-[3-(4-amino-2-butyl-1-methyl-imidazo[4,5-d]pyridazin-7-yl)-2,5-dihydropyrrole-1-carbonyl]-4-ureido-butyl]-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanamide;2,2,2-trifluoroacetic acid (Payload P37)

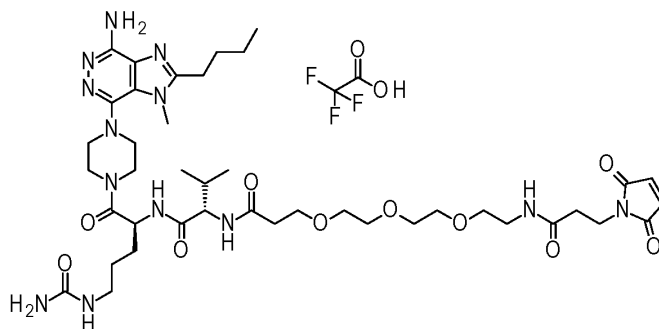


- 10 To a solution of (2S)-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid;2,2,2-trifluoroacetic acid, **Linker (L9)** (62 mg, 0.084 mmol) in DMF (1 mL) were added DIPEA (15 μ L, 0.085 mmol), a catalytic amount of DMAP, 2-butyl-7-(2,5-dihydro-1H-pyrrol-3-yl)-1-methyl-imidazo[4,5-d]pyridazin-4-amine;hydrochloride, **Example**
- 15 **(TLR8)** (26 mg, 0.084 mmol), HATU (32 mg, 0.084 mmol), followed by dropwise addition of DIPEA (30 μ L, 0.170 mmol). The resulting brown solution was stirred at rt, under argon for 1 h. Then, TFA (30 μ L) was added, and the reaction mixture was concentrated under vacuum then purified by reverse phase column chromatography using as eluent a mixture of H_2O + 0.1% TFA/ CH_3CN (95:5 to 0:100). The compound containing fractions were pooled,
- 20 concentrated under vacuum then freeze dried to give **Example 48** (23 mg, 27% yield).
 1H NMR (500 MHz, δ in ppm, $DMSO-d_6$): 0.77 - 0.87 (m, 6 H); 0.95 (t, $J=7.4$ Hz, 3 H); 1.29 - 1.50 (m, 4 H); 1.51 - 1.63 (m, 1 H); 1.64 - 1.74 (m, 1 H); 1.74 - 1.85 (m, 2 H); 1.90 - 2.00 (m, 1 H); 2.26 - 2.54 (m partially hidden , 4 H); 2.92 - 3.04 (m, 4 H); 3.11 - 3.18 (m, 2 H); 3.29 - 3.74 (m partially hidden, 14 H); 3.83 - 3.88 (m, 3 H); 4.19 - 4.28 (m, 1 H); 4.31 - 5.03
- 25 (m, 5 H); 5.06 - 5.60 (m, 2 H); 5.89 - 6.00 (m, 1 H); 6.50 (br s, 1 H); 6.99 (s, 2 H); 7.74 -

7.84 (m, 1 H); 7.96 - 8.04 (m, 1 H); 8.17 (br d, J=6.3 Hz, 1 H); 8.68 - 9.05 (m, 2 H); 14.41 - 14.81 (m, 1 H)

MS method N1: t_R (min): 0.94; $[M+H]^+$ 883.2, $[M+2H]^{2+}$ 442.4

- 5 **Example 49:** Preparation of (2S)-N-[(1S)-1-[4-(7-amino-2-butyl-3-methyl-imidazo[4,5-d]pyridazin-4-yl)piperazine-1-carbonyl]-4-ureido-butyl]-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanamide;2,2,2-trifluoroacetic acid (Payload P38)



- 10 To a round-bottom flask containing EDCI (17 mg, 0.091 mmol) in DCM (1 mL) and DMF (1 mL), was added at 4°C ethyl cyanoglyoxylate-2-oxime (14 mg, 0.010 mmol), and (2S)-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanoic acid;2,2,2-trifluoroacetic acid **Linker (L9)** (64 mg, 0.086 mmol) in DMF (1 mL), followed by addition of 2-butyl-3-methyl-4-piperazin-1-yl-imidazo[4,5-d]pyridazin-7-amine, **Example (TLR9)** (25 mg, 0.087 mmol). The reaction was stirred at 4°C for 1 h then warmed to rt and left overnight. TFA (10 μ L) was added, and the solution was concentrated to dryness before purification by reverse phase column chromatography using as eluent a mixture of H₂O + 0.1% TFA/CH₃CN (95:5 to 20:80). The compound containing fractions were pooled, concentrated under vacuum then freeze dried to give **Example 49** (42 mg, 49% yield).

- ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.77 - 0.88 (m, 6 H); 0.95 (t, J=7.3 Hz, 3 H); 1.31 - 1.58 (m, 5 H); 1.59 - 1.71 (m, 1 H); 1.79 (quin, J=7.6 Hz, 2 H); 1.90 - 2.04 (m, 1 H); 2.27 - 2.50 (m, 4 H); 2.88 - 3.23 (m, 6 H); 3.36 (t, J=5.9 Hz, 1 H); 3.43 - 3.53 (m, 8 H); 3.53 - 4.18 (m, 12 H); 4.02 (s, 3 H); 4.22 (br dd, J=8.8, 6.6 Hz, 1 H); 4.70 - 4.79 (m, 1 H); 5.04 - 5.70 (m, 2 H); 5.87 - 6.00 (m, 1 H); 7.00 (s, 2 H); 7.82 (d, J=8.9 Hz, 1 H); 8.01 (br t, J=5.5 Hz, 1 H); 8.10 (br d, J=7.6 Hz, 1 H); 8.61 (br s, 2 H); 13.93 (br s, 1 H)

25 MS method N: t_R (min): 1.37; [M+H]⁺ 1522.5

[illegible]

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7 Hz, 3 H); 1.24 (d, J=6 Hz, 6 H); 1.33 (sxt, J=7 Hz, 2 H); 1.64 (quin, J=8 Hz, 2 H); 2.27 - 2.39 (m, 4 H); 2.88 (t, J=8 Hz, 2 H); 3.14

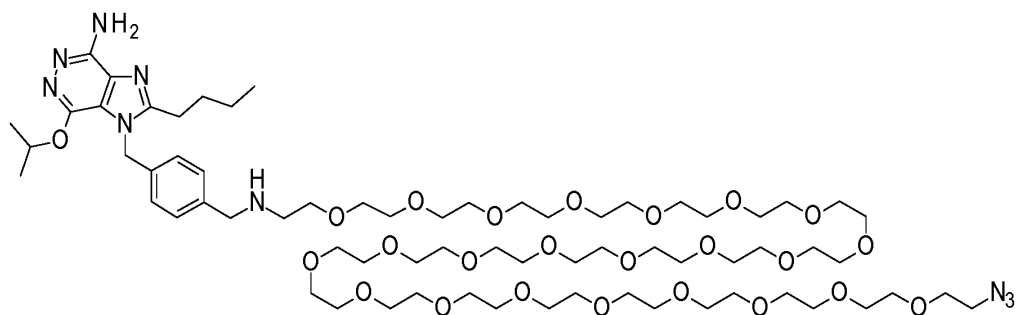
To a stirred solution of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]

[illegible]

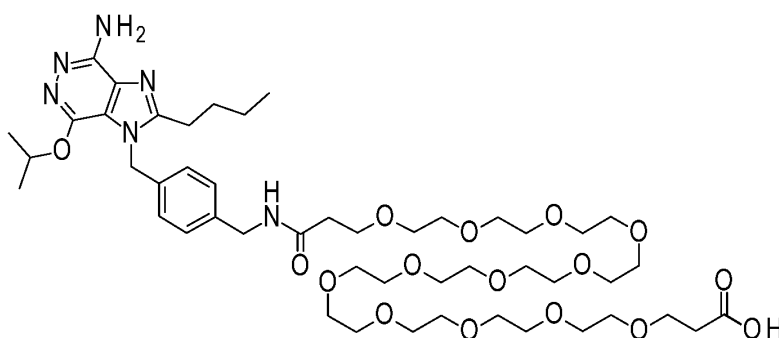
gel chromatography (Merck, 10 g, silica gel 15-40 μ m), with eluent DCM//DCM/MeOH (9/1), from 1/0 to 0/1 to afford the expected compound (71 mg, 87% yield) as colorless oil. ^1H NMR (400 MHz, δ in ppm, DMSO- d_6): 2.42 (s, 3 H); 3.36 - 3.41 (m, 2 H); 3.42 - 3.71 (m, 92 H); 4.11 (dd, J=5, 4 Hz, 2 H); 7.48 (d, J=8 Hz, 1 H); 7.78 (d, J=8 Hz, 1 H)

5

Step 2: Preparation of **Example 53** 3-[[[4-[[2-(2-azidoethoxy)ethoxy]ethylamino)methyl]phenyl)methyl]-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-7-amine



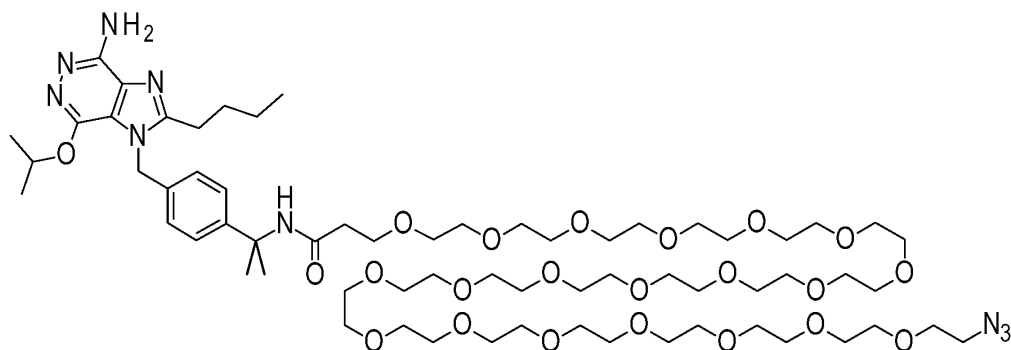
To a solution of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, **Example (TLR3)** (20 mg, 0.054 mmol) in anhydrous DCM (2 mL), at 0 °C, under argon, was added a solution of 71-azido-3,6,9,12,15,18,21,24,27,30,33,36,39, 42,45,48,51,54,57,60,63,66,69-tricosaoxahenheptacontyl 4-methylbenzene-sulfonate from **Step 1** (68.1 mg, 0.054 mmol) in anhydrous DCM (6 mL), followed by addition of TEA (45 µL, 0.327 mmol). The ice bath was removed, the reaction mixture was stirred overnight at rt, and heated at 50°C, for 4 h. DCM was then evaporated at reduced pressure (40°C, 600 mb) and replaced with THF (6 mL). The reaction mixture was then heated under reflux at 80°C for 72 h. The mixture was evaporated to dryness to give 132 mg of crude material, which was purified twice by HPLC using a C18 Waters Xbridge OBD column (19 x 150 mm 5µm), with mobile phase A: 10 mM ammonium bicarbonate and mobile phase B: MeCN, under UV detection (222 nm). The compound containing fractions were combined, concentrated under vacuum (25°C, 100 mb to 30 mb), and freeze-dried to give **Example 53** (15.7 mg, 20% yield).



To a solution of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methylamino]-3-oxo-propoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid from **Step 3** (6.66 mg, 6.68 μmol) in DCM (1 mL), were added tetrafluorophenol (143 μL,

To a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol (2 g, 2.162 mmol) in anhydrous THF (15 mL) was added sodium(0) (16 mg, 0.696 mmol) and *tert*-butyl acrylate (400 μ L, 2.162 mmol) 30 min later. The mixture was reacted for 8 h at rt then, under LCMS monitoring, new additions of *tert*-butyl acrylate (a total of 300 μ L, 3.243 mmol) and sodium (11 mg, 0.478 mmol) were needed to go to reaction completion. After a total of 24 h of

[illegible]

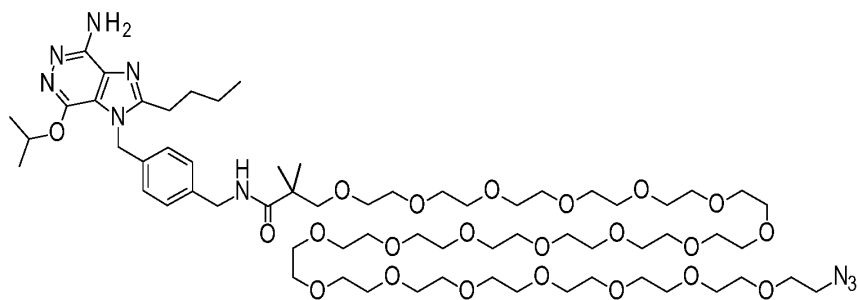
[illegible]

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.1 Hz, 6 H); 1.25 - 1.39 (m, 2 H); 1.47 (s, 6 H); 1.56 - 1.68 (m, 2 H); 2.27 - 2.37 (m, 2 H); 2.78 - 2.85 (m, 2 H); 3.33 - 3.74 (m, 82 H); 5.24 - 5.39 (m, 1 H); 5.53 (s, 2 H); 5.89 (s, 2 H); 6.98 (d, J=8.4 Hz, 2 H); 7.27 (d, J=8.4 Hz, 2 H); 7.99 (s, 1 H)

MS method N1: t_R (min): 1.38; $[M+H]^+$ 1374.2; $[M+2H]^{2+}$ 688.0

Example 56: Preparation of N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]

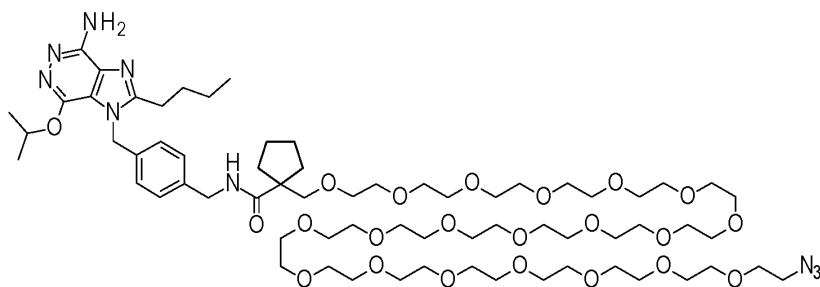
[illegible]



To a round-bottom flask containing EDCI (11.3 mg, 0.0579 mmol) was added anhydrous DMF (1 mL), the reaction mixture was stirred at rt till the end of dissolution. It was then placed in an ice bath, and to it, were added ethyl cyanoglyoxylate-2-oxime (7.6 mg, 0.0519 mmol), and 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]-2,2-dimethyl-propanoic acid, **Linker (L7)** (38.5 mg, 0.0342 mmol) in DCM (1 mL), followed by addition of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (**Example (TLR3)**) (15.2 mg, 0.0413 mmol) in DCM (0.4 mL). The reaction mixture was stirred 1 h at 0°C then overnight at rt. The reaction mixture was concentrated then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O (40:5:0.5), to give 21 mg (45% yield) of **Example 57**, as a yellowish oil.

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.3 Hz, 3 H); 1.06 (s, 6 H); 1.21 (d, J=6.1 Hz, 6 H); 1.27 - 1.39 (m, 2 H); 1.58 - 1.67 (m, 2 H); 2.74 - 2.83 (m, 2 H); 3.35 - 3.64 (m, 82 H); 4.23 (d, J=6.0 Hz, 2 H); 5.32 (quin, J=6.2 Hz, 1 H); 5.54 (s, 2 H); 5.87 (s, 2 H); 7.00 (d, J=8.3 Hz, 2 H); 7.18 (d, J=8.3 Hz, 2 H); 7.91 (t, J=5.9 Hz, 1 H)

MS method N1: t_R (min): 1.40; $[M+HCOO]^-$ 1419[illegible]



To a round-bottom flask containing EDCI (6.3 mg, 0.0323 mmol) was added anhydrous DMF (0.5 mL), the reaction mixture was stirred at rt till the end of dissolution. It was then placed in an ice bath, and to it, were added ethyl cyanoglyoxylate-2-oxime (4.7 mg, 0.0327 mmol), and 1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclopentanecarboxylic acid **Linker (L6)** (21.4 mg, 0.0204 mmol) in DCM (1 mL), followed by addition of 1-(4-(aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (**Example (TLR3)**) (9.5 mg, 0.0259 mmol) in DCM (0.2 mL). The reaction mixture was stirred 30 min at 0°C then overnight at rt. The reaction mixture was concentrated then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O (40:5:0.5), to give 17 mg (62% yield) of **Example 58**, as a yellowish oil.

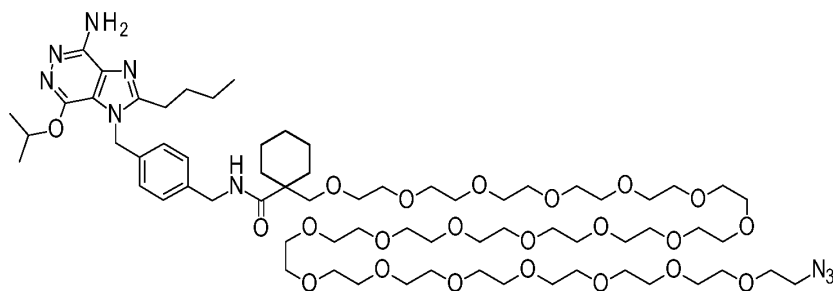
¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.3 Hz, 3 H); 1.20 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.44 - 1.57 (m, 6 H); 1.58 - 1.69 (m, 2 H); 1.86 - 1.95 (m, 2 H); 2.75 - 2.82 (m, 2 H); 3.36 - 3.65 (m, 80 H); 3.43 (s, 2 H); 4.24 (d, J=6.2 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.89 (s, 2 H); 7.00 (d, J=8.0 Hz, 2 H); 7.19 (d, J=8.0 Hz, 2 H); 7.95 (t, J=6.1 Hz, 1 H)

MS method N1: t_R (min): 1.46; [M+H]⁺ 1401.3; [M+2H]²⁺ 701.0

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Example 59: Preparation of N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclohexanecarboxamide (Payload P57)

25



To a round-bottom flask containing EDCI (6.3 mg, 0.0323 mmol) was added anhydrous DCM (0.5 mL), the reaction mixture was stirred at rt till the end of dissolution. It was then placed in an ice bath, and to it, were added ethyl cyanoglyoxylate-2-oxime (4.7 mg, 0.0327

5 mmol), and 1-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]cyclohexanecarboxylic acid **Linker (L5)** (21.4 mg, 0.0204 mmol) in DCM (0.5 mL), followed by addition of 1-(4-

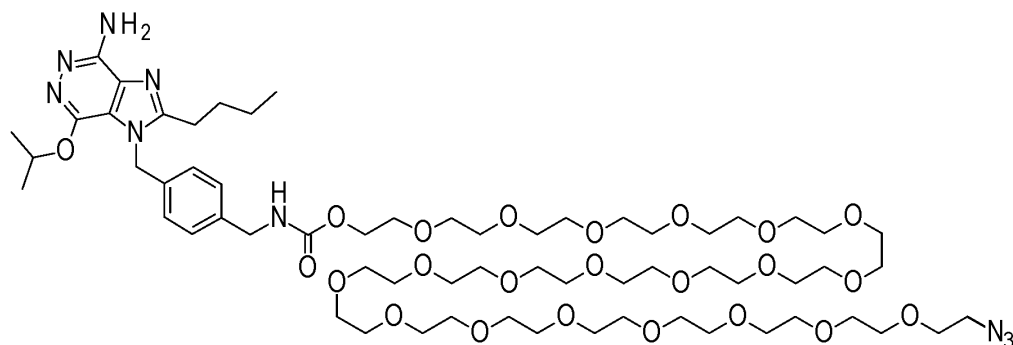
10 (aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine (**Example (TLR3)**) (9.5 mg, 0.0259 mmol) in DCM (0.5 mL). The reaction mixture was stirred 30 min at 0°C then overnight at rt. The reaction mixture was concentrated then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O (95:5:0.5), to give 18 mg (71% yield) of **Example 59**, as a colorless glassy solid.

¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.1 Hz, 6 H);

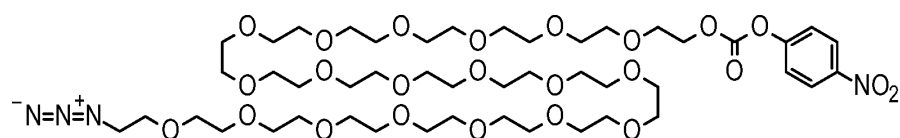
15 1.22 - 1.52 (m, 10 H); 1.59 - 1.66 (m, 2 H); 1.86 - 1.94 (m, 2 H); 2.76 - 2.80 (m, 2 H); 3.35 - 3.65 (m, 80 H); 4.25 (d, J=6.1 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.90 (s, 2 H); 7.00 (d, J=8.5 Hz, 2 H); 7.20 (d, J=8.2 Hz, 2 H); 7.94 (br t, J=6.1 Hz, 1 H)

MS method N1: t_R (min): 1.45; [M+H]⁺ 1416.4; [M+2H]²⁺ 708.5

20 **Example 60:** Preparation of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]phenyl]methyl]carbamate (Payload P58)



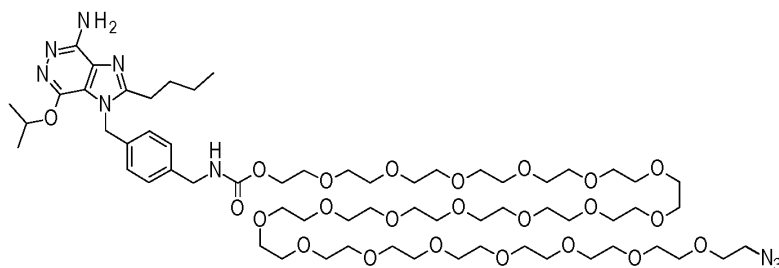
Step 1: 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl) carbonate



5

To a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl) carbonate (108 mg, 0.117 mmol) in DCM (5 mL) were successively added DIPEA (40 μ L, 0.230 mmol) and bis(4-nitrophenyl) carbonate (330 mg, 1.085 mmol). The reaction mixture was stirred overnight at rt then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O, 60:5:0.1 to give 114 mg (90% yield) of the expected compound.

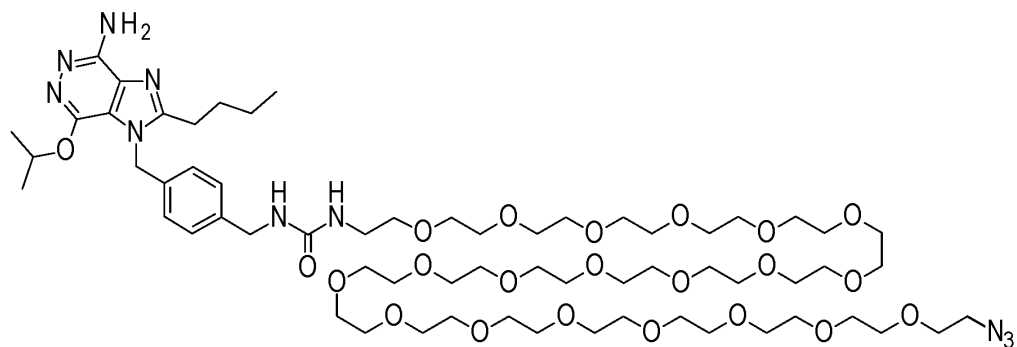
Step 2: Preparation of **Example 60** 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]carbamate



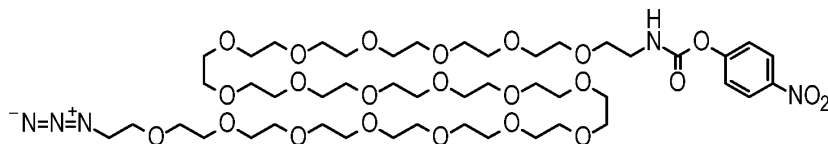
To a cooled solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl) carbonate from **Step 1** (112 mg, 0.103 mmol) in DCM (2.5 mL) was added 1-(4-aminomethyl)benzyl)-2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, (**Example (TLR3)**) (19 mg, 0.052 mmol) and DIPEA (27 μ L, 0.155 mmol). The mixture was reacted overnight at room temperature then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O, 40:5:0.5. The compound collected fractions were pooled, concentrated then further purified by reverse phase column chromatography reverse phase column chromatography using as eluent a mixture of H₂O, 0.1% TFA/CH₃CN (95:5 to 5:95). The compound containing fractions were pooled and concentrated under vacuum to give **Example 60** (10 mg, 14% yield), as a colorless glassy solid.

¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.4 Hz, 3 H); 1.20 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.57 - 1.68 (m, 2 H); 2.75 - 2.82 (m, 2 H); 3.34 - 3.70 (m, 78 H); 4.02 - 4.07 (m, 2 H); 4.13 (br d, J=6.1 Hz, 2 H); 5.32 (spt, J=6.1 Hz, 1 H); 5.55 (s, 2 H); 5.90 (s, 2 H); 7.02 (d, J=8.1 Hz, 2 H); 7.20 (d, J=8.1 Hz, 2 H); 7.73 (t, J=6.1 Hz, 1 H)
MS method N1: t_R (min): 1.36; [M+HCOO]⁻ 1362.3

Example 61: Preparation of 1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]urea (Payload P59)

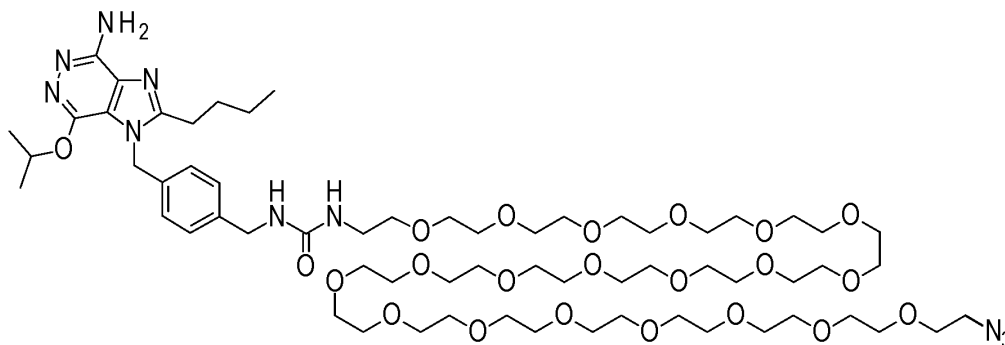


Step 1: 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl)carbonate



- 5 To a solution of bis(4-nitrophenyl)carbonate (195 mg, 0.641 mmol) in DCM (2 mL) was added a solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl)carbonate **Linker (L8)** (30 mg, 0.031 mmol) in DCM (2 mL) followed by DIPEA (30 μ L, 0.172 mmol) dropwise.
- 10 The reaction mixture was stirred overnight at rt then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O, 60:5:0.1 to give 33 mg (90% yield) of the expected compound.
- MS method N5: t_R (min): 1.8; $[M+H]^+$ 1088.7

- 15 **Step 2:** Preparation of **Example 61** 1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]-3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]urea



- 20 To a cooled solution of 2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl (4-nitrophenyl)carbonate from **Step 1** (30 mg, 0.026 mmol) in DCM (1 mL) was added 1-(4-(aminomethyl)benzyl)-

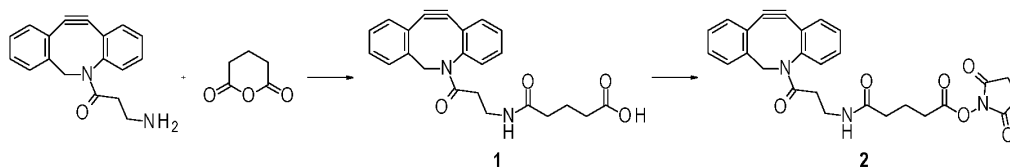
2-butyl-7-isopropoxy-1H-imidazo[4,5-d]pyridazin-4-amine, (**Example (TLR3)**) (5.5 mg, 0.015 mmol) in DCM (1 mL) and DIPEA (8 μ L, 0.046 mmol). The mixture was reacted 3 h at rt then purified by silica gel column chromatography using as eluent a mixture of DCM/MeOH/H₂O, 40:5:0.5. The compound containing fractions were pooled, concentrated then further purified by reverse phase column chromatography using as eluent a mixture of H₂O + 0.1% TFA/CH₃CN (95:5 to 5:95) to give **Example 61** (7.5 mg, 38% yield), as a colorless glassy solid.

¹H NMR (500 MHz, δ in ppm, DMSO-*d*₆): 0.84 (t, J=7.4 Hz, 3 H); 1.21 (d, J=6.2 Hz, 6 H); 1.27 - 1.38 (m, 2 H); 1.59 - 1.70 (m, 2 H); 2.76 - 2.82 (m, 2 H); 3.11 - 3.17 (m, 2 H); 3.22 - 3.68 (m partially hidden, 78 H); 4.15 (d, J=6.0 Hz, 2 H); 5.32 (quin, J=6.1 Hz, 1 H); 5.54 (s, 2 H); 5.89 (s, 2 H); 5.94 (t, J=5.7 Hz, 1 H); 6.37 (t, J=5.8 Hz, 1 H); 7.02 (d, J=8.0 Hz, 2 H); 7.19 (d, J=8.0 Hz, 2 H)

MS method N1: t_R (min): 1.33; [M+H]⁺ 1317.2; [M+2H]²⁺ 659.4

15 **D) Preparation of conjugates**

Synthesis of ADIBO-glutaryl-ONHS modifying agent



Compound 1: 5-[[3-(2-azatricyclo[10.4.0.04,9]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoic acid

20 To a solution of dibenzocyclooctyne-amine ([1255942-06-3], 77.5 mg, 280 μ mol) in anhydrous DCM (2 mL) was added glutaric anhydride (42.5 mg, 364 μ mol). The colorless reaction mixture was stirred overnight at RT and purified by flash chromatography on 5 g of silica gel (gradient elution EtOAc to EtOAc/MeOH/H₂O 7:2:1) to give 94 mg of **Compound 1** as a white foam (86%).

25 ¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.59 (quin, J=7 Hz, 2 H); 1.83 (ddd, J =6, 8 and 16 Hz, 1 H); 1.94 (t, J=8 Hz, 2 H); 2.11 (t, J=7 Hz, 2 H); 2.40 (ddd, J= 6, 8 and 16 Hz, 1 H); 2.87 (m, 1 H); 3.09 (m, 1 H); 3.63 (d, J=14 Hz, 1 H); 5.04 (d, J=14 Hz, 1 H); 7.26-7.42 (m, 3 H); 7.43-7.52 (m, 3 H); 7.54-7.67 (m, 3 H); 12.01 (large s, 1 H).

Compound 2: (2,5-dioxopyrrolidin-1-yl)-5-[[3-(2-azatricyclo[10.4.0.0⁴,9]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoate

Under argon, to a solution of **Compound 1** (31 mg, 79 μ mol) in anhydrous DCM (1.7 mL) was added N,N'-disuccinimidyl carbonate (43 mg, 159 μ mol) followed by DIEA (28 μ L, 159 μ mol). The reaction mixture was stirred overnight at RT and then purified by flash chromatography on 4.3 g of diol silica (gradient elution heptane/EtOAc) to give 18.5 mg of **Compound 2** as a white solid (47%).

¹H NMR (400 MHz, δ in ppm, DMSO-*d*₆): 1.65-1.77 (m, 2 H); 1.85 (ddd, J = 6, 8 and 16 Hz, 1 H); 2.04 (t, J = 7 Hz, 2 H); 2.40 (m, 1 H); 2.59 (t, J = 7 Hz, 2 H); 2.81 (s, 4 H); 2.95 (m, 1 H); 3.09 (m, 1 H); 3.63 (d, J = 14 Hz, 1 H); 5.05 (d, J = 14 Hz, 1 H); 7.26-7.54 (m, 6 H); 7.55-7.71 (m, 3 H)

MS method M1: *t*_R = 1.03, ES, *m/z* = 488 [M+H]⁺, *m/z* = 486 [M-H]⁻, *m/z* = 532 [M-H+HCO₂H]⁻.

15 Synthesis of mAb-DBCO

mAb 1: CEACAM5_Tusamitamab-DBCO

mAb 1 was prepared following general method M1; to **CEACAM5_Tusamitamab** (CAS [2349294-95-5], 29.55 mg at 19.7 mg/mL) in buffer B were added DPBS (0.771 mL) and 1M HEPES (14.3 μ L). This solution was treated with 500 μ L of a solution of (2,5-dioxopyrrolidin-1-yl)-5-[[3-(2-azatricyclo[10.4.0.0⁴,9]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoate in DMA (2.85 mM) and reacted for 1 h; **mAb 1** was obtained crude as a colorless limpid solution (30 mg at 10.5 mg/mL) with a LAR (SEC) of 4.0 and a monomeric purity of 100%.

25 mAb 2: PDL1_Avelumab_K76R-K100R-DBCO

mAb 2 was prepared following general method M1; to **PDL1_Avelumab_K76R_K100R** (51 mg at 11.34 mg/mL in DPBS) were added DMA (1 mL) and 1M HEPES (28 μ L). This solution was treated with 122 μ L of a solution of (2,5-dioxopyrrolidin-1-yl)-5-[[3-(2-azatricyclo[10.4.0.0⁴,9]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoate in DMA (20 mM) and reacted for 45 min; **mAb 2** was obtained crude as a colorless limpid solution (51 mg at 9 mg/mL) with a LAR (SEC) of 4.2 and a monomeric purity of 98.8%.

mAb 3: CEACAM5_769_VarB_VH7/VL2-DBCO

mAb 3 was prepared following general method M1; CEACAM5_769_VarB_VH7/VL2 (40 mg at 9.51 mg/mL) in DPBS was treated with 95 μ L of a solution of (2,5-dioxopyrrolidin-1-yl)-5-[[3-(2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoate in DMSO (20 mM) and reacted for 1 h; **mAb 3** was obtained crude as a colorless limpid solution (40 mg at 9.3 mg/mL) with a LAR (SEC) of 4.3 and a monomeric purity of 97%.

10 **mAb 4: CEACAM5_p01_CEA_1_07-DBCO**

mAb 4 was prepared following general method M1; CEACAM5_p01_CEA_1_07 (58 mg at 10.87 mg/mL) in DPBS was treated with 138 μ L of a solution of (2,5-dioxopyrrolidin-1-yl)-5-[[3-(2-azatricyclo[10.4.0.0^{4,9}]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-3-oxo-propyl]amino]-5-oxo-pentanoate in DMSO (20 mM) and reacted for 1 h; **mAb 4** was obtained crude as a colorless limpid solution (58 mg at 10.59 mg/mL) with a LAR (SEC) of 4.1 and a monomeric purity of 99%.

Synthesis of uncapped thiomAbs**Synthesis of uncapped thiomAb 1: PDL1_Avelumab_K360C-SH**

20 **Uncapped thiomAb 1** was prepared following general method M4a; a solution of **PDL1_Avelumab_K360C** (36 mg at 10.59 mg/mL) in DPBS was reacted with 60 μ L of a 280 mM DTT solution for 1 h at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Healthcare) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 73 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 1** was obtained crude as a colorless limpid solution (36 mg at 4.32 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 2: PDL1_Avelumab_LALA_K360C-SH

30 **Uncapped thiomAb 2** was prepared following general method M4a; a solution of **PDL1_Avelumab_LALA_K360C** (72 mg at 9.05 mg/mL) in DPBS was reacted with 120 μ L of a 280 mM DTT solution for 1 h at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Healthcare) pre-equilibrated

with DPBS buffer, the reduced antibody was reacted with 130 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 2** was obtained crude as a colorless limpid solution (72 mg at 5.23 mg/mL) and used as it is in the conjugation step.

5 **Synthesis of uncapped thiomAb 3:** CEACAM5_p01_CEA_1_07_A118C-SH

Uncapped thiomAb 3 was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_A118C (39 mg at 7.71 mg/mL) in DPBS was reacted with 60 μ L of a 280 mM DTT solution for 45 min at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Cytiva) pre-equilibrated
10 with DPBS buffer, the reduced antibody was reacted with 74 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 3** was obtained crude as a colorless limpid solution (35 mg at 3.87 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 4: CEACAM5_p01_CEA_1_07_E152C-SH

15 **Uncapped thiomAb 4** was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_E152C (31 mg at 6.21 mg/mL) in DPBS was reacted with 48 μ L of a 280 mM DTT solution for 45 min at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 56 μ L of a 50 mM DHAA solution
20 for 3 hours at RT. **Uncapped thiomAb 4** was obtained crude as a colorless limpid solution (27 mg at 2.84 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 5: CEACAM5_p01_CEA_1_07_K360C-SH

Uncapped thiomAb 5 was prepared following general method M4a; a solution of
25 CEACAM5_p01_CEA_1_07_K360C (38 mg at 7.73 mg/mL) in DPBS was reacted with 60 μ L of a 280 mM DTT solution for 45 min at 37°C then with 32 μ L of a 280 mM DTT solution for 30 min at 37°C to allow the reaction to go to completion (HRMS monitoring). Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 70 μ L
30 of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 5** was obtained crude as a colorless limpid solution (27 mg at 2.74 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 6: CEACAM5_p01_CEA_1_07_K414C-SH

Uncapped thiomAb 6 was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_K414C (36 mg at 7.21 mg/mL) in DPBS was reacted with 56 μ L of a 280 mM DTT solution for 60 min at 37°C. Following gel filtration using a
5 Sephadex™ G25 matrix (HiPrep™ 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 74 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 6** was obtained crude as a colorless limpid solution (34 mg at 3.96 mg/mL) and used as it is in the conjugation step.

10 **Synthesis of uncapped thiomAb 7:** CEACAM5_p07_CEA_3_50_VH1_A118C-SH

Uncapped thiomAb 7 was prepared following general method M4a; a solution of CEACAM5_p07_CEA_3_50_VH1_A118C (39 mg at 3.23 mg/mL) in DPBS was reacted with 60 μ L of a 280 mM DTT solution for 1 hour at 37°C then with a total of 175 μ L of a 280 mM DTT solution for 3.5 hours at 37°C to allow the reaction to go to completion (HRMS
15 monitoring). Following gel filtration using a Sephadex™ G25 matrix (HiPrep™ 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer and concentration, the reduced antibody was reacted with 70 μ L of a 50 mM DHAA solution for 2.5 hours at RT. **Uncapped thiomAb 7** was obtained crude as a colorless limpid solution (34 mg at 2.80 mg/mL) and used as it is in the conjugation step.

20

Synthesis of uncapped thiomAb 8: CEACAM5_p07_CEA_3_50_VH1_K360C-SH

Uncapped thiomAb 8 was prepared following general method M4a; a solution of CEACAM5_p07_CEA_3_50_VH1_K360C (39 mg at 3.31 mg/mL) in DPBS was reacted with 62 μ L of a 280 mM DTT solution for 1.5 hours at 37°C then with 62 μ L of a 280 mM
25 DTT solution for 1.5 hours at 37°C to allow the reaction to go to completion (HRMS monitoring). Following gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) with DPBS buffer, the reduced antibody was reacted with 81 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 8** was obtained crude as a colorless limpid solution (37 mg at 2.32 mg/mL) and used as it is in the conjugation step.

30

Synthesis of uncapped thiomAb 9: CEACAM5_p07_CEA_3_50_VH1_K414C-SH

Uncapped thiomAb 9 was prepared following general method M4a; a solution of

CEACAM5_p07_CEA_3_50_VH1_K414C (35 mg at 2.94 mg/mL) in DPBS was reacted with 54 μ L of a 280 mM DTT solution for 60 min at 37°C. Following gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) with DPBS buffer, the reduced antibody was reacted with 66 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 9** was obtained crude as a colorless limpid solution (33 mg at 2.76 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 10: CEACAM5_p01_CEA_1_07_A118C_K360C-SH

Uncapped thiomAb 10 was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_A118C_K360C (36 mg at 7.26 mg/mL) in DPBS was reacted with 70 μ L of a 280 mM DTT solution for 60 min at 37°C. Following gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) with DPBS buffer, the reduced antibody was reacted with 78 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 10** was obtained crude as a colorless limpid solution (35 mg at 3.17 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 11: CEACAM5_p01_CEA_1_07_E152C_K360C-SH

Uncapped thiomAb 11 was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_E152C_K360C (36 mg at 7.21 mg/mL) in DPBS was reacted with 70 μ L of a 280 mM DTT solution for 45 min at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 138 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 11** was obtained crude as a colorless limpid solution (33 mg at 2.98 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 12: CEACAM5_p01_CEA_1_07_K274C_K414C-SH

Uncapped thiomAb 12 was prepared following general method M4a; a solution of CEACAM5_p01_CEA_1_07_K274C_K414C (35 mg at 7.03 mg/mL) in DPBS was reacted with 83 μ L of a 280 mM DTT solution for 60 min at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiprepTM 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 75 μ L of a 50 mM DHAA solution for 4 hours at RT. **Uncapped thiomAb 12** was obtained crude as a colorless limpid solution

(34 mg at 3.86 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 13: CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-SH

- 5 **Uncapped thiomAb 13** was prepared following general method M4a; a solution of CEACAM5_p07_CEA_3_50_VH1_A118C_K360C (38 mg at 3.19 mg/mL) in DPBS was reacted with 74 μ L of a 280 mM DTT solution for 60 min at 37°C then with a total of 296 μ L of a 280 mM DTT solution for 6 hours at 37°C to allow the reaction to go to completion (HRMS monitoring). Following gel filtration (Sephadex G-25, PD-10 Desalting Columns,
- 10 Cytiva) with DPBS buffer, the reduced antibody was reacted with 133 μ L of a 50 mM DHAA solution for 3 hours at RT. **Uncapped thiomAb 13** was obtained crude as a colorless limpid solution (34 mg at 2.4 mg/mL) and used as it is in the conjugation step.

Synthesis of uncapped thiomAb 14: CEACAM5_p07_CEA_3_50_VH1_K274C_K414C-SH

- 15 **Uncapped thiomAb 14** was prepared following general method M4a; a solution of CEACAM5_p07_CEA_3_50_VH1_K274C_K414C (34 mg at 3.22 mg/mL) in DPBS was reacted with 115 μ L of a 280 mM DTT solution for 60 min at 37°C. Following gel filtration using a SephadexTM G25 matrix (HiPrepTM 26/10 desalting column, GE Cytiva) pre-equilibrated with DPBS buffer, the reduced antibody was reacted with 115 μ L of a 50 mM
- 20 DHAA solution for 2.5 hours at RT. **Uncapped thiomAb 14** was obtained crude as a colorless limpid solution (32 mg at 2.64 mg/mL) and used as it is in the conjugation step.

Example 13: CEACAM5_Tusamitamab-P5 ADC

- 25 **Example 13** was prepared following general method M2; a solution of **mAb 1** (31 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 1.2 mL of DPBS and 195 μ L of DMA was reacted overnight with 104 μ L of a solution of **P5** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a SephadexTM G25 matrix
- 30 (HitrapTM desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 13** (10 mg at 3 mg/mL, overall yield 31%) was finally obtained as a colorless limpid solution

with a DAR (HRMS) of 4.4 and a monomeric purity of 100%.

RP-HRMS: 147416 (naked mAb); 148709 (D1); 150003 (D2); 151298 (D3); 152592 (D4); 153887 (D5); 155182 (D6); 156476 (D7); 157770 (D8); 159063 (D9); 160361 (D10).

5 **Example 14: PDL1_Avelumab_K76R-K100R-P5 ADC**

Example 14 was prepared following general method M2; a solution of **mAb 2** (35.5 mg at 9 mg/mL) in 80:20 buffer B/DMA was reacted overnight with 101 µL of a solution of **P5** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel
10 filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 14** (22 mg at 2.6 mg/mL, overall yield 62%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.5 and a monomeric purity of 99.8%.
15 RP-HRMS: 144605 (D1); 145898 (D2); 147192 (D3); 148487 (D4); 149781 (D5); 151076 (D6); 152369 (D7); 153675 (D8); 154960 (D9).

Example 15: CEACAM5_Tusamitamab-P8 ADC

Example 15 was prepared following general method M2; a solution of **mAb 1** (32 mg at 20 10.5 mg/mL) in 88:12 buffer B/DMA was reacted overnight with 103 µL of a solution of **P8** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES
25 membrane, 50K, Millipore). **Example 15** (22 mg at 3 mg/mL, overall yield 68%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 99%.

RP-HRMS: 147389 (naked mAb); 148771 (D1); 150151 (D2); 151531 (D3); 152911 (D4); 154291 (D5); 155672 (D6); 157049 (D7); 158426 (D8); 159804 (D9).

30

Example 16: PDL1_Avelumab_K76R-K100R-P8 ADC

Example 16 was prepared following general method M2; a solution of **mAb 2** (25 mg at

9 mg/mL) in 80:20 buffer B/DMA, 868 μ L of DPBS and 126 μ L of DMA was reacted overnight with 91 μ L of a solution of **P8** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 16** (16.4 mg at 3.04 mg/mL, overall yield 65%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 98.9%.
RP-HRMS: 147575 (D1); 148954 (D2); 150336 (D3); 151717 (D4); 153098 (D5); 154477 (D6); 155859 (D7); 157236 (D8).

Example 17: CEACAM5_Tusamitamab-P3 ADC

Example 17 was prepared following general method M2; a solution of **mAb 1** (32 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 1.15 mL of DPBS and 191 μ L of DMA was reacted overnight with 96 μ L of a solution of **P3** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 17** (21 mg at 2.4 mg/mL, overall yield 66%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 98%.
RP-HRMS: 147403 (naked mAb); 148971 (D1); 150545 (D2); 152116 (D3); 153689 (D4); 155261 (D5); 156833 (D6); 158405 (D7); 159988 (D8).

Example 18: PDL1_Avelumab_K76R_K100R-P3 ADC

Example 18 was prepared following general method M2; a solution of **mAb 2** (30 mg at 10 mg/mL) in 88:12 buffer B/DMA was reacted overnight with 101 μ L of a solution of **P3** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 18** (20 mg at 1.84 mg/mL, overall yield 66%) was

finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.1 and a monomeric purity of 99.2%.

RP-HRMS: 146180 (naked mAb); 147751 (D1); 149324 (D2); 150897 (D3); 152470 (D4); 154041 (D5); 155608 (D6).

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Example 19: CEACAM5_Tusamitamab-Example 3 ADC

Example 19 was prepared following general method M2; a solution of **mAb 1** (33 mg at 10 mg/mL) in 80:20 buffer B/DMA and 442 µL of DPBS was reacted overnight with 110 µL of a solution of **Example 3** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore) and formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 19** (18.2 mg at 1.52 mg/mL, overall yield 55%) was finally obtained as a colorless limpid solution with a

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DAR (HRMS) of 4.2 and a monomeric purity of 99.6%.

RP-HRMS: 147409 (naked mAb); 148640 (D1); 149871 (D2); 151103 (D3); 152335 (D4); 153566 (D5); 154796 (D6); 156030 (D7); 157262 (D8); 158484 (D9).

Example 20: PDL1_Avelumab_K76R_K100R-Example 3 ADC

Example 20 was prepared following general method M2; a solution of **mAb 2** (23 mg at 9 mg/mL) in 80:20 buffer B/DMA, 869 µL of DPBS and 126 µL of DMA was reacted overnight with 126 µL of a solution of **Example 3** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 20** (12 mg at 2.63 mg/mL, overall yield 52%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.95 and a monomeric purity of 98.7%.

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RP-HRMS: 147420 (D1); 148653 (D2); 149884 (D3); 151118 (D4); 152348 (D5); 153580 (D6); 154810 (D7); 156046 (D8).

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Example 21: CEACAM5_Tusamitamab-Example 5 ADC

Example 21 was prepared following general method M2; a solution of **mAb 1** (32 mg at 10.5 mg/mL) in 80:20 buffer B/DMA was reacted overnight with 103 μ L of a solution of **Example 5** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Vivaspin® Turbo: PES membrane, 50K, Sartorius). **Example 21** (25 mg at 2.9 mg/mL, overall yield 77%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.8 and a monomeric purity of 98.8%.

RP-HRMS: 147416 (naked mAb); 148727 (D1); 150046 (D2); 151363 (D3); 152681 (D4); 153998 (D5); 155316 (D6); 156634 (D7); 157950 (D8); 159269 (D9); 160587 (D10).

Example 22: PDL1_Avelumab_K76R_K100R-Example 5 ADC

Example 22 was prepared following general method M2; a solution of **mAb 2** (32 mg at 9 mg/mL) in 80:20 buffer B/DMA, 869 μ L of DPBS and 126 μ L of DMA was reacted overnight with 126 μ L of a solution of **Example 5** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 22** (22 mg at 3.01 mg/mL, overall yield 68%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.5 and a monomeric purity of 98.9%.

RP-HRMS: 147514 (D1); 148828 (D2); 150146 (D3); 151465 (D4); 152783 (D5); 154100 (D6); 155418 (D7); 156732 (D8); 158054 (D9).

Example 23: PDL1_Avelumab_K360C-Example 6 ADC

Example 23 was prepared following general method M4b; a solution of crude **uncapped thiomAb 1** (37.2 mg at 5.1 mg/mL) in DPBS and 1.73 mL of cold DMA was reacted overnight with 38 μ L of a solution of **Example 6** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 23**

(25 mg at 3.85 mg/mL, overall yield 59%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99.6%.

RP-HRMS: 148521 (D2)

5 **Example 24: PDL1_Avelumab_LALA_K360C-Example 6 ADC**

Example 24 was prepared following general method M4b; a solution of crude **uncapped thiomAb 2** (32 mg at 5.23 mg/mL) in DPBS and 1 mL of cold DMA was reacted overnight with 64 µL of a solution of **Example 6** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 24** (13.3 mg at 2.05 mg/mL, overall yield 42%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.84 and a monomeric purity of 100%.
RP-HRMS: 147299 (D1); 148386 (D2).

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Example 25: CEACAM5_Tusamitamab-Example 2 ADC

Example 25 was prepared following general method M2; a solution of **mAb 1** (41 mg at 10.5 mg/mL) in 88:12 buffer B/DMA was reacted overnight with 112 µL of a solution of **Example 2** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 25** (28 mg at 3.18 mg/mL, overall yield 68%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.3 and a monomeric purity of 98.3%.
RP-HRMS: 147394 (naked mAb); 148904 (D1); 150417 (D2); 151926 (D3); 153434 (D4); 154944 (D5); 156453 (D6); 157964 (D7); 159473 (D8); 160984 (D9).

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Example 26: PDL1_Avelumab_K76R_K100R-Example 2 ADC

Example 26 was prepared following general method M2; a solution of **mAb 2** (30 mg at 9 mg/mL) in 89:11 buffer B/DMA was reacted overnight with 112 µL of a solution of **Example 2** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg

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HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 26** (22 mg at 2.2 mg/mL, overall yield 73%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity of 97.7%.

RP-HRMS: 146186 (naked mAb); 147696 (D1); 149206 (D2); 150715 (D3); 152224 (D4); 153733 (D5); 155243 (D6); 156749 (D7).

10 **Example 27: CEACAM5_Tusamitamab-Example 7 ADC**

Example 27 was prepared following general method M3; a solution of **mAb 1** (31 mg at 10 mg/mL) in 80:20 Buffer B/DMA and 442 µL of DPBS was reacted overnight with 110 µL of a solution of **Example 7** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), concentrated, formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). After final concentration and sterile filtration **Example 7** (18.5 mg at 3.2 mg/mL, overall yield 59.6%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99.5%.

RP-HRMS: 147403 (naked mAb); 149377 (D1); 151340 (D2); 153302 (D3); 155266 (D4); 157230 (D5); 159194 (D6); 161160 (D7); 163120 (D8); 165078 (D9).

Example 28: PDL1_Avelumab_K76R_K100R-Example 7 ADC

Example 28 was prepared following general method M2; a solution of **mAb 2** (25.2 mg at 9.02 mg/mL) in 80:20 DPBS/DMA and 868 µL of DPBS, and 126 µL of DMA was reacted overnight with 91 µL of a solution of **Example 7** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS pH 6.5 with 20% DMA) then formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). After concentration and sterile filtration, **Example 28** (18 mg at 2.55 mg/mL, overall yield 72%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.75 and a monomeric purity of 98.7%.

RP-HRMS: 148162 (D1); 150128 (D2); 152091 (D3); 154056 (D4); 156021 (D5); 157980 (D6); 159945 (D7).

Example 29: CEACAM5_Tusamitamab-Example 11b ADC

- 5 **Example 29** was prepared following general method M2; a solution of **mAb 1** (31 mg at 10.5 mg/mL) in 80:20 Buffer B/DMA, 1.15 mL of DPBS and 191 µL of DMA was reacted overnight with 90 µL of a solution of **Example 11b** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS pH 6.5 with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™
- 10 G25 matrix (Hitrap™ desalting columns, GE Healthcare). After concentration and sterile filtration **Example 29** (20.24 mg at 1.84 mg/mL, overall yield 66%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 99.7%.
RP-HRMS: 147401 (naked mAb); 149293 (D1); 151186 (D2); 153077 (D3); 154970 (D4); 156862 (D5); 158753 (D6); 160645 (D7); 162540 (D8); 164420 (D9).

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Example 30: PDL1_Avelumab_K76R_K100R-Example 11b ADC

- Example 30** was prepared following general method M2; a solution of **mAb 2** (30.6 mg at 9.96 mg/mL) in 88:12 DPBS/DMA was reacted overnight with 110 µL of a solution of **Example 11b** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200
- 20 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS pH 6.5 with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). After concentration and sterile filtration **Example 30** (25.65 mg at 2.85 mg/mL, overall yield 84%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.2 and a monomeric purity of 99.4%.
- 25 RP-HRMS: 146177 (naked mAb); 148070 (D1); 149963 (D2); 151853 (D3); 153745 (D4); 155636 (D5); 157525 (D6); 159419 (D7).

Example 31: CEACAM5_Tusamitamab-Example 8 ADC

- Example 31** was prepared following general method M2; a solution of **mAb1** (29.82 mg at 10.5 mg/mL) in 80:20 Buffer B/DMA, 1398 µL of DPBS and 510 µL of DMA was reacted overnight with 154 µL of a solution of **Example 8** in DMA (13.7 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare,
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DPBS with 10% DMA), concentrated then formulated in buffer E by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 31** (19 mg at 3.11 mg/mL, overall yield 63%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.11 and a monomeric purity of 99%.

- 5 RP-HRMS: 147408 (naked mAb); 149305 (D1); 151210 (D2); 153112 (D3); 155013 (D4); 156913 (D5); 158813 (D6); 160713 (D7); 162616 (D8); 164501 (D9).

Example 32: PDL1_Avelumab_K76R_K100R-Example 8 ADC

- Example 32** was prepared following general method M3; a solution of **mAb 2** (25.2 mg at 9 mg/mL) in 80:20 DPBS/DMA, 1.136 mL of DPBS and 127 µL of DMA was reacted overnight with 156 µL of a solution of **Example 8** in DMA (16.02 mM). The reaction mixture was purified by diafiltration then formulated in buffer A by gel filtration using PD10 desalting column (GE Healthcare). After additional purification by diafiltration with buffer A, **Example 32** (15 mg at 3.96 mg/mL, overall yield 60%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 98.5%.
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RP-HRMS: 148093 (D1); 149991 (D2); 151895 (D3); 153802 (D4); 155696 (D5); 157600 (D6); 159499 (D7).

- 20 **Example 33: PDL1_Avelumab_K360C-P16 ADC**

- Example 33** was prepared following general method M4b; a solution of **uncapped thiomAb 1** (37.9 mg at 5.65 mg/mL) in DPBS and 1mL of DMA was reacted overnight with 38 µL of a solution of **P16** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 26/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated then formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 33** (27.5 mg at 2.39 mg/mL, overall yield 73%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
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RP-HRMS: 149688 (D2).

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Example 34: PDL1_Avelumab_K360C-Example 10 ADC

Example 34 was prepared following general method M4b; a solution of **uncapped**

thiomAb 1 (37.9 mg at 5.65 mg/mL) in DPBS and 1 mL of DMA was reacted overnight with 38 μ L of a solution of **Example 10** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 26/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated then formulated in buffer D by gel filtration using a Sephadex™
5 G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 34** (28.2 mg at 2.56 mg/mL, overall yield 74%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
RP-HRMS: 149336 (D2).

10 **Example 35: PDL1_Avelumab_LALA_K360C-Example 10 ADC**

Example 35 was prepared following general method M4b; a solution of **uncapped thiomAb 2** (22.19 mg at 5.44 mg/mL) in DPBS and 0.75 mL of cold DMA was reacted overnight with 22.5 μ L of a solution of **Example 10** in DMA (20 mM). Then 0.25 mL of DMA and 11.9 μ L of a solution of **Example 10** in DMA (20 mM) were added and the
15 reaction was pursued for 3h. The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). **Example 35** (16.5 mg at 3.0 mg/mL, overall yield 72%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity
20 of 100%.
RP-HRMS: 149175 (D2).

Example 36: PDL1_Avelumab_K360C-P14 ADC

Example 36 was prepared following general method M4b; a solution of **uncapped thiomAb1** (9.9 mg at 4.41 mg/mL) in DPBS and 0.55 mL of cold DMA was reacted
25 overnight with 12 μ L of a solution of **P14** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). **Example 36** (8.6 mg at 2.53 mg/mL, overall yield 87%) was
30 finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99.6%.
RP-HRMS: 149639 (D2).

Example 37: PDL1_Avelumab_LALA_K360C-P14 ADC

Example 37 was prepared following general method M4b; a solution of **uncapped thiomAb 2** (11.11 mg at 5.34 mg/mL) in DPBS and 0.74 mL of cold DMA was reacted
5 overnight with 33.8 µL of a solution of **P14** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). **Example 37** (6.9 mg at 2.3 mg/mL, overall yield 61%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and
10 a monomeric purity of 100%.
RP-HRMS: 148470 (D2).

Example 38: PDL1_Avelumab_K76R_K100R-Example 12 ADC

Example 38 was prepared as follows: a solution of Avelumab K76R-K100R (11.34 mg/mL in DPBS), 720 µL of DPBS and 514 µL of cold DMA were reacted overnight with 15.2 µL
15 of a solution of crude tetrafluorophenyl ester of **Example 12** in DMA (20 mM). Then 16.8 µL of a solution of crude tetrafluorophenyl ester of **Example 12** in DMA (20 mM) were added and the reaction was pursued for 1.5 hours. The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10%
20 DMA), concentrated, formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). After additional purification by diafiltration with buffer D and sterile filtration **Example 38** (6.71 mg at 3.05 mg/mL, overall yield 42%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 1.78 and a monomeric purity of 99.2%.
RP-HRMS: 146214 (naked mAb); 147199 (D1); 148184 (D2); 149171 (D3); 150150 (D4);
25 151140 (D5).

Example 39: CEACAM5_Tusamitamab-Example 11a ADC

Example 39 was prepared following general method M2; a solution of **mAb 1** (31 mg at 10.5 mg/mL) in 80:20 Buffer B/DMA, 1.2 mL of DPBS and 196 µL of DMA was reacted
30 overnight with 104 µL of a solution of **Example 11a** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA) then formulated in buffer D by gel filtration using a Sephadex™

G25 matrix (HitrapTM desalting columns, GE Healthcare). **Example 39** (25 mg at 3.35 mg/mL, overall yield 77%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.6 and a monomeric purity of 99.8%.

RP-HRMS: 149243 (D1); 151069 (D2); 152899 (D3); 154728 (D4); 156558 (D5); 158387 (D6); 160216 (D7); 162045 (D8); 163871 (D9).

Example 40: PDL1_Avelumab_K76R_K100R-Example 11a ADC

Example 40 was prepared following general method M2; a solution of **mAb 2** (34 mg at 10.5 mg/mL) in 93:7 DPBS/DMA, 1.136 mL of DPBS and 127 µL of DMA was reacted overnight with 113.4 µL of a solution of **Example 11a** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA) then formulated in buffer D by gel filtration using a SephadexTM G25 matrix (HitrapTM desalting columns, GE Healthcare). **Example 40** (26.6 mg at 3.33 mg/mL, overall yield 78 %) was finally obtained after concentration and sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 99.9%.

RP-HRMS: 148021 (D1); 149852(D2); 151679 (D3); 153510 (D4); 155338 (D5); 157166 (D6); 158999 (D7); 160825 (D8); 162660 (D9).

Example 62: CEACAM5_Tusamitamab-Example 43 ADC

Example 62 was prepared following general method M2; a solution of **mAb 1** (27 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 1.17 mL of DPBS and 196 µL of DMA was reacted overnight with 96 µL of a solution of **Example 43** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), formulated in buffer D by gel filtration using a SephadexTM G25 matrix (HitrapTM desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 62** (17 mg at 2.78 mg/mL, overall yield 63%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.4 and a monomeric purity of 99.8%.

RP-HRMS: 148624 (D1); 149851 (D2); 151074 (D3); 152300 (D4); 153525 (D5); 154749 (D6); 155975 (D7); 157196 (D8); 158437 (D9).

Example 63: PDL1_Avelumab_K76R_K100R-Example 43 ADC

Example 63 was prepared following general method M3; a solution of **mAb 2** (25.2 mg at 9 mg/mL) in 80:20 DPBS/DMA, 877 µL of DPBS and 127 µL of DMA was reacted overnight with 92 µL of a solution of **Example 43** in DMA (20 mM). The crude mixture
5 was purified by diafiltration then formulated in buffer E by gel filtration. **Example 63** (13 mg at 3.43 mg/mL, overall yield 52%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 97.4%.
RP-HRMS: 146185 (naked mAb); 147411 (D1); 148639 (D2); 149864 (D3); 151096 (D4); 152314 (D5); 153547 (D6); 154748 (D7); 155983 (D8).

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Example 64: CEACAM5_Tusamitamab-Example 45 ADC

Example 64 was prepared following general method M3; a solution of **mAb 1** (25 mg at 10.5 mg/mL) in 88:12 DPBS/DMA, 1.17 mL of DPBS and 196 µL of DMA was reacted overnight with 96 µL of a solution of **Example 45** in DMA (20 mM). The crude mixture
15 was purified by diafiltration then formulated in buffer D by gel filtration. **Example 64** (16.5 mg at 2.4 mg/mL, overall yield 66%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.5 and a monomeric purity of 99%.
RP-HRMS: 147403 (naked mAb); 148702 (D1); 150021 (D2); 151334 (D3); 152647 (D4); 153957 (D5); 155269 (D6); 156582 (D7); 157891 (D8); 159199 (D9).

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Example 65: CEACAM5_769_VarB_VH7/VL2-Example 45 ADC

Example 65 was prepared following general method M2; a solution of **mAb 3** (20.6 mg at 9.3 mg/mL) in 97:3 DPBS/DMSO was reacted overnight with 71 µL of a solution of **Example 45** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg
25 HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 65** (15.8 mg at 2.6 mg/mL, overall yield 76%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of
30 4.1 and a monomeric purity of 100%.
RP-HRMS: 147759 (naked mAb); 149070 (D1); 150382 (D2); 151693 (D3); 153005 (D4); 154316 (D5); 155629 (D6); 156940 (D7); 158251 (D8); 159564 (D9).

Example 66: CEACAM5_p01_CEA_1_07-Example 45 ADC

Example 66 was prepared following general method M2; a solution of **mAb 4** (29 mg at 10.59 mg/mL) in 97.5:2.5 DPBS/DMSO was reacted overnight with 103 µL of a solution of **Example 45** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 66** (22 mg at 3.06 mg/mL, overall yield 76%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.4 and a monomeric purity of 100%.

RP-HRMS: 147375 (naked mAb); 148678 (D1); 149989 (D2); 151301 (D3); 152613 (D4); 153925 (D5); 155236 (D6); 156548 (D7); 157859 (D8); 159167 (D9).

Example 67: PDL1_Avelumab_K76R_K100R-Example 45 ADC

Example 67 was prepared following general method M3; a solution of **mAb 2** (26 mg at 9 mg/mL) in 80:20 DPBS/DMA, 877 µL of DPBS and 128 µL of DMA was reacted overnight with 92 µL of a solution of **Example 45** in DMA (20 mM). The crude mixture was purified by diafiltration then formulated in buffer E by gel filtration. **Example 67** (10.5 mg at 3.01 mg/mL, overall yield 40%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.5 and a monomeric purity of 98.5%.

RP-HRMS: 146184 (naked mAb); 147499 (D1); 148810 (D2); 150118 (D3); 151432 (D4); 152746 (D5); 154058 (D6); 155368 (D7).

Example 68: CEACAM5_p01_CEA_1_07-Example 46 ADC (DAR4)

Example 68 was prepared following general method M5; a solution of CEACAM5_p01_CEA_1_07 (94 mg at 10.87 mg/mL) in DPBS was reacted with 293 µL of a 280 mM solution of DTT in DPBS for 75 min at 37°C. **Reduced mAb 4** was obtained as a colorless limpid solution (92 mg at 5.43 mg/mL) after buffer exchange by gel filtration (Sephadex G-25, HiPrep 26/10 Desalting Column, Cytiva) pre-equilibrated with DPBS buffer containing 5 mM EDTA. 47 mg of **reduced mAb 4** from step 1 was then reacted overnight with 73 µL of a solution of **Example 46** in DMA (20 mM). The crude mixture

was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 68** (36 mg at 3.66 mg/mL, overall yield 76%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99%.

RP-HRMS (reduced sample): 23283 (LC); 24347 (LC1); 50406 (HC); 51471 (HC1); 51489 (HC1+18); 52535 (HC2); 52553 (HC2 + 18); 53599 (HC3); 53617 (HC3 + 18).

10 **Example 69: CEACAM5_p01_CEA_1_07-Example 46 ADC (DAR8)**

Example 69 was prepared following general method M5; a solution of CEACAM5_p01_CEA_1_07 (65 mg at 13.49 mg/mL) in DPBS was reacted with 200 µL of a 280 mM solution of DTT in DPBS for 60 min at 37°C. **Reduced mAb 4** was obtained as a colorless limpid solution (62 mg at 8.87 mg/mL) after buffer exchange by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) pre-equilibrated with DPBS buffer containing 5 mM EDTA and 5% DMA. 32 mg of **reduced mAb 4** from step 1 was then reacted for 1 hour with 110 µL of a solution of **Example 46** in DMA (20 mM) and kept at +4°C for 24 hours. The crude mixture (DAR(HRMS)<8) was buffer exchanged by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) pre-equilibrated with DPBS buffer then further reduced with 150 µL of a 280 mM solution of DTT in DPBS (75 min at 37°C). DTT was removed by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) pre-equilibrated with DPBS buffer containing 5 mM EDTA then further reacted for 4 hours with 55 µL of a solution of **Example 46** in DMA (20 mM). After purification by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentration by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore) and formulation in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva), **Example 69** (22 mg at 3.32 mg/mL, overall yield 68%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 8 and a monomeric purity of 98%.

30 RP-HRMS: 24346 (LC1); 53599 (HC3).

Example 70: CEACAM5_p01_CEA_1_07_A118C-Example 46 ADC

Example 70 was prepared following general method M4b; a solution of crude **uncapped thiomAb 3** (35 mg at 3.87 mg/mL) in DPBS was reacted overnight with 45 μ L of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 70** (25 mg at 3.7 mg/mL, overall yield 65%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.8 and a monomeric purity of 97%.

RP-HRMS (reduced sample): 23283 (LC); 50439 (HC); 51503 (HC1).

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Example 71: CEACAM5_p01_CEA_1_07_E152C-Example 46 ADC

Example 71 was prepared following general method M4b; a solution of crude **uncapped thiomAb 4** (28 mg at 2.84 mg/mL) in DPBS was reacted overnight with 33 μ L of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 71** (22 mg at 3.38 mg/mL, overall yield 70%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99%.

20 RP-HRMS (reduced sample): 23283 (LC); 51445 (HC1).

Example 72: CEACAM5_p01_CEA_1_07_K360C-Example 46 ADC

Example 72 was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (27 mg at 2.74 mg/mL) in DPBS was reacted overnight with 41 μ L of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 72** (22 mg at 3.33 mg/mL, overall yield 56%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.9 and a monomeric purity of 96%.

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RP-HRMS (reduced sample): 23282 (LC); 51444 (HC1).

Example 73: CEACAM5_p01_CEA_1_07_K414C-Example 46 ADC

Example 73 was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (27 mg at 2.74 mg/mL) in DPBS was reacted overnight with 41 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 73** (22 mg at 3.33 mg/mL, overall yield 56%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.9 and a monomeric purity of 96%.

10 RP-HRMS (reduced sample): 23283(LC); 24346 (LC1); 51446 (HC1).

Example 74: CEACAM5_p07_CEA_3_50_VH1_A118C-Example 46 ADC

Example 74 was prepared following general method M4b; a solution of crude **uncapped thiomAb 7** (34 mg at 2.8 mg/mL) in DPBS was reacted for 2 hours with 61 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 74** (29 mg at 4.3 mg/mL, overall yield 71%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.8 and a monomeric purity of 99%.

20 RP-HRMS (reduced sample): 23338 (LC); 51327 (HC1).

Example 75: CEACAM5_p07_CEA_3_50_VH1_K360C-Example 46 ADC

Example 75 was prepared following general method M4b; a solution of crude **uncapped thiomAb 8** (37 mg at 2.11 mg/mL) in DPBS was reacted overnight with 57 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 75** (26 mg at 2.6 mg/mL, overall yield 64%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.9 and a monomeric purity of 98%.

30 RP-HRMS (reduced sample): 23339 (LC); 50207 (HC); 51365 (HC1); 51288 (HC1+17).

Example 76: CEACAM5_p07_CEA_3_50_VH1_K414C-Example 46 ADC

Example 76 was prepared following general method M4b; a solution of crude **uncapped thiomAb 9** (33 mg at 2.76 mg/mL) in DPBS was reacted overnight with 66 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 76** (22 mg at 3.3 mg/mL, overall yield 59%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 98%.
RP-HRMS (reduced sample): 23338 (LC); 51271 (HC1).

Example 77: CEACAM5_p01_CEA_1_07_A118C_K360C-Example 46 ADC

Example 77 was prepared following general method M4b; a solution of crude **uncapped thiomAb 10** (35 mg at 3.17 mg/mL) in DPBS was reacted overnight with 90 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 77** (19 mg at 4.08 mg/mL, overall yield 51%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 99%.
RP-HRMS (reduced sample): 23283 (LC); 51476 (HC1); 52542 (HC2).

Example 78: CEACAM5_p01_CEA_1_07_E152C_K360C-Example 46 ADC

Example 78 was prepared following general method M4b; a solution of crude **uncapped thiomAb 11** (33 mg at 2.98 mg/mL) in DPBS was reacted overnight with 92 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 78** (30 mg at 4.5 mg/mL, overall yield 81%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99%.

RP-HRMS (reduced sample): 23283 (LC); 51419 (HC1); 52484 (HC2).

Example 79: CEACAM5_p01_CEA_1_07_K274C_K414C-Example 46 ADC

Example 79 was prepared following general method M4b; a solution of crude **uncapped thiomAb 12** (34 mg at 3.86 mg/mL) in DPBS was reacted overnight with 103 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 79** (26 mg at 4.11 mg/mL, overall yield 72%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.1 and a monomeric purity of 99%.

RP-HRMS (reduced sample): 23283 (LC); 24346 (LC1); 52485 (HC2); 53552 (HC3).

Example 80: CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-Example 46 ADC

Example 80 was prepared following general method M4b; a solution of crude **uncapped thiomAb 13** (34 mg at 2.4 mg/mL) in DPBS was reacted overnight with 100 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 80** (21 mg at 2.69 mg/mL, overall yield 53%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.6 and a monomeric purity of 98%.

RP-HRMS (reduced sample): 23338 (LC); 51300 (HC1); 52364 (HC2).

Example 81: CEACAM5_p07_CEA_3_50_VH1_K274C_K414C-Example 46 ADC

Example 81 was prepared following general method M4b; a solution of crude **uncapped thiomAb 14** (32 mg at 2.64 mg/mL) in DPBS was reacted overnight with 98 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 81** (24 mg at 3.55 mg/mL, overall yield 62%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity

of 98%.

RP-HRMS (reduced sample): 23338 (LC); 51246 (HC1); 52309 (HC2).

Example 82: PDL1_Avelumab_K360C-Example 46 ADC

- 5 **Example 82** was prepared following general method M4b; a solution of crude **uncapped thiomAb 1** (35.7 mg at 4.32 mg/mL) in DPBS and 2.03 mL of DMA was reacted overnight with 37 µL of a solution of **Example 46** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using a Sephadex™
- 10 G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 82** (26.9 mg at 3.49 mg/mL, overall yield 77%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
RP-HRMS: 148513 (D2).

15 **Example 83: PDL1_Avelumab_LALA_K360C-Example 46 ADC**

- Example 83** was prepared following general method M4b; a solution of crude **uncapped thiomAb 2** (31 mg at 5.23 mg/mL) in DPBS and 1 mL of cold DMA was reacted overnight with 32 µL of a solution of **Example 7** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS
- 20 with 10% DMA), concentrated, formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 83** (22 mg at 2.25 mg/mL, overall yield 70%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
RP-HRMS: 148347 (D2).

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Example 84: CEACAM5_p01_CEA_1_07_K360C-Example 47 ADC

- Example 84** was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (18 mg at 1.87 mg/mL) in DPBS was reacted with a total of 24 µL of a solution of **Example 47** in DMA (20 mM) for a total of 21 hours. The reaction mixture was then
- 30 purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated and formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 84** (15 mg at 4.54 mg/mL, overall yield 60%)

was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.95 and a monomeric purity of 99%.

RP-HRMS (reduced sample): 23282 (LC); 50387 (HC); 51361 (HC1).

5 **Example 85: CEACAM5_p01_CEA_1_07_K360C-P36 ADC**

Example 85 was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (13.9 mg at 5.12 mg/mL) in DPBS was reacted overnight with 20 µL of a solution of **P36** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 85** (11 mg at 3.15 mg/mL, overall yield 80%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 98%.

RP-HRMS (reduced sample): 23280 (LC); 50377 (HC); 51357 (HC1).

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Example 86: PDL1_Avelumab_K360C-P36 ADC

Example 86 was prepared following general method M4b; to a solution of crude **uncapped thiomAb 1** (30 mg at 4.26 mg/mL) in DPBS were added 747 µl of cold DMA and 30 µL of a solution of **P36** in DMA (20 mM). The mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 86** (17 mg at 3.29 mg/mL, overall yield 65%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.9 and a monomeric purity of 98%.

25 RP-HRMS: 147490 (D1 + Cys); 148352 (D2)

Example 87: CEACAM5_p01_CEA_1_07_K360C-Example 48 ADC

Example 87 was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (13.9 mg at 5.12 mg/mL) in DPBS was reacted overnight with 20 µL of a solution of **Example 48** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting

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Columns, Cytiva). **Example 87** (11.2 mg at 3.19 mg/mL, overall yield 80%) was finally obtained after sterile filtration as a colorless limp solution with a DAR (HRMS) of 2 and a monomeric purity of 99%.

RP-HRMS (reduced sample): 23280 (LC); 50377 (HC); 51260 (HC1)

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Example 88: PDL1_Avelumab_K360C-Example 48 ADC

Example 88 was prepared following general method M4b; to a solution of crude **uncapped thiomAb 1** (23.7 mg at 4.74 mg/mL) in DPBS were added 531 µl of cold DMA and 24 µL of a solution of **Example 48** in DMA (20 mM). The mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 88** (17 mg at 2.21mg/mL, overall yield 56%) was finally obtained after sterile filtration as a colorless limp solution with a DAR (HRMS) of 2 and a monomeric purity of 98%.

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15 RP-HRMS: 148163 (D2).

Example 89: CEACAM5_p01_CEA_1_07_K360C-Example 49 ADC

Example 89 was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (13.9 mg at 5.12 mg/mL) in DPBS was reacted overnight with 20 µL of a solution of **Example 49** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 89** (12 mg at 3.43 mg/mL, overall yield 79%) was finally obtained after sterile filtration as a colorless limp solution with a DAR (HRMS) of 2 and a monomeric purity of 98%.

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25 RP-HRMS (reduced sample): 23280 (LC); 50377 (HC); 51277 (HC1).

Example 90: PDL1_Avelumab_K360C-Example 49 ADC

Example 90 was prepared following general method M4b; to a solution of crude **uncapped thiomAb 1** (23.7 mg at 4.74 mg/mL) in DPBS were added 531 µl of cold DMA and 20 µL of a solution of **Example 49** in DMA (20 mM). The mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS

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with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 90** (12.6 mg at 3.7 mg/mL, overall yield 56%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 98%.

5 RP-HRMS: 148196 (D2).

Example 91: CEACAM5_Tusamitamab-Example 42 ADC

Example 91 was prepared following general method M2; a solution of **mAb 1** (27.5 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 1.23 mL of DPBS and 120 µL of DMA was reacted
10 overnight with 186 µL of a solution of **Example 42** in DMA (10 mM). After addition of 4 µL of the solution of **Example 42** and 1 h reaction at RT, the crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer E by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 91** (14 mg at
15 2.6 mg/mL, overall yield 51%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 98.9%.

RP-HRMS: 147399 (naked mAb); 148922 (D1); 150415 (D2); 151922 (D3); 153423 (D4); 154928 (D5); 156427 (D6); 157936 (D7); 159443 (D8).

20 **Example 92: PDL1_Avelumab_K76R_K100R-Example 42 ADC**

Example 92 was prepared following general method M2; a solution of **mAb 2** (22.5 mg at 9 mg/mL) in 80:20 buffer B/DMA and 646 µL of DMA was reacted overnight with 162 µL of a solution of **Example 42** in DMA (10 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10%
25 DMA), concentrated and formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 92** (6.7 mg at 3.42 mg/mL, overall yield 40%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity of 99.6%.

RP-HRMS: 147682 (D1); 149193 (D2); 150696 (D3); 152196 (D4); 153705 (D5); 155213
30 (D6).

Example 93: CEACAM5_Tusamitamab-Example 41 ADC

Example 93 was prepared following general method M3; a solution of **mAb 1** (26 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 1.17 mL of DPBS and 196 μ L of DMA was reacted overnight with 96 μ L of a solution of **Example 41** in DMA (20 mM). After addition of 10 μ L of the solution of **Example 41** and 1.5 h reaction at RT, the crude mixture was purified by diafiltration then formulated in buffer E by gel filtration. **Example 93** (14 mg at 2.76 mg/mL, overall yield 54%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 98.6%.
RP-HRMS: 148995 (D1); 150587 (D2); 152178 (D3); 153765 (D4); 155353 (D5); 156946 (D6); 158540 (D7).

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Example 94: PDL1_Avelumab_K76R_K100R-Example 41 ADC

Example 94 was prepared following general method M2; a solution of **mAb 2** (24.5 mg at 9 mg/mL) in 80:20 buffer B/DMA, 877 μ L of DPBS and 108 μ L of DMA was reacted overnight with 91 μ L of a solution of **Example 41** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 94** (15 mg at 2.18 mg/mL, overall yield 61%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.5 and a monomeric purity of 98.5%.
RP-HRMS: 147751 (D1); 149362 (D2); 150952 (D3); 152544 (D4); 154131 (D5); 155726 (D6).

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Example 95: CEACAM5_Tusamitamab-P29 ADC

Example 95 was prepared following general method M2; a solution of **mAb 1** (31 mg at 9.7 mg/mL) in 93:7 buffer B/DMA was reacted overnight with 104 μ L of a solution of **P29** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 95** (24.8 mg at 3.1 mg/mL, overall yield 79%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.2 and a monomeric

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purity of 99.9%.

RP-HRMS: 147412 (naked mAb); 148629 (D1); 149847 (D2); 151064 (D3); 152281 (D4); 153499 (D5); 154716 (D6); 155933 (D7); 157151 (D8); 158673 (D9).

5 **Example 96: PDL1_Avelumab_K76R_K100R-P29 ADC**

Example 96 was prepared following general method M2; a solution of **mAb 2** (31 mg at 9 mg/mL) in 80:20 buffer B/DMA, 729 µL of DPBS and 89 µL of DMA was reacted overnight with 94 µL of a solution of **P29** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with
10 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 96** (22 mg at 2.4 mg/mL, overall yield 70%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.6 and a monomeric purity of 98.8%.
15 RP-HRMS: 146194 (naked mAb); 147415 (D1); 148632 (D2); 149849 (D3); 151067 (D4); 152284 (D5); 153500 (D6); 154725 (D7); 155938 (D8).

Example 97: CEACAM5_Tusamitamab-P32 ADC

Example 97 was prepared following general method M2; a solution of **mAb 1** (29 mg at
20 9.7 mg/mL) in 93:7 buffer B/DMA was reacted overnight with 104 µL of a solution of **P32** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES
25 membrane, 50K, Millipore). **Example 97** (22.1 mg at 3.0 mg/mL, overall yield 76%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.4 and a monomeric purity of 99.9%.
RP-HRMS: 147414 (naked mAb); 148715 (D1); 150019 (D2); 151322 (D3); 152626 (D4); 153930 (D5); 155234 (D6); 156537 (D7); 157835 (D8); 159145 (D9); 160398 (D10).

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Example 98: PDL1_Avelumab_K76R_K100R-P32 ADC

Example 98 was prepared following general method M2; a solution of **mAb 2** (32 mg at

9 mg/mL) in 80:20 buffer B/DMA, 795 μ L of DPBS and 97 μ L of DMA was reacted overnight with 102 μ L of a solution of **P32** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 98** (23 mg at 2.85 mg/mL, overall yield 72%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 100%.
RP-HRMS: 146195 (naked mAb); 147500 (D1); 148804 (D2); 150107 (D3); 151411 (D4);
10 152714 (D5); 154020 (D6); 155320 (D7); 156627 (D8).

Example 99: CEACAM5_Tusamitamab-P27 ADC

Example 99 was prepared following general method M2; a solution of **mAb 1** (31 mg at 10.5 mg/mL) in 80:20 buffer B/DMA and 471 μ L of DPBS was reacted overnight with
15 104 μ L of a solution of **P27** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore) and formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 99** (16 mg at
20 3.22 mg/mL, overall yield 51%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.6 and a monomeric purity of 100%.
RP-HRMS: 147423 (naked mAb); 148914(D1); 150410(D2); 151906 (D3); 153402 (D4);
154897 (D5); 156393 (D6); 157888 (D7); 159384 (D8); 160879 (D9); 162378 (D10).

Example 100: PDL1_Avelumab_K76R_K100R-P27 ADC

Example 100 was prepared following general method M2; a solution of **mAb 2** (32 mg at 9 mg/mL) in 80:20 buffer B/DMA and 423 μ L of DPBS was reacted overnight with 106 μ L of a solution of **P27** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA),
30 concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore) and formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 100** (8 mg at

3.04 mg/mL, overall yield 25%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 4.3 and a monomeric purity of 100%.

RP-HRMS: 146207 (naked mAb); 147697 (D1); 149194 (D2); 150689 (D3); 152185 (D4); 153682 (D5); 155176 (D6); 156671 (D7); 158168 (D8); 159660 (D9).

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Example 101: CEACAM5_Tusamitamab-P25 ADC

Example 101 was prepared following general method M2; a solution of **mAb 1** (32 mg at 9.7 mg/mL) in 93:7 buffer B/DMA was reacted overnight with 104 µL of a solution of **P25** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 101** (22 mg at 2.76 mg/mL, overall yield 70%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.5 and a monomeric purity of 99.1%.

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RP-HRMS: 147414 (naked mAb); 148995 (D1); 150577 (D2); 152158 (D3); 153740 (D4); 155321 (D5); 156903 (D6); 158487 (D7); 160065 (D8); 161655 (D9).

Example 102: PDL1_Avelumab_K76R_K100R-P25 ADC

Example 102 was prepared following general method M2; a solution of **mAb 2** (34 mg at 10.5 mg/mL) in 93:7 buffer B/DMA was reacted overnight with 113 µL of a solution of **P25** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare) and concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore). **Example 102** (23 mg at 3.3 mg/mL, overall yield 68%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.55 and a monomeric purity of 100%.

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RP-HRMS: 146191 (naked mAb); 147780 (D1); 149361 (D2); 150944 (D3); 152525 (D4); 154107 (D5); 155691 (D6); 157265 (D7); 158852 (D8).

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Example 103: CEACAM5_p07_CEA_3_50_VH1_K360C-P39 ADC

Example 103 was prepared following general method M4b; a solution of crude **uncapped thiomAb 8** (111 mg at 3.33 mg/mL) in DPBS was reacted overnight with 113 µL of a solution of **P39** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 26/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, Hiprep® 26/10 Desalting Columns, Cytiva). **Example 103** (100 mg at 6.03 mg/mL, overall yield 77%) was finally obtained after concentration and sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.95 and a monomeric purity of 99%.
RP-HRMS (reduced sample): 23339 (LC); 50207 (HC); 51152 (HC1).

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Example 104: CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-P39 ADC

Example 104 was prepared following general method M4b; a solution of crude **uncapped thiomAb 13** (22 mg at 2.30 mg/mL) in DPBS was reacted overnight with 90 µL of a solution of **P39** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 104** (17 mg at 3.64 mg/mL, overall yield 63%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 98%.
RP-HRMS (reduced sample): 23339 (LC); 51183 (HC); 52129 (HC1).

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Example 105: CEACAM5_p07_CEA_3_50_VH1_K360C-P40 ADC

Example 105 was prepared following general method M4b; a solution of crude **uncapped thiomAb 8** (116 mg at 3.29 mg/mL) in DPBS was reacted overnight with 198 µL of a solution of **P40** in DMA (20 mM). The reaction mixture was concentrated then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, Hiprep® 26/10 Desalting Columns, Cytiva). **Example 105** (107 mg at 6.8 mg/mL, overall yield 76%) was finally obtained after concentration and sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99%.
RP-HRMS: 149030 (D2).

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Example 106: CEACAM5_p07_CEA_3_50_VH1_A118C_K360C-P40 ADC

Example 106 was prepared following general method M4b; a solution of crude **uncapped thiomAb 13** (31 mg at 2.93 mg/mL) in DPBS was reacted overnight with 129 μ L of a solution of **P40** in DMA (20 mM). The reaction mixture was concentrated then purified by
5 SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 106** (18 mg at 3.12 mg/mL, overall yield 65%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 98%.
10 RP-HRMS (reduced sample): 23339 (LC); 24310 (LC1); 51210 (HC1); 52181 (HC2).

Example 107: CEACAM5_Tusamitamab-Example 50 ADC

Example 107 was prepared following general method M3; a solution of **mAb 1** (15 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 858 μ L of DPBS and 148 μ L of DMA was reacted
15 overnight with 66.75 μ L of a solution of **Example 50** in DMA (16.02 mM). The crude mixture was purified by diafiltration then formulated in buffer A by gel filtration using PD10 desalting column (GE Healthcare). After additional purification by diafiltration with buffer A and sterile filtration, **Example 107** (6.84 mg at 2.28 mg/mL, overall yield 46%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity
20 of 98.63%.
RP-HRMS: 147412 (naked mAb); 149306 (D1); 151202 (D2); 153096 (D3); 154992 (D4); 156887 (D5); 158781 (D6); 160680 (D7); 162577 (D8).

Example 108: PDL1_Avelumab_K76R_K100R-Example 50 ADC

Example 108 was prepared following general method M3; a solution of **mAb 2** (25.2 mg at 6.2 mg/mL) in 80:20 DPBS/DMA was reacted overnight with 123 μ L of a solution of
25 **Example 50** in DMA (16.02 mM). The crude mixture was purified by diafiltration then formulated in buffer A by gel filtration using PD10 desalting column (GE Healthcare). **Example 108** (19.1 mg at 2.81 mg/mL, overall yield 76%) was finally obtained after sterile
30 filtration as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity of 98.8%.
RP-HRMS: 146196 (naked mAb); 148082 (D1); 149976 (D2); 151879 (D3); 153771 (D4);

155666 (D5); 157566 (D6); 159468 (D7).

Example 109: PDL1_Avelumab_K360C-P45 ADC

Example 109 was prepared following general method M4b; a solution of crude **uncapped thiomAb 1** (37 mg at 6.58 mg/mL) in DPBS and 1 mL of DMA was reacted overnight with 38 µL of a solution of **P45** in DMSO (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). **Example 109** (25.5 mg at 2.66 mg/mL, overall yield 69%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
RP-HRMS: 149677 (D2).

Example 110: CEACAM5_769_VarB_VH7/VL2-P47 ADC

Example 110 was prepared following general method M2; a solution of **mAb 3** (20 mg at 9.3 mg/mL) in 98:2 DPBS/DMSO was reacted for 1.5 hours with 71 µL of a solution of **P47** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 110** (10.1 mg at 1.42 mg/mL, overall yield 50%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.7 and a monomeric purity of 94%.
RP-HRMS: 147766 (naked mAb); DAR 149478 (D1), 151197 (D2); 152916 (D3); 154634 (D4); 156354 (D5); 158073 (D6); 159791 (D7), 161512 (D8); 163241 (D9).

Example 111: CEACAM5_p01_CEA_1_07-P47 ADC

Example 111 was prepared following general method M2; a solution of **mAb 4** (29 mg at 10.59 mg/mL) in 98:2 DPBS/DMSO was reacted for 5 hours with 103 µL of a solution of **P47** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore),

formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 111** (21.8 mg at 3.03 mg/mL, overall yield 70%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and a monomeric purity of 100%.

- 5 RP-HRMS: 149083 (D1), 150805 (D2); 152524 (D3); 154243 (D4); 155963 (D5); 157680 (D6); 159400 (D7); 161114 (D8); 162835 (D9).

Example 112: CEACAM5_p01_CEA_1_07-Example 51 ADC (DAR4)

- Example 112** was prepared following general method M5; a solution of
10 CEACAM5_p01_CEA_1_07 (41 mg at 10.87 mg/mL) in DPBS was reacted with 170 µL of a 280 mM solution of DTT in DPBS for 75 min at 37°C. **Reduced mAb 4** was obtained as a colorless limpid solution (37 mg at 5.39 mg/mL) after buffer exchange by gel filtration (Sephadex G-25, HiPrep 26/10 Desalting Column, Cytiva) in DPBS buffer containing 5mM EDTA. **Reduced mAb 4** from step 1 was then reacted overnight with 55 µL of a solution of
15 **Example 51** in DMA (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 112** (33 mg at 3.3 mg/mL, overall yield 80%) was finally
20 obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity of 99%.
RP-HRMS (reduced sample): 23283 (LC); 24754 (LC1); 50407 (HC); 51878 (HC1); 53350 (HC2); 54821 (HC3).

25 **Example 113: CEACAM5_p01_CEA_1_07-Example 51 ADC (DAR8)**

- Example 113** was prepared following general method M5; a solution of
CEACAM5_p01_CEA_1_07 (64 mg at 10.87 mg/mL) in DPBS was reacted with 200 µL of a 280 mM solution of DTT in DPBS for 60 min at 37°C. **Reduced mAb 4** was obtained as a colorless limpid solution (32 mg at 9.25 mg/mL) after gel filtration (Sephadex G-25, PD-
30 10 Desalting Columns, Cytiva) in DPBS buffer containing 5mM EDTA. **Reduced mAb 4** from step 1 was then reacted for 1 hour with 110 µL of a solution of Example 51 in DMA (20 mM). In order to get full reaction completion the crude mixture was then buffer exchanged

in DPBS by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva) then further reduced with 150 μ L of a 280 mM solution of DTT in DPBS for 60 min at 37°C, buffer exchanged by gel filtration (Sephadex G-25, HiPrep 26/10 Desalting Column, Cytiva) in DPBS buffer containing 5 mM EDTA and reacted for 4 hours with with 55 μ L of a solution of **Example 51** in DMA (20 mM). The reaction mixture was concentrated, purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 113** (23 mg at 3.64 mg/mL, overall yield 73%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 8 and a monomeric purity of 98%.
RP-HRMS: 24754 (LC1); 54821 (HC3).

Example 114: PDL1_Avelumab_K360C-Example 51 ADC

Example 114 was prepared following general method M4b; a solution of crude **uncapped thiomAb 1** (37 mg at 6.58 mg/mL) in DPBS and 1 mL of DMA was reacted overnight with 48 μ L of a solution of **Example 51** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). After sterile filtration **Example 114** (26.84 mg at 2.44 mg/mL, overall yield 72.5%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.
RP-HRMS: 149325 (D2).

Example 115: PDL1_Avelumab_LALA_K360C-Example 51 ADC

Example 115 was prepared following general method M4b; a solution of crude **uncapped thiomAb 2** (16.4 mg at 5.44 mg/mL) in DPBS and 0.736 mL of DMA was reacted overnight with 16 μ L of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer D using PD10 desalting column (GE Healthcare). **Example 115** (12.26 mg at 2.31 mg/mL, overall yield 72%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and

a monomeric purity of 100%.

RP-HRMS: 149166 (D2).

Example 116: CEACAM5_p01_CEA_1_07_A118C-Example 51 ADC

- 5 **Example 116** was prepared following general method M4b; a solution of crude **uncapped thiomAb 3** (27 mg at 5.41 mg/mL) in DPBS was reacted overnight with 32 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva).
- 10 **Example 116** (17 mg at 2.53 mg/mL, overall yield 60%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 1.6 and a monomeric purity of 98%.

RP-HRMS (reduced sample): 23282 (LC); 50437 (HC); 51909 (HC1).

15 **Example 117: CEACAM5_p01_CEA_1_07_E152C-Example 51 ADC**

- Example 117** was prepared following general method M4b; a solution of crude **uncapped thiomAb 4** (20 mg at 4.13 mg/mL) in DPBS was reacted overnight with 28 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated,
- 20 formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 117** (17 mg at 2.45 mg/mL, overall yield 74%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99%.

RP-HRMS: 150277 (DAR 2).

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Example 118: CEACAM5_p01_CEA_1_07_K360C-Example 51 ADC

- Example 118** was prepared following general method M4b; a solution of crude **uncapped thiomAb 5** (28 mg at 5.60 mg/mL) in DPBS was reacted overnight with 35 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated,
- 30 formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 118** (19 mg at 2.76 mg/mL, overall yield 68%) was finally obtained after sterile

filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99%.

RP-HRMS: 150278 (DAR 2).

5 **Example 119: CEACAM5_p01_CEA_1_07_K414C-Example 51 ADC**

Example 119 was prepared following general method M4b; a solution of crude **uncapped thiomAb 6** (25 mg at 5.09 mg/mL) in DPBS was reacted overnight with 35 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 119** (18 mg at 2.59 mg/mL, overall yield 70%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 97%.

RP-HRMS: 150279 (DAR 2).

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Example 120: CEACAM5_p01_CEA_1_07_A118C_K360C-Example 51 ADC

Example 120 was prepared following general method M4b; a solution of crude **uncapped thiomAb 10** (26 mg at 5.21 mg/mL) in DPBS was reacted overnight with 70 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 120** (17 mg at 2.44 mg/mL, overall yield 60%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2.7 and a monomeric purity of 97%.

25 RP-HRMS (reduced sample): 23282 (LC); 50411 (HC); 51883 (HC1); 53355 (HC2); 54841 (HC3).

Example 121: CEACAM5_p01_CEA_1_07_E152C_K360C-Example 51 ADC

Example 121 was prepared following general method M4b; a solution of crude **uncapped thiomAb 11** (26 mg at 5.16 mg/mL) in DPBS was reacted overnight with 72 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated,

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formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva).

Example 121 (17 mg at 2.67 mg/mL, overall yield 65%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4 and a monomeric purity of 96%.

5 RP-HRMS: 153165 (D4).

Example 122: CEACAM5_p01_CEA_1_07_K274C_K414C-Example 51 ADC

Example 122 was prepared following general method M4b; a solution of crude **uncapped thiomAb 12** (27 mg at 5.29 mg/mL) in DPBS was reacted overnight with 74 µL of a solution of **Example 51** in DMA (20 mM). The reaction mixture was then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 122** (17 mg at 2.58 mg/mL, overall yield 62%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 95%.

15 RP-HRMS (reduced sample): 23281 (LC); 51824 (HC1); 53296 (HC2).

Example 123: CEACAM5_Tusamitamab-Example 57 ADC

Example 123 was prepared following general method M2; to a solution of **mAb 1** (50 mg at 10.5 mg/mL) in 80:20 DPBS/DMA were added 2.88 mL of DPBS, 541 µL of cold DMA and 180 µL of a solution of **Example 57** in DMSO (20 mM). The crude mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 123** (41.5 mg at 4.89 mg/mL, overall yield 83%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99%.

25 RP-HRMS: 149165 (D1); 150913 (D2); 152660 (D3); 154407 (D4); 156156 (D5); 157901 (D6); 159645 (D7); 161395 (D8).

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Example 124: CEACAM5_Tusamitamab-Example 58 ADC

Example 124 was prepared following general method M2; a solution of **mAb 1** (45 mg at

8.61 mg/mL) in 98:2 DPBS/DMSO was reacted overnight with 172 μ L of a solution of **Example 59** in DMSO (20 mM). 15 μ L of DBCO-NHS reagent were then added and the reaction was pursued for 2 hours. The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by
5 diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 125** (37 mg at 3.6 mg/mL, overall yield 82%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and monomeric purity of 99%.

10 RP-HRMS (reduced sample): 23467 (LC); 25240 (LC1); 27013 (LC2); 28785 (LC3); 50237 (HC); 52010 (HC1); 53783 (HC2); 55557 (HC3); 57330 (HC4).

Example 125: CEACAM5_Tusamitamab-Example 59 ADC

Example 125 was prepared following general method M2; a solution of **mAb 1** (45 mg at
15 8.61 mg/mL) in 98:2 DPBS/DMSO was reacted overnight with 172 μ L of a solution of **Example 59** in DMSO (20 mM). 15 μ L of DBCO-NHS reagent were then added and the reaction was pursued for 2 hours. The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by
20 diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 125** (37 mg at 3.6 mg/mL, overall yield 82%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.9 and monomeric purity of 99%.

RP-HRMS (reduced sample): 23467 (LC); 25254 (LC1); 27041 (LC2); 28828 (LC3); 50237
25 (HC); 52024 (HC1); 53811 (HC2); 55599 (HC3); 57393 (HC4).

Example 126: CEACAM5_Tusamitamab-Example 60 ADC

Example 126 was prepared following general method M2; to a solution of **mAb 1** (50 mg at 10.5 mg/mL) in 80:20 DPBS/DMA were added 2.82 mL of DPBS, 592 μ L of cold DMA
30 and 176 μ L of a solution of **Example 60** in DMSO (20 mM). The crude mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin

columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 126** (42 mg at 4.68 mg/mL, overall yield 82%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99%.

5 RP-HRMS: 149109 (D1); 150802 (D2); 152491 (D3); 154182 (D4); 155874 (D5); 157563 (D6); 159254 (D7), 160953 (D8).

Example 127: CEACAM5_Tusamitamab-Example 61 ADC

Example 127 was prepared following general method M2; a solution of **mAb 1** (43 mg at 8.32 mg/mL) in 98:2 DPBS/DMSO was reacted overnight with 169 µL of a solution of **Example 61** in DMSO (20 mM). 12 µL of DBCO-NHS reagent were then added and the reaction was pursued for 2 hours. The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 127** (30 mg at 3.04 mg/mL, overall yield 70%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.1 and monomeric purity of 100%.

15 RP-HRMS (reduced sample): 23467 (LC); 25156 (LC1); 26846 (LC2); 28536 (LC3); 30225 (LC4); 50237 (HC); 51927 (HC1); 53617 (HC2); 55307 (HC3); 56966 (HC4).

Example 128: PDL1_Avelumab_K360C-P52 ADC

Example 128 was prepared following general method M4b; a solution of crude **uncapped thiomAb 1** (11.72 mg at 5.21 mg/mL) in DPBS and 0.55 mL of cold DMA was reacted overnight with 24 µL of a solution of **P52** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). **Example 128** (5.81 mg at 2.53 mg/mL, overall yield 50%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 100%.

30 RP-HRMS: 148627 (D2).

Example 129: PDL1_Avelumab_LALA_K360C-P52 ADC

Example 129 was prepared following general method M4b; a solution of crude **uncapped thiomAb 2** (16.45 mg at 5.44 mg/mL) in DPBS and 0.736 mL of DMA was reacted overnight with 34 μ L of a solution of **P52** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). **Example 129** (12.6 mg at 2.11 mg/mL, overall yield 76%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2 and a monomeric purity of 99.45%.

10 RP-HRMS: 148461 (D2).

Example 130: PDL1_Avelumab_K76R_K100R-Example 54 ADC

Example 130 was prepared as follows: a solution of **PDL1_Avelumab_K76R_K100R** (15.87 mg at 11.34 mg/mL in DPBS) and 210 μ L of DMA were reacted overnight with 15 μ L of a solution of crude tetrafluorophenyl ester of **Example 54** in DMA (20 mM). Then 17.5 μ L of a solution of crude tetrafluorophenyl ester of **Example 54** in DMA (20 mM) were added and the reaction was pursued for 2 hours. The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 10% DMA), concentrated and formulated in buffer D by gel filtration using PD10 desalting column (GE Healthcare). After additional purification by diafiltration with buffer D and sterile filtration, **Example 130** (7.48 mg at 2.2 mg/mL, overall yield 47%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 1.9 and a monomeric purity of 99.8%.

20 RP-HRMS: 146208 (naked mAb); 147187 (D1); 148167 (D2); 149145 (D3); 150124 (D4); 151107 (D5); 152065 (D6).

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Example 131: CEACAM5_Tusamitamab-Example 53 ADC

Example 131 was prepared following general method M3; a solution of **mAb 1** (15 mg at 10.5 mg/mL) in 80:20 buffer B/DMA, 858 μ L of DPBS and 118 μ L of DMA was reacted overnight with 96.64 μ L of a solution of **Example 53** in DMA (11.07 mM). The reaction mixture was purified by diafiltration then formulated in buffer A by gel filtration using PD10 desalting column (GE Healthcare). **Example 131** (10.89 mg at 3.3 mg/mL, overall yield

30

66%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.0 and a monomeric purity of 97.79%.

RP-HRMS: 147412 (naked mAb); 149235 (D1); 151055 (D2); 152878 (D3); 154702 (D4); 156521 (D5); 158350 (D6); 160158 (D7).

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Example 132: PDL1_Avelumab_K76R_K100R-Example 53 ADC

Example 132 was prepared following general method M3; a solution of **mAb 2** (25.2 mg at 6.2 mg/mL) in 80:20 DPBS/DMA was reacted overnight with 163 µL of a solution of **Example 53** in DMA (11.07 mM). The reaction mixture was purified by diafiltration then formulated in buffer A by gel filtration using PD10 desalting column (GE Healthcare). After additional purification by diafiltration with buffer A and sterile filtration, **Example 132** (15.4 mg at 2.8 mg/mL, overall yield 61%) was finally obtained as a colorless limpid solution with a DAR (HRMS) of 3.3 and a monomeric purity of 98.1%.

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RP-HRMS: 148009 (D1); 149826 (D2); 151657 (D3); 153488 (D4); 155304 (D5); 157094 (D6).

15

Example 133: CEACAM5_Tusamitamab-Example 55 ADC

Example 133 was prepared following general method M2; a solution of **mAb 1** (43 mg at 8.32 mg/mL) in 98:2 DPBS/DMSO was reacted overnight with 169 µL of a solution of **Example 55** in DMSO (20 mM). The crude mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 133** (31 mg at 3.17 mg/mL, overall yield 72%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.6 and monomeric purity of 100%.

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RP-HRMS (reduced sample): 23467 (LC); 25214 (LC1); 26961 (LC2); 28707 (LC3); 50237 (HC); 51984 (HC1); 53731 (HC2); 55477 (HC3).

30

Example 134: CEACAM5_Tusamitamab-Example 56 ADC

Example 134 was prepared following general method M2; to a solution of **mAb 1** (50 mg at 10.5 mg/mL) in 80:20 DPBS/DMA were added 2.82 mL of DPBS, 592 µL of cold DMA

and 176 μ L of a solution of **Example 56** in DMSO (20 mM). The crude mixture was reacted overnight then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated by diafiltration with ultrafiltration spin columns (Amicon® Ultra: PES membrane, 50K, Millipore), formulated in buffer D by gel
5 filtration using a Sephadex™ G25 matrix (HiPrep 26/10 Desalting, Cytiva). **Example 134** (35 mg at 3.94 mg/mL, overall yield 69%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 3.8 and a monomeric purity of 99%.
RP-HRMS: 147428 (naked mAb); 149152 (D1), 150884 (D2); 152619 (D3); 154352 (D4); 156085 (D5); 157818 (D6); 159548 (D7), 161285 (D8).

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Example 135: PDL1_Avelumab_K360C-Example 52 ADC

Example 135 was prepared following general method M4b; to a solution of crude **uncapped thiomAb 1** (30 mg at 4.26 mg/mL) in DPBS were added 720 μ L of cold DMA and a total of 120 μ L of a solution of **Example 52** in DMA (20 mM). The reaction mixture was reacted
15 for 21 hours then purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Cytiva, DPBS with 10% DMA), concentrated, formulated in buffer D by gel filtration (Sephadex G-25, PD-10 Desalting Columns, Cytiva). **Example 135** (12 mg at 1.78 mg/mL, overall yield 40%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 2.1 and a monomeric purity of 99%.
20 RP-HRMS: 148000 (D1 + cyst); 149370 (D2); 152351 (D4).

Example 136: CEACAM5_Tusamitamab-P42 ADC

Example 136 was prepared following general method M2; a solution of **mAb 1** (31.5 mg at 10.5 mg/mL) in 80:20 Buffer B/DMA, 1.2 mL of DPBS and 191 μ L of cooled DMA was
25 reacted overnight with 90 μ L of a cooled solution of **P42** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS with 20% DMA), formulated in buffer D by gel filtration using a Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). After concentration, 20 μ L of a solution of **P42** in DMA (20 mM) were added and reacted overnight. After
30 purification by diafiltration with buffer D, **Example 136** (20.85 mg at 2.64 mg/mL, overall yield 66.2%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.1 and a monomeric purity of 98.3%.

RP-HRMS: 147407 (naked mAb); 149300 (D1); 151187 (D2); 153075 (D3); 154961 (D4); 156849 (D5); 158736 (D6); 160625 (D7); 162510 (D8); 164400 (D9).

Example 137: PDL1_Avelumab_K76R_K100R- P42 ADC

- 5 **Example 137** was prepared following general method M2; a solution of **mAb 2** (31.7 mg at 9.06 mg/mL) in 80:20 DPBS/DMA, 0.824 mL of DPBS and 100 µL of cooled DMA was reacted overnight with 106 µL of a cooled solution of **P42** in DMA (20 mM). The reaction mixture was purified by SEC (Superdex 200 pg HiLoad® 16/60 desalting column, GE Healthcare, DPBS pH 6.4 with 20% DMA), formulated in buffer D by gel filtration using a
- 10 Sephadex™ G25 matrix (Hitrap™ desalting columns, GE Healthcare). After concentration **Example 137** (24 mg at 2.53 mg/mL, overall yield 73%) was finally obtained after sterile filtration as a colorless limpid solution with a DAR (HRMS) of 4.37 and a monomeric purity of 98.4%.

- RP-HRMS: 146201 (naked mAb); 148097 (D1); 149976 (D2); 151864 (D3);
- 15 153752 (D4); 155639 (D5); 157528 (D6); 159415 (D7); 161301 (D8); 163204 (D9); 165076 (D10); 166960 (D11).

PHARMACOLOGICAL TESTS

- EXAMPLE A: Evaluation of stimulation of human TLR7 by the**
- 20 **compounds/conjugates by *in-vitro* assay**

The compounds/conjugates were subjected to pharmacological testing for determining their activity as TLR7 agonists. Our reference, R848, is a well-known TLR7/8 agonist.

- The below tests involving measuring the *in vitro* activity of some compounds of the disclosure on human TLR7 pathway were carried out using parental HEK-Blue™ hTLR7
- 25 cells and engineered Tumor Associated Antigen or TAA (CEACAM5/PDL1)-expressing HEK-Blue™ hTLR7 cells as reporter cells.

- The activity described below, with respect to human TLR7, is given by the half maximal effective concentration (EC50), which corresponds to the concentration required to obtain a
- 30 50% of the activation. The lower the EC50, the less the concentration of the compound is required to produce 50% of the maximum activation and the higher the potency. For

compounds/conjugates, the below EC50 between 1 to 500 nM are considered as demonstrating a pathway activation and $EC50 \geq 500$ nM are considered as demonstrating an absence of pathway activation.

- 5 The EC50 of reference R848 has been tested according to the below test and the results are in Table 3 below.

Table 3

Reference TLR7/8 agonist	EC50 on HEK-Blue™ hTLR7 cells
R848	162.6 nM

HEK-Blue™ hTLR7 Cells (# hkb-htrl7) cells from InvivoGen and engineered TAA
10 (CEACAM5/PDL1) expressing cells were used according to the manufacturer's instructions. The HEK-Blue™ hTLR7 cells were obtained by co-transfection of the hTLR7 gene in HEK293 cells and an optimized Secreted Embryonic Alkaline Phosphatase (SEAP) reporter gene which is placed under the control of the IFNβ minimal promoter fused to five NF-κB and AP-1-binding sites. The human CEACAM5 or human PDL1 expressing HEK-Blue™
15 hTLR7 cells were obtained by transfection of the tumor associated antigen genes in the parental cells followed by a polyclonal selection to enrich in cells overexpressing CEACAM5 or PDL1. Stimulation with a TLR7(/8) ligand activates NF-κB and AP-1 which induce the production of SEAP. The cells are seeded in 96-well plate at 3.10E4 cells/well and incubated 24 hours at 37°C, 5% CO2 before adding the conjugates to be evaluated. For
20 CEACAM5- HEK-Blue™ hTLR7 cells, the cells are subsequently incubated with 3-fold serial dilutions of each of the compounds (0.01 nM to 1 μM) for 48 or 96 hours at 37 °C, 5% CO2. For PDL1- HEK-Blue™ hTLR7 cells, the cells are subsequently incubated with 4-fold serial dilutions of each of the compounds (0.01 nM to 500 nM) for 72 hours at 37 °C, 5% CO2.
25
After incubation, 20 μL of the supernatant is harvested and incubated with 200 μL of QUANTI-Blue™ Solution. After a 3-hour incubation at 37°C, 5% CO2, SEAP activity is assessed by reading the optical density (OD) at 620 nm with an Envision microplate reader and a half maximal effective concentration (EC50) is calculated and used to rank the
30 compounds.

The results are summarized in Tables 4, 4', 5 and 5' below.

Table 4:

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 15	> 500 nM	33.4 nM
Example 13	> 500 nM	44 nM
Example 17	> 500 nM	15.8 nM
Example 21	> 500 nM	2.7 nM
Example 19	> 500 nM	27.9 nM
Example 25	> 500 nM	38.9 nM

5 **Table 4':**

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 29	> 500 nM	20.6 nM
Example 27	> 500 nM	22.3 nM
Example 31	> 500 nM	17.4 nM

Table 4'':

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 62	> 500 nM	18.4 nM
Example 64	> 500 nM	11.4 nM
Example 65	> 500 nM	12.4 nM

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 66	> 500 nM	12.4 nM
Example 68	> 500 nM	11.6 nM
Example 69	> 500 nM	4.7 nM
Example 70	> 500 nM	10.7 nM
Example 71	> 500 nM	5.4 nM
Example 72	> 500 nM	4.3 nM
Example 73	> 500 nM	3. 6 nM
Example 75	> 500 nM	11.9 nM
Example 77	nd	3.4 nM*
Example 78	nd	3.7 nM*
Example 79	nd	0.8 nM*
Example 80	> 500 nM	7.2 nM
Example 84	> 500 nM	12.4 nM
Example 85	> 500 nM	> 500 nM
Example 89	> 500 nM	> 500 nM
Example 91	> 500 nM	21.8 nM
Example 93	> 500 nM	30.7 nM
Example 95	> 500 nM	130 nM
Example 97	> 500 nM	60.4 nM
Example 99	> 500 nM	84.6 nM

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 101	> 500 nM	42.2 nM
Example 103	> 500 nM	> 500 nM
Example 105	> 500 nM	18.6 nM

*48 h incubation

Table 4''':

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7- CEACAM5
Example 107	> 500 nM	5.3 nM
Example 110	> 500 nM	9.8 nM
Example 111	> 500 nM	7.3 nM
Example 112	> 500 nM	4.2 nM
Example 113	> 500 nM	1.8 nM
Example 116	> 500 nM	5.3 nM
Example 117	> 500 nM	4.9 nM
Example 118	> 500 nM	10.6 nM
Example 119	> 500 nM	8.7 nM
Example 120	> 500 nM	5.3 nM
Example 121	> 500 nM	6.6 nM
Example 122	> 500 nM	1.8 nM
Example 131	> 500 nM	> 500 nM
Example 136	> 500 nM	26 nM

Table 5:

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7 (in nM)	EC50 HEK-Blue- hTLR7- PDL1 (in nM)
Example 14	>500	8.03
Example 16	>500	13.53
Example 18	>500	32.05
Example 20	>500	16.5
Example 23	>500	5.09
Example 26	>500	13.1

Table 5'

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7-PDL1
Example 28	>500 nM	6.98 nM
Example 32	>500 nM	5.6 nM
Example 33	>500 nM	6.6 nM
Example 34	>500 nM	7.87 nM
Example 35	>500 nM	4.86 nM
Example 36	>500 nM	3.69 nM
Example 37	>500 nM	5.44 nM
Example 40	>500 nM	5.38 nM

5

Table 5''

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7-PDL1
Example 63	>500 nM	11.2 nM
Example 82	>500 nM	3.3 nM
Example 86	>500 nM	>500 nM
Example 88	>500 nM	>500 nM
Example 90	>500 nM	>500 nM
Example 96	>500 nM	3.1 nM
Example 98	>500 nM	5.2 nM
Example 100	>500 nM	4.6 nM
Example 102	>500 nM	6.1 nM

Table 5'''

Compounds /Conjugates or references	EC50 HEKBlue- hTLR7	EC50 HEK-Blue- hTLR7-PDL1
Example 108	>500 nM	3.31 nM
Example 109	>500 nM	3.40 nM
Example 114	>500 nM	4.57 nM
Example 115	>500 nM	3.53 nM
Example 128	>500 nM	3.23 nM
Example 129	>500 nM	4.47 nM
Example 132	>500 nM	5.28 nM
Example 135	>500 nM	>500 nM
Example 137	>500 nM	4.75 nM

The above results show that the compounds/conjugates (II) of the disclosure well activate the TLR7 pathway in the engineered tumor antigen expressing HEK-Blue™ hTLR7 cells, but not in the parental cells that do not express CEACAM5 or PDL1. The TLR7 pathway activation by the compounds/conjugates (II) of the disclosure is dependent on the expression of the tumor antigen at the surface membrane of the cells.

EXAMPLE B: Evaluation of the stimulation of human TLR7/8 using -Dual™ monocytic cells by *in vitro* assay

The compounds/conjugates according to the disclosure were subjected to pharmacological testing for determining their activity on human monocytic cells that express at their membrane surface Fc receptors that show binding specificity for the Fc part of the compounds/conjugates.

The below tests involving measuring the *in vitro* activity of some compounds/conjugates of the disclosure were carried out using THP1-Dual™ cells as reporter monocyte cells. THP1-Dual™ cells induce the activation of NF-κB in response to certain TLR agonists, such as TLR7 and TLR8.

The activity described below is given by the half maximal effective concentration (EC50), which corresponds to the concentration required to obtain a 50% of the activation. The lower the EC50, the less the concentration of the compound is required to produce 50% of maximum activation and the higher the potency.

The below EC50 between 0.3 to 500 nM are considered as demonstrating an activation; EC50 ≥ 500 nM are considered as demonstrating an absence of activation.

THP1-Dual™ cells (# thpd-nfis) from InvivoGen were used according to the manufacturer's instructions. The cells were obtained by stable integration of two inducible reporter constructs in human THP-1 monocyte cells: an optimized Secreted Embryonic Alkaline Phosphatase (SEAP) reporter gene which is placed under the control of the IFNβ minimal promoter fused to five NF-κB and three c-Rel binding sites and a Secreted Lucia luciferase gene which is placed under the control of an ISG54 minimal promoter fused with five interferon (IFN)-stimulated response elements.

As a result, THP1-Dual™ cells allow to study the activation of the NF-κB pathway by monitoring the activity of SEAP, and the interferon regulatory factor pathway, by assessing the activity of Lucia luciferase. Stimulation of THP1-Dual™ cells with TLR7/8 ligand activates NF-κB and c-Rel which induce the production of SEAP as documented below.

5 **Table 6:**

Reference TLR7/8 agonist	EC50 NFκB pathway THP1-Dual cells
R848	367.1 nM

For CEACAM5 ADC, the cells are subsequently incubated with 3-fold serial dilutions of each of the compounds (0.01 nM to 1 μM) for 96 hours at 37 °C, 5% CO₂. For PDL1 ADC, the cells are subsequently incubated with 4-fold serial dilutions of each of the compounds
10 (0.01 nM to 500 nM) for 72 hours at 37 °C, 5% CO₂.

After incubation, 20 μL of the supernatant is harvested and incubated with 200 μL of QUANTI-Blue™ Solution. After a 3 hours incubation at 37°C, 5% CO₂, SEAP activity is assessed by reading the optical density (OD) at 620 nm with an Envision microplate reader
15 and a half maximal effective concentration (EC50) is calculated and used to rank the compounds/conjugates.

The results are summarized in Tables 7, 7', 8 and 8' below.

Table 7:

Conjugates	EC50 NFκB pathway THP1-Dual cells
Example 15	0.4 nM
Example 13	0.5 nM
Example 17	0.8 nM
Example 21	0.6 nM
Example 19	1.3 nM
Example 25	1.7 nM

Table 7’:

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 29	0.5 nM
Example 27	0.6 nM
Example 39	1.4 nM
Example 31	0.9 nM

5 Table 7’’:

Compounds /Conjugates or references	EC50 NFkB pathway THP1-Dual cells
Example 62	0.5 nM
Example 64	0.3 nM
Example 65	0.5 nM
Example 66	0.8 nM
Example 68	2.3 nM
Example 69	0.4 nM
Example 70	1.0 nM
Example 71	1.6 nM
Example 72	0.8 nM
Example 73	0.5 nM
Example 75	1.2 nM
Example 77	0.2 nM*

Compounds /Conjugates or references	EC50 NFkB pathway THP1-Dual cells
Example 78	0.4 nM*
Example 79	0.1 nM*
Example 80	0.5 nM
Example 84	1.1 nM
Example 85	0.4 nM
Example 89	1.1 nM
Example 91	0.5 nM
Example 93	0.6 nM
Example 95	1.7 nM
Example 97	1.7 nM
Example 99	2.7 nM
Example 101	1.7 nM
Example 103	0.4 nM
Example 105	0.5 nM

Table 7''':

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 107	0.5 nM
Example 110	1.0 nM
Example 111	2.2 nM
Example 112	3.3 nM

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 113	0.5 nM
Example 116	1.9 nM
Example 117	2.5 nM
Example 118	2.8 nM
Example 119	1.4 nM
Example 120	0.8 nM
Example 121	0.6 nM
Example 122	0.2 nM
Example 123	0.4 nM
Example 124	0.4 nM
Example 125	0.3 nM
Example 126	0.4 nM
Example 127	0.4 nM
Example 131	0.8 nM
Example 133	0.5 nM
Example 134	0.5 nM
Example 136	1.7 nM

Table 8:

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 14	0.22 nM

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 16	0.35 nM
Example 18	0.444 nM
Example 20	0.14 nM
Example 22	18.25 nM
Example 23	0.57 nM
Example 26	0.33 nM

Table 8’

Conjugates	EC50 NFkB pathway THP1-Dual cells
Example 28	0.35 nM
Example 30	0.24 nM
Example 32	3.46 nM
Example 33	0.41 nM
Example 34	0.73 nM
Example 35	>500 nM
Example 36	0.65 nM
Example 37	>500 nM
Example 40	0.14 nM

Table 8’’

Compounds /Conjugates or references	EC50 NFkB pathway THP1-Dual cells
Example 63	0.19 nM
Example 67	0.44 nM
Example 82	0.40 nM
Example 86	0.1 nM
Example 88	3.9 nM
Example 90	0.20 nM
Example 92	0.20 nM
Example 94	0.36 nM
Example 96	0.09 nM
Example 98	0.11 nM
Example 100	0.24 nM
Example 102	0.24 nM

Table 8'''

Compounds /Conjugates or references	EC50 NFkB pathway THP1-Dual cells
Example 108	0.31 nM
Example 109	0.57 nM
Example 114	1.13 nM
Example 115	>500 nM
Example 128	0.82 nM
Example 129	>500 nM

Compounds /Conjugates or references	EC50 NFkB pathway THP1-Dual cells
Example 132	0.28 nM
Example 135	0.7 nM
Example 137	0.10 nM

The above results show that the compounds/conjugates of formula (II), (II1) or (II2) of the disclosure activate the NF-kB pathway in THP1-Dual™ cells. It means that the compounds/conjugates of the disclosure bind the immune cell via the Fc part of the antibody, internalize and activate the NF-kB pathway through TLR7/8 activation.

EXAMPLE C: Evaluation of the cytokines IFNα, IL-6, IP10 and TNFα using the Meso Scale Discovery (MSD) *in vitro* assay

The tested ADCs (**Example 75**, **Example 103**, and **Example 105**) were incubated at various doses (5, 50, and 500 μM final) with CEACAM5-high MKN45 tumor cells and a DC-enriched primary cells preparation according to the manufacturer's instructions (Miltenyi #130-100-777) at a E:T ratio of 10:1 in 96 wells plates. After 24 h treatment at 37 °C, 5% CO₂, the plates are centrifuged and the supernatant were collected and assayed for cytokines IFNα, IL-6, IP10 and TNFα using the Meso Scale Discovery (MSD) assay platform according to the manufacturer's instructions. Briefly, 25 μL of cell culture supernatants were incubated for 1 h at RT in custom Multi-Spot 96-well plates prior coated with capture antibodies. After washing, SULFO-TAG® labeled detection antibodies were added to each of the wells and incubated for 1 h at RT before adding a read buffer. The subsequent reaction resulted in the emission of light which was then quantified to approximate the concentration (pg/mL) of each cytokine.

As compared with a naked mAb and as illustrated in Figures 1a, 1b, 1c and 1d, in co-culture assay with CEACAM5-high MKN45 cells and a DC-enriched primary cells preparation, **Examples 75, 105** (and **103** to a lesser extent) induce the production of IFNα and IP10 immune-stimulating cytokines. TNFα and IL6 pro-inflammatory cytokines are also produced but to a lesser extent with IFNα / TNFα ratio higher than 1. In vivo, this cytokine

response may promote polarization of the tumor microenvironment, immune cell recruitment, T cell priming, expansion and migration leading to tumor eradication by innate and adaptive immunity without induction of drastic elevation of pro-inflammatory cytokines, suggesting an improvement in the therapeutic index.

5

EXAMPLE D: Evaluation of the anti-tumoral activity by tumor cell killing assay

The below test involving measuring the *in vitro* tumor cell killing activity of some compounds of the present disclosure were carried out using Incucyte®. This assay is consisting in co-culture of human peripheral blood mononuclear cells (hPBMC) and
10 NucLight red-labelled human MKN-45 tumor cells. This Immune Cell Killing Assay allows direct measurements of immune cell-mediated killing of MKN-45 tumor cells with the combination of real-time automated analysis of tumor cell number. MKN-45 were used as they present a high expression of CEACAM5. To count living MKN-45 cells without altering their function, these cells have been stained thanks to a lentiviral-based labeling
15 reagent enabling expression of a nuclear-restricted Nuclight Red fluorescent protein. NucLight red-labeled human MKN-45 cells were seeded in 96-well plate at $7.5 \cdot 10^3$ cells/well and subsequently incubated for 24 hours at 37 °C, 5% CO₂. Then, conjugates of the disclosure were added in wells diluted in RPMI with 20% FBS at a final concentration of 0.06 to 1000 nM. hPBMCs were isolated by Ficoll density gradient
20 centrifugation from healthy donor peripheral blood samples. Freshly isolated hPBMCs were resuspended in RPMI-1640 medium supplement with 20% FBS and added to Nuclight red MKN-45 tumor cells at a ratio of 1:30 ($2.25 \cdot 10^5$ PBMC) at the same time than the compounds. The plates were then placed in the Incucyte® live-cell analysis apparatus. The Incucyte® Cell-by-Cell Analysis module was used to quantify the number of NucLight red-
25 labeled MKN-45 tumor cells over-time.

The activity described below is given by the area under the curve (AUC) at 138h of co-culture (with the curve representing the Nuclight Red cell count/image normalized at 0 h) on the y axis in function of compounds concentration (0.06 to 1000 nM) on the x axis. The
30 higher decrease in AUC, the higher the killing activity is. Figure 2 shows the result with one representative healthy hPBMC donor but was confirmed on 3 other donors which vary in the level of the intensity but give the same conclusion (data not shown), i.e. all the compounds

show tumor cell killing activity in co-culture assay of hPBMC and CEACAM5+ tumor cells equivalent or higher than Tusamitamab-R848 ADC.

5 The compounds/payloads of formula (I), (II) or (III) of the present disclosure are able to be conjugated, and the compounds/conjugates of formula (II), (III) or (IV) show a good solubility and a good stability.

10 The compounds/conjugates of formula (II), (III) or (IV) of the present disclosure are able to stimulate TLR7 and/or 8 pathways, bind to the tumoral target through the antibody and to internalize into the targeted cell, stimulate the immune cells in a FcγR-dependent binding and TLR7 and/or 8 activation and finally provide tumor cell killing by activated immune cells.

 Furthermore, the compounds/conjugates of the formula (II), (III) or (IV) are good candidates for therapeutic uses, and especially to prevent and/or treat cancer.

15 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

20 Accordingly, in another of its aspects, the disclosure provides medicaments which comprise at least one compound/conjugate of formula (II), (III) or (IV) according to the present disclosure, or a pharmaceutically acceptable salt thereof.

 The present disclosure, according to another of its aspects, also relates to the compounds/conjugates of formula (II), (III) or (IV) according to the present disclosure, or a pharmaceutically acceptable salt thereof, for use as medicaments.

25 The present disclosure, according to another of its aspects, also relates to the compounds/conjugates of formula (II), (III) or (IV) according to the present disclosure, or a pharmaceutically acceptable salt thereof, for use in therapy.

30 These medicaments are employed therapeutically, especially in the prevention and/or treatment of diseases or disorders that could benefit from an activation of the immune system.

 These medicaments are employed therapeutically, especially in the prevention and/or treatment of a disease or a disorder that could benefit from an activation of the

immune system, for instance in the prevention and/or treatment of a cell-proliferative disease, a cancer, a chronic myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer (for example an external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic keratosis, a basal cell carcinoma, a cutaneous T-cell lymphoma), an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy (for example an allergic rhinitis or a respiratory allergy), an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, for instance in the prevention and/or treatment of cancers.

In an embodiment, is a compound/conjugates of formula (II), (III) or (II2) of the instant disclosure, or a pharmaceutically acceptable salt thereof, for use in the prevention and/or treatment of a cancer.

It is possible to prevent and/or treat solid or liquid cancers.

In an embodiment, the cancers are selected from bone, brain, kidney, liver, adrenal gland, colorectal, urinary bladder, breast, stomach, ovaries, colon, rectum, prostate, pancreas, lung, vagina, thyroid, the neck and head cancers.

In another embodiment is a compound/conjugate of formula (II), (III) or (II2) of the instant disclosure or a pharmaceutically acceptable salt thereof, for use in an anti-tumoral vaccine.

The present disclosure, according to another of its aspects, also relates to a method of preventing and/or treating the pathological conditions indicated above, comprising administering to a subject in need thereof a therapeutically effective amount of a compound/conjugate of formula (II), (III) or (II2), or or a pharmaceutically acceptable salt thereof.

In an embodiment of this method of treatment, the subject is a human.

The present disclosure also relates to the use of a compound/conjugate of formula (II), (III) or (II2), or a pharmaceutically acceptable salt thereof according to the present disclosure, for the manufacture of a medicament useful in preventing and/or treating any of the pathological conditions indicated above, for example useful as anti-tumoral vaccine or for preventing and/or treating cancer.

The compounds/conjugates of formula (II), (III) or (II2) of the present disclosure may also be used in monotherapy or combination with radiotherapy or chemotherapy. For example, targeted chemotherapy, molecularly targeted therapy such as drugs interfering with

specific targeted molecules needed for carcinogenesis and tumor growth, drugs interfering with cancer cell metabolism, immunotherapy including but not limiting to checkpoint inhibitors, cellular immunotherapy, antibody therapy and cytokine therapy, radiotherapy-based modalities, antiangiogenic therapy or adjuvant or neoadjuvant therapy.

5 The compound/conjugate of formula (II), (II1) or (II2) may be used alone or in combination with at least one other anticancer agent.

 According to another of its aspects, the present disclosure relates to pharmaceutical compositions comprising an effective dose of at least one compound/conjugate of formula (II), (II1) or (II2) according to the disclosure or a
10 pharmaceutically acceptable salt thereof, and also at least one pharmaceutically acceptable excipient.

 The said excipients are selected, in accordance with the pharmaceutical form and method of administration desired, from the customary excipients, which are known to a person skilled in the art.

15 The unit administration forms appropriate include intra-ocular and intra-nasal administration forms, forms for inhalative, topical, transdermal, subcutaneous, intra-muscular or intravenous administration, rectal administration forms and implants. For topical application it is possible to use the compounds according to the disclosure in creams, gels, ointments or lotions.

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Sequence listing

Tusamitamab

5 >LC, AntiCEACAM5_hyb_769_4DVL1c_4DVH1a_IgG1
DIQMTQSPASLSASVGDRVTITCRASENIFSYLAWYQQKPGKSPKLLVYNTRTLAE
GVPSRFSGSGSGTDFTSLTISSLPEDFATYYCQHHYGTPFTFGSGTKLEIKRTVAA
PSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
10 NO.1)

>HC, AntiCEACAM5_hyb_769_4DVL1c_4DVH1a_IgG1
EVQLQESGPGGLVKPGGSLSLSCAASGFVFSYDMSWVRQTPERGLEWVAYISSGGG
ITYAPSTVKGRFTVSRDNAKNTLYLQMNSLTSEDVAVYYCAAHYFGSSGPFAYWGQ
15 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
20 NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLSP
G (SEQ ID NO. 2)

Avelumab LALA-K360C

25 > LC
TPP-28327|Anti-
PDL1_Avelumab_IgG1_LALA_K360C_dK|light_chain|
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNR
PSGVSNRFSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGKVTVLGQ
30 PKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKP
SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID
NO. 3)

>HC

TPP-28327 | IgG1_LALA_K360C_dK |

EVQLLES GGG LV QP GGS LRL SCA ASG FT FSS YIM MWVR QAP GKGLEWVSSIYPSGG
ITFYADTVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARIKLGTVTTVDYWGQ
5 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQPEN
10 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSP
G (SEQ ID NO. 4)

Avelumab K360C

15 >LC

TPP-28326 | Anti-PDL1_Avelumab_IgG1_K360C_dK | light_chain |
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNR
PSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGKVTVLGQ
PKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKP
20 SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID
NO. 5)

>HC

TPP-28326 | IgG1_K360C_dK |

25 EVQLLES GGG LV QP GGS LRL SCA ASG FT FSS YIM MWVR QAP GKGLEWVSSIYPSGG
ITFYADTVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARIKLGTVTTVDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
30 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSP
G (SEQ ID NO. 6)

Avelumab K76R-K100R

5 >LC
TPP-4384|Anti-PDL1_Avelumab_K76R-K100R-_IGHG1-
01_dK|light_chain| ; clone of 267065
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVSNR
PSGVSNRFSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVLGQ
10 PKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKP
SKQSNKNYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ
ID NO. 7)

>HC
15 TPP-4384|K76R-K100R-_IGHG1-01_dK|
EVQLLESQGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKLEWVSSIYPSGG
ITFYADTVKGRFTISRDNSRNTLYLQMNSLRAEDTAVYYCARIRLGTVTTVDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
20 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHENHNTQKSLSLSP
G (SEQ ID NO. 8)

25

Anti-CEACAM5_hyb_769graft_VHg2-7_VLg2-2

>LC, Anti-CEACAM5_hyb_769graft_VHg2-7_VLg2-2_IgG1
(SEQ ID NO.9)
30 DIQMTQSPSSLSASVGDRVTITCRASENIFSYLAWYQQKPGKAPKLLIYNTKTLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHHYGTFPTFGQGTKLEIKRTVAA
PSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSYISLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_hyb_769graft_VHg2-7_VLg2-2_IgG1

(SEQ ID NO.10)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVRQAPGKGLEWVSYISGYGG
5 ITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAHYFGSSGPFAYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
10 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

15 **Anti-CEACAM5_p01_CEA-1_07_IgG1_dK**

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_dK

(SEQ ID NO.11)

AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAAS TLQS
GVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
20 PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_dK

(SEQ ID NO.12)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSYGMHWVRQAPGKGLEWVAVISYDGS
25 NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYGSSTPSLDYWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
30 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_dK

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_dK

(SEQ ID NO.13)

5 AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAASTLQS
GVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_dK

10 **(SEQ ID NO.14)**

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGGTPSLDYWGQ
GTTVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
15 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

20

Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_dK

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_dK

(SEQ ID NO.15)

25 AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAASTLQS
GVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

30 >HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_dK

(SEQ ID NO.16)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGGTPSLDYWGQ

5 GTT V T V S S A S T K G P S V F L A P S S K S T S G G T A A L G C L V K D Y F P C P V T V S W N S G A L T S
G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D
K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E
K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N
N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P
G

10 **Anti-CEACAM5_p01_CEA-1_07_IgG1_K360C_dK**
>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K360C_dK
(SEQ ID NO.17)
A I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S N Y L A W Y Q Q K P G K V P R L L I Y A A S T L Q S
G V P S R F S G S G S G T D F T L T I S S L Q P E D V A T Y Y C Q K Y N S V P Y T F G Q G T K V E I K R T V A A
15 P S V F I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S
K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K360C_dK
(SEQ ID NO.18)
20 Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S S Y G M H W V R Q A P G K G L E W V A V I S Y D G S
N E Y Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A I P Y G S G T P S L D Y W G Q
G T T V T V S S A S T K G P S V F L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S
G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D
K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
25 Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E
K T I S K A K G Q P R E P Q V Y T L P P S R D E L T C N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N
N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P
G

30 **Anti-CEACAM5_p01_CEA-1_07_IgG1_K414C_dK**
>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K414C_dK
(SEQ ID NO.19)

AIQMTQSPSSLSASVGDRTTITCRASQGISNYLAWYQQKPGKVPRLLIYAASLTQS
GVPSRFSGSGSGTDFTLTITSSLPEDVATYYCQKYNVSPYTFGGQGTKVEIKRTVAA
PSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

5

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K414C_dK

(SEQ ID NO.20)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGSTPSLDYWGQ
10 GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQOPEN
15 NYKTTTPVLDSDGSFFLYSKLTVDCSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_dK

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>LC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_dK

(SEQ ID NO.21)

EIVMTQSPATLSVSPGERATLSCRASQSGSSNLAWYQQKPGQAPRLLIYGSSARAT
GIPARFSGSGSGTEFTLTITSLQSEDFAVYYCQQYNNWPYTFGGQGTKLEIKRTVAA
PSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
25 KDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_dK

(SEQ ID NO.22)

QVQLVESGGGVVQPGRSLRLSCVASGFTFSIYGMNWVRQAPGKGLEWVAVISYDGS
30 YKNYADSVKGRFTISRDSKNTLFLQMNSLRTEDTAVYYCASGSTGTPAVDFWGQG
TTVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPG

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Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K360C_dK

>LC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K360C_dK

(SEQ ID NO.23)

10

EIVMTQSPATLSVSPGERATLSCRASQSGSSNLAWYQQKPGQAPRLLIYGSSARAT
GIPARFSGSGSGTEFTLTITSLQSEDFAVYYCQQYNNWPYTFGQGTKLEIKRTVAA
PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

15

>HC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K360C_dK

(SEQ ID NO.24)

20

QVQLVESGGGVVQPGRSLRLSCVASGFTFSIYGMNWVRQAPGKGLEWVAVISYDGS
YKNYADSVKGRFTISRDKSKNTLFLQMNSLRTEDTAVYYCASGSTGTTPAVDFWGQG
TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPG

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Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K414C_dK

>LC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K414C_dK

(SEQ ID NO.25)

30

EIVMTQSPATLSVSPGERATLSCRASQSGSSNLAWYQQKPGQAPRLLIYGSSARAT
GIPARFSGSGSGTEFTLTITSLQSEDFAVYYCQQYNNWPYTFGQGTKLEIKRTVAA
PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K414C_dK

(SEQ ID NO.26)

QVQLVESGGGVVQPGRSLRLSCVASGFTFSIYGMNWVRQAPGKGLEWVAVISYDGS
YKNYADSVKGRFTISRQSKNTLFLQMNSLRTEDTAVYYCASGSTGTPAVDFWGQG
5 TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
10 YKTTTPVLDSDGSFFLYSKLTVDCSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPG

Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_K360C_dK

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_K360C_dK

(SEQ ID NO.27)

AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAASLTQS
GVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
PSVFIFFPSDEQLKSGTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_A118C_K360C_dK

(SEQ ID NO.28)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGSTPSLDYWGQ
25 GTTVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQOPEN
30 NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSP
G

Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_K360C_dK

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_K360C_dK

(SEQ ID NO.29)

5 AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAASTLQS
GVPSRFSGSGSGTDFTLTISLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_E152C_K360C_dK

10 **(SEQ ID NO.30)**

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGSTPSLDYWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPCPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
15 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

20

Anti-CEACAM5_p01_CEA-1_07_IgG1_K274C_K414C_dK

>LC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K274C_K414C_dK

(SEQ ID NO.31)

25 AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPRLLIYAASTLQS
GVPSRFSGSGSGTDFTLTISLQPEDVATYYCQKYNVSPYTFGGGTKVEIKRTVAA
PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

30 >HC, Anti-CEACAM5_p01_CEA-1_07_IgG1_K274C_K414C_dK

(SEQ ID NO.32)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS
NEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIPYSGSTPSLDYWGQ

5 GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVCFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLYSKLTVDCSRWQQGNVSCSVMHEALHNHYTQKSLSLSP
G

10 **Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_K360C_dK**
>LC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_K360C_dK
(SEQ ID NO.33)
EIVMTQSPATLSVSPGERATLSCRASQSGSSNLAWYQQKPGQAPRLLIYGSSARAT
GIPARFSGSGSGTEFTLTITSLQSEDFAVYYCQQYNNWPYTFGQGTKLEIKRTVAA
15 PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>HC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_A118C_K360C_dK
(SEQ ID NO.34)
20 QVQLVESGGGVVQPGRSLRLSCVASGFTFSIYGMNWVRQAPGKGLEWVAVISYDGS
YKNYADSVKGRFTISRDKSKNTLFLQMNSLRTEDTAVYYCASGSTGTTPAVDFWQQG
TTVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
25 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTCNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPG

30 **Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K274C_K414C_dK**
>LC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K274C_K414C_dK
(SEQ ID NO.35)

EIVMTQSPATLSVSPGERATLSCRASQSGSSNLAWYQQKPGQAPRLLIYGSSARAT
GIPARFSGSGSGTEFTLTITSLQSEDFAVYYCQQYNNWPYTFGQGTKLEIKRTVAA
PSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

5

>HC, Anti-CEACAM5_p07_CEA-3_50_IgG1_VH1_K274C_K414C_dK
(SEQ ID NO.36)

QVQLVESGGGVVQPGRSLRLSCVASGFTFSIYGMNWVRQAPGKGLEWVAVISYDGS
YKNYADSVKGRFTISRDKSKNTLFLQMNSLRTEDTAVYYCASGSTGTPAVDFWQQG
10 TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVCFNWF
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
15 YKTTTPVLDSDGSFFLYSKLTVDCSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

In case of discrepancy between the above list of sequences and sequences disclosed
in an appended sequence listing, the correct sequences are those disclosed above in
20 the present disclosure.

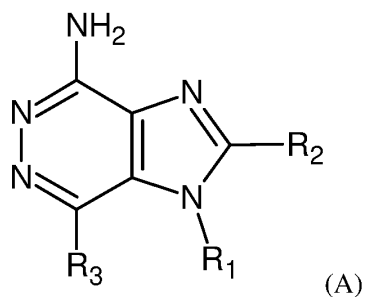
CLAIMS

1. A compound of formula (I) or a pharmaceutically acceptable salt thereof:

A-L1-RCG1 (I)

5 wherein:

- **A** is a drug unit of formula (A)



wherein:

10 **R₁** represents:

- a hydrogen atom, or
- a group selected from:

a)

- 15
- a (C₁-C₆)alkyl- group;
 - a hydroxy-(C₁-C₆)alkyl- group;
 - a NH₂-(C₁-C₆)alkyl- group;
 - a NH-(C₁-C₆)alkyl-(C₁-C₆)alkyl- group;
 - a N((C₁-C₆)alkyl)₂-(C₁-C₆)alkyl- group;
- 20
- a (C₂-C₆)alkenyl- group;
 - a (C₂-C₆)alkynyl- group;

b)

- a phenyl(C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent
- 25 selected from:
- b1) - a (C₁-C₆)alkoxy- group;
 - b2) - a hydroxyl group;

b3) a -C(O)-H group; and

b4) - a (C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from:

b4.1) - a hydroxyl group; and

5 b4.2) - a -NR₄R₅ group wherein R₄ and R₅, being independently from each other selected from:

b4.2.1) - a hydrogen atom;

b4.2.2) - a (C₁-C₁₆)alkyl- group;

10 b4.2.3) - a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30, a (C₁-C₆)alkoxy(C₁-C₆)alkyl- group, or a (C₁-C₆)alkoxy(C₁-C₆)alkoxy(C₁-C₆)alkyl- group;

b4.2.4) - a (C₁-C₆)alkyl-S(O)₂- group;

b4.2.5) - a (C₁-C₆)alkyl-NH-C(O)- group;

b4.2.6) - a (C₁-C₁₆)alkyl-C(O)- group;

b4.2.7) - a (C₁-C₁₆)alkyl-O-C(O)- group;

15 b4.2.8) - a CH₃-[O-(CH₂)₂]_n-C(O)- group with n being an integer from 1 to 30;

b4.2.9) - a (C₃-C₁₀)cycloalkyl- group being unsubstituted or substituted by at least one substituent selected from:

- a hydroxyl group; and

- a (C₁-C₆)alkyl- group; or

20 b4.2.10) - a (C₃-C₁₀)membered heterocycloalkyl- group comprising from one to four heteroatoms selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-;

b4.2.11) - a phenyl-C(O)- group;

b4.2.12) - a (C₁-C₆)alkoxy-phenyl-(C₁-C₆)alkyl-O-C(O)- group;

b4.2.13) - a (C₁-C₁₆)alkyl-C(O)-NH-phenyl-(C₁-C₆)alkyl-O-C(O)- group;

25 b4.2.14) - a (C₁-C₁₆)alkyl-O-C(O)-(C₁-C₆)alkyl- group;

or R₄ and R₅ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, said (C₃-C₁₀)membered heterocycloalkyl- group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group, and a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30;

30

- c) - a (C₃-C₁₀)cycloalkyl(C₁-C₆)alkyl- group being unsubstituted or substituted by at least one substituent selected from -NH₂ and a NH₂-(C₁-C₆)alkyl- group;
- d) - a (C₃-C₁₀)membered heterocycloalkyl(C₁-C₆)alkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,
5 said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group and a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30; and
- e) - a (C₅-C₁₀)membered heteroaryl(C₁-C₆)alkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,
10 said heteroaryl being unsubstituted or substituted by at least one substituent selected from:
- a (C₁-C₆)alkyl- group;
 - a NH₂-(C₁-C₆)alkyl- group and
 - 15 - a cyano group;
- f) - a (C₃-C₁₀)membered heterocycloalkyl-NH-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;
20
- g) - a (C₃-C₁₀)membered heterocycloalkyl-N(C(O)-(C₁-C₆)alkyl)-(C₁-C₁₆)alkyl- group, said heterocycloalkyl group comprising one to four heteroatoms selected from oxygen, nitrogen, S(O), SO₂ and sulfur;
- 25 **R₂** represents a halogen atom,
or a group selected from:
- a (C₁-C₆)alkyl- group;
 - a (C₂-C₆)alkenyl- group;
 - a (C₂-C₆)alkynyl- group;
 - 30 - a (C₁-C₆)alkylthio- group;
 - a (C₁-C₆)alkylthio(C₁-C₆)alkyl- group;
 - a (C₁-C₆)alkyl-S(O)- group;

- a (C₁-C₆)alkyl-S(O₂)- group;
- a (C₁-C₆)alkyl-S(O)-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-S(O₂)-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkoxy- group;
- 5 - a (C₁-C₆)alkoxy(C₁-C₆)alkyl- group;
- a (C₁-C₆)haloalkoxy(C₁-C₆)alkyl- group;
- a (C₃-C₅)cycloalkyl-O-(C₁-C₆)alkyl- group;
- a (C₁-C₆)alkyl-NH-(C₁-C₆)alkyl- group;
- a ((C₁-C₆)alkyl)₂-N-(C₁-C₆)alkyl- group;
- 10 - a (C₁-C₆)alkyl-NH- group; and
- a ((C₁-C₆)alkyl)₂N- group;

R₃ represents:

- a deuterium atom;
 - 15 - a hydrogen atom
- or a group selected from:

a)

- a (C₁-C₆)alkyl- group;
- 20 - a (C₂-C₆)alkenyl- group;
- a (C₂-C₆)alkynyl- group; and
- a (C₁-C₆)alkylthio- group;

b)

- 25 - a -OR₆ group wherein R₆ is selected from:
 - a hydrogen atom;
 - a (C₁-C₆)alkyl- group;
 - a CH₃-[O-(CH₂)₂]_n- group with n being an integer from 1 to 30;
 - a (C₂-C₆)alkenyl- group;
- 30 - a (C₂-C₆)alkynyl- group;
- a (C₃-C₁₀)cycloalkyl- group;
- a phenyl group;

- a phenyl(C₁-C₆)alkyl- group; and
 - a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, sulfur, -S(=O)- and -S(=O)₂-;
- 5 c)
- a -NR₇R₈ group wherein R₇ and R₈ being, independently from each other, selected from:
 - a hydrogen atom;
 - a CH₃-[O-(CH₂)₂]_n- with n being an integer from 1 to 30;
 - a (C₁-C₆)alkyl- group unsubstituted or substituted by
- 10 - a (C₅-C₁₀)membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur; or
- a phenyl group being unsubstituted or substituted by at least one substituent selected from:
 - a cyano group and
- 15 - a NR₉R₁₀-(C₁-C₆)alkyl- group wherein:
- R₉ and R₁₀ being, independently from each other, selected from:
- a hydrogen atom;
 - a (C₁-C₆)alkyl- group;
 - a CH₃-[O-(CH₂)₂]_n- with n being an integer from 1 to 30, or
- 20 R₉ and R₁₀ together form with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,
- said (C₃-C₁₀)membered heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from a (C₁-C₆)alkyl- group, and a CH₃-[O-(CH₂)₂]_n- group with n
- 25 being an integer from 1 to 30;
- or R₇ and R₈ form together with the nitrogen atom to which they are attached a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected from oxygen, nitrogen, and sulfur,
- 30 said heterocycloalkyl group being unsubstituted or substituted by at least one substituent selected from:
- a phenyl group and

- a hydroxy(C₁-C₆)alkyl-phenyl- group;

d)

- a (C₃-C₁₀)membered heterocycloalkyl- group comprising one to four heteroatoms selected
5 from oxygen, nitrogen and sulfur;

e)

- a (C₅-C₁₀)membered heteroaryl- group comprising one to four heteroatoms selected from
oxygen, nitrogen, and sulfur,
10 said (C₅-C₁₀)membered heteroaryl- group being unsubstituted or substituted by at least one
(C₁-C₆)alkyl- group;

f)

- a (C₆-C₁₀)membered aryl- group; and
15

g)

- a (C₃-C₁₀)cycloalkyl- group;

- **L1** represents a linker; and

20 - **RCG1** represents a reactive chemical group that is reactive towards a chemical group
present on a binding agent, for instance on a protein, a polypeptide, a ligand, a peptide, an
oligonucleotide or an oligosaccharide, for instance on a targeting agent for instance on a cell-
binding agent such as an antibody such as a monoclonal antibody or a VHH antibody.

25 **2.** The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to
claim 1, wherein **L1** represents a cleavable linker or a non - cleavable linker.

3. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to
claim 1 or claim 2, wherein:

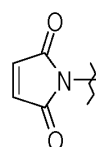
30 - when L1 is a cleavable linker, it is a peptidic linker; or
- when L1 is a non-cleavable linker, it is a PEGylated linker.

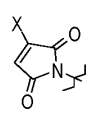
4. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to any one of claims 1 to 3, wherein **RCG₁** represents:

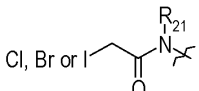
(i) a $R_aZ_a-C(=O)-$ reactive group for which:

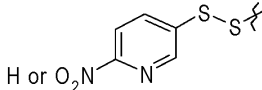
Z_a represents a single bond, $-O-$ or $-NH-$, such as $-O-$, and

5 R_a represents a hydrogen atom, a $-(C_1-C_6)$ alkyl group, a $-(C_3-C_7)$ cycloalkyl group, a $-(C_2-C_6)$ alkenyl group, a $-(C_6-C_{10})$ aryl group, a $-(C_5-C_{10})$ heteroaryl group comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a $-(C_3-C_7)$ heterocycloalkyl group comprising 2 to 6 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, sulfur, $-S(O)-$ and $-SO_2-$, said $-(C_6-C_{10})$ aryl group, $-(C_5-C_{10})$ heteroaryl group and/or $-(C_3-C_7)$ heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a $-(C_1-C_6)$ alkyl group, a $-(C_1-C_6)$ alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

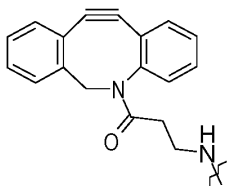
(ii) one of the following reactive groups: a maleimido  group; a

15 substituted maleimido group such as  with $X = Br, O-\text{C}_6\text{H}_4-CN$; a haloacetamido

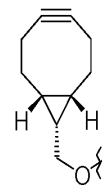
 group with R_{21} representing a hydrogen atom or a (C_1-C_6) alkyl group, such as a methyl group; $Cl-$; N_3- ; $HO-$; $HS-$; an activated disulfide such as

 ; H_2N- ; $HC\equiv C-$ or an activated $C\equiv C$ such as a cyclooctyne moiety

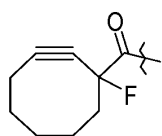
for instance a DBCO-amine



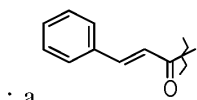
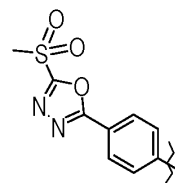
or BCN



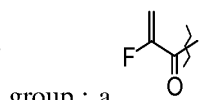
or MFCO



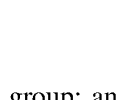
; a phenyloxadiazolyl methylsulfone group (PODS) such as



; a

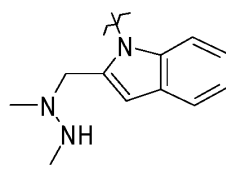


group; a

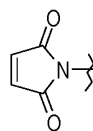


group; an O-alkyl hydroxylamine or a Pictet-Spengler

reaction substrate such as

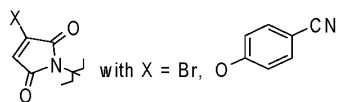


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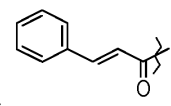
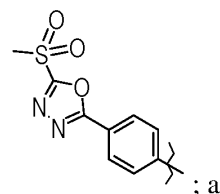
for instance RCG₁ is N₃-, a maleimido group

, a substituted maleimido group

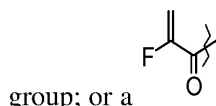


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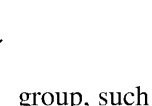
, a COOH group, a phenyloxadiazolyl methylsulfone group



; a

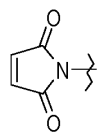


group; or a



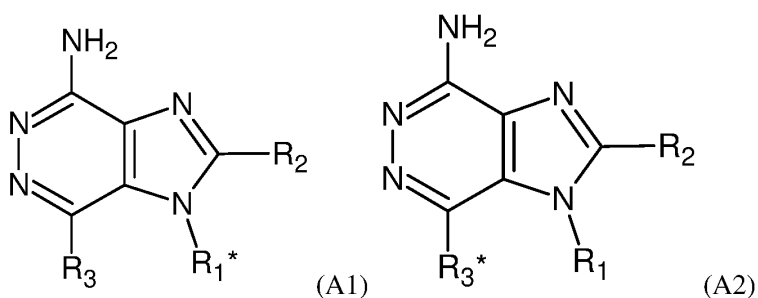
group, such as N₃-, a -COOH group,

and a maleimido group



.

5. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to
10 claim 1, wherein A is of formula (A1) or of formula (A2)



in which:

R₁, R₂ and R₃ are as defined in claim 1;

the single asterisk (*) indicates the site of covalent attachment to -L1-RCG1;

5 **R₁*** represents:

- either a single bond, which means that -L1-RCG1 is directly linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R₁ as defined in the present disclosure, which means that -L1-RCG1 is linked to the nitrogen atom of the imidazo[4,5-d]pyridazine ring
10 *via* R₁;

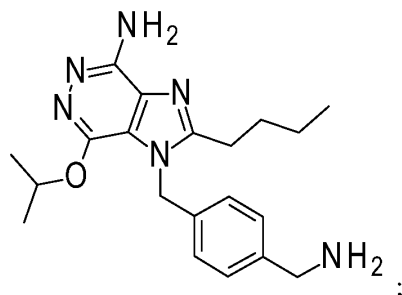
R₃* represents:

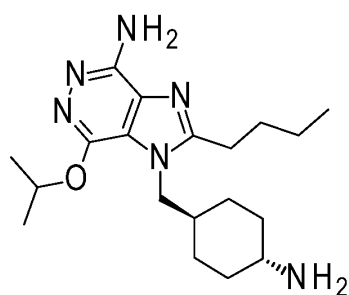
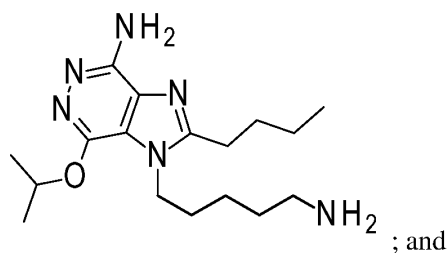
- either a single bond, which means that -L1-RCG1 is directly linked to the carbon atom of the imidazo[4,5-d]pyridazine ring,

- or a divalent radical formed from R₃ as defined in the present disclosure, which
15 means that -L1-RCG1 is linked to the carbon atom of the imidazo[4,5-d]pyridazine ring *via* R₃.

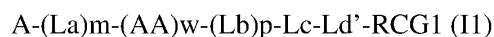
6. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to claim 1, wherein **A** is chosen among:

20





- 5 7. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to any one of claims 1 to 6, wherein said compound is chosen among a compound of formula (I1) and a compound of formula (I2):



10 wherein:

- **A** is as defined in any one of claims 1, 5 and 6;

- **La** represents a self immolative linker, for instance a *-C(O)-O-(CR₁₄R₁₅)-aryl-NR₁₆-** group, *-C(O)-O-(CR₁₄R₁₅)-heteroaryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-**, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-aryl-NR₁₆-** group, a *-C(O)-NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, a *-CR₁₅R₁₄-aryl-NR₁₆-** group or a *-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, aryl being a (C₆-C₁₀) membered aryl group and heteroaryl being a (C₅-C₁₀) membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, for instance a *-C(O)-O-CH₂-phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the
- 15 NR₁₇-(C₂-C₆)alkyl-NR₁₈-C(O)-O-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, a *-CR₁₅R₁₄-aryl-NR₁₆-** group or a *-CR₁₅R₁₄-heteroaryl-NR₁₆-** group, aryl being a (C₆-C₁₀) membered aryl group and heteroaryl being a (C₅-C₁₀) membered heteroaryl group comprising one to four heteroatoms selected from oxygen, nitrogen and sulfur, for instance a *-C(O)-O-CH₂-phenyl-NH-** group, the single asterisk (*) indicating the site of covalent attachment to the
- 20 A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the (AA)_w radical;

R₁₄, R₁₅, R₁₆, R₁₇ and R₁₈ being a hydrogen atom or a (C₁-C₆)alkyl group;

group;

- **m** represents an integer from 0 to 1;

- 5 - **(AA)^w** represents a sequence of **w** amino acids **AA** connected together *via* peptide bonds;
w representing an integer ranging from 1 to 12, for instance from 1 to 6, such as 2 and 3;

AA denoting a natural or unnatural amino acid, of configuration D or L, chosen from: alanine (Ala), β -alanine, γ -aminobutyric acid, 2-amino-2-cyclohexylacetic acid, 2-amino-2-phenylacetic acid, arginine (Arg), asparagine (Asn), aspartic acid (Asp),
 10 citrulline (Cit), cysteine (Cys), α,α -dimethyl- γ -aminobutyric acid, β,β -dimethyl- γ -aminobutyric acid, glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), ϵ -acetyl-lysine (AcLys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan
 15 (Trp), tyrosine (Tyr), and valine (Val), for instance valine (Val), citrulline (Cit), alanine (Ala), and glutamic acid (Glu) ;

- **Lb** represents a spacer, for instance a $^*-C(O)-(C_1-C_6)alkyl-C(O)-NH-^{**}$ group or a $^*-C(O)-^{**}$ group, such as a $^*-C(O)-(CH_2)_3-C(O)-NH-^{**}$ group or a $^*-C(O)-^{**}$ group, the single
 20 asterisk (*) indicating the site of covalent attachment to the (AA)^w radical and the double asterisk (**) indicating the site of covalent attachment to the Lc radical;

- **p** represents an integer from 0 to 1;

25 - **Lc** represents a spacer, for instance a $^*-(CH_2-CH_2-O)_n-^{**}$ group in which n represents an integer from 2 to 12, for instance from 3 to 7, such as 3 and 4 , the single asterisk (*) indicating the site of covalent attachment to the Lb radical if present (that is to say if p is 1) or directly to the (AA)^w radical if Lb is not present (that is to say if p is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld' radical;

30

- **Ld'** represents a spacer, for instance a $^*-(C_2-C_6)alkyl-^{**}$ group, a $^*-(C_2-C_6)alkyl-NH-C(O)-(C_2-C_6)alkyl-^{**}$ group or a $^*-(C_2-C_6)alkyl-C(O)-NH-(C_2-C_6)alkyl-^{**}$ group, such as a

*-(CH₂)₂-** group, a *-(CH₂)₂-NH-C(O)-(CH₂)₂-** group or a *-(CH₂)₂-C(O)-NH-(CH₂)₂-** group, the single asterisk (*) indicating the site of covalent attachment to the Lc radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical; and

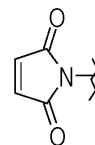
5 - **RCG1** is as defined in claim 1, for instance **RCG1** represents:

(i) a R_aZ_a-C(=O)- reactive group for which:

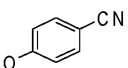
Z_a represents a single bond, -O- or -NH, such as -O-, and

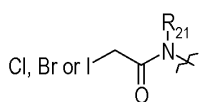
R_a represents a hydrogen atom, a -(C₁-C₆)alkyl group, a -(C₃-C₇)cycloalkyl group, a -(C₂-C₆)alkenyl group, a -(C₆-C₁₀)aryl group, a -(C₅-C₁₀)heteroaryl group
 10 comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a -(C₃-C₇)heterocycloalkyl group comprising 2 to 6 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-, said -(C₆-C₁₀)aryl group, -(C₅-C₁₀)heteroaryl group and/or -(C₃-C₇)heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a -
 15 (C₁-C₆)alkyl group, a -(C₁-C₆)alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

(ii) one of the following reactive groups: a maleimido

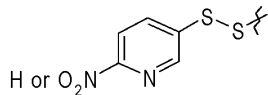


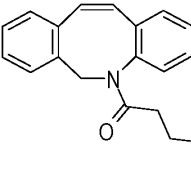
group; a

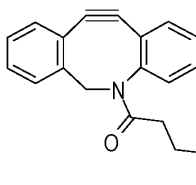
substituted maleimido group such as  with X = Br, ; a haloacetamido



group with R₂₁ representing a hydrogen atom or a (C₁-C₆)alkyl group,
 20 such as a methyl group; Cl-; N₃-; HO-; HS-; an activated disulfide such as

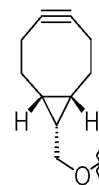


H or O₂N-; ; H₂N-; HC≡C- or an activated C≡C such as a cyclooctyne moiety

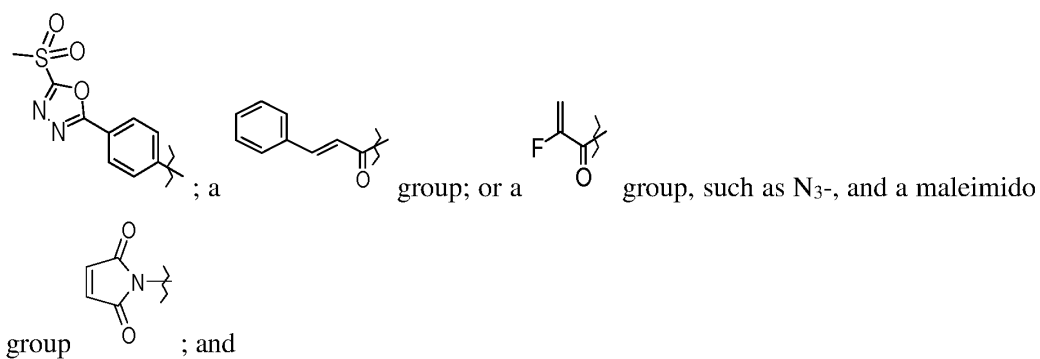
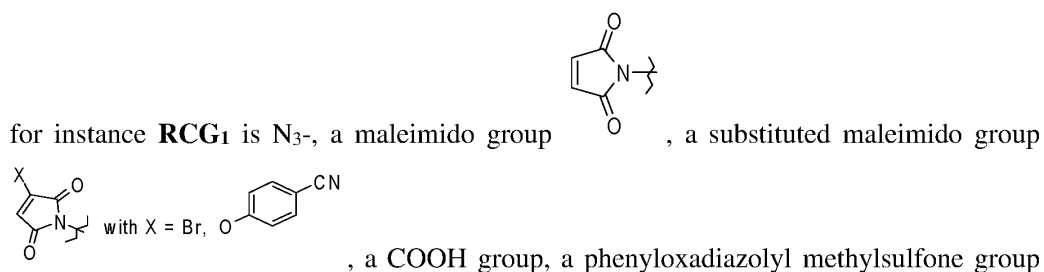
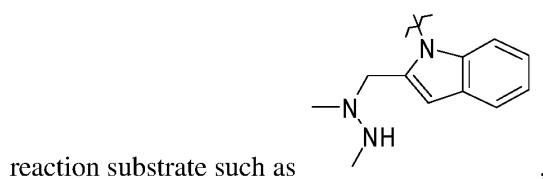
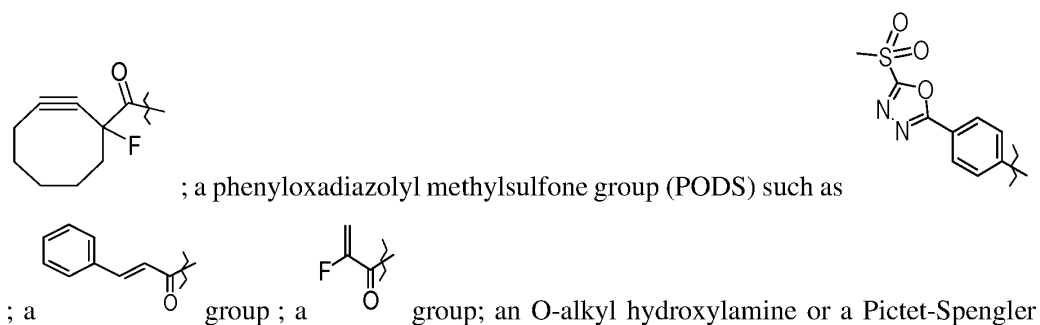


for instance a DBCO-amine

or BCN



or MFCO

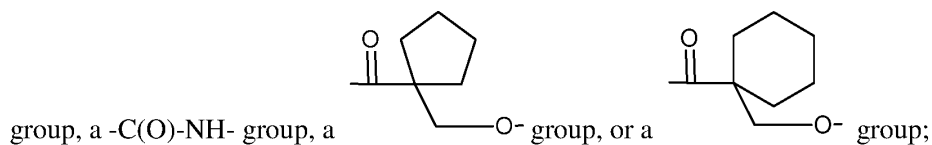


A-(P)o-L-Ld-RCG1 (I2)

10 wherein

- **A** is as defined in any one of claims 1, 5 and 6;

- **P** represents a -C(O)- group, a -C(O)-O- group, a -S(O₂)- group, a -C(O)-C(CH₃)₂-CH₂-O-



- **o** represents an integer from 0 to 1;

- **L** represents a $^{*}-(\text{CH}_2-\text{CH}_2-\text{O})_n-^{**}$ group in which n represents an integer from 1 to 30, for instance from 8 to 25, such as 8, 12, 16, 20, 23 and 24, the single asterisk (*) indicating the site of covalent attachment to the P radical if present (that is to say if o is 1) or directly to the A radical if P is not present (that is to say if o is 0) and the double asterisk (**) indicating the site of covalent attachment to the Ld radical;

- **Ld** represents a spacer, for instance a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{NH}-\text{C}(\text{O})-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group or a $^{*}-(\text{C}_2-\text{C}_6)\text{alkyl}-\text{C}(\text{O})-\text{NH}-(\text{C}_2-\text{C}_6)\text{alkyl}-^{**}$ group, such as a $^{*}-(\text{CH}_2)_2-^{**}$ group, a $^{*}-(\text{CH}_2)_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_2-^{**}$ group or a $^{*}-(\text{CH}_2)_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_2-^{**}$ group, the single asterisk (*) indicating the site of covalent attachment to the L radical and the double asterisk (**) indicating the site of covalent attachment to the RCG1 radical; and

15

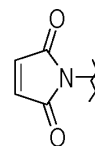
- **RCG1** is as defined in claim 1, for instance **RCG1** represents:

(i) a $\text{R}_a\text{Z}_a-\text{C}(=\text{O})-$ reactive group for which:

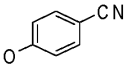
Z_a represents a single bond, -O- or -NH, such as -O-, and

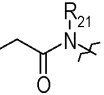
R_a represents a hydrogen atom, a $-(\text{C}_1-\text{C}_6)\text{alkyl}$ group, a $-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ group, a $-(\text{C}_2-\text{C}_6)\text{alkenyl}$ group, a $-(\text{C}_6-\text{C}_{10})\text{aryl}$ group, a $-(\text{C}_5-\text{C}_{10})\text{heteroaryl}$ group comprising 4 to 9 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, and sulfur, or a $-(\text{C}_3-\text{C}_7)\text{heterocycloalkyl}$ group comprising 2 to 6 carbon atoms and 1 to 4 heteroatom(s) selected from oxygen, nitrogen, sulfur, -S(O)- and -SO₂-, said $-(\text{C}_6-\text{C}_{10})\text{aryl}$ group, $-(\text{C}_5-\text{C}_{10})\text{heteroaryl}$ group and/or $-(\text{C}_3-\text{C}_7)\text{heterocycloalkyl}$ group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, such as a fluorine atom, a $-(\text{C}_1-\text{C}_6)\text{alkyl}$ group, a $-(\text{C}_1-\text{C}_6)\text{alkoxy}$ group, a hydroxyl group, an oxo group, a nitro group and a cyano group; or

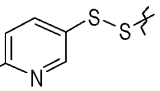
(ii) one of the following reactive groups: a maleimido

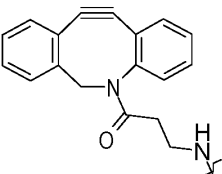
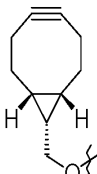


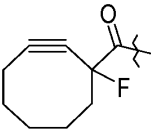
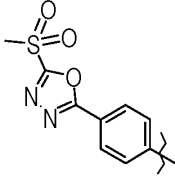
group; a

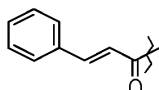
substituted maleimido group such as  with X = Br,  ; a haloacetamido

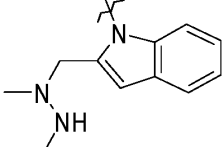
Cl, Br or I  group with R₂₁ representing a hydrogen atom or a (C₁-C₆)alkyl group, such as a methyl group; Cl-; N₃-; HO-; HS-; an activated disulfide such as

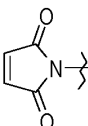
 ; H₂N-; HC≡C- or an activated C≡C such as a cyclooctyne moiety

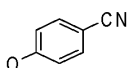
5 for instance a DBCO-amine  or BCN  or MFCO 

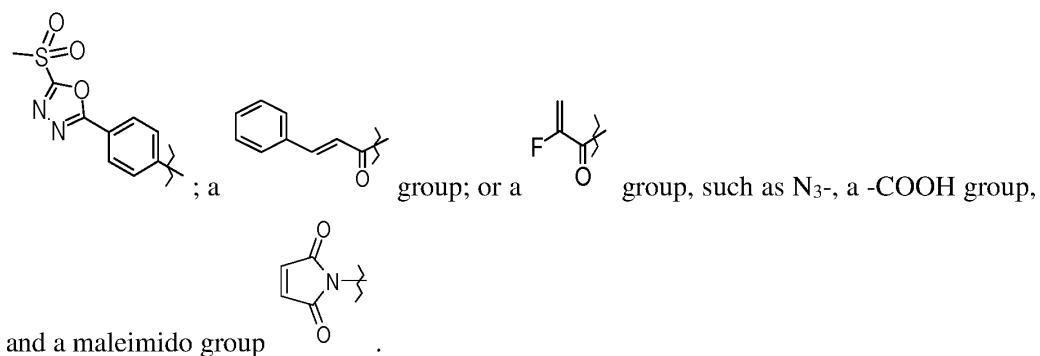
 ; a phenyloxadiazolyl methylsulfone group (PODS) such as 

; a  group ; a  group; an O-alkyl hydroxylamine or a Pictet-Spengler

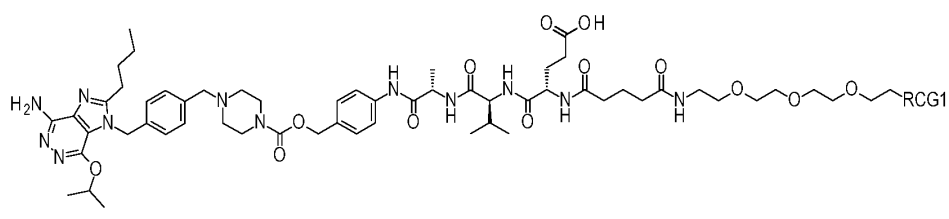
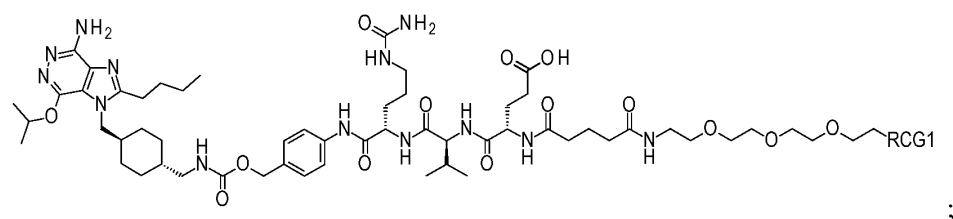
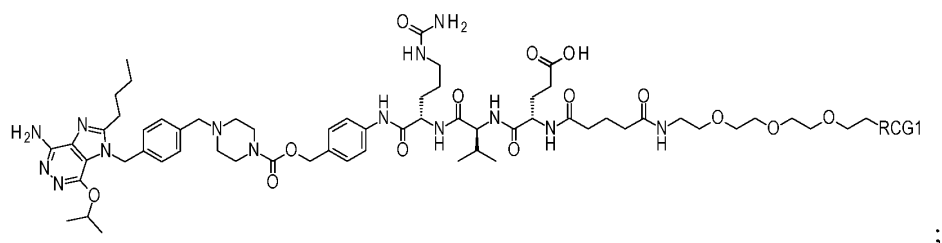
reaction substrate such as ,

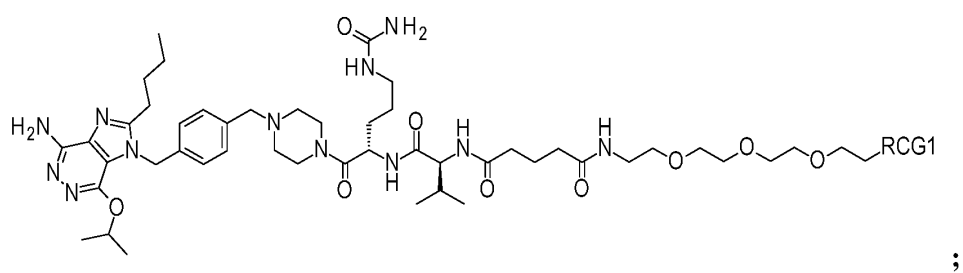
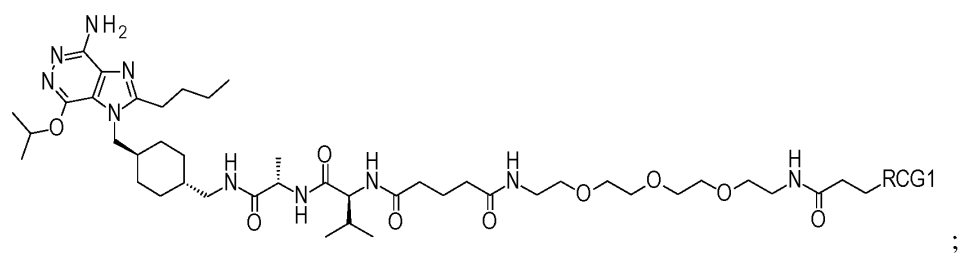
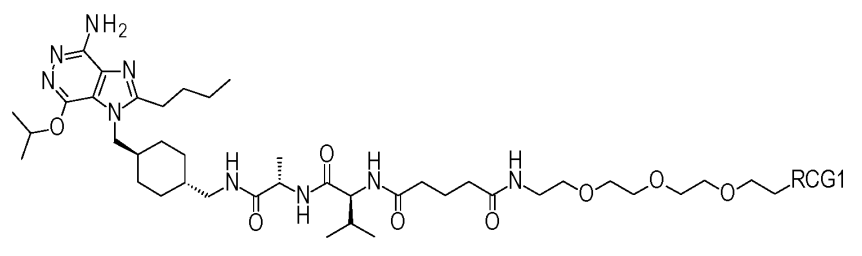
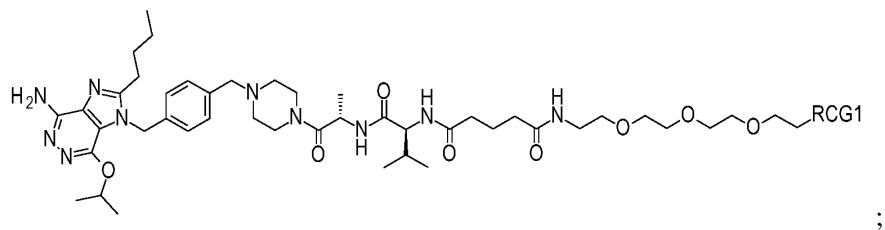
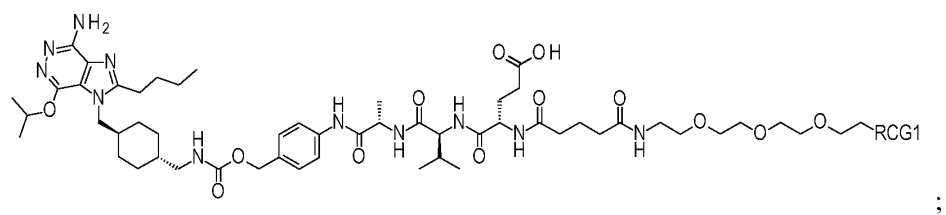
for instance **RCG₁** is N₃-, a maleimido group , a substituted maleimido group

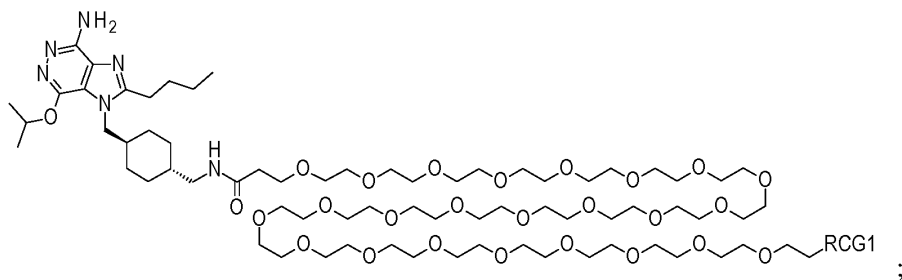
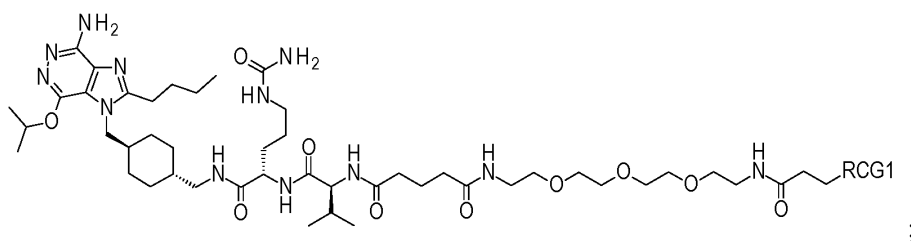
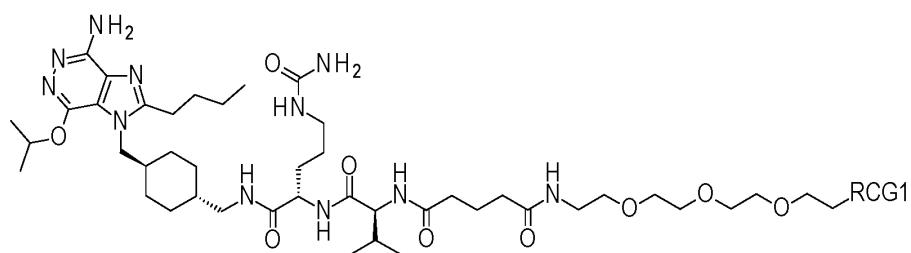
10  with X = Br, , a COOH group, a phenyloxadiazolyl methylsulfone group



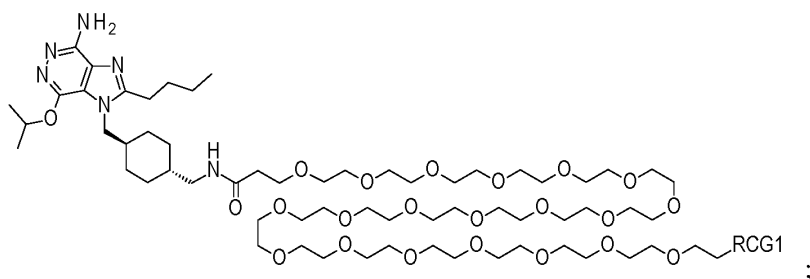
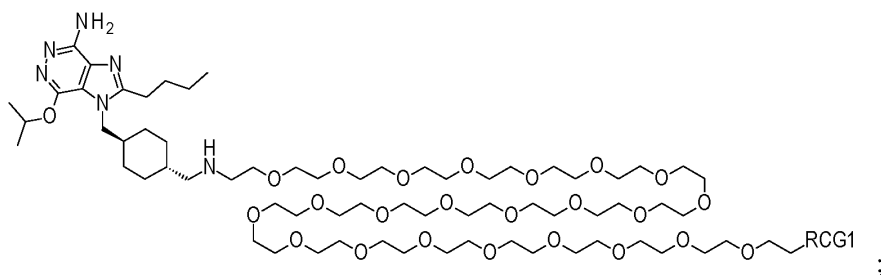
8. The compounds of formula (I) or a pharmaceutically acceptable salt thereof according to any one of claims 1 to 7, wherein said compound is chosen among:

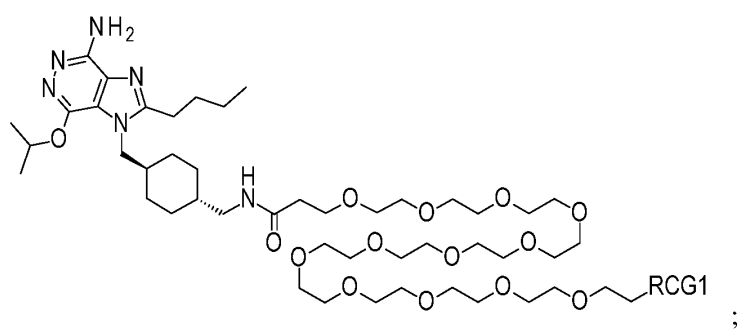
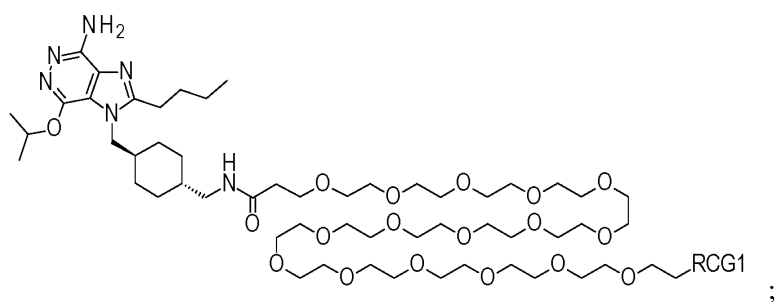




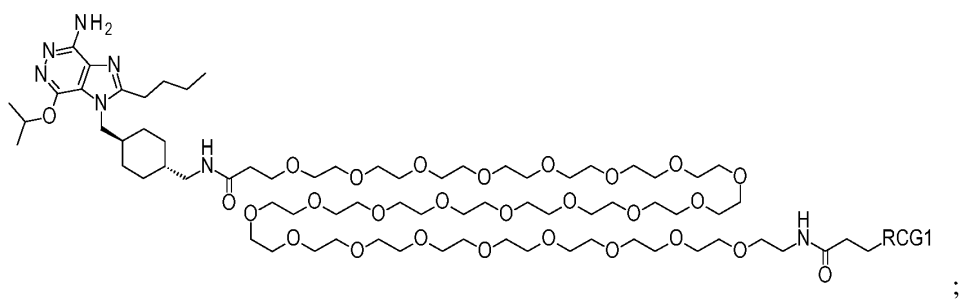
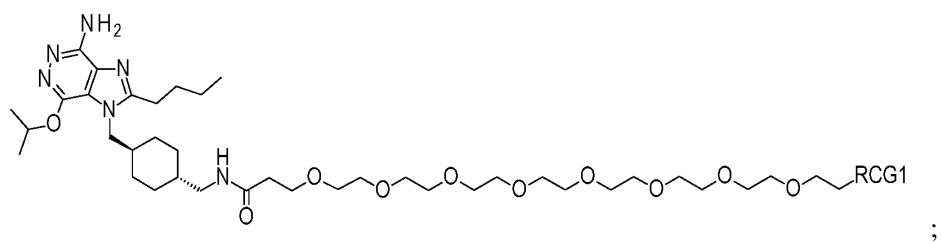


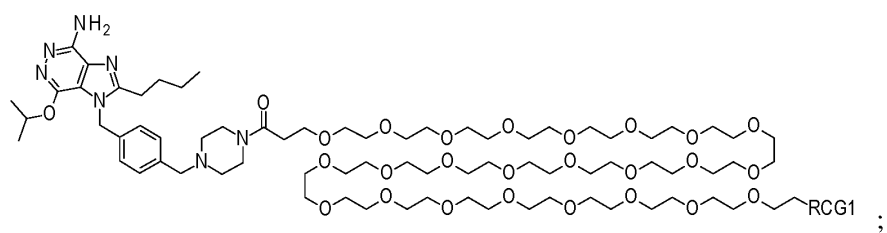
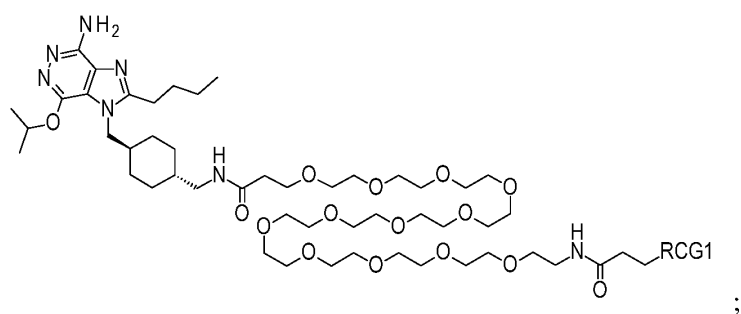
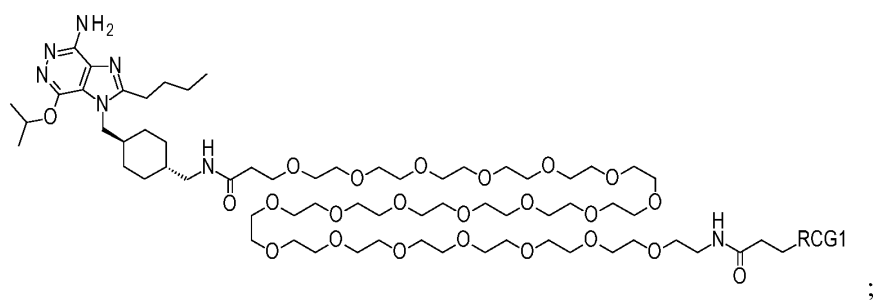
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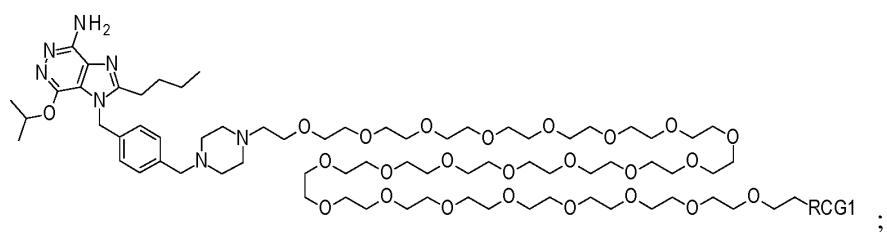


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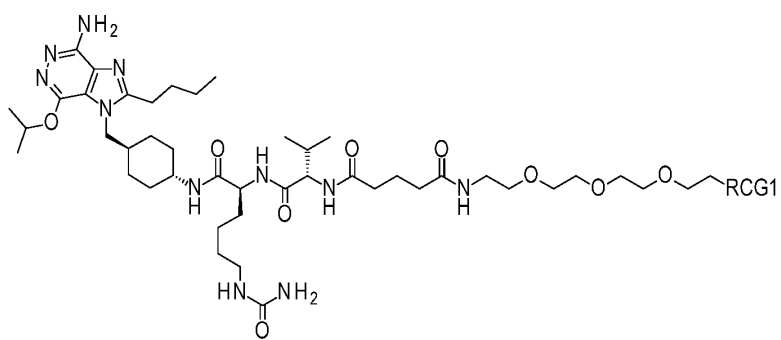
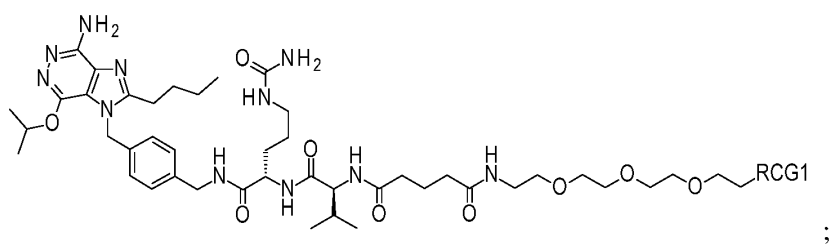
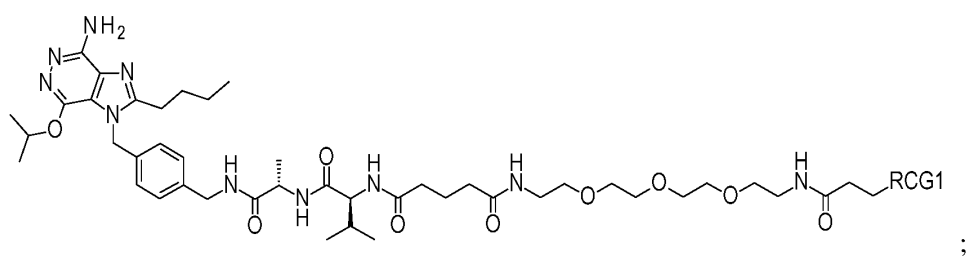
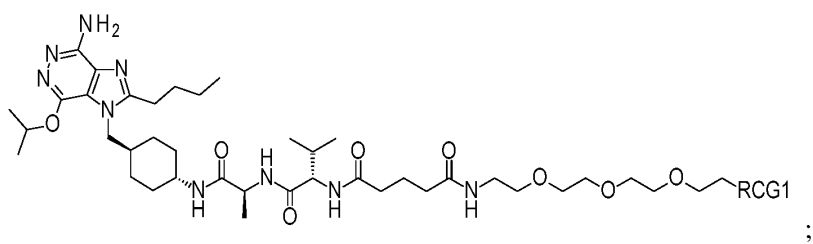
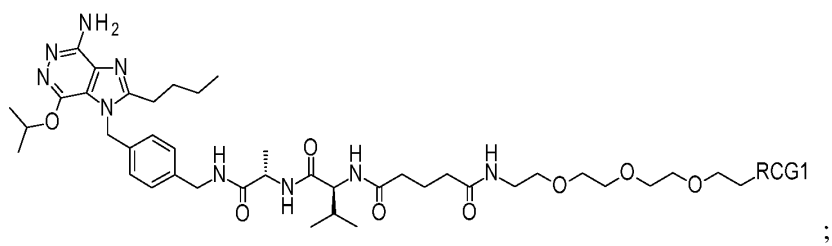


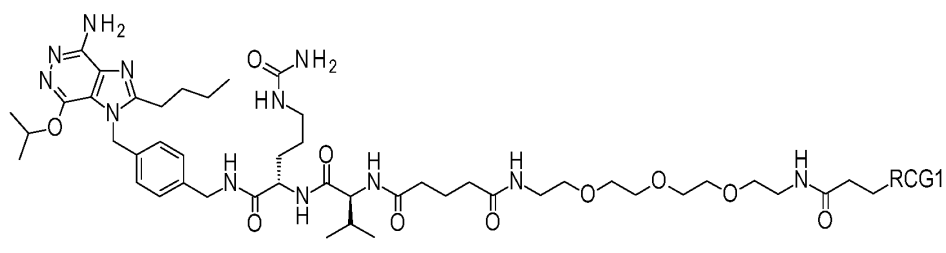


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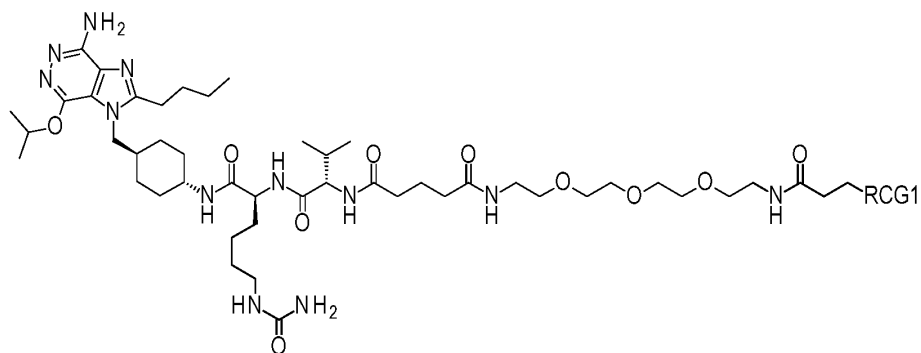




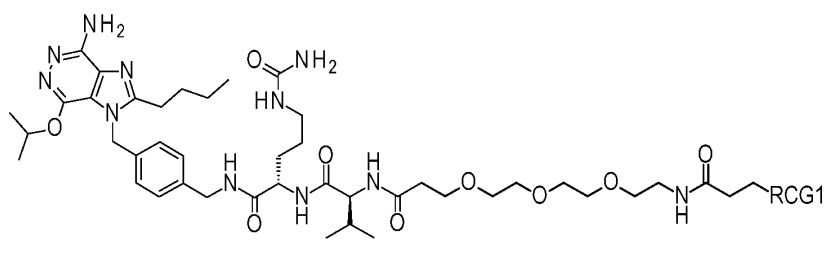




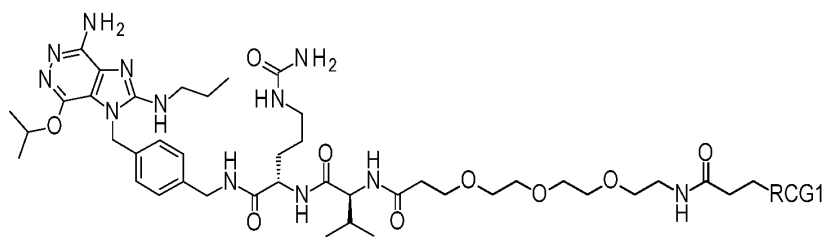
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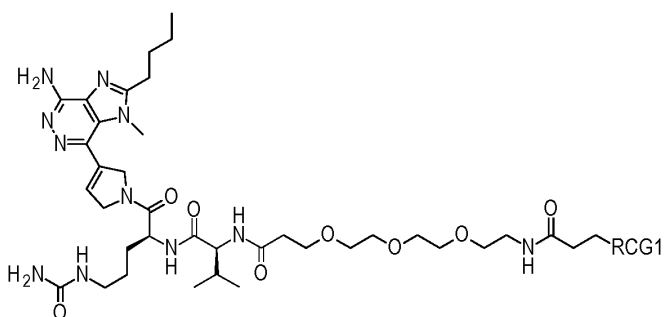
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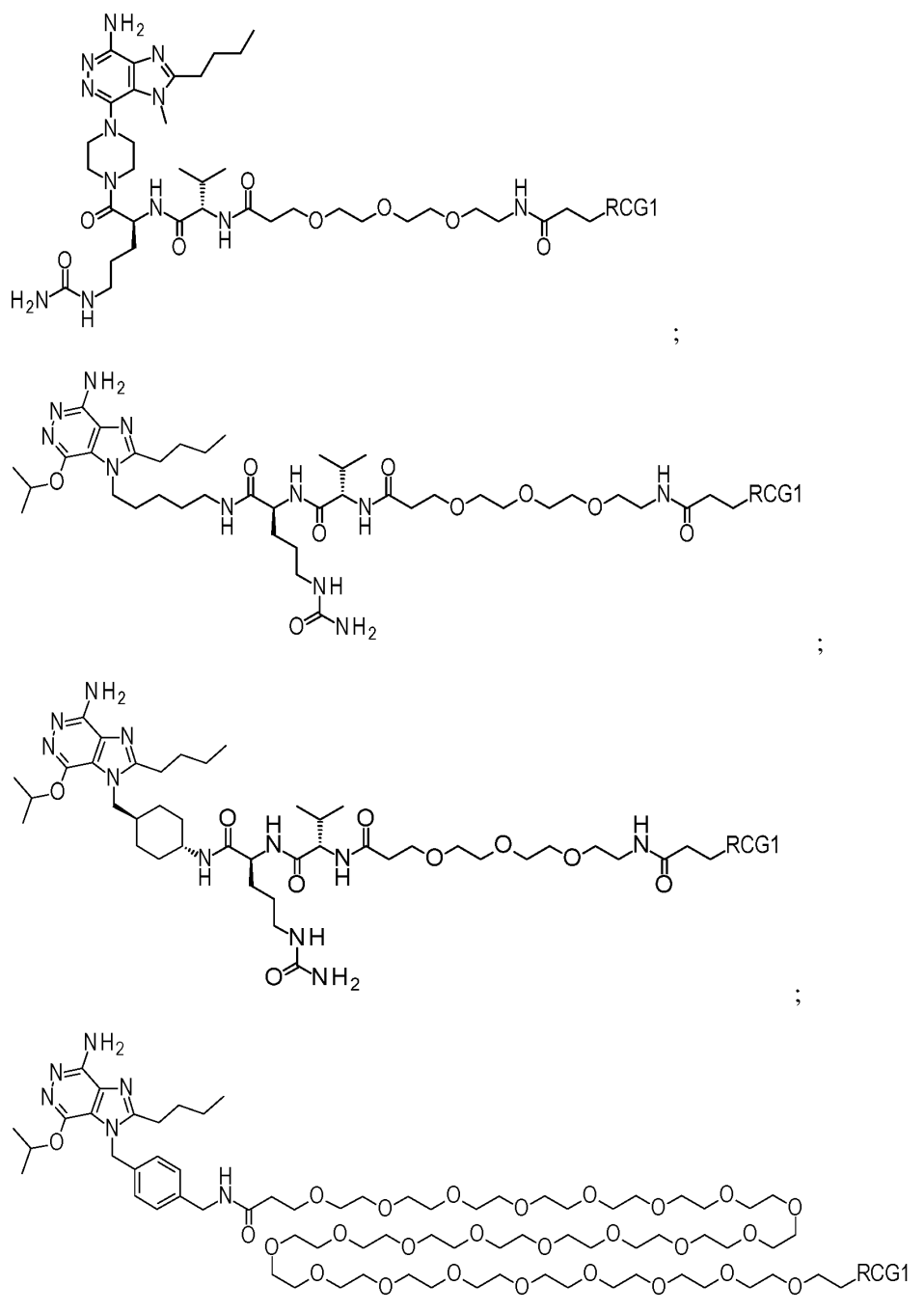
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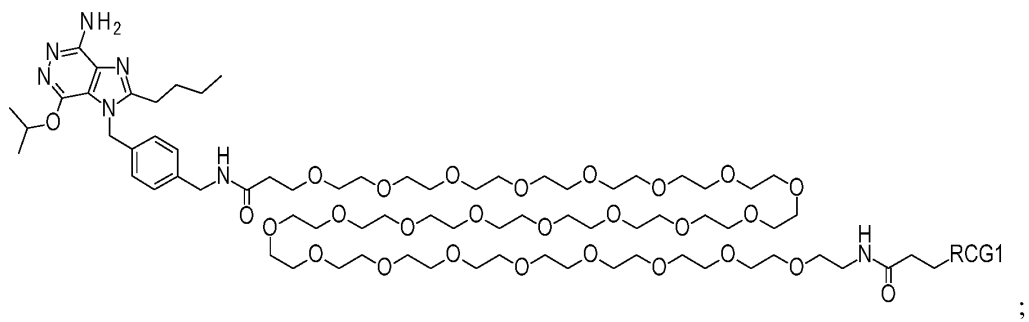
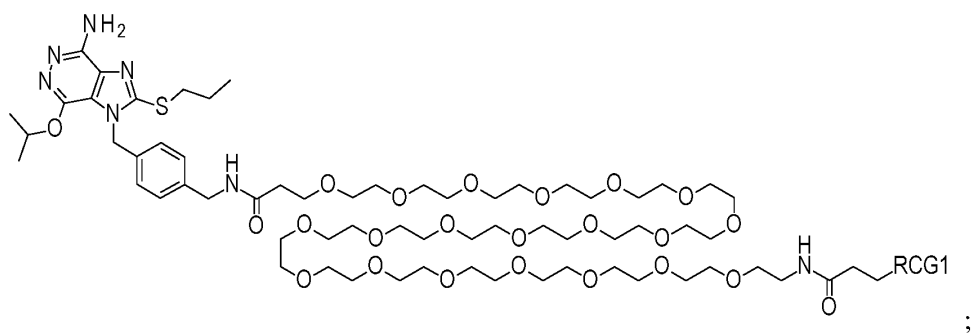
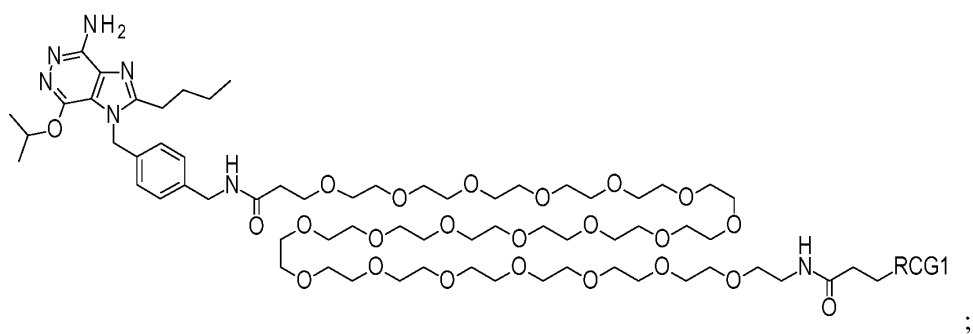
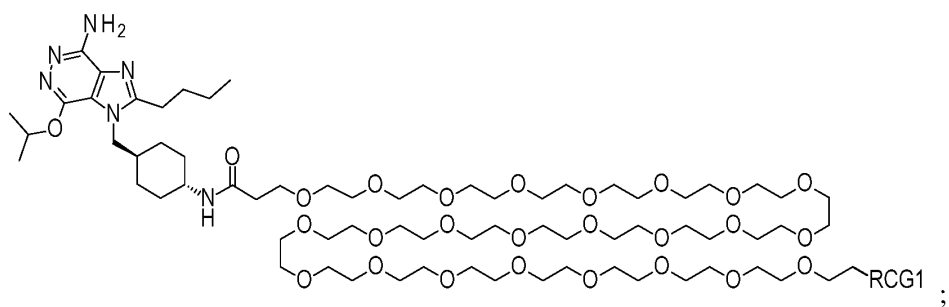


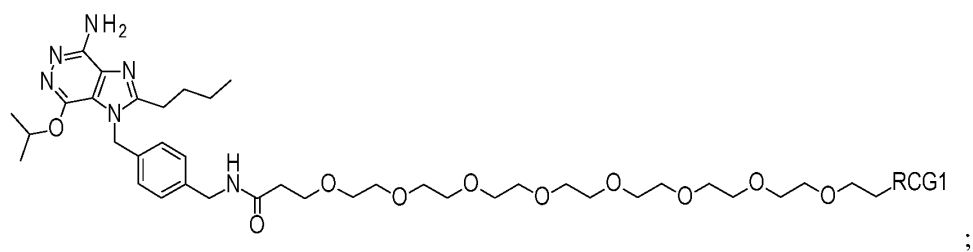
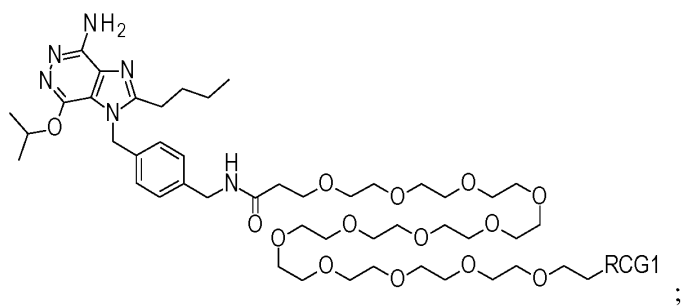
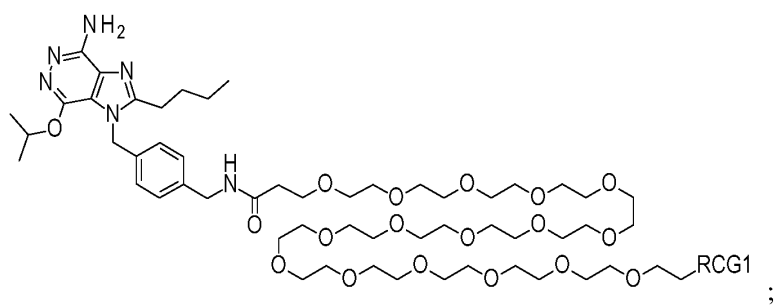
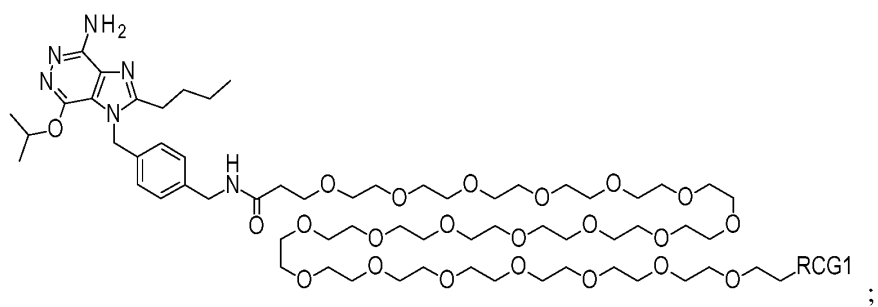
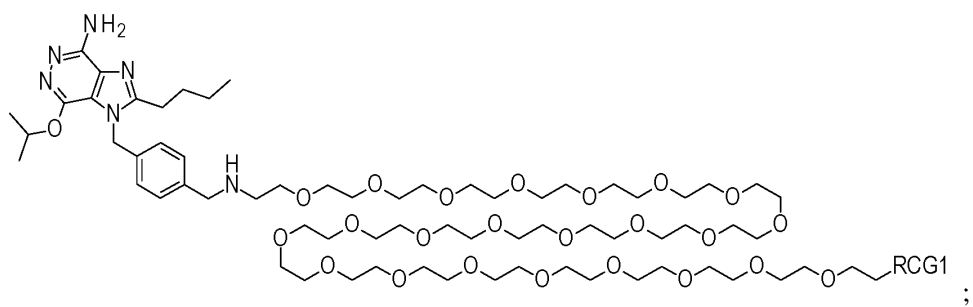
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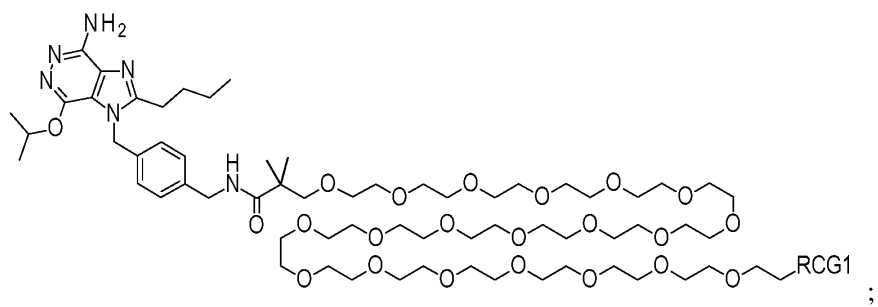
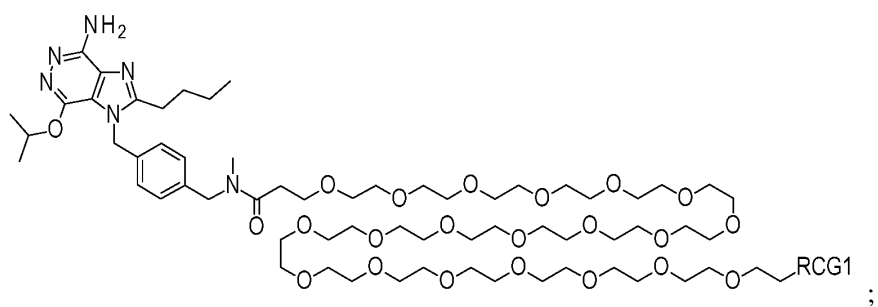
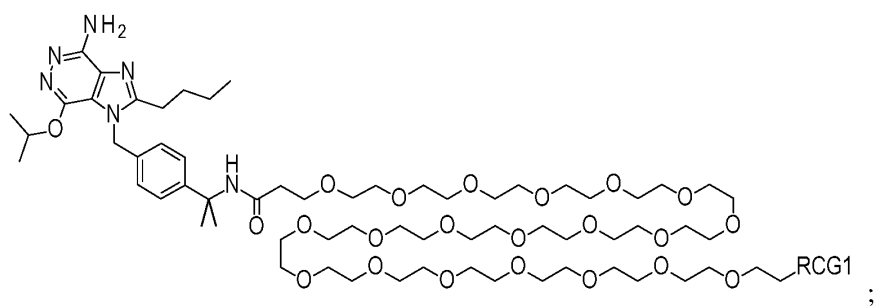
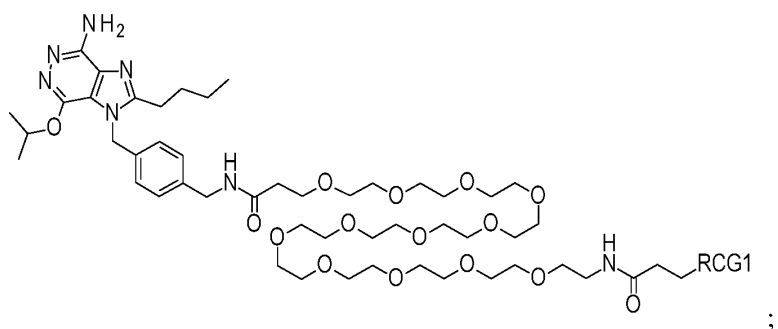


;









oxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P2) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]carbamoyloxymethyl]anilino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P3) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazine-1-carbonyl]oxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P4) (4S)-5-[[[(2S)-1-[[[(2S)-1-[4-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methyl]carbamoyloxymethyl]anilino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-4-[[5-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethylamino]-5-oxopentanoyl]amino]-5-oxopentanoic acid ;

(P5) N'-[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazin-1-yl]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide ;

(P6) N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

(P7) N'-[(2S)-1-[[[(2S)-1-[[*trans*-4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl)methylamino]-1-oxopropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide;

(P8) N'-[(2S)-1-[[[(2S)-1-[4-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl]piperazin-1-yl]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide;

(P31) N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide;

(P32) N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]amino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate;

(P33) N'-[(2S)-1-[[[(2S)-1-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methylamino]-5-(carbamoylamino)-1-oxopentan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide; 2,2,2-trifluoroacetate;

(P34) N'-[(1S)-1-[[[(1S)-1-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]cyclohexyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide, 2,2,2-trifluoroacetic acid;

(P35) (2S)-N-[[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]methyl]-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide, 2,2,2-trifluoroacetic acid;

(P36) (2S)-N-[[4-[[7-amino-4-isopropoxy-2-(propylamino)imidazo[4,5-d]pyridazin-3-yl]methyl]phenyl]methyl]-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide, 2,2,2-trifluoroacetic acid;

(P37) N'-[(1S)-1-[[[(1S)-1-[3-(4-amino-2-butyl-1-methyl-imidazo[4,5-d]pyridazin-7-yl)-2,5-dihydropyrrole-1-carbonyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]pentanediamide, 2,2,2-trifluoroacetic acid;

(P38) N'-[(1S)-1-[[[(1S)-1-[4-(7-amino-2-butyl-3-methyl-imidazo[4,5-d]pyridazin-4-yl)piperazine-1-carbonyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]-N-[2-

[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]ethoxy]ethyl]

pentanediamide,2,2,2-trifluoroacetic acid;

(P39) (2S)-N-[5-(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-yl)]

pentyl]-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethoxy]ethoxy]

5 ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-pentanamide,2,2,2-trifluoro
acetic acid;

(P40) (2S)-N-[4-[(7-amino-2-butyl-4-isopropoxy-imidazo[4,5-d]pyridazin-3-

yl)methyl]cyclohexyl]-2-[[[(2S)-2-[3-[2-[2-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]

ethoxy]ethoxy]ethoxy]propanoylamino]-3-methyl-butanoyl]amino]-5-ureido-

10 pentanamide,2,2,2-trifluoroacetic acid;

(P41) N-[[4-[(7-amino-2-butyl-4-propan-2-yloxy)imidazo[4,5-d]pyridazin-3-

[illegible]

[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]

[illegible]

15 |ethoxy|ethoxy|propanamide;

(P42) N-[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-

[illegible]

[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]

[illegible]

20]ethoxy]ethoxy]propanamide;

(P43) N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[4-[7-

amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl)methyl

[illegible]

ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethyl|-

25 3-(2,5-dioxopyrrol-1-yl)propenamide, 2,2,2-trifluoroacetic acid;

(P44) N-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[3-[[4-(7-

amino-4-isopropoxy-2-propylsulfanyl-imidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]

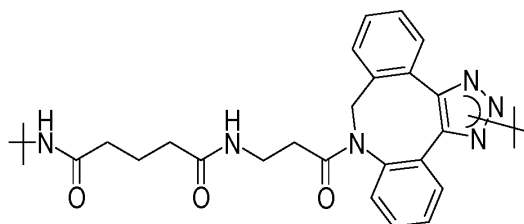
methy|amino|-3-oxo-propoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|ethoxy|

[illegible]

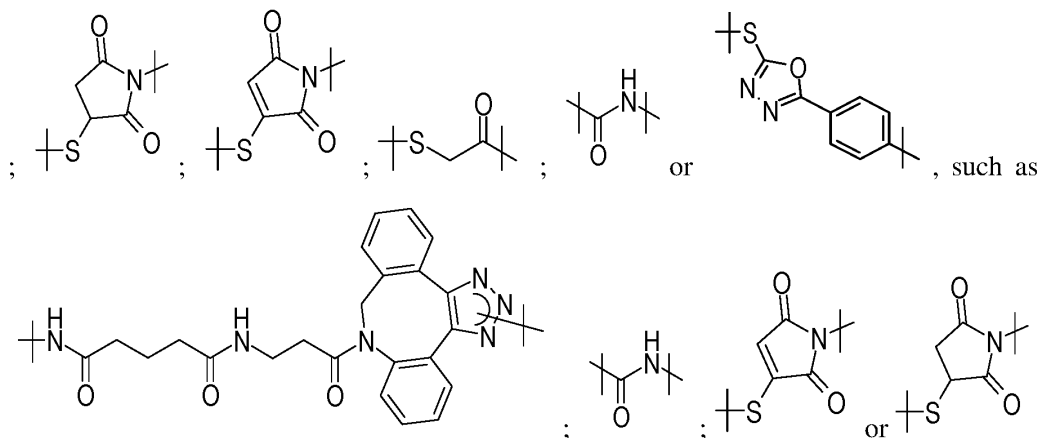
30 [ethyl]-3-(2,5-dioxopyrrol-1-yl)propanamide;

(P45) N-[2-

[3-[[4-[(7-amino-2-butyl-4-propan-2-yloxyimidazo[4,5-d]pyridazin-3-yl)methyl]phenyl]



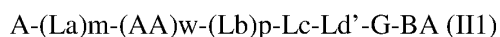
instance G represents the following groups:



5

11. The compounds of formula (II) or a pharmaceutically acceptable salt thereof according to claim 10, wherein said compound is chosen among a compound of formula (II1) and a compound of formula (II2):

10

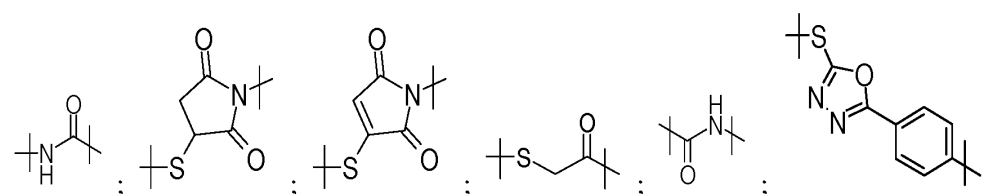


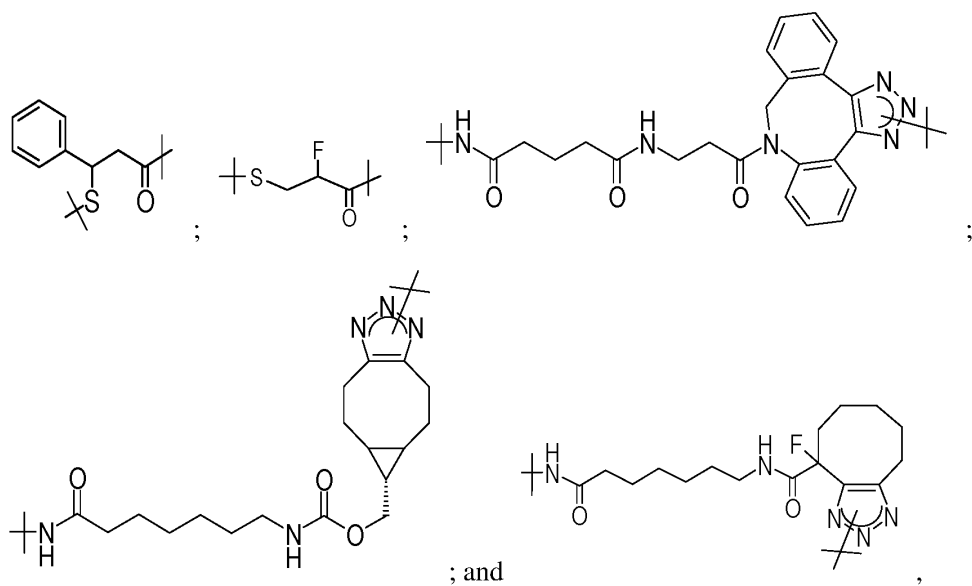
wherein:

A is as defined in any one of claims 1, 5 and 6, **La**, **m**, **(AA)_w**, **AA**, **w**, **Lb**, **p**, **Lc** and **Ld'** are as defined in claim 7, and **BA** is as defined in claim 10;

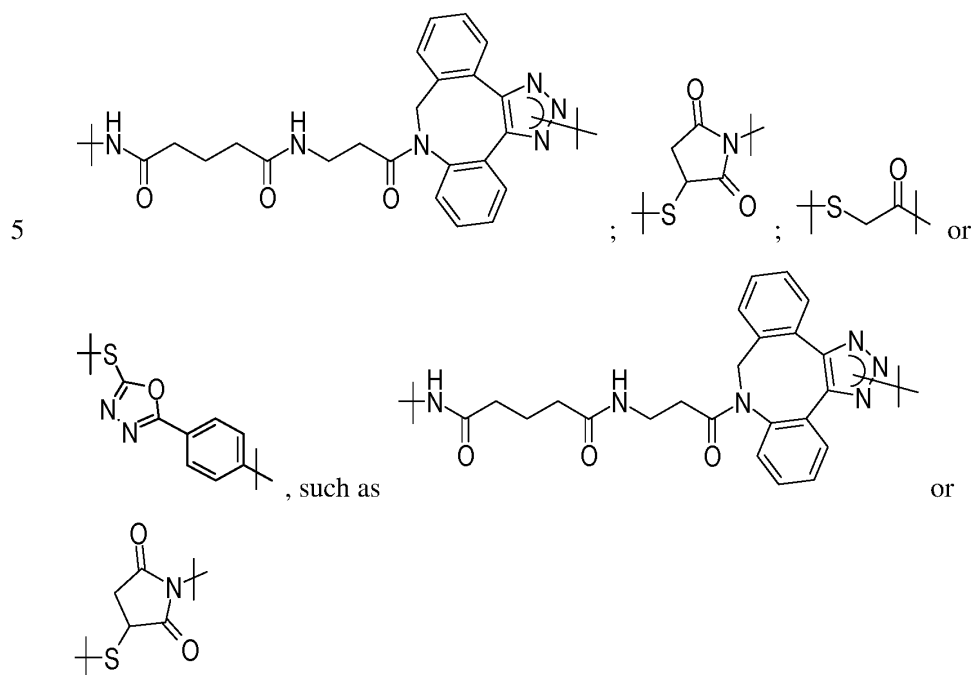
G represents the product of reaction between RCG1 as defined in claim 1 or claim 4 and

15 RCG2, a reactive group present on the binding agent as defined in claim 10 for instance an antibody, for instance G is selected from the group consisting of :





for instance, the left side of the G groups being linked to BA and the right side of the G groups being linked to Ld', for instance G represents the following groups:



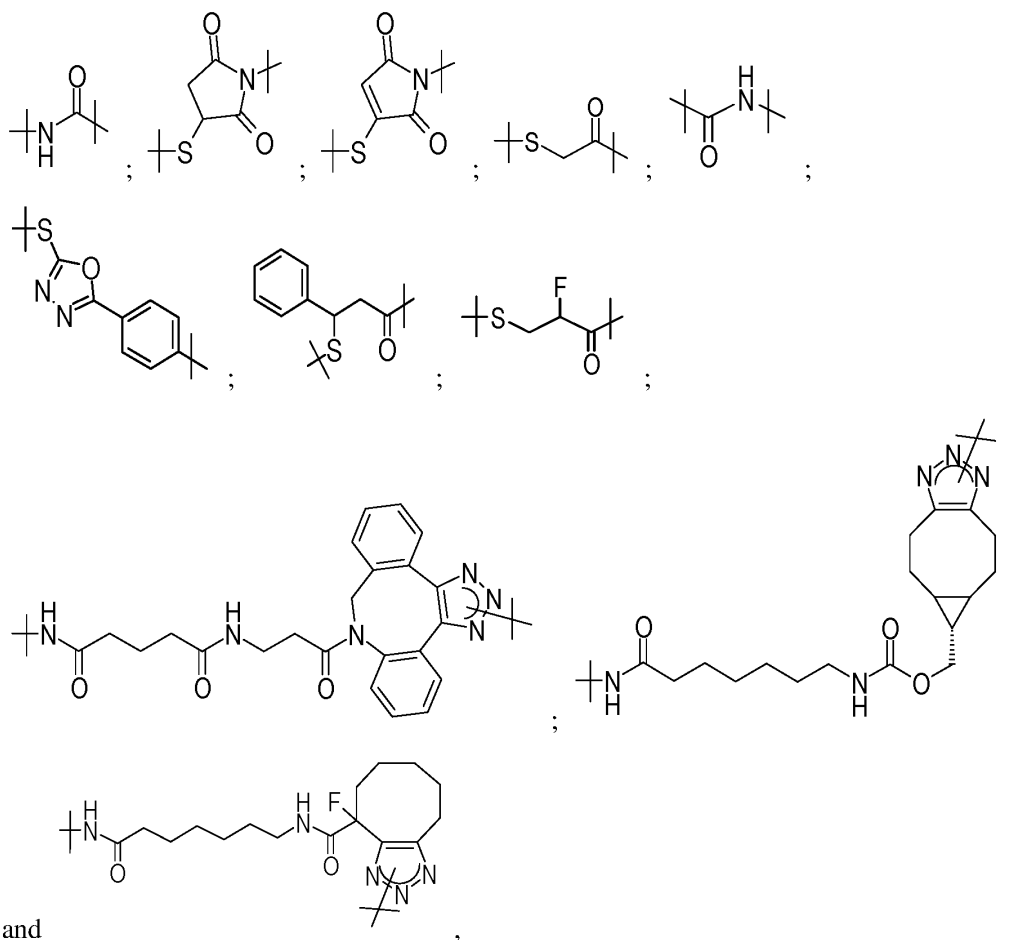
A-(P)o-L-Ld-G-BA (II2)

10 wherein:

A is as defined in any one of claims 1, 5 and 6, and **P**, **o**, **L** and **Ld** are as defined in claim 7, and **BA** is as defined in claim 10;

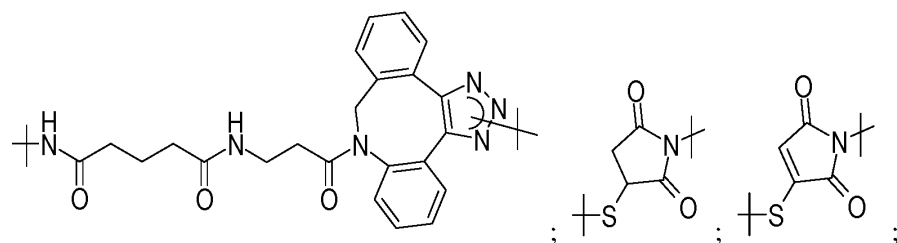
G represents the product of reaction between RCG1 as defined in claim 1 or in claim 4 and RCG2, a reactive group present on the binding agent as defined in claim 10 for instance an

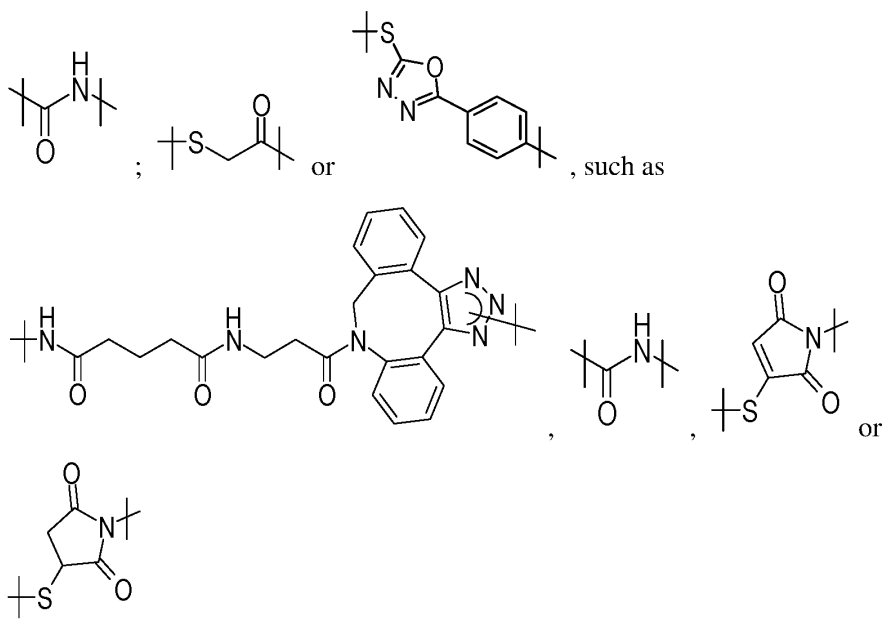
5 antibody, for instance **G** is selected from the group consisting of:



and

10 for instance, the left side of the **G** groups being linked to **BA** and the right side of the **G** groups being linked to **Ld**, for instance **G** represents the following groups:





- 5 **12.** The compound of formula (II) or a pharmaceutically acceptable salt thereof according to claim 10 or 11, wherein the binding agent BA is an antibody Ab, for instance a monoclonal antibody or a VHH antibody.

- 13.** The compound of formula (II) or a pharmaceutically acceptable salt thereof according to any one of claims 10 to 12, wherein:

- when **L1** is $-(\text{La})_m-(\text{AA})_w-(\text{Lb})_p-\text{Lc}-\text{Ld}'-$, **L1** is selected from the group consisting of:

*-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-** ;

- 15 *-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

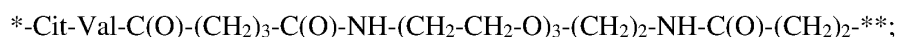
*-Cit-Val-C(O)-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;

*-Cit-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

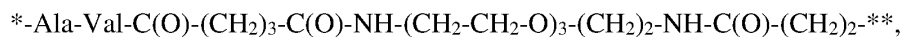
*-Ala-Val-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-**;

- 20 *-C(O)-O-CH₂-para-phenyl-NH-Cit-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-** ;

*-C(O)-O-CH₂-para-phenyl-NH-Ala-Val-Glu-C(O)-(CH₂)₃-C(O)-NH-(CH₂-CH₂-O)₃-(CH₂)₂-NH-C(O)-(CH₂)₂-**;

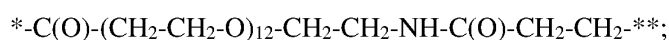
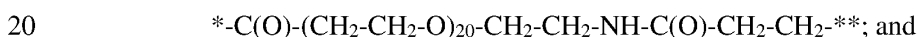
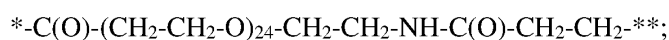
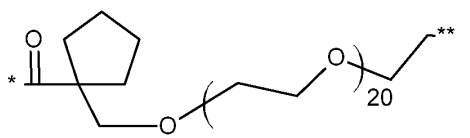
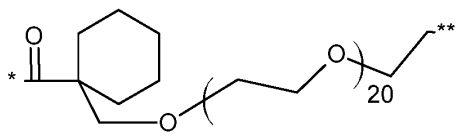
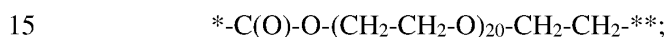
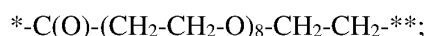
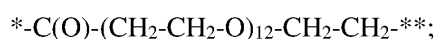
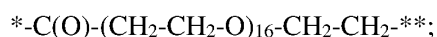
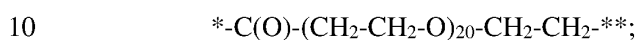
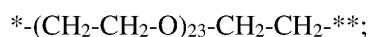
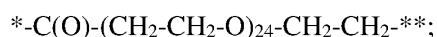


and



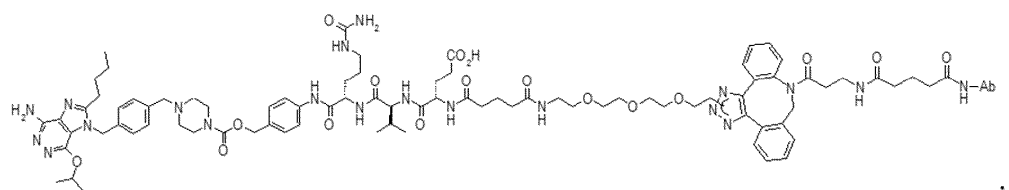
the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group; or

- when **L1** is -(P)o-L-Ld-, **L1** is selected from the group consisting of:

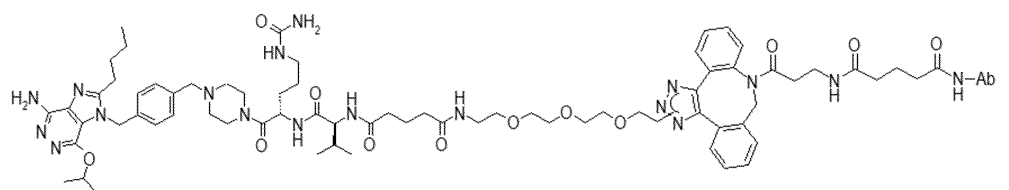


the single asterisk (*) indicating the site of covalent attachment to the A drug unit radical and the double asterisk (**) indicating the site of covalent attachment to the G radical belonging to the -G-BA group.

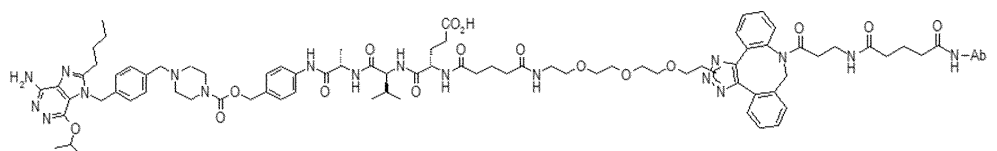
14. The compound of formula (II) or a pharmaceutically acceptable salt thereof according to any one of claims 10 to 12, wherein said compound is chosen among:



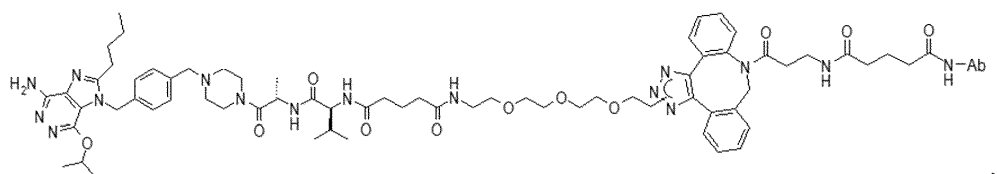
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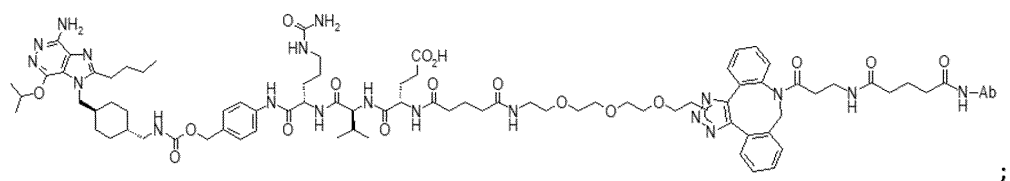
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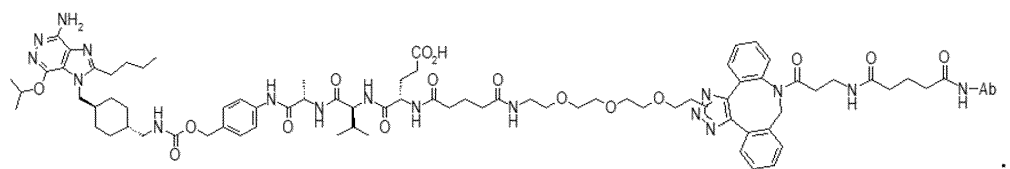
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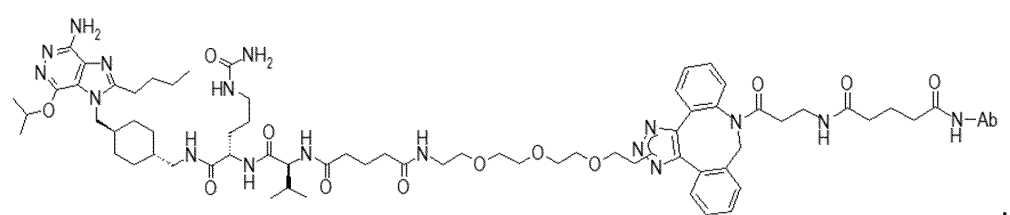
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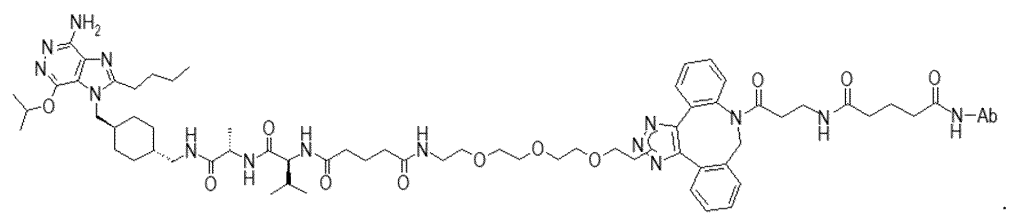
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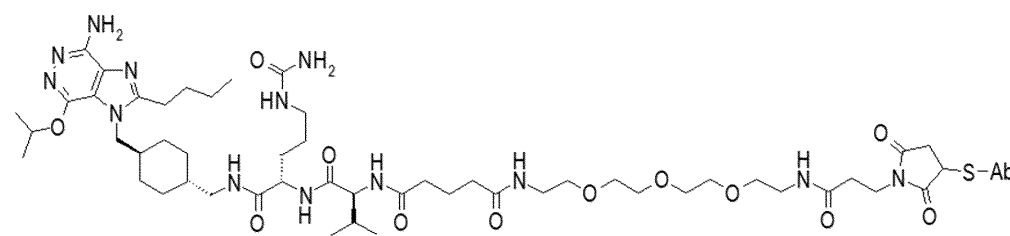
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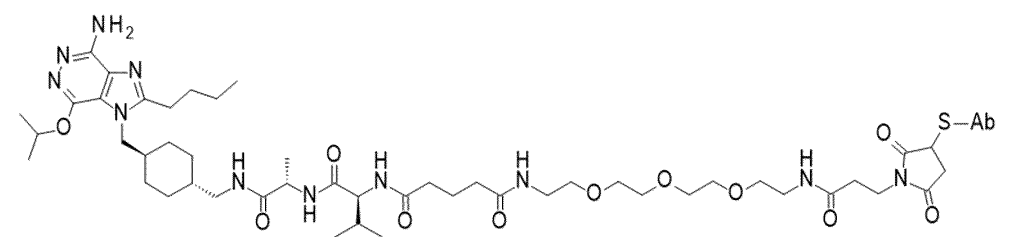
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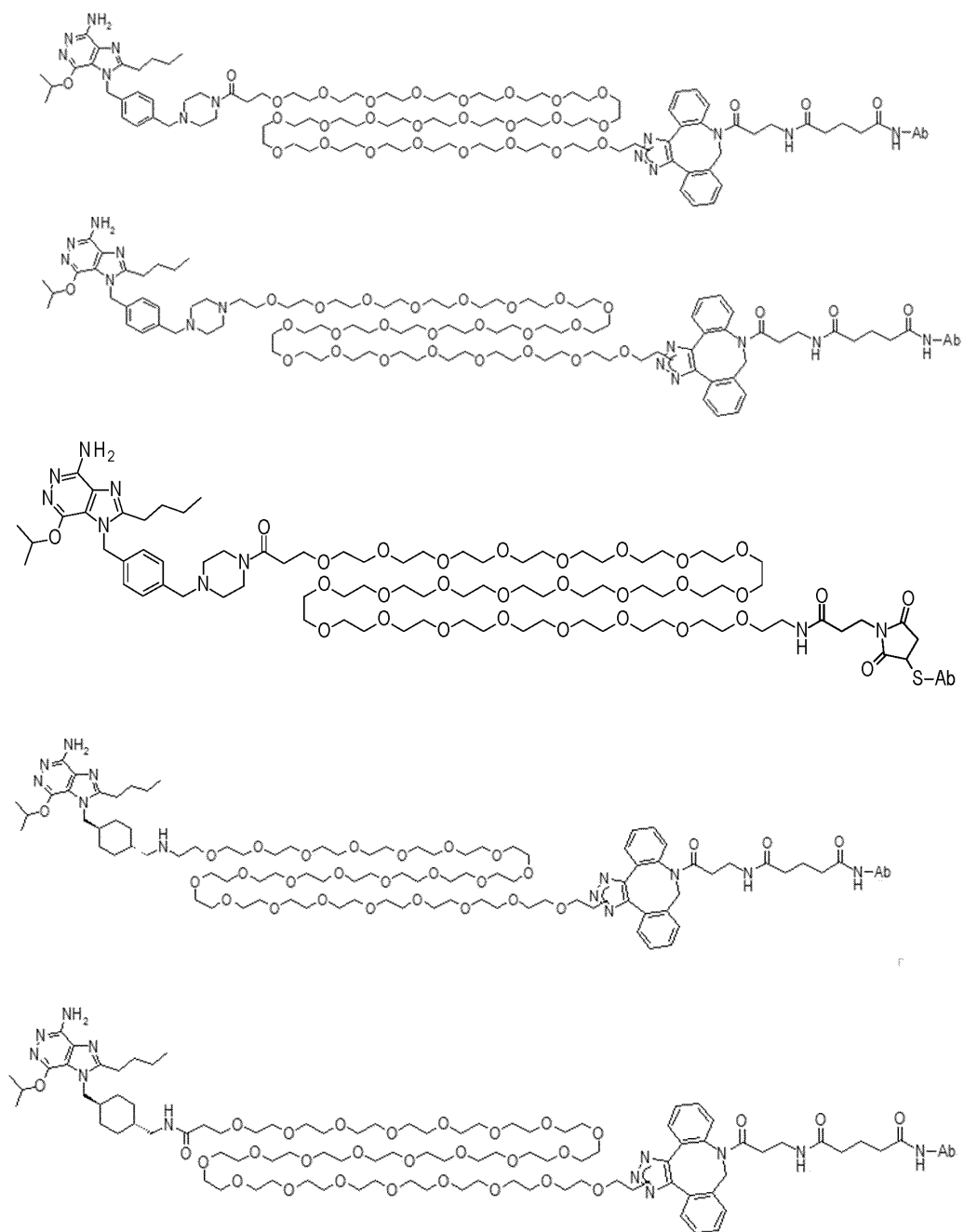
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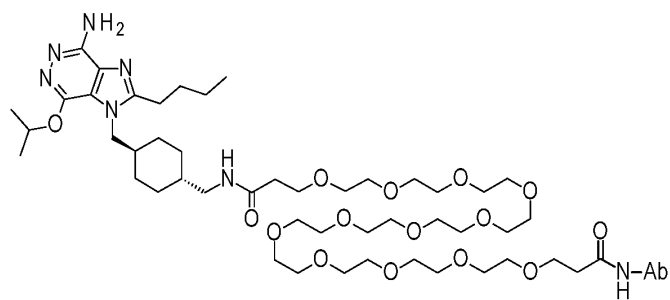
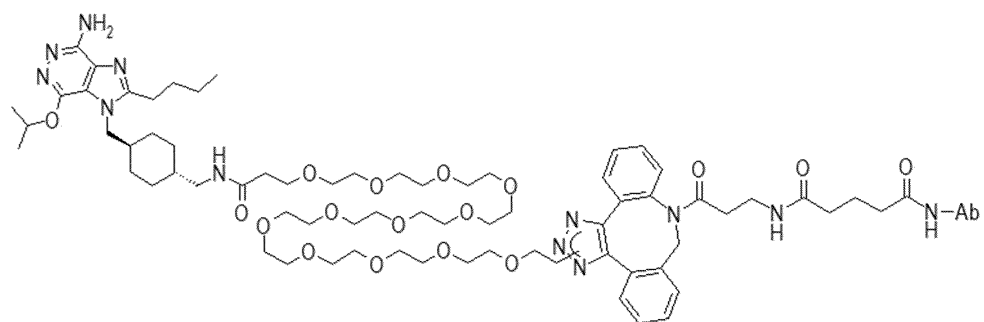
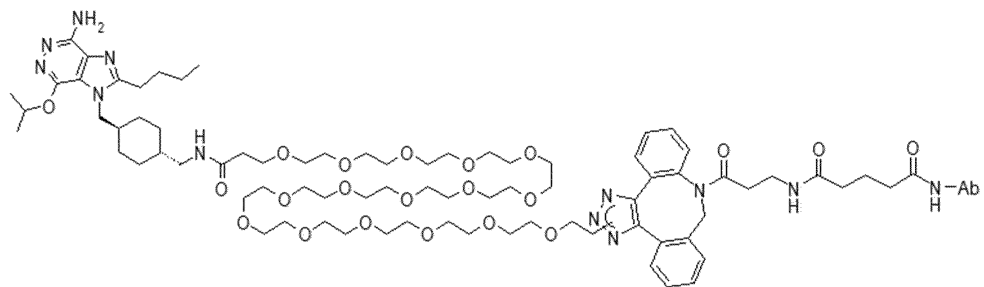
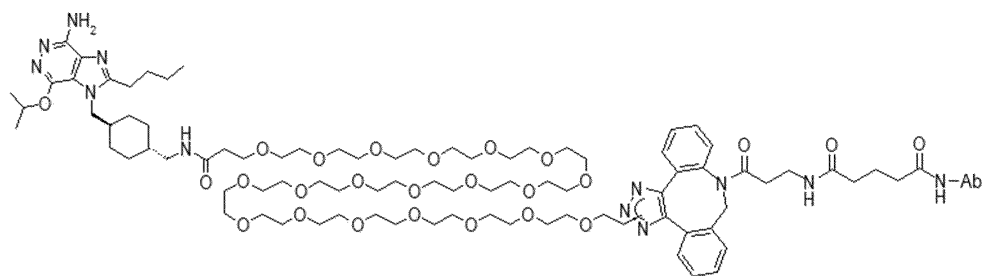


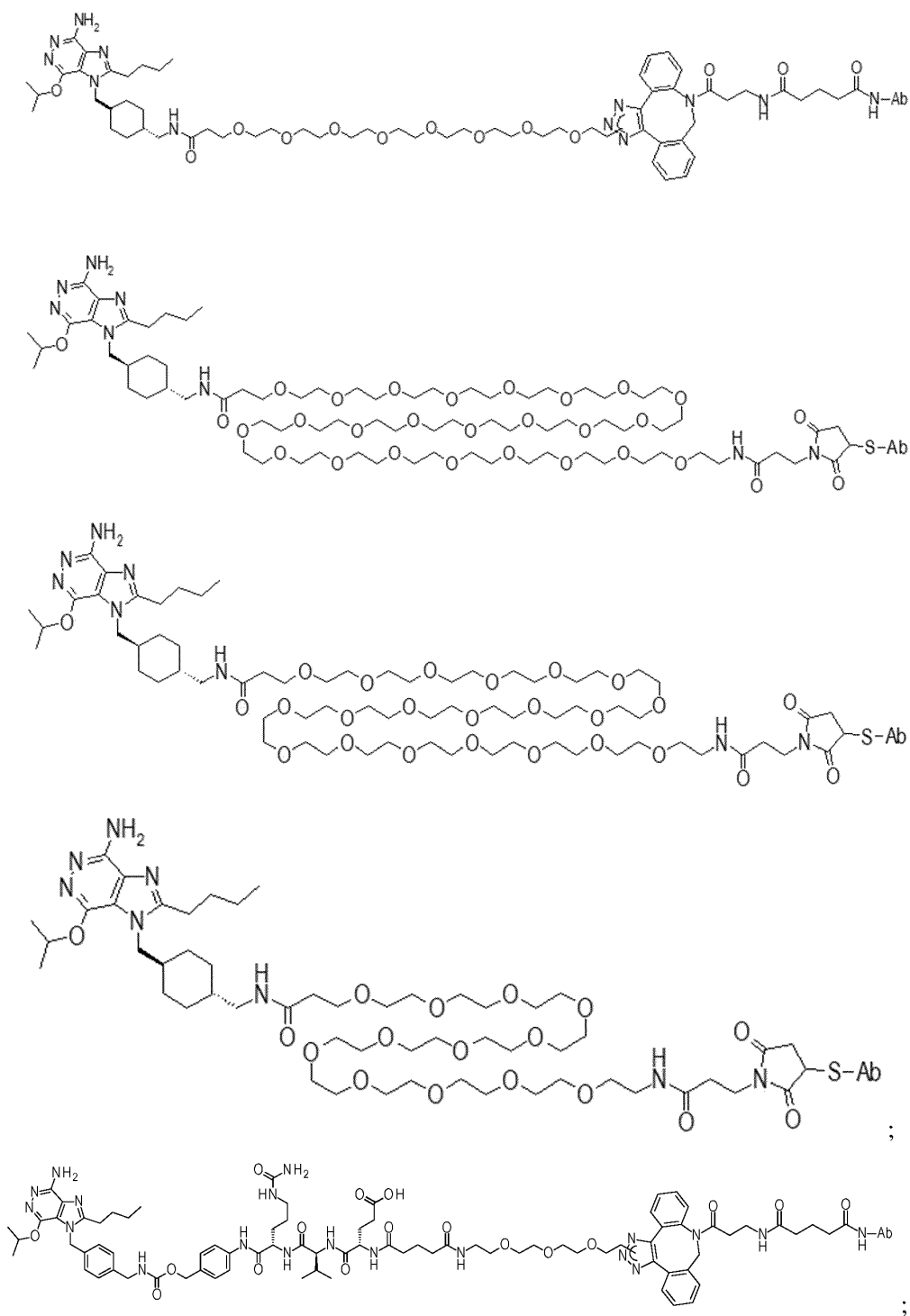
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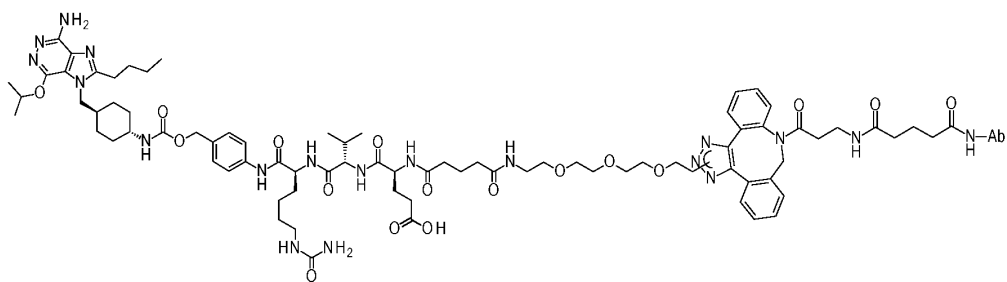


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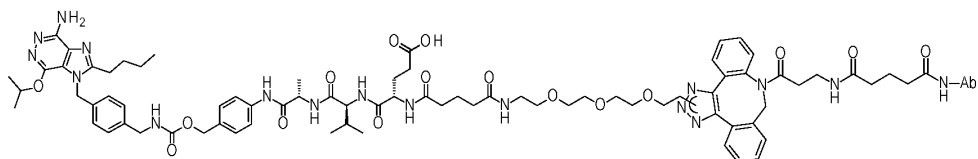




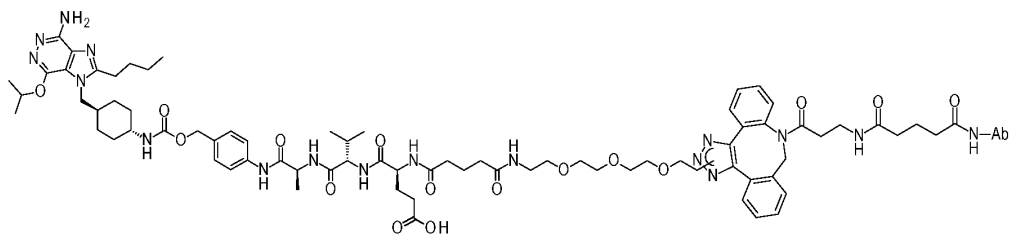




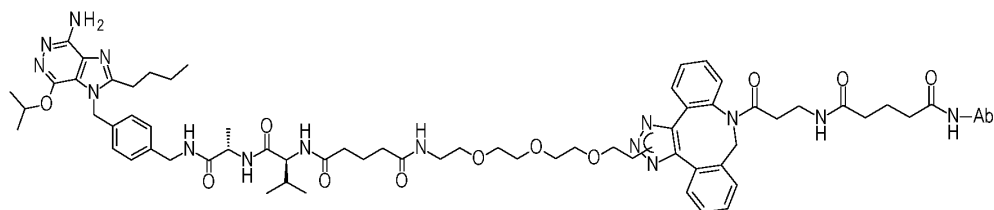
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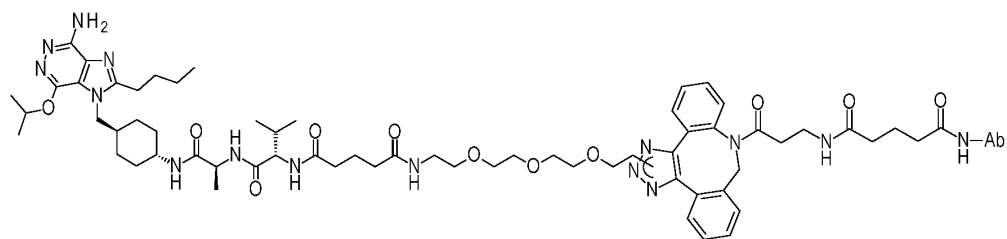
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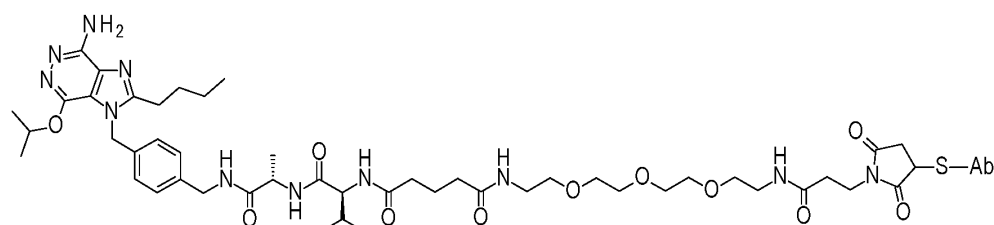


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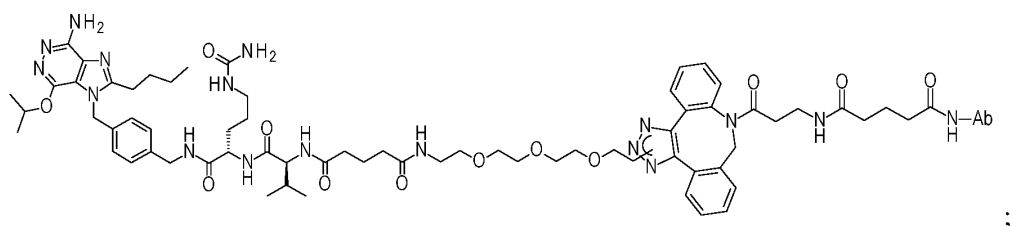


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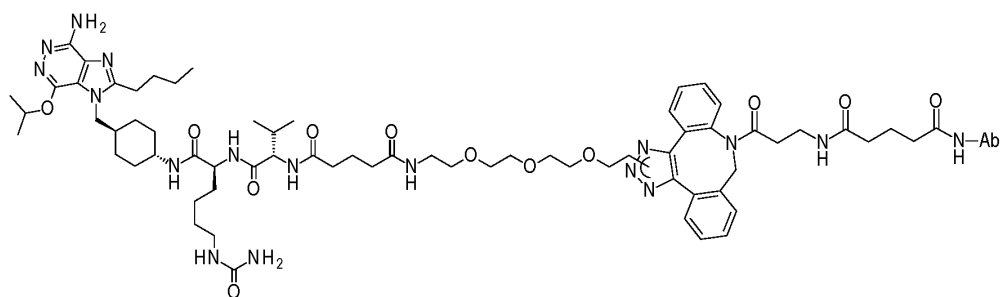
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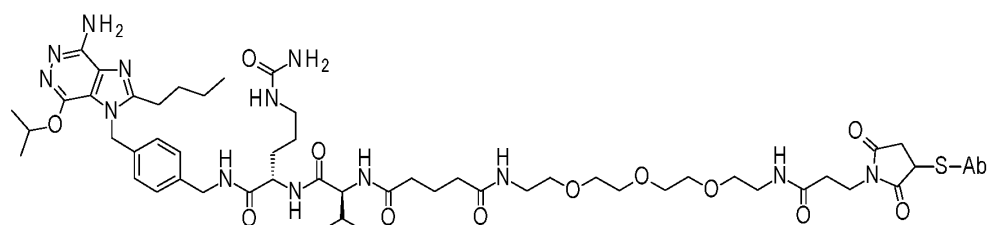
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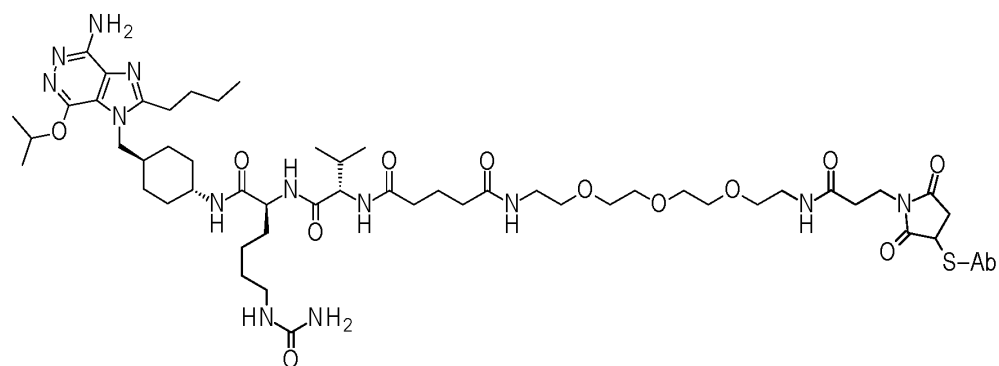
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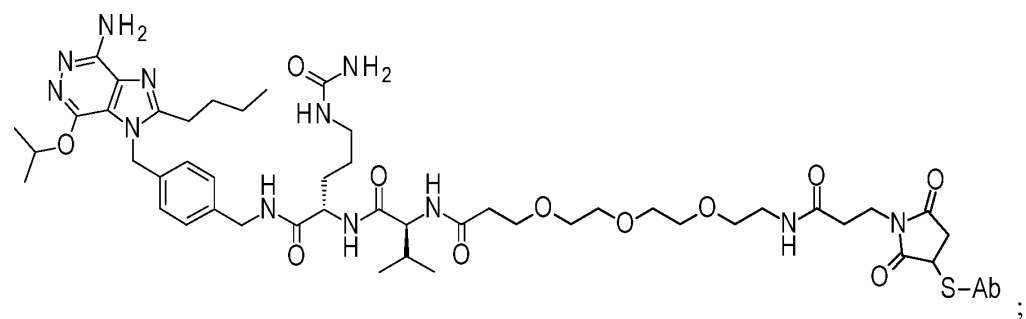
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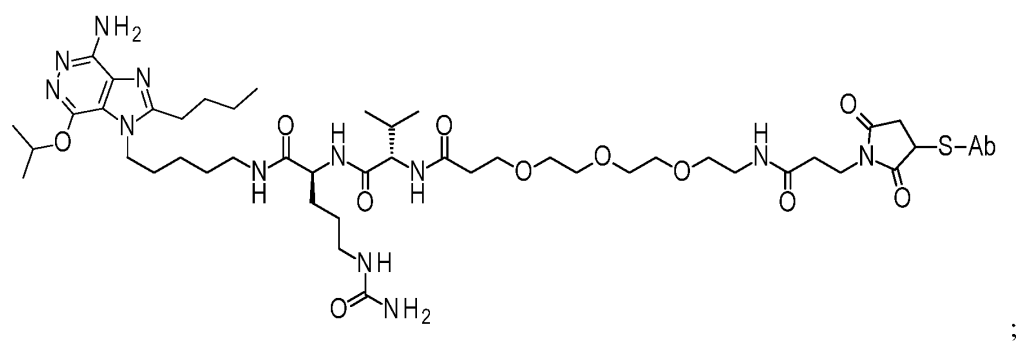
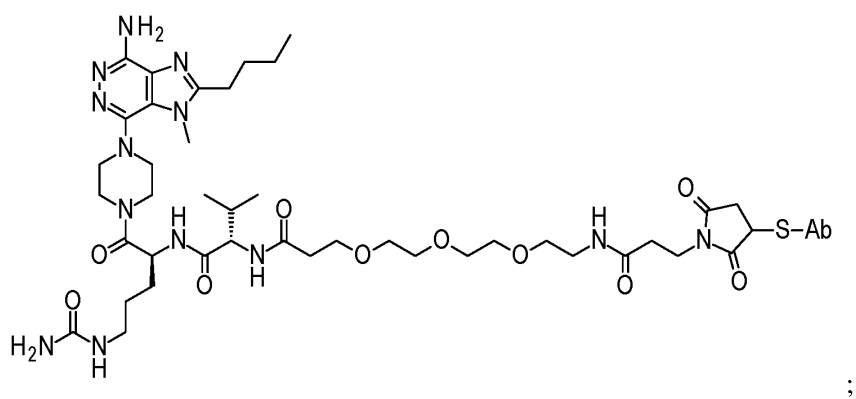
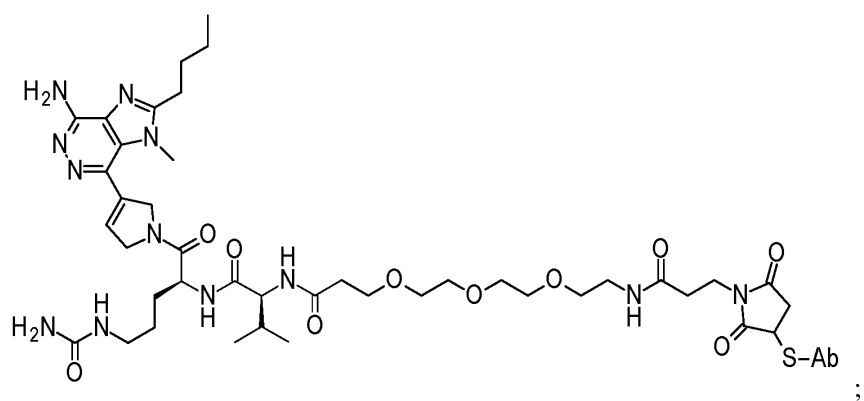
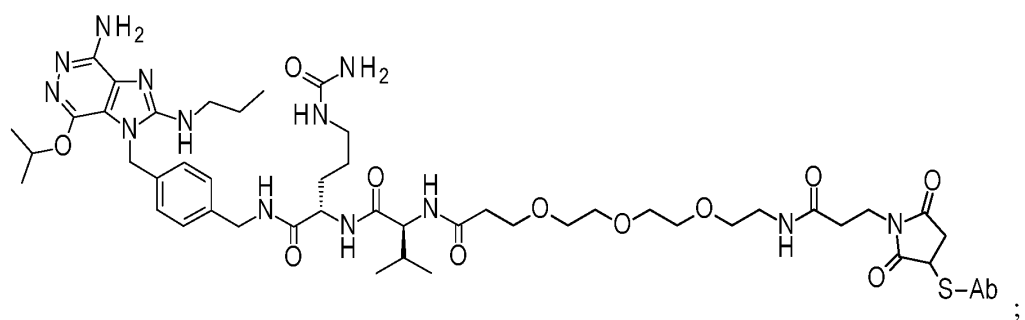
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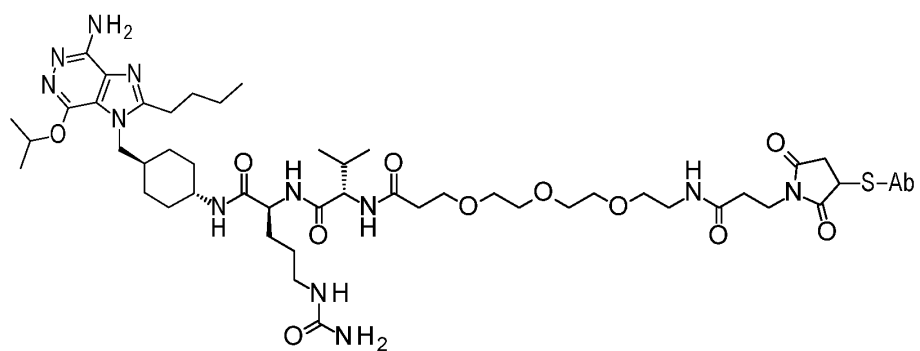


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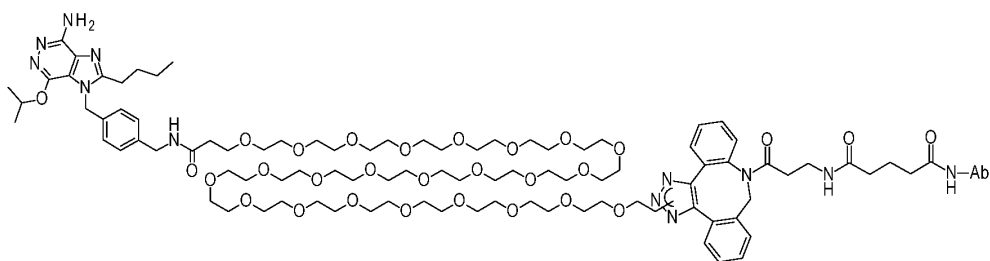


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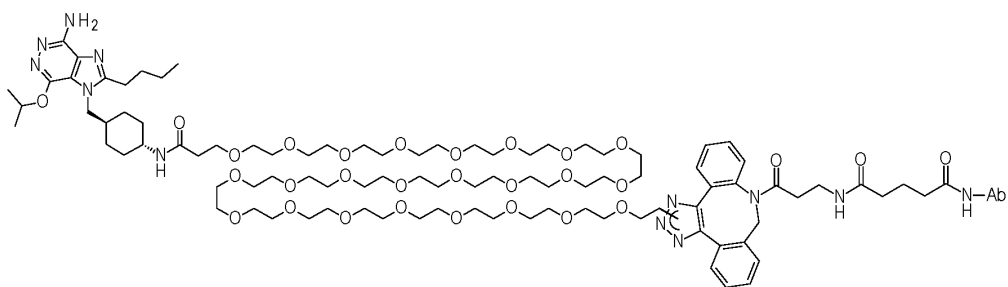




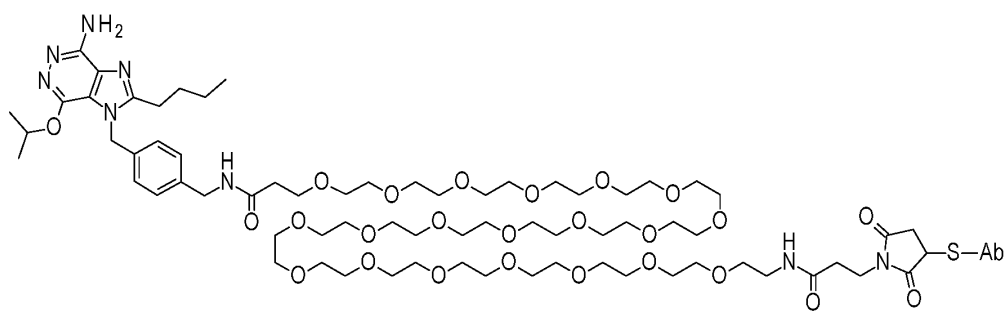
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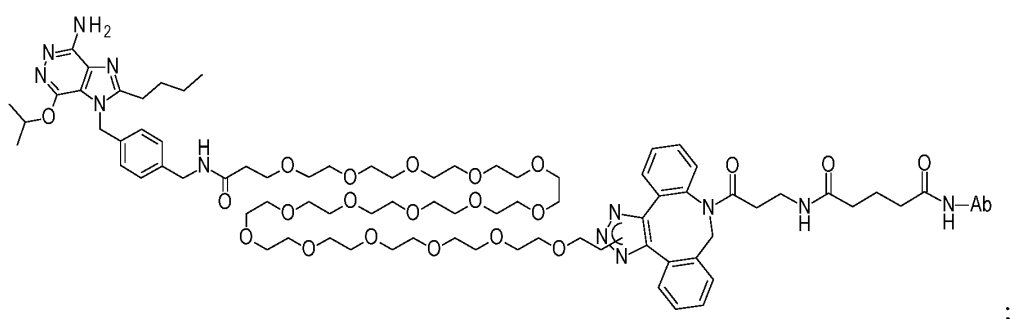
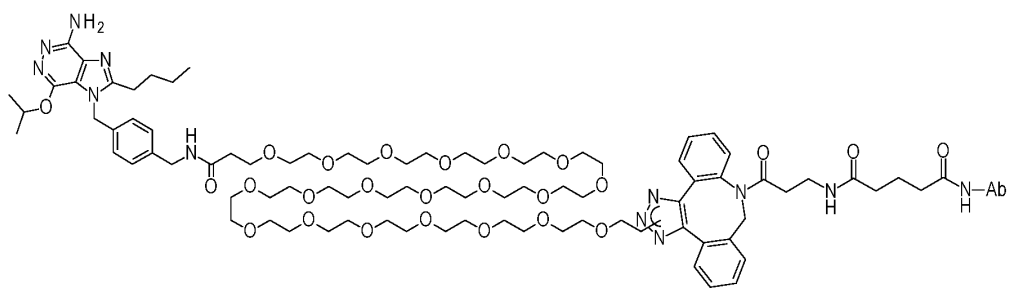
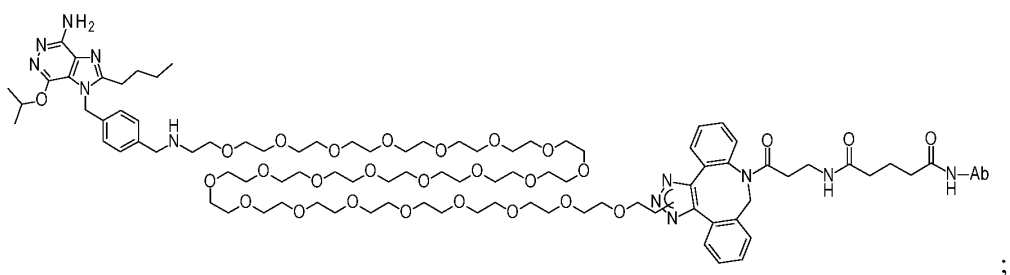
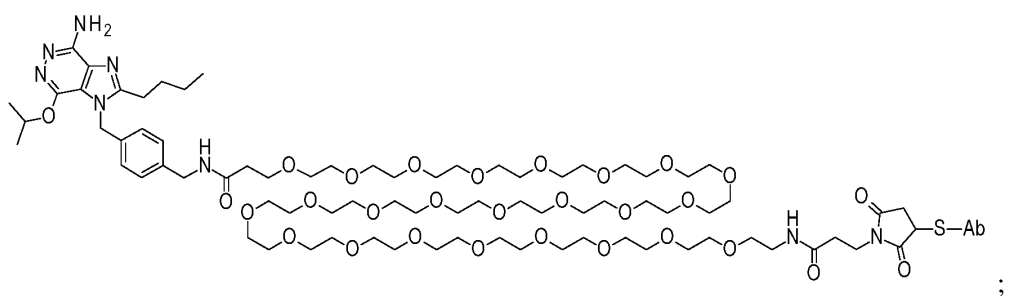
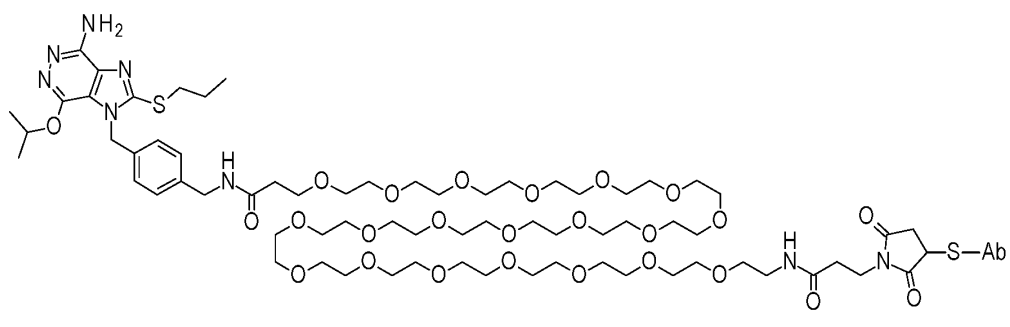
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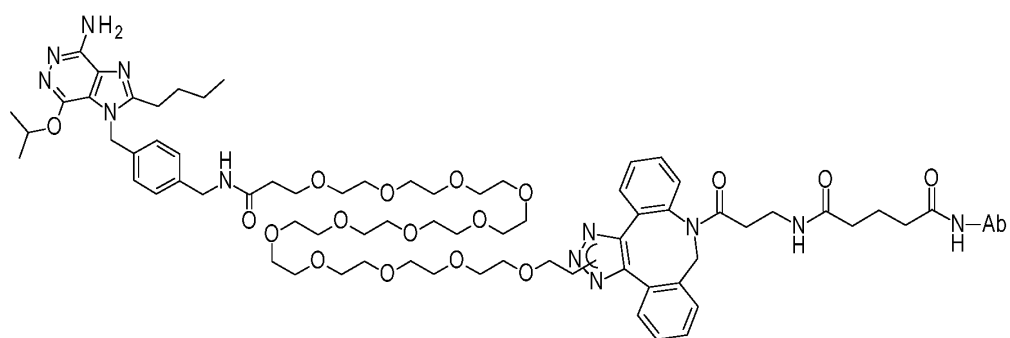


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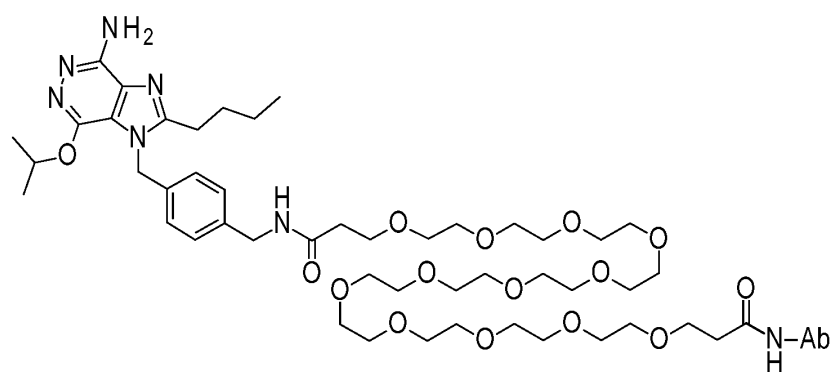


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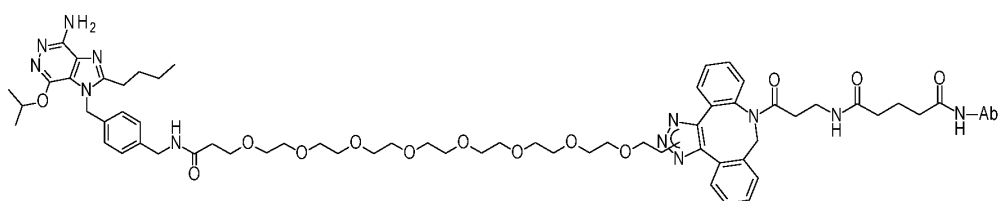




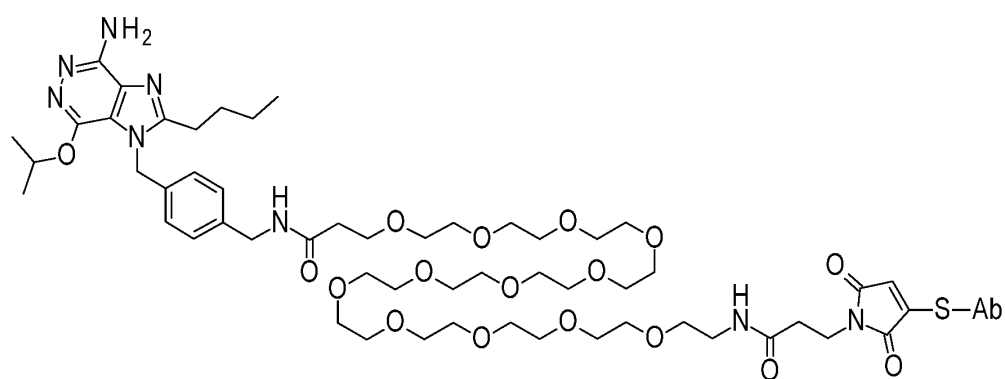
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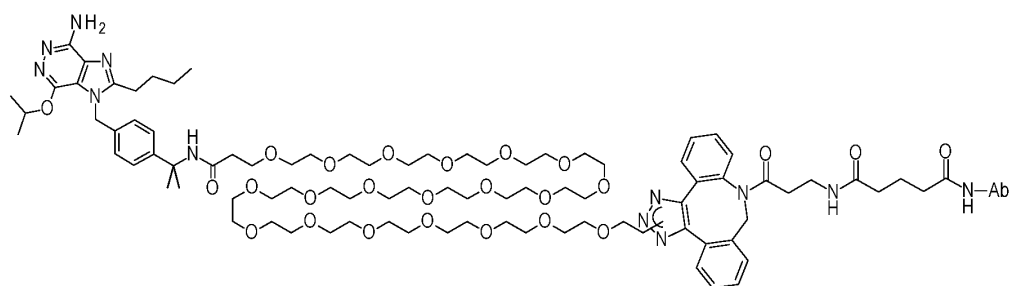
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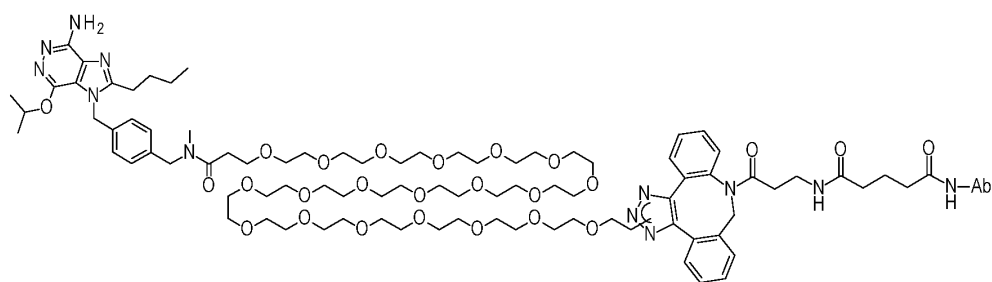
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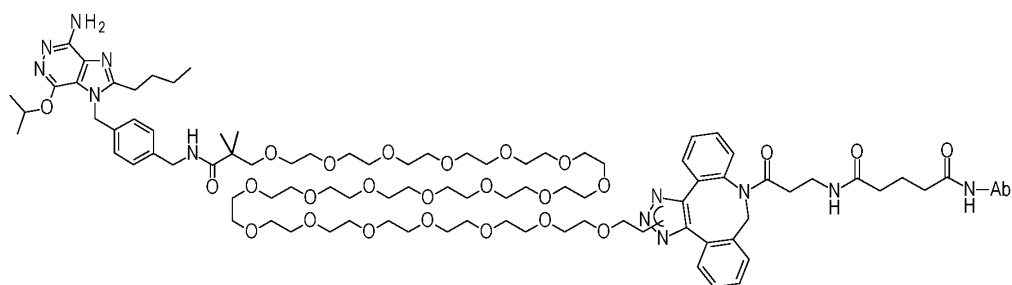
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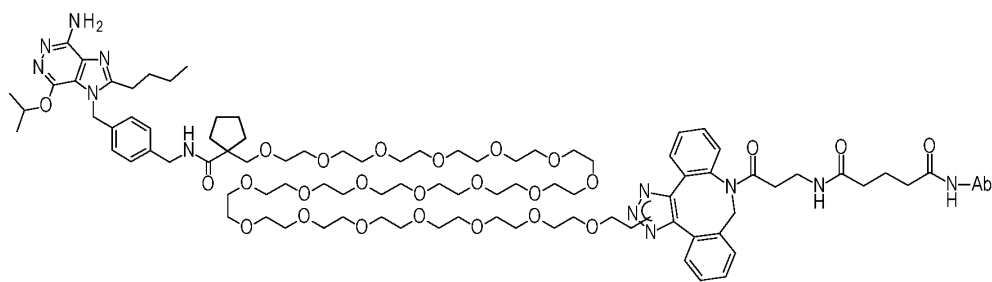
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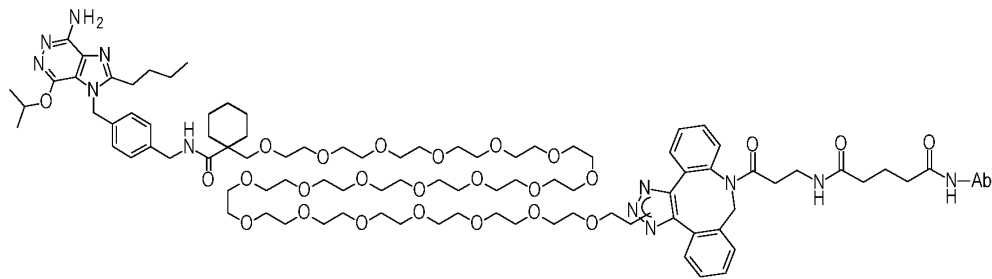
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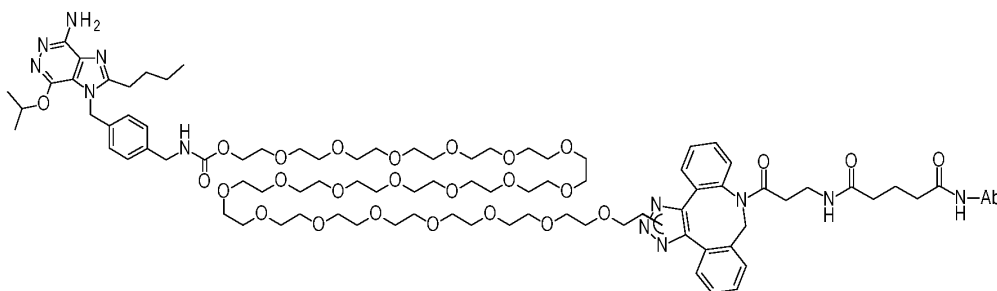
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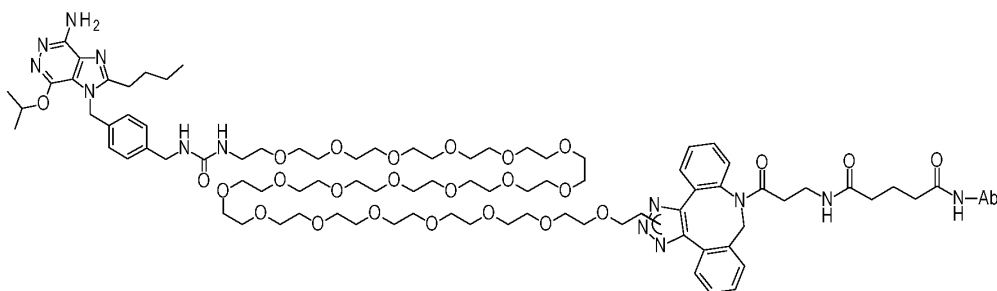
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and



wherein Ab represents an antibody, for instance a monoclonal antibody or a VHH antibody.

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15. The compound of formula (II) or a pharmaceutically acceptable salt thereof according to any one of claims 10 to 12, wherein **RCG2** is selected from the group consisting of:

- (i) a ϵ -amino group (ϵ -NH₂ group) borne by the side chain of a lysine residue that is present at the surface of the binding agent as defined in claim 10 for instance of the antibody;
- 10 (ii) an α -amino group (α -NH₂ group) of an N-terminal amino acid of a heavy chain or a light chain of the binding agent as defined in claim 10 for instance of the antibody;
- (iii) the saccharide groups of the hinge region;
- (iv) a thiol (a -SH group) of a cysteine residue generated by reducing an intra-chain disulfide bond of the binding agent as defined in claim 10 for instance of the antibody or a -SH group
- 15 of an engineered cysteine residue of the binding agent as defined in claim 10 for instance of the antibody;
- (v) an amide group (a -C(O)NH₂ group) borne by the side chains of a glutamine residue that is present at the surface of the binding agent as defined in claim 10 for instance of the antibody;
- 20 (vi) an aldehyde group (a -C(O)H group) introduced using formylglycine generating enzyme; and

(vii) **RCG2** group, optionally introduced by means of a modifying agent.

16. The compound of formula (II) or a pharmaceutically acceptable salt thereof according to any one of claims 10 to 15, wherein

- 5 - when **RCG1** represents a N-hydroxysuccinimidyl ester, **RCG2** represents a -NH₂ group;
- when **RCG1** represents a maleimido function, a haloacetamido function, a chlorine atom or an activated disulfide function, **RCG2** represents a -SH group;
- when **RCG1** represents a -N₃ group, **RCG2** represents a HC≡C- or an activated C≡C function such as a cyclooctyne moiety;
- 10 - when **RCG1** represents a -OH or -NH₂ group, **RCG2** represents a carboxylic acid or amide function;
- when **RCG1** represents a -SH group, **RCG2** represents a maleimido function, a haloacetamido function or an activated disulfide function;
- when **RCG1** represents a HC≡C- function or an activated C≡C function, **RCG2**
- 15 represents a -N₃ group;
- when **RCG1** represents a O-alkyl hydroxylamine function or a Pictet-Spengler reaction substrate, **RCG2** represents an aldehyde or a ketone function.

17. The compounds of formula (I) according to any one of claims 1 to 9 or the compounds
20 of formula (II) according to any one of claims 10 to 16, wherein the drug unit A of formula (A) as defined in claim 1 or claim 6, or of formula (A1) or of formula (A2) as defined in claim 5 or claim 6 is a TLR7 agonist.

18. The compounds of formula (I) according to any one of claims 1 to 9 or the compounds
25 of formula (II) according to any one of claims 10 to 16, wherein the drug unit A of formula (A) as defined in claim 1 or claim 6, or of formula (A1) or of formula (A2) as defined in claim 5 or claim 6 is a TLR7/8 agonist.

19. The compounds of formula (I) according to any one of claims 1 to 9, 17 and 18, or of
30 formula (II) according to any one of claims 10 to 18, wherein the drug unit A is further low or non - cell permeable.

20. The compounds of formula (I) or of formula (II) according to claim 19, wherein the low or non - cell permeable drug unit A is defined by a PAMPA below 25.

21. The compounds of formula (II) according to claim 7 or of formula (III) according to claim 11, wherein the amino acid sequence (AA)_w comprises a citrulline.

22. The compounds of formula (II) or of formula (III) according to claim 21, wherein the amino acid sequence (AA)_w is Val-Cit.

23. The compounds of formula (II), (II1) or (II2) according to any one of claims 10 to 22, wherein RCG2 is a reactive group of a cysteine engineered amino acid residue of an antibody.

24. The compounds of formula (II), (II1) or (II2) according to claim 23, wherein the cysteine engineered amino acid residue is at least one position according to the numbering of the EU numbering index, selected from an interchain cysteine, 118, 152, 360, 274, 414 of the heavy chain constant (C_H) domain.

25. The compounds of formula (II), (II1) or (II2) according to claim 24, wherein the cysteine engineered amino acid residue is at position 360 of the heavy chain constant (C_H) domain according to the numbering of the EU numbering index.

26. A pharmaceutical composition comprising a compound of formula (II), (II1) or (II2) according to any one of claims 10 to 25, and at least one pharmaceutically acceptable excipient.

27. A compound of formula (II), (II1) or (II2) according to any one of claims 10 to 25, for use as a medicine.

28. A compound of formula (II), (II1) or (II2) according to any one of claims 10 to 25, for use to induce high amount of immune stimulating cytokines with low or no pro-inflammatory cytokines.

29. A compound of formula (II), (II1) or (II2) according to any one of claims 10 to 25, for use to stimulate the cytokine induction of IFN α and IP10 with low or no stimulation of IL6 and TNF α .

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30. A compound of formula (II), (II1) or (II2) according to claim 29, wherein IFN α / TNF α ratio is higher than 1.

31. A compound of formula (II), (II1) or (II2) according to any one of claims 10 to 25, for use in the prevention and/or in the treatment of a disease or a disorder that may benefit of an activation of the immune system, for instance for use in the prevention and/or treatment of a cell-proliferative disease, a cancer, a chronic myelogenous, a hairy cell leukemia, a dermatological disease such as a skin lesion or a skin cancer for example an external genital and perianal warts/condyloma acuminata, a genital herpes, an actinic keratosis, a basal cell carcinoma, or a cutaneous T-cell lymphoma, an autoimmune disease, an inflammatory disease, a respiratory disease, a sepsis, an allergy for example an allergic rhinitis or a respiratory allergy, an asthma, a graft rejection, a graft-versus-host disease, and an immunodeficiency, for instance in the prevention and/or in the treatment of cancers.

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