

ANTIBODY-DRUG CONJUGATE COMPRISING AN ANTI-GUCY2C ANTIBODY
AS WELL AS METHODS OF PRODUCTION AND USES THEREOF

FIELD

The present disclosure relates to new antibody-drug-conjugates (ADCs) comprising a favorable modular linker linking the pharmaceutically active drug substance with the antibody. The present disclosure further relates to methods of producing the antibody-drug conjugate as well as to uses thereof and pharmaceutical compositions comprising the same. Embodiments of the present disclosure have been particularly developed as antibody-drug conjugates for use in the treatment of Guanylate Cyclase 2C (GUCY2C) positive gastrointestinal cancers and will be described hereinafter with reference to this application. However, it will be appreciated that the present disclosure is not limited to this particular field of use.

BACKGROUND

Aside from surgical resection and radiation therapy, chemotherapy remains as the therapeutic option predominantly utilized in the treatment of cancer. However, it is also well-established that chemotherapy – through its limited selectivity and specificity towards cancer cells – typically offers only a small therapeutic window for the administration of highly toxic doses of chemotherapeutic agents, which regularly lead to severe side effects for the patients through the toxic effect on non-cancer cells, while still only conveying a low efficacy towards malign cancer cells.

Targeted drug therapy solutions have been developed to increase the specific delivery of cytotoxic drug substances directly to malign cancer cells. In particular, antibody-drug conjugates (ADCs) provide new treatment options that allow for the delivery of drug substances to malign cancer cells with high specificity and with very limited or no effects on non-cancer cells.

Antibody-drug conjugates (ADCs) typically consist of an antibody covalently linked to a cytotoxic drug substance via a synthetic linker. While (a) the antibody ensures the high degree of specificity and (b) the cytotoxic drug substance ensures the effect on the cancer cells once delivered, (c) the linker compound used to conjugate the

antibody and the drug substance plays an important role as it can significantly affect the therapeutic index, efficacy and pharmacokinetics of an ADC.

In particular, the linker compound must (i) ensure high plasma stability of the conjugate in circulation to prevent premature drug release and consequential undesired systemic off-target effects; (ii) maintain the properties of the antibody as well as of the cytotoxic drug substance, (iii) provide a high degree of aqueous solubility of the conjugate to allow for the delivery of hydrophobic/lipophilic drug substances and to prevent aggregation of the ADCs in a pharmaceutical composition; and (iv) ensure appropriate exposure of the targeted cancer cells to the cytotoxic drug substance to maximize the therapeutic effect.

The lipophilic nature of many cytotoxic drug substances can adversely affect the properties of an ADC to the extent that the payloads are not efficiently delivered to the target cells. Modulating ADC properties by affixing polar groups in linkers may reduce aggregation during conjugation and improve physiochemical properties of the ADCs leading to improved pharmacokinetics (PK) and an improved therapeutic index of the ADC (see, for example, *Nature Biotechnology*, 2015, 33, 733-735). Modulation of the linker to effect a change in the polarity or charge of the final metabolite may improve activity toward multidrug resistant (MDR) cells owing to better retention of the payloads inside the cells (see, for example, *Cancer Res*; 70(6) Mar. 15, 2010, p2528). The chemical moieties on linkers, e.g. polyethylene glycol (PEG), sugar, or sulfuric acid groups (see, for example, WO2014062697, US20100323973), not only affect the conjugation efficiency and the ease of production of ADCs, but also often remain as part of the metabolites and thus affect the ADC's activity and safety.

The therapeutic efficacy of an ADC is not only dependent on the appropriate release of the cytotoxic drug substance but also on the effective bio-conjugation connecting the drug substance to the antibody and, further, on the number molecules of the cytotoxic drug substance successfully linked to a single antibody, i.e. the Drug-Antibody-Ratio (DAR). A low DAR is known to decrease ADC efficacy and requires a highly toxic drug substance to be conjugated, whereas a high DAR has been linked to potential ADC instability leading to increased systemic effects, a reduced ADC half-life and a general alteration of the pharmacokinetics of the molecule. However, given that stochastic chemistries have typically been applied to conjugate drug substances

to antibodies, even FDA-approved ADCs are composed of a mixture of conjugates, which are heterogeneous with respect to their DAR.

Furthermore, selection of the appropriate target and high antibody specificity greatly influence the likelihood of a new ADC being therapeutically effective. In this respect, cancer-specific or cancer-associated antigens must be identified that allow for the generation of antibodies that can target malign cancer cells with high specificity and substantially no off-target binding of non-cancer or healthy cells.

With respect to gastrointestinal cancers, it is noteworthy that they account for approximately 26% of all cancer diagnoses and are responsible for approximately 35% of cancer deaths worldwide. There were an estimated 4.8 million new cases of gastrointestinal (GI) cancers and 3.4 million related deaths, worldwide, in 2018.

Gastrointestinal cancers comprise esophageal, gastric, colorectal, liver, esophageal and pancreatic tumors, whereby colorectal cancer (CRC) is most frequent and contributes to about 10.2% of all new cases, followed by gastric cancer (5.7%), liver cancer (4.7%), esophageal cancer (3.2%) and pancreatic cancer (2.5%). CRC contributes to about 9% of all cancer-related deaths, followed by gastric cancer (8.2%), liver cancer (8.2%), esophageal cancer (5.3%) and pancreatic cancer (4.5%) with about 3.4 million GI cancer deaths globally in 2018 (Arnold et al. (2020) *Gastroenterology* 159:335-349). Colorectal cancer (CRC) is thus, the third most common cancer worldwide after lung and breast cancer but the second leading cause of cancer death.

In women, CRC is the second most common adult cancer and the third most common in men, and it is the fourth leading cause of cancer death.

The 5-year and 10-year survival rates are 65% and 58%, respectively, and incidence and mortality rates are 25% higher in men than in women. From 1975 to 2013, CRC incidence rate increased from 10-15 cases per 100,000 population of Americans between the age of 20-49 years of age. This is paralleled by an increase in CRC cases of about 20% in developing countries such as Argentina, Brazil, and China. Furthermore, 30% of all patients with CRC experience metastasis for which the

prognosis still remains poor with a median 5-year survival of only 18.5% in the United States and 27.7% in Europe.

Even though the incidence rate has declined over the past 30 years due to improvements in screening and treatment paradigms it is alarming that incidence rates in individuals younger than 50 years have been steadily increasing, with a 2.2% annual overall incidence rate rise between 2012-2016. The exact etiology for this paradigm shift is unknown, however, it is likely that this is attributable to changes in diet and other lifestyle risk factors or may be attributable to increased nonsystematic screening of young adults.

10-20% of all patients with CRC possess a positive family history and ~5% of all cases of CRC are linked to a known hereditary CRC syndrome detectable by germline. In some cases, increased sporadic CRC incidence is associated with longstanding inflammatory bowel disease and variable lifestyle factors such as physical inactivity, unhealthy diet, smoking, obesity, and heavy alcohol consumption.

Differences in clinical outcomes and drug responsiveness of CRC treatment are dependent on the location of cancer along the colon and rectum. Relevant contributing factors include their distinct physiological functions, gut microbiome, regionally resident immune cell types, dietary carcinogens, and timing of disease detection. In addition, ontogeny factors may contribute to disease severity and treatment outcome:

The proximal (right) large intestine (cecum, ascending colon, and the transverse colon) derives from the embryonic midgut, whereas the distal (left) large intestine (splenic flexure, descending colon, sigmoid colon, and rectum) derives from the embryonic hindgut. These ontogenetic differences are associated with differential gene expression patterns along the proximal–distal axis. In women and in African-Americans, proximal sporadic colon tumors are more frequently diagnosed with an incidence of 51-62% of cases which also show a higher TNM stage at first diagnosis, display a pattern with high levels of genome-wide promoter hypermethylation referred to as CpG island methylator phenotype (CIMP), exhibit microsatellite instability (MSI) due to deficient DNA mismatch repair mechanisms (dMMR), are more frequently mutated in KRAS and BRAF and have a worse prognosis in terms of survival. Distal

colorectal tumors are more likely to present with chromosomal instability (CIN) and show a more favorable prognosis.

Genomic profiling of CRC has revealed significant intratumoral and intertumoral heterogeneity resulting from the accumulation of genetic mutations and chromosomal aberrations during disease initiation and progression. Genomic instability in CRC presents as one of two major forms: CIN and MSI. CRC lacking CIN or MSI is classified as genome stable (GS) CRC.

In GS CRC, the DNA repair genes and tumor suppressors are likely to be transcriptionally silenced through CIMP, though a large proportion of MSI CRCs and a small population of CIN CRCs are also CIMP-positive, and about 10% of CRCs are negative for CIN, MSI, or CIMP.

Chromosome instability (CIN) CIN is characterized by chromosomal numerical alterations (aneuploidy) and structural alterations (somatic copy number alterations, deletions, insertions, amplifications, or loss of heterozygosity), occurring in 65-70% of sporadic CRCs. Nearly all CIN tumors show activated Wnt signaling, and 80% harbor mutational inactivation of APC, a negative regulator of the Wnt pathway. Mutational inactivation/deletion of TP53 occurs in 60% of CIN tumors and p53 loss of function directly drives CIN and provides a permissive context for genome instability mechanisms.

With respect to genome instability, combined telomere dysfunction and p53 deficiency is a major CIN mechanism, as revealed by the occurrence of anaphase bridges in early-stage carcinomas of human CRC and spontaneous CRC occurrence in telomerase-deficient p53 mutant mice.

Microsatellite instable (MSI) CRC is characterized by the presence of microsatellites, DNA sequences which contain repetitive motifs that tend to accumulate higher mutation rates than other genomic regions. MSI is the phenotypic manifestation of deficient mismatch repair (dMMR) resulting from mutational inactivation of MMR genes, including MLH1, MSH2, MSH3, MSH6, PMS2 and Exo1 (De' Angelis et al. Acta Biomed 2018 Dec 17;89(9-S):97-101).

Other important factors that contribute to the etiology of CRC are somatic genetic alterations which activate key signal transduction pathways that promote the proliferative state of CRC cancer cells. In CRC, the major proliferative signaling pathways are the EGFR-RAS and WNT- β -catenin pathways.

EGFR signaling EGFR activation triggers downstream RAS/RAF/MEK/ERK and PI3K/AKT signaling cascades which ultimately lead to proliferation. Mutations in EGFR itself are rare in CRC with 1% of cases of CRC being attributable to a mutation in CRC (Barber et al N Engl J Med 351: 2883) and instead shows overexpression in ~80% of CRCs. Enhanced EGFR activation can occur via post-translational modifications involving methylation of R198 and R200 by protein arginine methyltransferase 1 (PRMT1), which enhances its binding to EGF and consequent signaling activation, even in the presence of the EGFR inhibitor.

It has recently been demonstrated that hepatocyte growth factor (HGF), the ligand of the MET-receptor, can fully replace EGF in the outgrowth of single Lgr5+ mouse intestinal stem cells (ISCs) to intestinal organoids, comprising all differentiated intestinal cell lineages. In addition, HGF and EGF were equally effective in promoting expansion of *Apc*-mutant mouse organoids, while *Met* deletion in ISCs in vivo attenuated stem-cell fitness and the formation of *Apc*-driven intestinal adenomas. These findings indicate that EGFR and MET signaling have overlapping and partially redundant functionality in the mouse intestinal mucosa. Recent results also indicate that HGF/MET signaling can fully overcome EGFR inhibition in human ISCs and CRC stem cells, allowing expansion of single normal ISCs, as well as *APC*-mutant cells into organoids, even in the presence of EGFR-inhibition (Joosten et al Gastroenterology 2019 Oct;157(4):1153-1155).

Another factor that is decisive for the clinical effectiveness of EGFR blockade is the mutational status of its downstream signaling components, specifically, gain-of-function mutations in RAS, RAF, MEK, or ERK, which can maintain cancer cell proliferation and survival upon EGFR inhibition. Activating mutations in KRAS, NRAS, or HRAS are collectively present in ~50% of cases of CRC; these mutations involve codons 12 or 13 and, less frequently, codons 61, 117, or 146. With respect to the RAS pathway in CRC, the RAS effector, BRAF, is mutated in 10-15% of early-stage CRC and around 5% in stage IV CRC, in the hotspot codon 600 (V600E). Clinically, the

inhibition of BRAF alone showed limited activity in metastatic BRAFV600E CRC owing to EGFR-mediated reactivation of MAPK signaling leading to the approval of combinations of EGFR inhibitors with BRAF and MEK inhibitors that have now become the standard of care in patients with metastatic BRAF mutant tumors.

Screening for CRC can be done via either direct visualization method such as colonoscopy, CT colonography, flexible sigmoidoscopy, flexible sigmoidoscopy with fecal immunochemical test (FIT) or via stool-based tests such as guaiac-based fecal occult blood test, FIT, or multi-targeted stool DNA test or via serology test such as SEPT9 DNA test.

Once the diagnosis of CRC is made, staging is an important factor as it determines treatment options for the patient. For tumor staging the Tumor, node, metastasis (TNM) system from the combined American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) is a commonly used staging system which defines the following cancer stages:

Standard conventional treatments for CRC are surgery, chemotherapy and radiotherapy. Depending on the localization and progression of the disease, these treatments can be used in combination. Treatment options include for example for stage I surgical resection alone. For stage III curative surgery is followed with adjuvant chemotherapy as standard of care. Total mesorectal excision (TME) through laparoscopic and transanal surgery approaches are often the options for localized cancer and whenever the tumor location is easy to access. However, complete removal of all cancer cells is often not possible. About 66% of stage II and 61% of stage III colon and rectal patients must undergo further treatments with adjuvant chemotherapy and/or radiotherapy. These treatments have many side effects due to their lack of specificity and cytotoxicity toward any cells that are growing and dividing. Despite recent improvements in the diagnostic and treatment options, 54% of patients relapse even after neoadjuvant treatment. Thus, it is crucial to have more alternative and effective treatments to treat CRC patients.

Current chemotherapy for CRC includes both single-agent therapy, which is mainly fluoropyrimidine (5-FU)-based, and multiple-agent regimens containing one or several drugs, including oxaliplatin (OX), irinotecan (IRI), and capecitabine (CAP or

XELODA or XEL), whereby the combined therapy regimens FOLFOX (5-FU+OX), FOXFIRI (5-FU+IRI), XELOX or CAPOX (CAP+OX), and CAPIRI (CAP+OX) represent the main approaches in first-line treatment. However, chemotherapy is associated with certain limitations, such as systemic toxicity, unsatisfying response rate, as well as unpredictable innate and acquired resistance, and low tumor-specific selectivity.

Currently, the main chemotherapy agents used for the treatment of the affected patients are fluoropyrimidine (intravenous or oral)-based as either single-agent treatments, such as intravenous 5-fluorouracil (5-FU) and oral capecitabine (CAP) or as multiple-agent regimens, including the combinations FOLFOX (5-FU and oxaliplatin), FOLFIRI (5-FU and irinotecan), XELOX/CAPOX (CAP and oxaliplatin), CAPIRI (CAP and irinotecan). Beyond traditional chemotherapy, the EGFR-targeted agents, cetuximab and panitumumab, are approved for first-line treatment of mCRC combinations of cetuximab with FOLFIRI or panitumumab with FOLFOX have significantly enhanced therapeutic efficacy. However, treatment decisions for early-stage mCRC do not consider *BRAF* or *KRAS* mutations, given the dramatically poor prognosis conferred by these mutations as seen in clinical trials. Although the survival rate of patients with mCRC has improved in recent years, the response and prognosis of patients with the aforementioned mutations are still poor.

The current lack of effective therapies and non-specific cytotoxic limitations have led investigators to transition towards the development of predictive, preventative, and personalized medicine strategies to improve the treatment of CRC and mCRC given the substantial unmet need for prospective therapies for patients with *BRAF*- or *KRAS*-mutant mCRC. Even today, patients with metastatic colorectal cancer (mCRC) have a 5-year overall survival rate of <10%.

Gastric cancer is the second leading cause of death from malignant disease worldwide, with especially high mortality rates in East, South, and Central Asia; Central and Eastern Europe; and South America. Treatment options for gastric cancer vary with cancer stages: Very early cancer can typically be treated by surgery. Potentially resectable cancer may typically be treated by surgery as first treatment, with either subtotal gastrectomy or total gastrectomy. Nearby lymph nodes and possibly parts of nearby organs may be removed as well. In addition, patients may

receive chemotherapy alone or chemo plus radiation therapy (chemoradiation), for example patients may receive 5-fluorouracil (5-FU) in combination with cisplatin (CDDP) (FP therapy). Other possible treatment regimens include epirubicin, cisplatin, and fluorouracil (ECF therapy), or regimens in which fluorouracil was replaced by capecitabine (ECX therapy), or ECF therapy in which cisplatin is replaced by oxaliplatin (EOF therapy) may be used. Alternatively, both cisplatin and fluorouracil can be replaced by oxaliplatin and capecitabine (EOX therapy).

In metastatic gastric cancers treatment is aimed at controlling the growth of the cancer and may include chemotherapy alone, chemotherapy plus immunotherapy, or chemotherapy along with radiation therapy. The combination of a platinum and fluoropyrimidine (5-FU) is the global standard first-line chemotherapy regimen within a non-curative setting. After platinum and 5-FU failure paclitaxel plus ramucirumab has been established as standard second-line therapy. However, treatment-related neuropathy, progression during or rapid recurrence following perioperative FLOT regimen (fluorouracil, oxaliplatin, docetaxel) have raised the demand for a taxane-free second-line therapy. Trifluridine/tipiracil has recently been approved for patients with metastatic gastric cancer. In addition to chemotherapy, patients may be treated with immune checkpoint inhibitors, such as ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), or atezolizumab (anti-PD-L1).

Diagnostically gastrointestinal endoscopy is indispensable for the diagnosis of gastric cancer, as well as staging laparoscopy (SL), which is a minimally invasive, brief procedure that only requires a small incision. The advantages of SL include providing an accurate diagnosis of peritoneal dissemination and extraserosal invasion, and the ability to perform peritoneal lavage for cytology. In patients with advanced gastric cancer for whom imaging does not yield a diagnosis, peritoneal lavage cytology obtained before treatment can be very important for treatment planning. Peritoneal lavage cytology obtained by SL for evaluation of peritoneal dissemination is thought to be useful for assessing the effects of neoadjuvant chemotherapy and/or immunotherapy.

The most frequent liver tumor is hepatocellular carcinoma (HCC) among all primary liver cancers, accounting for 75-85% of cases. HCC is usually diagnosed at an advanced stage, for which there remain limited effective treatment options. Until 2007,

there were no effective treatment options for patients diagnosed with advanced-stage disease or patients who transitioned into advanced-stage disease after other treatments failed. Sorafenib was the first systemic drug approved by the U.S. Food and Drug Administration (FDA) as standard treatment for advanced HCC between 2007 and 2016. In recent years, other small molecule drugs have been developed which include lenvatinib, regorafenib, or cabozantinib which have either been approved or are still undergoing clinical trials. These drugs are currently also tested in combination with immune checkpoint inhibitors, such as nivolumab, or pembrolizumab. In addition, combinations of immune checkpoint inhibitors and angiogenesis inhibitors, such as atezolizumab and bevacizumab are being tested.

Esophageal cancer as one form of GI cancer is being treated according to its stage, which may include endoscopic treatments, as well as chemotherapy and radiation therapy. Common drugs and drug combinations that are being used to treat esophageal cancer but are usually not given with radiation include ECF: epirubicin (Ellence), cisplatin, and 5-FU (especially for gastroesophageal junction tumors), DCF: docetaxel (Taxotere), cisplatin, and 5-FU, or trifluridine and tipiracil (Lonsurf), a combination drug in pill form.

For pancreatic cancer several chemotherapy regimens have been approved the most widely used and best-studied agent is Gemcitabine. Gemcitabine is often administered in combination with albumin-bound (Nab) Paclitaxel, which improved survival time compared to Gemcitabine monotherapy. Alternative treatment options include the multi-drug regimen FOLFIRINOX (5-Fluorouracil, Leucovorin, Irinotecan, and Oxaliplatin). Also, for pancreatic cancer, combination therapies using immune checkpoint inhibitors such as pembrolizumab, or nivolumab in combination with gemcitabine, nab-paclitaxel, or capecitabine are being clinically assessed.

Guanylyl Cyclase 2C (GUCY2C, EC: 4.6.1.2) is a member of a family of receptor-enzyme proteins synthesizing guanosine 3',5'-cyclic monophosphate (cyclic GMP; cGMP). GUCY2C is a transmembrane receptor for the endogenous hormonal ligands: guanylin and uroguanylin. Ligand binding to the extracellular receptor catalyzes the conversion of GTP into cyclic GMP (cGMP) and initiates downstream cGMP-related signaling pathways, which are implicated in the regulation of intestinal homeostatic

processes such as epithelial cell proliferation, differentiation, and apoptosis (Lisby et al. *Expert Rev Precis Med Drug Dev.* 2021; 6(2): 117–129.).

GUCY2C is specifically expressed by intestinal epithelial cells. GUCY2C regulates the dynamic progression of cells along the crypt–villus and crypt–surface axis, coordinating homeostatic processes including proliferation, DNA repair, metabolic programming, lineage-specific cell fate, and epithelial–mesenchymal interactions organizing that axis. Given the major role of GUCY2C in maintaining epithelial regeneration, dysregulation of the GUCY2C–cGMP axis promotes pathologies that include inflammatory bowel disease and bowel transit disorder, in addition to colorectal cancer. Importantly, silencing the GUCY2C signaling axis is associated with colorectal tumorigenesis through the loss of ligand binding.

GUCY2C protein can be detected near-universally (>95%) in all primary and metastatic human colorectal tumors regardless of anatomical location or grade, as well as a subset of gastroesophageal and pancreatic tumors, but not in tumors arising outside the GI tract. GUCY2C was also shown to be expressed in esophageal, gastric and pancreatic tumors (Danaee et al (2017) *PLoS One.* 2017; 12(12): e0189953).

Given its unique expression pattern in colorectal cancer (CRC) and metastatic colorectal cancer (mCRC) various approaches have been taken to utilize GUCY2C in the treatment of these diseases which have included cancer vaccines using GUCY2C, its use as a target for CAR-T cell therapy (Magee et al. *Cancer Immunol Res.* 6(5), 509–516 (2018)), or the use of therapeutic antibodies and antibody–drug conjugates targeting GUCY2C. WO2013/163633 A1 discloses anti-GUCY2C antibodies and anti-GUCY2C ADCs comprising auristatin E (MMAE) and auristatin F MMAE (MMAF) which inhibit mitosis by inhibiting tubulin polymerization. Clinical trials of the anti-GUCY2C ADC TAK264 (MLN0264, 5F9vcMMAE) which employed MMAE as payload have been terminated. WO2021/205325 A1 discloses anti-CD3-GUCY2C bispecific antibodies and respective use in cancer therapy to induce a cytolytic T cell response in GUCY2C positive target cells.

While these efforts indicate that GUCY2C-directed therapies may be effective, a lack of approved GUCY2C-directed therapies, and in particular of approved GUCY2C-directed ADCs still exists.

Accordingly, there is a need in the art for improved antibody-drug conjugates (ADCs), which mediate stable conjugation of a cytotoxic drug substance to the antibody, while allowing for easy adaptation of the ADC's Drug-Antibody-Ratio (DAR) and/or aqueous solubility depending on the cytotoxicity and hydrophobicity of the drug substance to be conjugated, respectively. Even more so, there is a need for such improved ADC's having high potency and a good efficacy profile in the context of treating gastrointestinal cancer.

It is an object of the present disclosure to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative. In particular, it is an object of the present disclosure to provide such improved ADCs having high potency and a good efficacy profile in the context of treating gastrointestinal cancer.

SUMMARY

As indicated above, the present disclosure aims to provide improved ADCs having a linker structure that is particularly suitable to provide ADCs with high potency and a good efficacy profile in the context of treating gastrointestinal cancer.

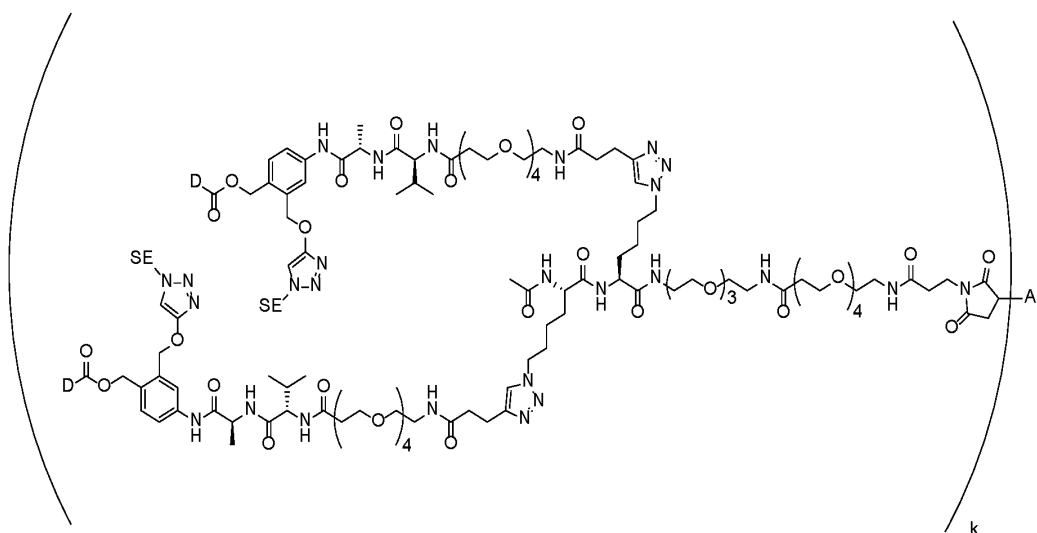
The here-described ADCs have a linker with an overall modular architecture providing a selection of beneficial linker functionalities for a high potency ADC. Specifically, the modular linker used in the ADC of the present disclosure comprises two functional units of a cleavable peptide sequence and a self-immolative spacer, wherein a solubility enhancing group and the cytotoxic drug substance are bound to each one of the self-immolative spacers in physical proximity. Further, each functional unit is connected to a corresponding branching unit in the multimeric core of the linker via a defined chemical spacer. Given that the multimeric core of the ADC of the present disclosure comprises two branching units each, which are connected to one cytotoxic drug substance, respectively, the linker has a defined Degree-of-Labeling (DOL) of 2.

Finally, the linker is conjugated to the antibody via a further chemical spacer extending from the multimeric core. Conjugation of the drug substance-carrying linker to the antibody occurs via the terminus of the spacer extending from the multimeric core by

way of a reaction of a terminal maleimide group of the spacer with a sulfur atom of a cysteine residue in the constant heavy chain of the antibody.

Beneficially, thiol reactions are highly efficient and typically proceed rapidly under mild conditions. This means that thiol-reactive groups can form conjugates quickly and with a high degree of completeness. Further, the thioether bonds formed between thiol groups and thiol-reactive groups are stable under physiological conditions. While they are less prone to hydrolysis or degradation compared to some other types of chemical bonds, further stability of the conjugate can, e.g., be achieved through incorporation of a basic amino group adjacent to the maleimide as described in Lyon *et al.* Nat. Biotechnol. 2014, 32, 1059–1062. Accordingly, in the ADCs of the invention, the reaction product is typically a succinimidyl thioether or a derivative thereof and stably links the antibody with the drug substance-carrying linker.

Accordingly, in a first aspect the present disclosure relates to an antibody-drug conjugate (ADC) of Formula (I)



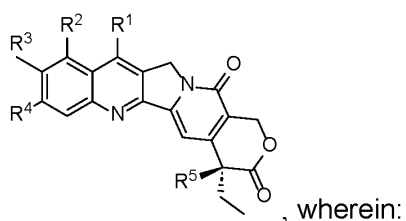
Formula (I)

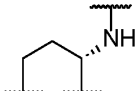
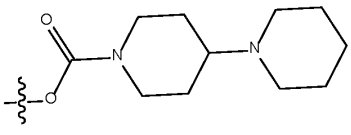
or a pharmaceutically acceptable salt or solvate thereof; wherein:

k is an integer from 2 to 4;

Ab is an anti-GUCY2C antibody;

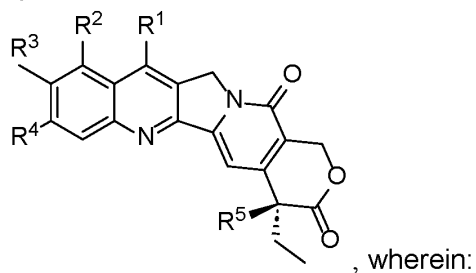
D is a pharmaceutically active substance selected from



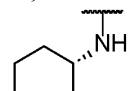
- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH;
or
- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H
and R⁵ is –O–; or
- R¹ is –H, R² is –NO₂, R³ –H, and R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₂NHCH(CH₃)₂, R² is –H, R³ –H, and R⁴ is –H and R⁵ is –O–;
or
- R¹ is –CH₂CH₃, R² is –H, R³ –OH, and R⁴ is –H and R⁵ is –O–;

SE is a solubility enhancing group and comprises an alpha cyclodextrin, a beta-cyclodextrin, a gamma-cyclodextrin, a polysarcosine, a PEG, or a polyalcohol made from carbohydrates.

Preferably, D is

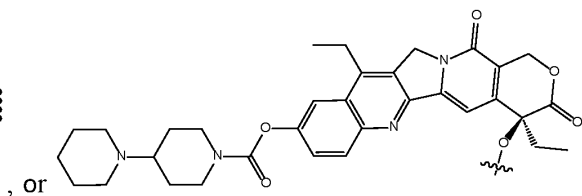
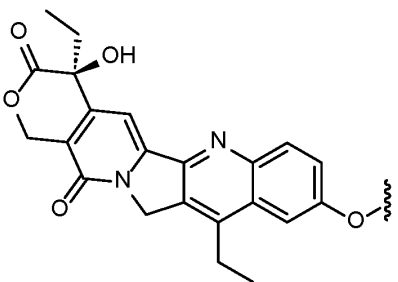
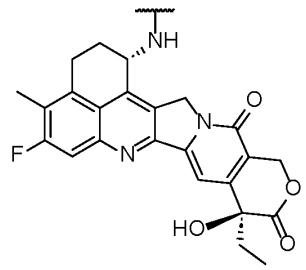
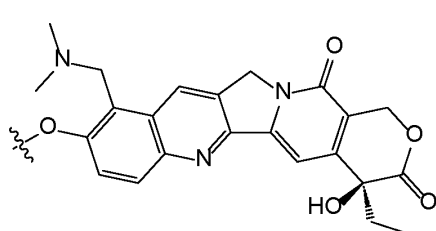


, wherein:

- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH; or
- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or

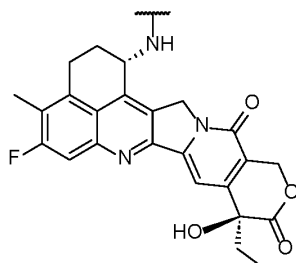
- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H and R⁵ is –O–.

In preferred embodiments D is



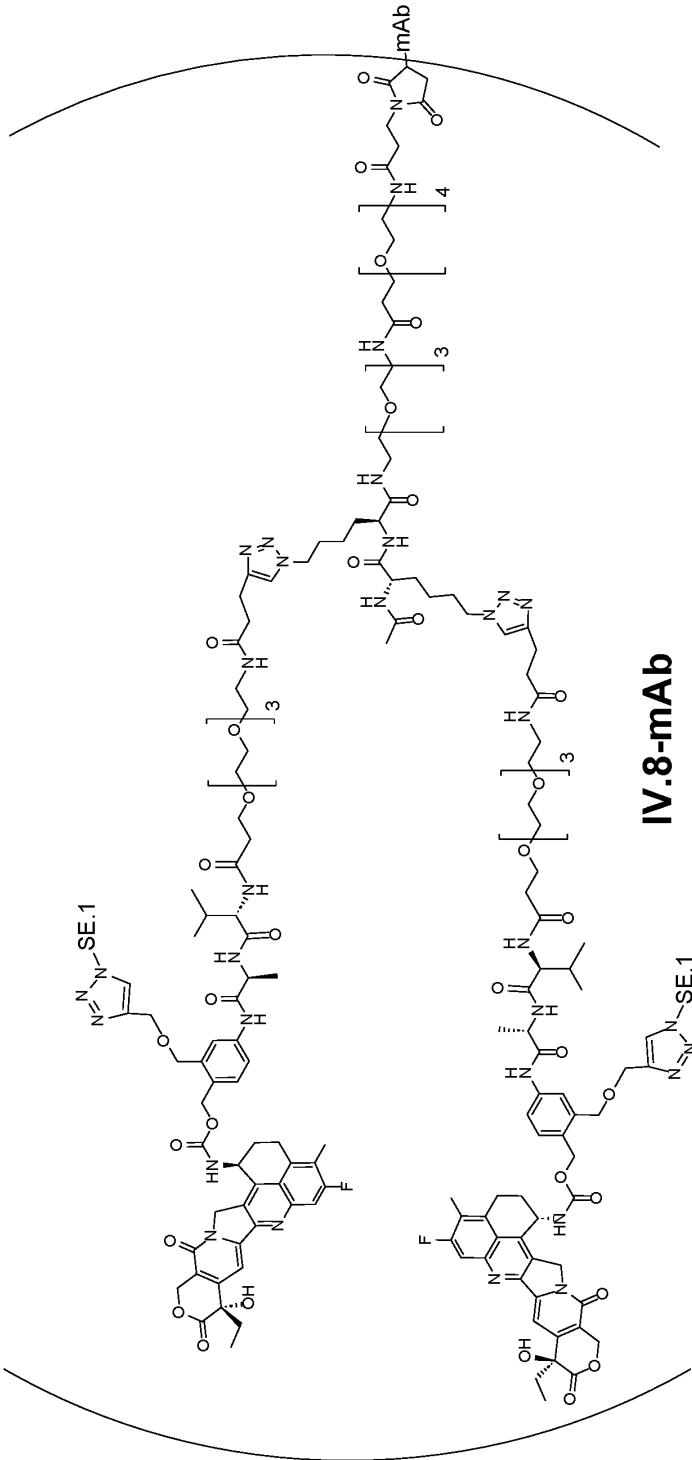
wherein the wavy line indicates the attachment site of D to the linker as disclosed herein.

Most preferably, D is



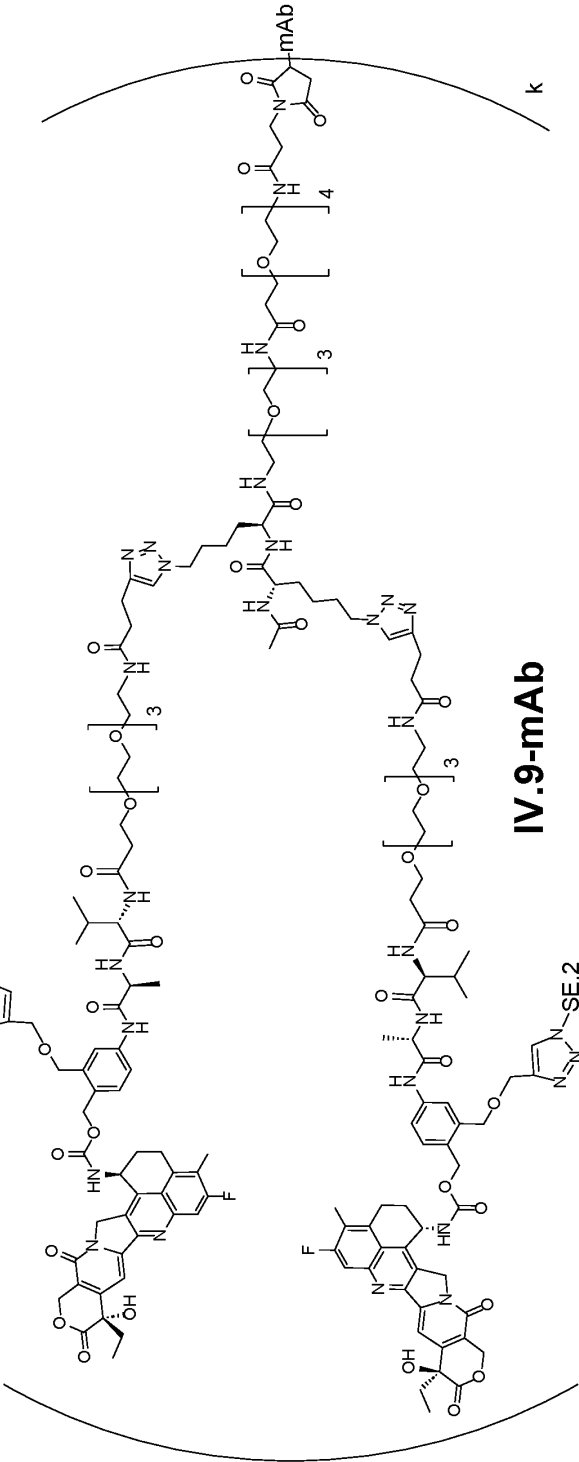
In some embodiments, the antibody is a monoclonal antibody that recognizes and binds a target sequence or epitope of Guanylate Cyclase 2C (GUCY2C).

One specifically preferred embodiment of the antibody-drug conjugate of Formula (I) of the first aspect is:



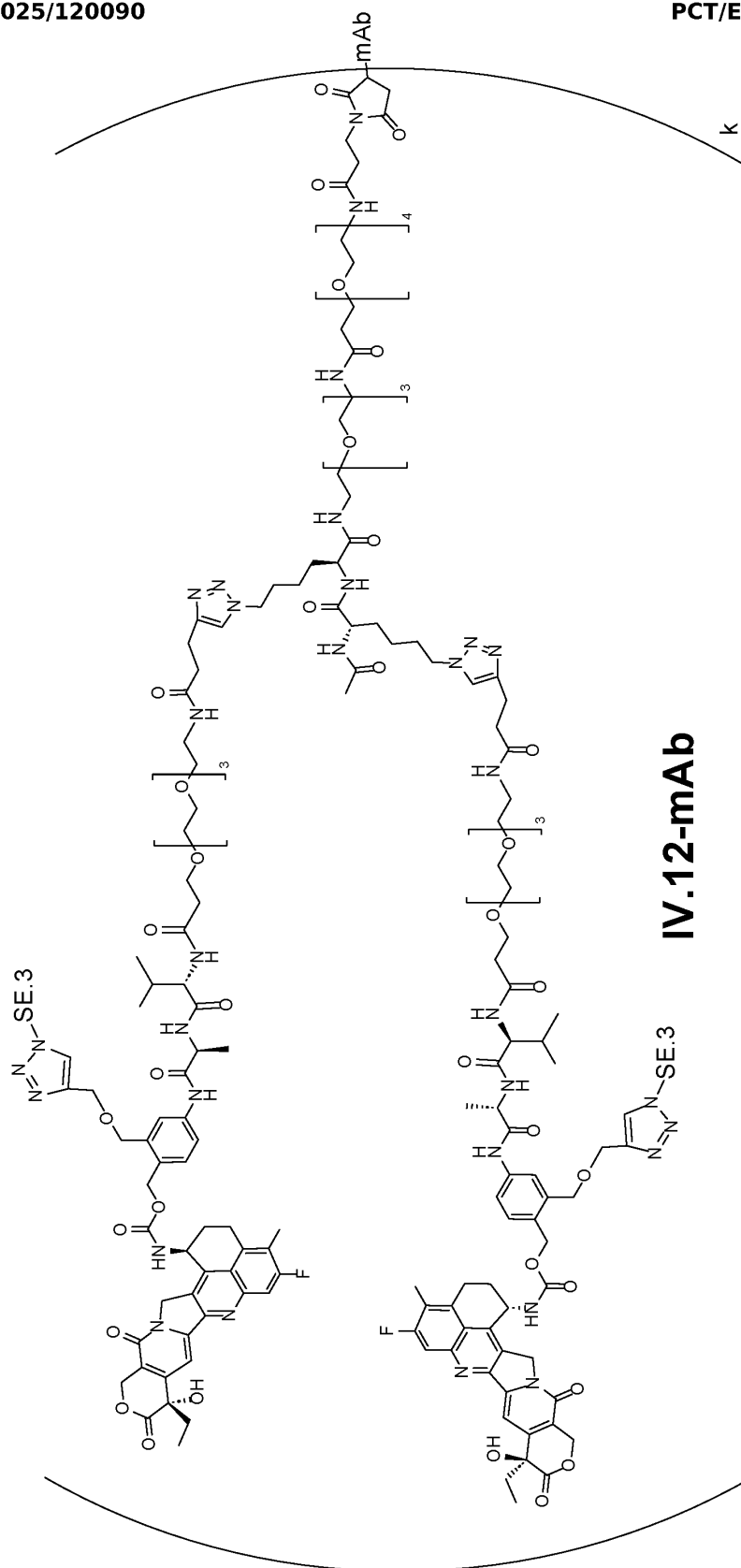
wherein k is 2, and mAb is an anti-GUCY2C antibody selected from the group of mAb1, mAb8 and mAb41, all as disclosed herein, preferably, mAb is mAb8

Another specifically preferred embodiment of the antibody-drug conjugate of Formula (I) of the first aspect is:



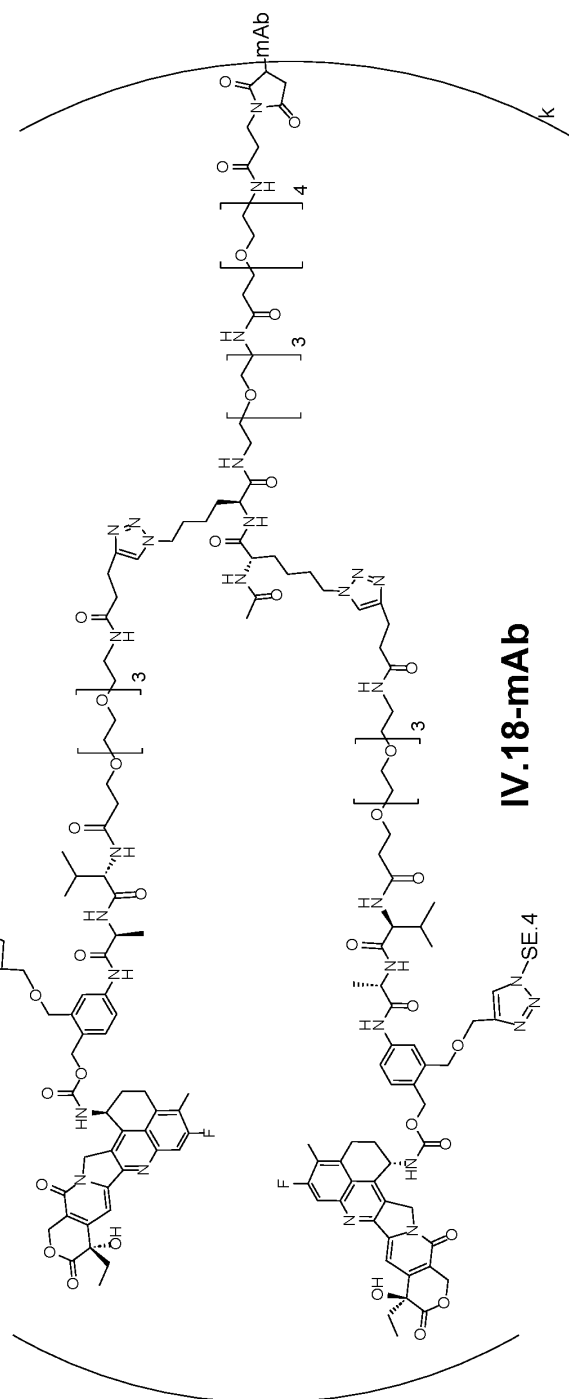
wherein k is 2, and mAb is an anti-GUCY2C antibody selected from the group of mAb1, mAb8 and mAb41, all as disclosed herein, preferably, mAb is mAb8.

Another specifically preferred embodiment of the antibody-drug conjugate of Formula (I) of the first aspect is:



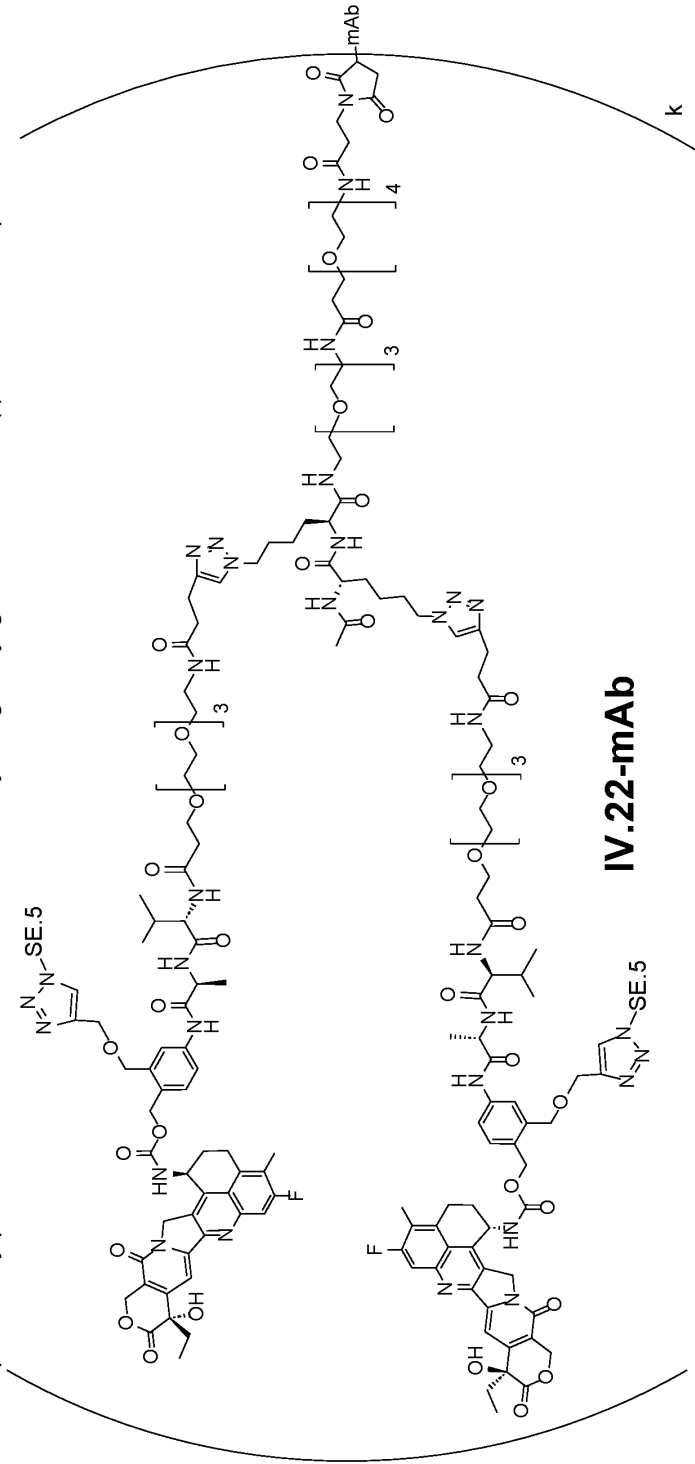
wherein k is 2 and mAb is an anti-GUCY2C antibody selected from the group of mAb1, mAb8 and mAb41, all as disclosed herein, preferably, mAb is mAb8.

Another specifically preferred embodiment of the antibody-drug conjugate of Formula (I) of the first aspect is:



wherein k is 2 and mAb is an anti-GUCY2C antibody selected from the group of mAb1, mAb8 and mAb41, all as disclosed herein, preferably, mAb is mAb8.

Another specifically preferred embodiment of the antibody-drug conjugate of Formula (I) of the first aspect is:



wherein k is 2 and mAb is an anti-GUCY2C antibody selected from the group of mAb1, mAb8 and mAb41, all as disclosed herein, preferably, mAb is mAb8.

In a second aspect, the present disclosure relates to the antibody-drug conjugate of the first aspect for use as a medicament, preferably for use in the treatment of cancer, more preferably for the treatment of gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer.

In a third aspect, the present disclosure relates to a pharmaceutical composition comprising the antibody-drug conjugate of the first aspect.

Accordingly, the second and third aspects of the present disclosure also encompass methods of treating cancer, preferably of treating gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer, wherein the method comprises administering a therapeutically effective amount of the antibody-drug conjugate of the first aspect or of the pharmaceutical composition of the third aspect to a patient in need thereof. Similarly, the second aspect of the present disclosure also encompasses use of the antibody-drug conjugate of the first aspect in the manufacture of a medicament for the treatment of cancer, preferably for the treatment of gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer.

In such embodiments, the gastrointestinal cancer is typically characterized by the expression of a target sequence or epitope of a Guanylate Cyclase 2C (GUCY2C) antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present disclosure will now be described, by way of example only, with reference to the accompanying drawings in which:

Fig. 1 In vitro cytotoxicity of an anti-GCC ADC of the present disclosure comprising the linker-payload (IV.8), in comparison to anti-GCC ADCs of the present disclosure comprising the linker-payloads (IV.17) and (IV.19) on **(A)** HEK cells overexpressing human GUCY2C (HEK293-GUCY2C-HDP-2B3 cells, or **(B)** HEK293 wildtype cells. SEC indicates purification of ADC by size-exclusion chromatography. "N/A" indicates that no EC50 calculation was done. The ADCs tested were all characterized by a DAR=4.

- Fig. 2** In vitro cytotoxicity of anti-GCC ADCs of the present disclosure comprising the linker-payloads (IV.9), (IV.12) and (IV.18) on (A) HEK cells overexpressing human GUCY2C (HEK293-GUCY2C-HDP-2B3 cells, or (B) HEK293 wildtype cells. Anti-GCC ADCs tested are DAR=4 conjugates.
- Fig. 3** shows mean tumor volume [mm³] depicted from day 0 to day 67 post group allocation in mice treated either with vehicle control or with the Exatecan-TBDCs of the present disclosure IV.12 and IV.11, as a single dose of 15 mg/kg on day 1 (n = 10 animals/group, mean depicted $\pm$ SD).
- Fig. 4** shows mean tumor volume [mm³] depicted from day 0 to day 66 post group allocation in mice treated either with vehicle control or with the Exatecan-TBDC of the present disclosure IV.9 either as a single dose of 15 mg/kg on day 1 or 1x/week for 3 weeks with 10 or 15 mg/kg (n = 10 animals/group, mean depicted $\pm$ SD).
- Fig. 5** shows mean tumor volume [mm³] depicted from day 0 to day 66 post group allocation in mice treated either with vehicle control or with the mAb8-based Exatecan-TBDC of the present disclosure conjugated to linker-payload IV.8 as a single dose of 15 mg/kg on day 1 (n = 10 animals/group, mean depicted $\pm$ SD).
- Fig. 6** shows mean tumor volume [mm³] depicted from day 0 to day 66 post group allocation in mice treated either with vehicle control or with the Exatecan-TBDC of the present disclosure IV.18 as a single dose of 15 mg/kg on day 1 (n = 10 animals/group, mean depicted $\pm$ SD).
- Fig. 7** shows mean tumor volume [mm³] depicted from day 0 to day 22 post group allocation in mice treated either with vehicle control or with the mAb8-based Exatecan-TBDCs of the present disclosure conjugated to linker-payloads IV.9, IV.8, IV.12 or IV.18 or IV.20 (all DAR=4) 1x/week for 3 weeks with 5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD). "hD" denotes high DAR (DAR10).

Fig. 8 shows mean tumor volume [mm³] depicted from day 0 to day 22 post group allocation in mice treated either with vehicle control or with mAb8-based Exatecan-TBDCs of the present disclosure conjugated to linker-payloads IV.9, IV.8, IV.12 or IV.18 or IV.20 (all DAR=4) 1x/week for 3 weeks with 2.5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD).

Fig. 9 shows an *in vitro* bystander assay. Target-negative HEKwt cells, stained with CellTracer FarRed, and GUCY2C-expressing HEK-GUCY2C cells, stained with CFDA, were co-cultured and treated with anti-GCC ADCs. After 72h, bystander killing of compounds was assessed by analyzing apoptotic (7AAD+) target-negative HEKwt cells. All three anti-GCC ADCs resulted in around 22.5% to 32.8 % apoptotic HEKwt cells if these cells were cultured with GUCY2C-expressing HEK-GUCY2C cells, while no effect was observed on HEKwt cells without target-expressing HEK-GUCY2C cells.

Fig. 10 shows mean tumor volume [mm³] depicted from day 0 to day 100 post group allocation, measured by caliper. Mice were treated with vehicle control, or with mAb8-based anti-GUCY2C ADCs conjugated to linker-payloads IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) 1x/week for 3 weeks with 5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD).

Fig. 11 shows mean tumor volume [mm³] depicted from day 0 to day 100 post group allocation, measured by caliper. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs conjugated to linker-payloads IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) 1x/week for 3 weeks with 2.5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD).

Fig. 12 shows overall survival depicted from day 0 to day 100 post group allocation. Survival was monitored daily. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs conjugated to linker-payload conjugates IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) 1x/week for 3 weeks with 5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD).

Fig. 13 shows overall survival depicted from day 0 to day 100 post group allocation.

Survival was monitored daily. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs comprising linker-payload conjugates IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) 1x/week for 3 weeks with 2.5 mg/kg each (n = 10 animals, mean depicted $\pm$ SD).

Fig. 14 shows mean tumor volume [mm³] depicted from day 0 to day 87 post group allocation, measured by caliper. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs comprising linker-payload conjugates IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR4 conjugates) with a single intravenous dose of 10 mg/kg (n = 10 animals, mean depicted $\pm$ SD).

Fig. 15 shows mean tumor volume [mm³] depicted from day 0 to day 87 post group allocation, measured by caliper. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs conjugated to linker-payloads IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) with a single intravenous dose of 5 mg/kg (n = 10 animals, mean depicted $\pm$ SD).

Fig. 16 shows overall survival depicted from day 0 to day 87 post group allocation. Survival was monitored daily. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs conjugated to linker-payloads IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) with a single intravenous dose of 10 mg/kg (n = 10 animals, mean depicted $\pm$ SD).

Fig. 17 shows overall survival depicted from day 0 to day 87 post group allocation. Survival was monitored daily. Mice were treated with vehicle control, mAb8-based anti-GUCY2C ADCs conjugated to linker-payloads IV.9, IV.8, IV.12, IV.18 or IV.22 (all DAR=4 conjugates) with a single intravenous dose of 5 mg/kg (n = 10 animals, mean depicted $\pm$ SD).

DETAILED DESCRIPTION

Before the present disclosure is described in detail below, it is to be understood that this disclosure is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present disclosure which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kolbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland). The definitions of the chemical groups as used herein unless specified otherwise shall have the meaning and be defined as provided in "Compendium of Chemical Terminology" ("Gold Book") published by the International Union of Pure and Applied Chemistry (IUPAC) version 2.3.3, goldbook.iupac.org, ISBN: 0-9678550-9-8), the content of which is hereby incorporated by reference.

Notwithstanding, to provide a clear and consistent understanding of the specification and claims, as well as of the scope to be given to certain terms, the following definitions are provided.

Definitions

The term "**modular linker**" in the context of the present disclosure refers to a linker that comprises at least one molecule of a cytotoxic drug substance (also referred to simply as drug or payload), several further structural as well as functional modules (also referred to as spacers or functional units or groups), including at least one solubility enhancing group and a branching unit, as well as a reactive group suitable to stably connect the modular linker to a target-binding moiety such as an antibody.

The term "**solubility enhancing group**" in the context of the present disclosure refers to a module within the modular linker, which at least counters – at best negates – hydrophobic/lipophilic properties of a cytotoxic drug substance such as to provide

the modular linker comprising a specific payload with a high degree of aqueous solubility. A variety of solubility enhancing groups suitable for the incorporation into the modular linker of the present disclosure are well-known to the skilled person and include, without limitation: (a) polar functional groups, such as hydroxyl (-OH), amine (-NH₂), and carboxyl (-COOH) groups; polar sulfonate (-SO₃H) groups; polymeric chains, such as hydrophilic polyethylene glycol (PEG) chains; surfactant groups, such as alkyl chains; aromatic ring structures; halogen substituents such as the addition of fluorine or chlorine; ester (-COO-) or ether (-O-) groups; and cyclodextrins/cyclic oligosaccharides.

In the context of the present disclosure, the term **"self-immolative spacer"** refers to a spacer that is stably connected to the cytotoxic drug substance but undergoes triggered self-immolation, i.e. decomposition, such that its connection to the cytotoxic drug substance is abolished and the drug substance is released without any adducts or traces of the self-immolative spacer itself. In some embodiments a change in pH can trigger self-immolation of the spacer and release of the payload. In other embodiments, the self-immolative spacer is also connected to an enzymatically cleavable peptide sequence and enzymatic cleavage of the peptide triggers self-immolation of the spacer and release of the payload. An illustrative example of an enzymatically triggered self-immolative spacer is a p-aminobenzyl (PAB) spacer connected to an enzymatically cleavable Val-Ala dipeptide.

In the context of the present disclosure, the term **"cleavable linker"** refers to a linker that is cleavable (i) by an enzyme, or (ii) in a reducing environment. Further, the terms "enzymatically cleavable" or "cleavable by an enzyme" or the likes convey that the peptide, structure, sequence or spacer in question can be cleaved by an enzyme, particularly by a lysosomal protease, such as Cathepsin B, resulting in the release of the pharmaceutically active drug substance/payload.

In the context of the present disclosure, the term **"branching unit"** refers to a structure within a modular linker that allows for a furcation of the compound into a branched structure, wherein each branch is typically connected to at least one molecule of the cytotoxic drug substance via a self-immolative spacer. Therefore, a modular linker typically comprises as many drug molecules as branches. As such, a modular linker comprising between 1 and 10, preferably between 2 and 8, 2 and 6 or

2 and 4 branches, typically comprises between 1 and 10, preferably between 2 and 8, 2 and 6 or 2 and 4 cytotoxic drug substance molecules, respectively.

As used herein, the term “**antibody**” refers to a protein consisting of one or more polypeptide chains encoded by immunoglobulin genes or fragments of immunoglobulin genes or cDNAs derived from the same. Said immunoglobulin genes include the light chain kappa, lambda and heavy chain alpha, delta, epsilon, gamma and mu constant region genes as well as any of the many different variable region genes.

The basic immunoglobulin (antibody) structural unit is usually a tetramer composed of two identical pairs of polypeptide chains, the light chains (L, having a molecular weight of about 25 kDa) and the heavy chains (H, having a molecular weight of about 50-70 kDa). Each heavy chain is comprised of a heavy chain variable region (abbreviated as V_H or V_H) and a heavy chain constant region (abbreviated as C_H or C_H). The heavy chain constant region is comprised of three domains, namely CH1, CH2 and CH3. Each light chain contains a light chain variable region (abbreviated as V_L or V_L) and a light chain constant region (abbreviated as C_L or C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, which are also called complementarity determining regions (CDR) interspersed with regions that are more conserved called framework regions (FR). Each V_H and V_L region is composed of three CDRs and four FRs arranged from the amino terminus to the carboxy terminus in the order of FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains form a binding domain that interacts with an antigen. The constant regions (Fc regions) are not directly involved in the binding of the antibody to the antigen but exhibit various effector functions such as participation in antibody dependent cell- mediated cytotoxicity (ADCC), phagocytosis via binding to Fc γ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn) and complement activation via the C1q component, leading to the chemotactic, opsonic and, potentially in the case of a viable cellular antigen target, cytolytic actions of complement. Human antibodies of the IgG1 class are the most potent in activating the complement system and are therefore the desirable isotype for the therapeutic antibodies of the ADCs of the present invention.

Human Fc γ receptors include Fc γ R (I), Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa and neonatal FcRn for which it was demonstrated that a common set of IgG1 residues is involved in binding all Fc γ Rs, while Fc γ RII and Fc γ RIII utilize distinct sites outside of this common set (Shields et al. (2001) J. Biol. Chem 276: 6591-6604). One group of IgG1 residues reduced binding to all Fc γ Rs when altered to alanine: Pro-238, Asp-265, Asp-270, Asn-297 and Pro-239 (numbering according to EU numbering system, Edelman et al., Proc. Natl. Acad. Sci. USA; 63 (1969) 78-85.). All are in the IgG CH2 domain and clustered near the hinge joining CH1 and CH2. While Fc γ R1 utilizes only the common set of IgG1 residues for binding, Fc γ RII and Fc γ RIII interact with distinct residues in addition to the common set. Alteration of some residues reduced binding only to Fc γ RII (e.g. Arg-292) or Fc γ RIII (e.g. Glu- 293). Some variants showed improved binding to Fc γ RII or Fc γ RIII but did not affect binding to the other receptor. The neonatal FcRn receptor is believed to be involved in both antibody clearance and the transcytosis across tissues (see: Junghans (1997) Immunol. Res 16: 29-57; and Ghetie et al. (2000) Annu. Rev. Immunol. 18: 739-766). Human IgG1 residues determined to interact directly with human FcRn includes Ile253, Ser254, Lys288, Thr307, Gln311, Asn434 and His435.

The terms "**CDR**", "**CDRL1**", "**CDRL2**", "**CDRL3**", "**CDRH1**", "**CDRH2**", "**CDRH3**" as used herein follow the Kabat numbering convention (Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)). However, although the Kabat numbering convention for amino acid residues in variable domain sequences and full length antibody sequences is used throughout this specification, it will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full length antibody sequences. There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia et al. (1989) Nature 342: 877-883. The structure and protein folding of the antibody may mean that other residues based on the numbering system used are considered part of the CDR sequence and would be understood to be so by a skilled person, however, these differences functionally do not imply altered or different antigen-binding of the respective antibody. Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods.

The CDRs are most important for binding of the antibody or the antigen binding portion thereof. The FRs can be replaced by other sequences, provided the three-dimensional structure which is required for binding of the antigen is retained. Structural changes of the construct most often lead to a loss of sufficient binding to the antigen.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} M to 10^{-11} M or less. Any K_D greater than about 10^{-4} M is generally considered to indicate nonspecific binding.

As used herein, an antibody that “**specifically recognizes and binds**” to an antigen refers to an antibody that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of 10^{-7} M or less, preferably 10^{-8} M or less, even more preferably 5×10^{-9} M or less, and most preferably from about 10^{-8} , 10^{-9} M to about 10^{-10} M, 10^{-11} M or less, or e.g. from about 10^{-10} M to about 10^{-11} M or less, but does not bind to unrelated (e.g. structurally or sequence unrelated) antigens with an affinity equal to the affinity for the specific target.

The antibody of the ADC of the present disclosure which may e.g. also be referred to as immunoglobulin may be from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. The IgG isotype is divided in subclasses in certain species: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice.

The term “**antibody fragment**” or “**antigen-binding fragment**” as used herein refers to an antibody fragment or analog of an antibody which retains the binding specificity of the parent anti-GUCY2C antibody as disclosed herein and comprises a portion (for example, one or more CDRs) or variable region of the antigen binding region of the parent antibody. The antibody fragment is, for example, Fab, Fab', F(ab')₂, Fv fragment, sc-Fv, unibody, diabody, linear antibody, nanobody, domain antibody, or multispecific antibody fragment formed from the antibody fragment. A Fab fragment consists of the CH1 and variable regions of one light chain and one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A Fab' fragment as contains a light chain and a portion of a heavy chain that contains the VH domain, the CH1 domain, and the region

between the CH1 and CH2 domains. A $F(ab')_2$ fragment contains two light chains and two heavy chains containing a portion of the constant region between the CH1 and CH2 domains, thereby forming an interchain disulfide bond between the two heavy chains. A $F(ab')_2$ fragment is composed of two Fab' fragments held together by the disulfide bond between the two heavy chains. A **"Fv fragment"** contains variable regions from both the heavy and light chains but lacks the constant region. The term **"single-chain antibody"** is a single-chain recombinant protein formed by connecting the heavy chain variable region VH and the light chain variable region VL of an antibody through a connecting peptide. It is the smallest antibody fragment with a complete antigen-binding site. The term **"domain antibody fragment"** is an immunoglobulin fragment with immunological functions that only contains a heavy chain variable region or a light chain variable region chain. In some cases, two or more VH regions are covalently linked to a peptide linker to form a bivalent domain antibody fragment. The two VH regions of the bivalent domain antibody fragment can target the same or different antigens.

In the context of the present disclosure, the term **"reactive moiety for conjugation to an antibody"** refers to a chemically reactive group within the linker that can react with a correspondingly reactive group of the antibody under suitable conditions such that the resulting reaction product covalently conjugates the remaining linker compound to the antibody.

In the context of the present disclosure, the term **"reaction product of the modular linker and a correspondingly reactive group of an antibody"** refers to a moiety formed as the result of a reaction between the reactive moiety of the modular linker of the present disclosure and a correspondingly reactive group of an antibody. For example, if the reactive moiety of the modular linker of the present disclosure is a maleimide, the reaction product resulting from the reaction of the maleimide with the sulphur atom of the cysteine residue of the target-binding moiety is a succinimidyl thioether or a derivative thereof. Further, if the reactive moiety of the modular linker of the present disclosure is a group that selectively reacts with the thiol group of a free cysteine of the target-binding moiety, i.e. if the reactive moiety of the modular linker is a "thiol-reactive moiety", the reaction product is typically selected from: thiol-substituted acetamide; thiol-substituted succinimide; thiol-substituted succinamic acid; thiol-substituted heteroaryl, particularly thiol-substituted benzothiazole, thiol-substituted phenyltetrazole and thiol-substituted phenyloxadiazole; and a disulfide,

wherein one sulphur atom is derived from a cysteine residue of the target-binding moiety.

As used herein, a **"derivative"** of a compound refers to a species having a chemical structure that is similar to the compound, yet containing at least one chemical group not present in the compound and/or deficient of at least one chemical group that is present in the compound. The compound to which the derivative is compared is known as the "parent" compound. Typically, a "derivative" may be produced from the parent compound in one or more chemical reaction steps.

In the context of the present disclosure, the term a **"pharmaceutically acceptable salt or solvates"** includes acid addition salts, formed with inorganic acids such as hydrochloric acid, hydro bromic acid, sulphuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as glycolic acid, pyruvic acid, lactic acid, malonic acid, malic acid, inaleic acid, fumaric acid, tartaric acid, citric acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methane sulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, lauryl sulphuric acid, gluconic acid, glutamic acid, salicylic acid, muconic acid, and the like. The term further includes basic addition salts formed with the conjugate bases of any one of the above-listed inorganic acids, wherein the conjugate bases comprise a cationic component selected from Na^+ , K^+ , Mg^{2+} , Ca^{2+} , and $\text{NH}_g\text{R}'_{4-g}^+$; in which R' is a C_{1-3} alkyl and g is a number selected from among 0, 1, 2, 3, or 4 as well as solvent addition forms (i.e. solvates) of the respective acid addition salt.

As used herein, **"treat"**, **"treating"** or **"treatment"** of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

As used herein, a **"patient"** means any mammal who may benefit from a treatment with the target-binding drug conjugates described herein. Preferably, a "patient" is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, chicken, pig, sheep, goat, camel, cow, horse, donkey, cat, or dog), or primates including human beings. It is particularly preferred that the "patient" is a human being.

A **"therapeutically effective amount"** is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

In addition to the above definitions, and unless the context clearly requires otherwise, throughout the description and the claims, the words **"comprise"**, **"comprising"**, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

Further, reference throughout this specification to **"one embodiment"**, **"some embodiments"** or **"an embodiment"** means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment", "in some embodiments" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to one of ordinary skill in the art from this disclosure, in one or more embodiments.

As used herein, unless otherwise specified the use of the ordinal adjectives **"first"**, **"second"**, **"third"**, etc., to describe a common object, merely indicate that different instances of like objects are being referred to and are not intended to imply that the objects so described must be in a given sequence, either temporally, spatially, in ranking, or in any other manner.

As used herein, the term “**exemplary**” is used in the sense of providing examples, as opposed to indicating quality. That is, an “**exemplary embodiment**” is an embodiment provided as an example, as opposed to necessarily being an embodiment of exemplary quality.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra - or infra, is hereby incorporated by reference in its entirety to the extent possible under the respective patent law.

Antibody-drug conjugates (ADC)

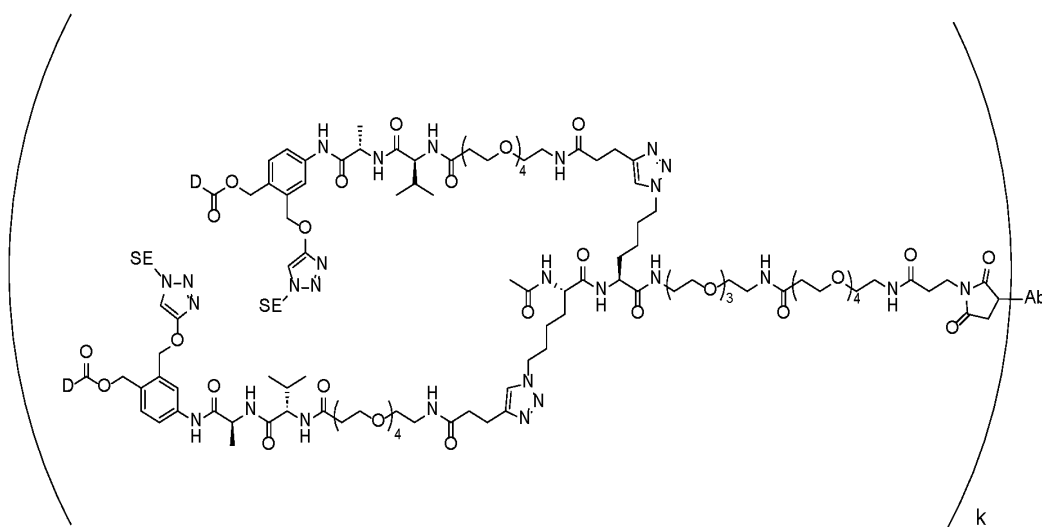
The inventors of the present disclosure have developed new antibody-drug conjugate (ADC) comprising a modular linker that comprises various modules (i.e. also referred to as groups, units, elements, etc.), and which have been implicated in ADC function and efficacy.

The modular architecture of the linker can accommodate pharmaceutically active drug substances having various physico-chemical properties as well as stably link the drug substance carrying linker to an antibody such that a robust and reliable ADC can be provided.

In addition, and as will become apparent from the further description below, through the conjugation chemistries employed in the ADC, both a homogeneous Degree-of-Labeling (DOL) of 2 per linker molecule and a Drug-Antibody-Ratio (DAR) of 4 to 8 is readily achievable.

Therefore, it was found that, in a relatively simple fashion, the ADC of the present disclosure provides for efficient targeting of selected, GUCY2C-positive cancer cells with the pharmaceutically active drug substance of the conjugate given the specificity of the antibody for a specific cancer-associated or cancer-specific antigen.

Accordingly, in a first aspect, the present disclosure relates to an antibody-drug conjugate (ADC) of Formula (I)



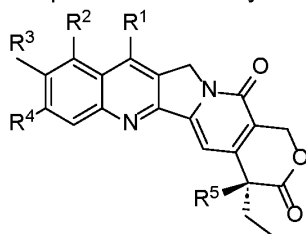
Formula (I)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

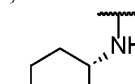
k is an integer from 2 to 4;

Ab is an anti-GUCY2C antibody;

D is a pharmaceutically active substance selected from

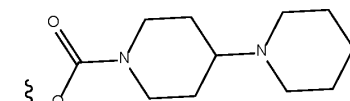


, wherein:

- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH;

or

- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or

- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H and R⁵ is –O–; or

- R¹ is –H, R² is –NO₂, R³ –H, and R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₂NHCH(CH₃)₂, R² is –H, R³ –H, and R⁴ is –H and R⁵ is –O–;
or
- R¹ is –CH₂CH₃, R² is –H, R³ –OH, and R⁴ is –H and R⁵ is –O–;

SE is a solubility enhancing group and comprises an alpha cyclodextrin, a beta-cyclodextrin, a gamma-cyclodextrin, a polysarcosine, a PEG, or a polyalcohol made from carbohydrates.

Particularly suitable pharmaceutically active drug substances D in the ADC of the present disclosure are camptothecins, a class of compounds that comprises both naturally-occurring as well as synthetic members, which have shown anti-cancer through inhibition of topoisomerase I thereby interfering with DNA replication and repair processes, leading to DNA damage and ultimately cell death in cancer cells.

In the ADC of the present invention, the pharmaceutically active drug substance D is a camptothecin compound selected from SN-38, topotecan, irinotecan, rubitecan, belotecan, SN38 and exatecan.

A notable member of this compound class is SN-38 (a derivative of camptothecin, generated as an active metabolite when the chemotherapeutic drug irinotecan / CPT-11 is metabolized in the body of the patient.

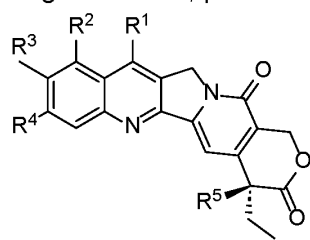
Topotecan and irinotecan are camptothecins also used as chemotherapeutic agents in the treatment of various cancers, including ovarian cancer, small cell lung cancer, and cervical cancer. As already indicated above, Irinotecan is a prodrug, which is administered in an inactive form and is converted into its active form (SN-38) within the body. Both Topotecan and irinotecan are typically administered intravenously.

Rubitecan remains an investigational chemotherapeutic agent of the camptothecin class, similar to irinotecan and topotecan. Rubitecan has been studied for its potential use in the treatment of various cancers, including colorectal cancer, ovarian cancer, and pancreatic cancer and has been extensively studied in cancer research.

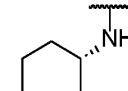
Belotecan is a further camptothecin compound shown to inhibit topoisomerase 1 thereby disrupting essential DNA replication and repair processes leading to cancer cell death.

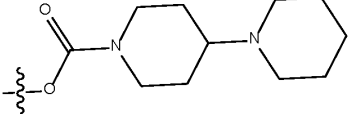
Exatecan is yet a further camptothecin compound (also known as DX-8951f or SG2000) suitable for inclusion in the ADC of the present invention.

Considering the above, preferably, D is

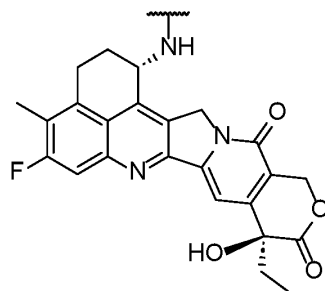


, wherein:

- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH; or
- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or

- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H and R⁵ is –O–.

Most preferably, D is



As disclosed above, both the pharmaceutically active substance D and the solubility enhancing group SE are connected to a self-immolative spacer of the modular linker of the ADC. Particularly, D is connected in para-position to the cleavable linker sequence of the self-immolate part of the aminobenzyl spacer, while the solubility enhancing group SE is connected in meta-position to the attached cleavable linker sequence. Accordingly, once the aminobenzyl spacer undergoes triggered self-immolation, i.e. decomposition, the pharmaceutically active drug substance D is

released as a metabolite of the self-immolation reaction without any chemical moieties from the linker compound remaining. In such embodiments, the amino group of a para-aminobenzyl spacer serves as the electron donor in the electron cascade of the 1,6-self-immolation reaction releasing the cytotoxic drug substance D. Advantageously, this traceless release mechanism ensures that the cytotoxic drug substance is not altered through its connection to and subsequent release from the linker compound.

The solubility enhancing group SE being linked to the self-immolative spacer in meta, while the cytotoxic drug substance is linked in the adjacent para position has been shown to be advantageous. Without wanting to be bound by theory, the close proximity of the solubility enhancing group SE and the cytotoxic drug substance D results in masking of the lipophilic/hydrophobic properties of the drug substance by forming a so-called "host-guest" complex, such as to further increase solubility of the modular linker compound.

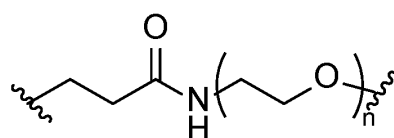
Once the ADC recognizes and binds the target sequence or target epitope of the cancer-specific antigen, it is internalized by the cancer cell and becomes a substrate for lysosomal proteases. In the ADC of the present invention, the self-immolative spacer is linked to a cleavable di-peptide sequence, which is a target for such lysosomal proteases, namely for cysteine proteases of the cathepsin family, including cathepsin B, C, D, H, L, S and Z. Typically, the di-peptide sequence is a valine-alanine (Val-Ala) sequence, which is cleavable by cathepsin B. Once the di-peptide sequence is cleaved, the electron cascade of the self-immolation reaction is triggered, leading to the traceless release of the drug substance from the self-immolative spacer of the ADC.

In alternative instances, the peptide sequence may be a dipeptide selected from valine-citrulline (Val-Cit), or phenylalanine-lysine (Phe-Lys), or a tripeptide selected from glutamic acid-valine-alanine (Glu-Val-Ala) or glutamic acid-valine-citrulline (Glu-Val-Cit).

As already indicated above, an advantageous feature of the modular linker of the ADC of the present disclosure is its multimeric core comprising two sequential amino acid-based branching units, each indirectly connected to the dipeptide or tripeptide

sequence and the self-immolative spacer carrying both the pharmaceutically active drug substance D and the solubility enhancing group SE.

In some embodiments, the ADC of the first aspect, the branching unit distal to the conjugation site of the drug-carrying linker to the antibody carries a terminal acyl cap, wherein said acyl cap constitutes the terminus of the final branching unit in the modular linker's multimeric core. Further, each branching unit is separated from the dipeptide or tripeptide sequence preceding the self-immolative spacer carrying both D and SE by a C₂-amide-PEG_n spacer. The term "C₂-amide-PEG_n" refers to a linker comprising ethylene glycol moieties covalently attached to a propylamide group,



, whereby n is from about 1 to about 10, e.g. 1, 2, 3,

4, 5, 6, 7, 8, 9, or 10.

As also mentioned above, pharmaceutically active drug substances, including camptothecins, typically have an unfavorably low solubility in aqueous solutions such as in physiologic/systemic solutions. Therefore, to avoid undesirable aggregation of the ADCs of the invention, the modular linker necessarily comprises one solubility enhancing group S connected to each self-immolative spacer. Solubility enhancing group SE may be an alpha-cyclodextrin, a beta-cyclodextrin, a gamma-cyclodextrin, a polysarcosine, a PEG, or a polyalcohol made from carbohydrates.

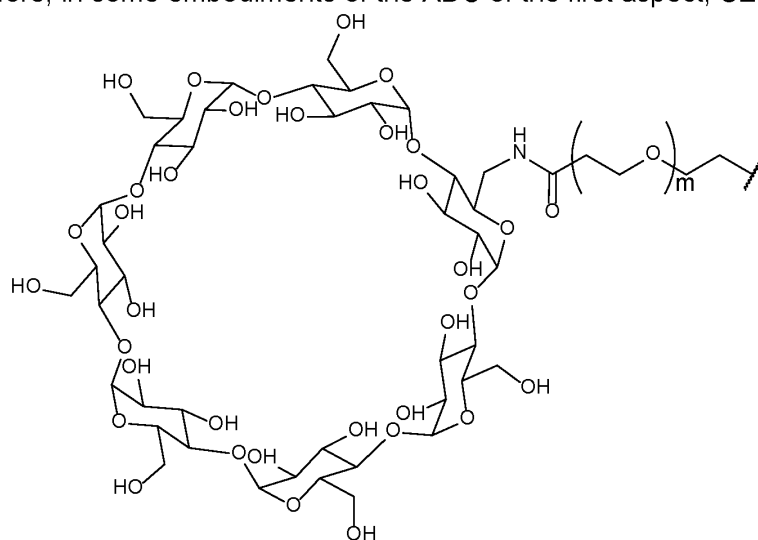
Cyclodextrins are cyclic oligosaccharides composed of glucose units arranged in a toroid (doughnut) shape. They have a hydrophobic interior and a hydrophilic exterior. This unique structure allows them to form inclusion complexes with hydrophobic or poorly soluble molecules and when a hydrophobic/lipophilic pharmaceutical drug substance is complexed with a cyclodextrin, its solubility in an aqueous solution is typically increased. Accordingly, a hydrophobic pharmaceutically active drug substance can be encapsulated within the hydrophobic pocket of the cyclodextrin, shielding it from the surrounding aqueous environment thereby enhancing its solubility. Notwithstanding, cyclodextrins are chemically compatible with a wide range of pharmaceutically active drug substances and typically are non-reactive with respect

to molecules encapsulated via the formation of an inclusion complex. This allows for the maintenance of complex stability and integrity of the pharmaceutically active drug substance at the same time.

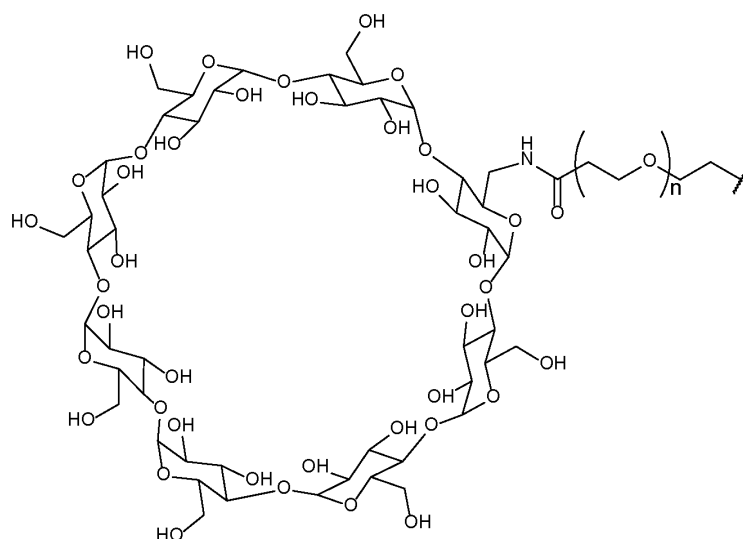
Further, cyclodextrins have been widely used in pharmaceuticals, food as well as cosmetics for many years and it is established that their overall safety and biocompatibility profiles are favorable.

Accordingly, cyclodextrins are particularly suitable solubility enhancing groups S in the context of the here-described linker compounds. For example, alpha-cyclodextrins (comprising six glucose molecules), beta-cyclodextrins (comprising seven glucose molecules) and gamma-cyclodextrins (comprising eight glucose molecules), when attached to an amide-PEG₁₋₁₂ spacer.

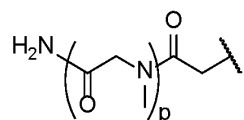
Therefore, in some embodiments of the ADC of the first aspect, SE is selected from:



wherein m is 2 to 12, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12, preferably m is 4, 5, 6, 7 or 8;

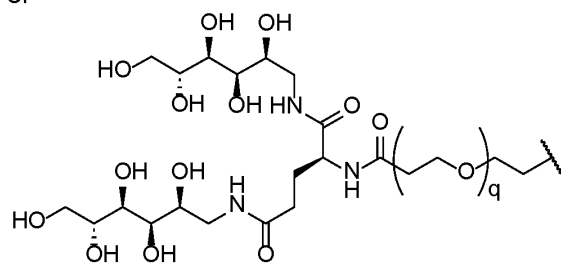


wherein n is 2 to 12, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12; preferably n is 4, 5, 6, 7 or 8,



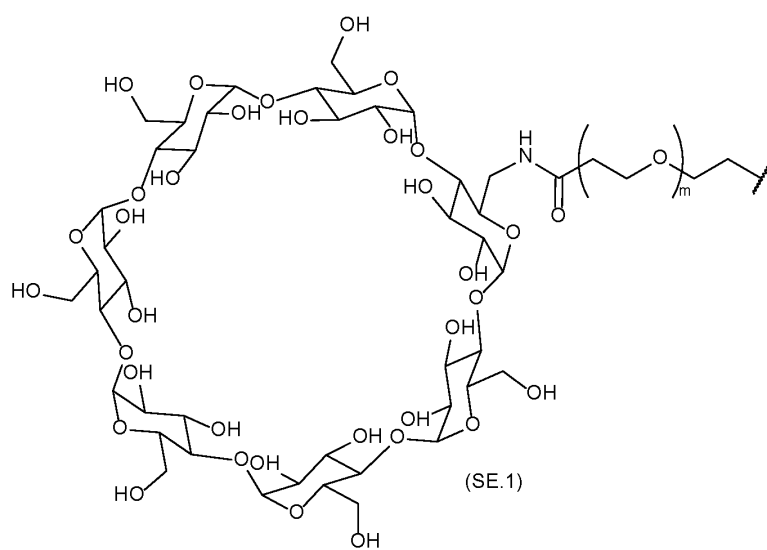
wherein p is 1 to 20, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20; preferably p is 8 to 14, even more preferred p is 10 to 12,

or



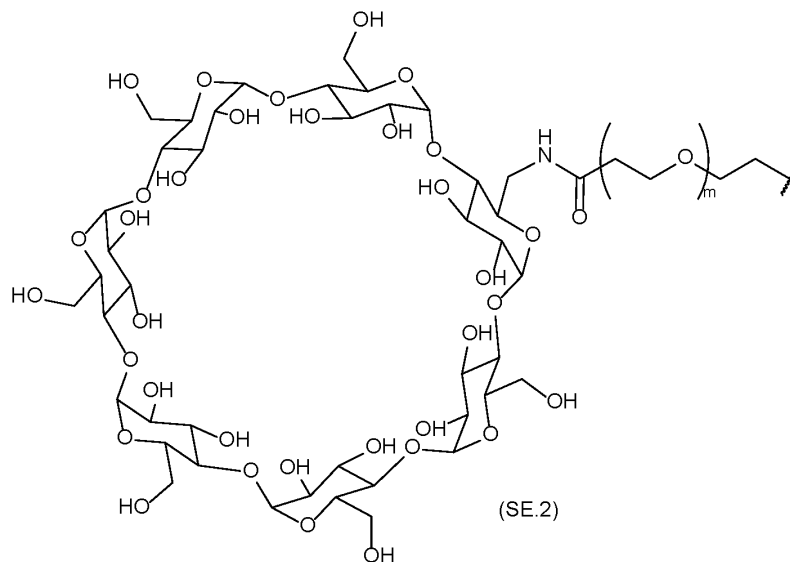
wherein q is 2 to 12, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12, preferably q is 4, 5, 6, 7 or 8.

In some embodiments, SE is:



and m is 4.

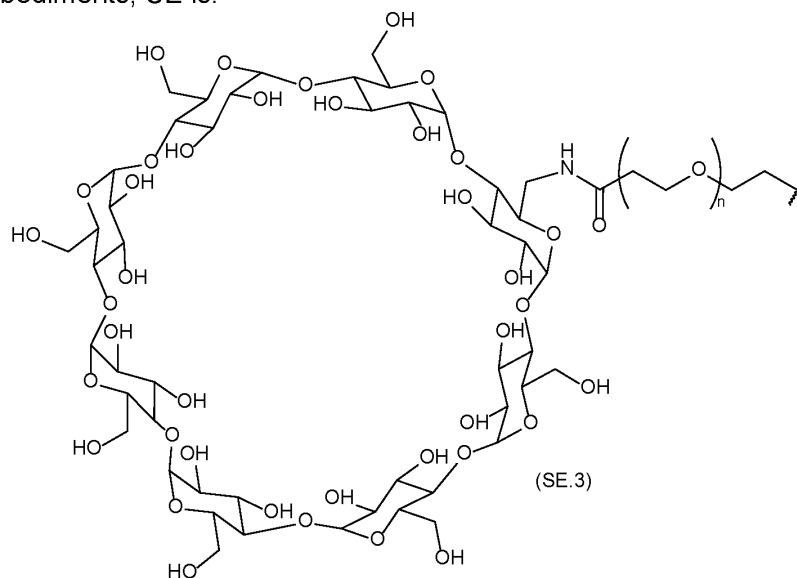
Alternatively, SE is:



and m is 8.

Selecting the length of the PEG spacers of $m = 4$ or $m = 8$ for the above-shown beta-cyclodextrins is particularly advantageous when the pharmaceutical drug substance D is a camptothecin.

In further embodiments, SE is:

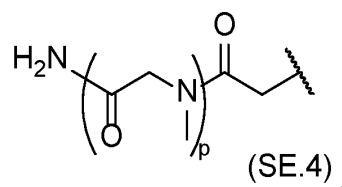


and n is 4.

Selecting the length of the PEG spacer of $n = 4$ for the above gamma-cyclodextrin is particularly advantageous when the pharmaceutical drug substance D is a camptothecin.

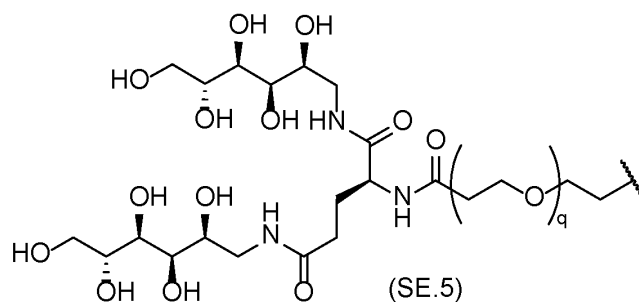
It is particularly advantageous to link a cyclodextrin-based solubility enhancing groups of the above illustrated types to the self-immolative spacer X via a PEG chain of the above indicated lengths. Such PEG chain lengths favor a conformational arrangement of the linker compound that allows for an inclusion complex placing the lipophilic drug substance into the pocket of the cyclodextrin ring structure substantially negating the hydrophobic/lipophilic properties of the camptothecin.

In yet further embodiments, SE is:



and p is 10.

In some embodiments, SE is:



and q is 8.

As already indicated, the antibody of the ADC specifically recognizes and binds a target sequence or epitope of an antigen, such as to mediate the ADC's cancer cell specificity. However, as also described above, preferably the antibody of the ADC of the first aspect is one of:

- a monoclonal antibody;
- a functional antibody fragment or antibody derivative such as a single-chain variable fragment (scFv), an **antigen**-binding fragment such as a Fab fragment, a F(ab'), fragment, a F(ab')₂ fragment or a bi- or tri-specific antibody construct,
- a Diabody,
- a Camelid Antibody,
- a Domain Antibody,
- a Nanobody,
- a bivalent homodimer with two chains consisting of scFvs,
- a shark antibody,
- an antibody consisting of new-world primate framework sequences plus non-new world primate complementarity-determining regions (CDR) or
- a dimerized construct comprising a constant heavy chain 3 (CH₃) domain, a variable light chain (V_L) and a variable heavy chain (V_H).

A diabody as disclosed above refers to an antibody-like molecule in which the variable region in the heavy chain (V_H) and the variable region in the light chain (V_L) derived from the same parent antibody will form a hetero-dimer through non-covalent bond. Diabodies and their generation are e.g. disclosed in Holliger, et al., Proc. Natl. Acad. Sci. USA 90, 6444-6448, 1993. Diabodies can e.g. be cysteine-engineered diabodies to facilitate conjugation to the linker-payloads as disclosed herein.

The term “nanobody®” (Nb) refers single variable domains (VHHs) of camelid heavy chain antibodies (hcAbs). Anti-GUCY2C nanobodies may e.g. be conjugated to the linker-payloads of the present disclosure by introducing a cysteine-comprising linker to which the linker-payloads as disclosed herein may be conjugated. Given the short plasma half-life of nanobodies, it is advantageous to fuse the anti-GUCY2C nanobody via a linker to a second nanobody which binds human serum albumin (HSA). Corresponding methods are e.g. disclosed in J Nanobiotechnol 21, 410 (2023).

The term “shark antibody” refers to IgNAR which represent the essential antibody of a shark's adaptive immune system. IgNARs contain a heavy chain homodimer not associated with light chains. Antigen-binding of IgNARs requires two independent and highly soluble VNARs (variable domain of IgNAR) with a molecular weight of around

12 kDa. The shark VNAR is the smallest antibody-like molecule occurring naturally. IgNARs which are directed against GUCY2C can e.g. be recombinantly engineered to comprise one, or more cysteine residues to facilitate conjugation to the linker-payloads of the present disclosure or can be modified to comprise a cysteine-comprising linker to which the linker-payloads as disclosed herein can be conjugated. Like Nanobodies, IgNARs can be fused to anti-HAS IgNARs to improve their pharmacokinetic properties, see e.g. mAbs 4:6, 673-685; November/December 2012. Preferably, the shark antibodies are de-immunized antibodies.

Each one of the above-mentioned antibody formats must ensure highly specific recognition and binding of the target sequence or target epitope of the cancer-specific antigen such as to avoid undesirable off-target effects.

In the context of the present invention, where gastrointestinal cancer patients, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer patients are to benefit from the ADC of the first aspect, predominantly, the antibody typically is a monoclonal antibody that recognizes and binds a target sequence or epitope of Guanylate Cyclase 2C (GUCY2C), preferably human, or cynomolgus Guanylate Cyclase 2C, more preferably human Guanylate Cyclase 2C.

Highly specific anti-GUCY2C have been described in PCT/EP2023/080350 (published as WO2024/094688 A1), which is hereby incorporated by reference in its entirety.

Accordingly, in one embodiment of the first aspect, the antibody of the ADC is the monoclonal anti-GUCY2C antibody mAb1, which includes an antigen binding region comprising:

- an mAb1 light chain variable region (mAb1 V_L) complementarity-determining region (CDR_L) sequences CDR_L1 of SEQ ID NO: 1, CDR_L2 of SEQ ID NO: 2 and CDR_L3 of SEQ ID NO: 3 and
- an mAb1 heavy chain variable region (mAb1 V_H) CDR_H1 of SEQ ID NO: 4, CDR_H2 of SEQ ID NO: 5 and CDR_H3 of SEQ ID NO: 6.

More specifically, the mAb1 antibody includes an antigen binding region comprising:

- the mAb1 V_L comprises a framework region 1 (FR_{L1}) of SEQ ID NO: 14, CDR_{L1} of SEQ ID NO: 1, a FR_{L2} of SEQ ID NO: 15, CDR_{L2} of SEQ ID NO: 2, a FR_{L3} of SEQ ID NO: 16, CDR_{L3} of SEQ ID NO: 3 and a FR_{L4} of SEQ ID NO: 17; and
- the mAb1 V_H comprises a framework region 1 (FR_{H1}) of SEQ ID NO: 18, CDR_{H1} of SEQ ID NO: 4, a FR_{H2} of SEQ ID NO: 19, CDR_{H2} of SEQ ID NO: 5, a FR_{H3} of SEQ ID NO: 20, CDR_{H3} of SEQ ID NO: 6 and a FR_{H4} of SEQ ID NO: 21.

Further, the mAb1 antibody includes an antigen binding region comprising:

- the mAb1 V_L of SEQ ID NO: 32 and
- the mAb1V_H of SEQ ID NO: 33.

In another embodiment of the first aspect, the antibody of the ADC is the monoclonal anti-GUCY2C antibody mAb8, which includes an antigen binding region comprising:

- an mAb8 light chain variable region (mAb8 V_L) complementarity-determining region (CDR_L) sequences CDR_{L1} of SEQ ID NO: 1, CDR_{L2} of SEQ ID NO: 2 and CDR_{L3} of SEQ ID NO: 7 and
- an mAb8heavy chain variable region (mAb8 V_H) CDR_{H1} of SEQ ID NO: 4, CDR_{H2} of SEQ ID NO: 5 and CDR_{H3} of SEQ ID NO: 6.

More specifically, the mAb8 antibody includes an antigen binding region comprising:

- the mAb8 V_L comprises a framework region 1 (FR_{L1}) of SEQ ID NO: 22, CDR_{L1} of SEQ ID NO: 1, a FR_{L2} of SEQ ID NO: 15, CDR_{L2} of SEQ ID NO: 2, a FR_{L3} of SEQ ID NO: 23, CDR_{L3} of SEQ ID NO: 7 and a FR_{L4} of SEQ ID NO: 24; and
- the mAb8 V_H comprises a framework region 1 (FR_{H1}) of SEQ ID NO: 18, CDR_{H1} of SEQ ID NO: 4, a FR_{H2} of SEQ ID NO: 19, CDR_{H2} of SEQ ID NO: 5, a FR_{H3} of SEQ ID NO: 20, CDR_{H3} of SEQ ID NO: 6 and a FR_{H4} of SEQ ID NO: 21.

Further, the mAb8 antibody includes an antigen binding region comprising:

- the mAb8 V_L of SEQ ID NO: 34 and
- the mAb8 V_H of SEQ ID NO: 35.

In yet another embodiment of the first aspect, the antibody of the ADC is the monoclonal anti-GUCY2C antibody mAb41, which includes an antigen binding region comprising:

- an mAb41 light chain variable region (mAb41 V_L) complementarity-determining region (CDR_L) sequences CDR_L1 of SEQ ID NO: 8, CDR_L2 of SEQ ID NO: 9 and CDR_L3 of SEQ ID NO: 10 and
- an mAb41 heavy chain variable region (mAb41 V_H) CDR_H1 of SEQ ID NO: 11, CDR_H2 of SEQ ID NO: 12 and CDR_H3 of SEQ ID NO: 13.

More specifically, the mAb41 antibody includes an antigen binding region comprising:

- the mAb41 V_L comprises a framework region 1 (FR_L1) of SEQ ID NO: 25, CDR_L1 of SEQ ID NO: 8, a FR_L2 of SEQ ID NO: 26, CDR_L2 of SEQ ID NO: 9, a FR_L3 of SEQ ID NO: 27, CDR_L3 of SEQ ID NO: 10 and a FR_L4 of SEQ ID NO: 28; and
- the mAb41 V_H comprises a framework region 1 (FR_H1) of SEQ ID NO: 29, CDR_H1 of SEQ ID NO: 11, a FR_H2 of SEQ ID NO: 30, CDR_H2 of SEQ ID NO: 12, a FR_H3 of SEQ ID NO: 31, CDR_H3 of SEQ ID NO: 13 and a FR_H4 of SEQ ID NO: 21.

Further, the mAb41 antibody includes an antigen binding region comprising:

- the mAb41 V_L of SEQ ID NO: 36 and
- the mAb41 V_H of SEQ ID NO: 37.

In some embodiments of the antibody-drug conjugate according to the first aspect, the antibody is: (a) a humanized or human antibody; and/or (b) an IgG type antibody, preferably an IgG1 antibody; and/or (c) a recombinant antibody.

According to one embodiment, the anti-GUCY2C antibody of the ADC is a recombinant antibody or antigen-binding fragment thereof. The term “recombinant” or “recombinantly produced” as used herein refers to a protein, such as the anti-GUCY2C antibody or antigen-binding fragment of the present disclosure as disclosed herein, that have been expressed in heterologous cells. For example, the anti-GUCY2C antibody or antigen-binding fragment of the present disclosure may be expressed in prokaryotic or eukaryotic cells. Prokaryotic cells for the expression of the antibody of the ADC of the present disclosure include e.g. gram-negative bacteria

such as *E.coli*, or gram-positive bacteria such as *Bacillus subtilis*. The use of heterologous prokaryotic expression systems may be particularly useful for the expression of antigen-binding fragments of the anti-GUCY2C antibody of the ADC of the present disclosure and may be done according to established protocols known in the art, such as e.g. Kwong and Rader, *Curr. Protoc. Protein Sci.* 55:6.10.1-6.10.14. for the expression of Fab fragments.

The use of eukaryotic cells for the expression of the antibody or antigen-binding fragment of the present disclosure is, however, preferred due to the glycosylation of the heterologously expressed antibody. It is even more preferred to use mammalian cells for the expression of the antibody or antigen-binding fragment of the invention. For example, Chinese hamster ovary (CHO) cells, or any of its genetically different progeny such as K1-, DukX B11-, DG44-cell lines may be used for the expression of the antibody of the invention. Other mammalian cell lines that may be used to produce the antibody or antigen-binding fragment of the present disclosure include e.g. NS0 cells, embryonic kidney (HEK) 293 cells, PER.C6 cells, MCF7 cells. Cells can e.g. be transfected with an expression vector comprising the coding sequence for the anti-GUCY2C antibody or antigen-binding fragment according to the invention. The expression vector or recombinant plasmid is produced by placing the coding antibody sequences under control of suitable regulatory genetic elements, including promoter and enhancer sequences like, e.g. a CMV promoter. Heavy and light chain sequences can e.g. be expressed from individual expression vectors which are co-transfected, or from dual expression vectors. Said transfection may be a transient transfection or a stable transfection. The transfected cells are subsequently cultivated to produce the transfected antibody construct. When stable transfection is performed, then stable clones secreting antibodies with properly associated heavy and light chains are selected by screening with an appropriate assay, such as, e.g. ELISA, subcloned, and propagated for future production. Corresponding methods are e.g. disclosed in WO03/018771.

According to one embodiment, the anti-GUCY2C antibody of the ADC of the present disclosure comprises an Fc region, which comprises the amino acid sequence according to SEQ ID NO: 38. Antibodies of the present disclosure which comprise an Fc region comprising the amino acid sequence according SEQ ID NO: 38 are able to induce antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent

cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC). It may, however, be desirable to reduce or eliminate effector function of the antibody of the ADC of the present disclosure as disclosed herein for example to prevent target cell death, unwanted cytokine secretion, or killing of cells that express the Fcγ receptor such as macrophages.

Accordingly, the anti-GUCY2C antibody of the ADC of the present disclosure as described herein may also include modifications and/or mutations that alter the properties of the antibodies and/or fragments, such as those which decrease ADCC, ADCP, or complement-dependent cytotoxicity CDC as known in the art. Preferably, ADCC, ADCP and CDC are reduced by at least 90% or more, more preferably by at least 95%, more preferably by at least 97.5%, even more preferred by at least 98%, 99% compared to an antibody comprising a wild type Fc region comprising e.g. the amino acid sequence according to SEQ ID NO: 38.

The binding of IgG1 to activating and inhibitory Fcγ receptors (FcγRs) or the first component of complement (C1q) depends on residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain are critical for FcγRs and complement C1q binding and have unique sequences. Substitution of human IgG1 and IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 greatly reduced ADCC and CDC (Armour, et al., Eur. J. Immunol. 29(8) (1999) 2613-2624; Shields, et al., J. Biol. Chem. 276(9) (2001) 6591-6604, WO 2021/234402 A2).

Accordingly, in one embodiment, the anti-GUCY2C antibody of the ADC of the present disclosure comprises a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, such that said molecule has a reduced affinity for IgG1 Fc receptors FcγRI, FcγRII and FcγRIII as well as to complement component C1q compared to a wild-type Fc region (e.g. comprising an amino acid sequence according to SEQ ID NO: 38).

Affinity to an Fc region, such as the binding of IgG1 to FcγRs, can be determined using a variety of techniques known in the art, for example but not limited to, equilibrium methods (e.g., enzyme-linked immunoabsorbent assay (ELISA); KinExA, Rathanaswami et al. Analytical Biochemistry, Vol. 373:52-60, 2008; or

radioimmunoassay (RIA)), or by a surface plasmon resonance assay as disclosed in e.g. Wilkinson et al. PLoS One. 2021; 16(12): e0260954 or other mechanism of kinetics-based assay (e.g., BIACORETM analysis or OctetTM analysis (forteBIO)), and other methods such as indirect binding assays, competitive binding assays fluorescence resonance energy transfer (FRET), gel electrophoresis and chromatography (e.g., gel filtration).

Thus, in some embodiments the anti-GUCY2C antibody of the ADC of the present disclosure have been genetically engineered to comprise a variant Fc region which comprise modification of at least one amino acid residue that directly contacts FcγRs based on structural and crystallographic analysis. The term “genetically engineered” or “genetic engineering” as used herein relates to the modification of the amino acid sequence or part thereof of a given or natural polypeptide or protein, such as e.g. the Fc region of an antibody, in the sense of nucleotide and/or amino acid substitution, insertion, deletion or reversion, or any combinations thereof, by gene technological methods, such as, e.g., site-directed mutagenesis as described in Carter, Biochem. J. (1986) Vol. 237: 1-7, or J Biol Chem. (2015) Vol. 290(5): 2577-2592. As used herein, the term “amino acid substitution” or “mutation” relates to modifications of the amino acid sequence of the protein, wherein one or more amino acids are replaced with the same number of different amino acids, producing a protein which contains a different amino acid sequence than the original protein. A conservative amino acid substitution is understood to relate to a substitution which due to similar size, charge, polarity and/or conformation does not significantly affect the structure and function of the protein. Groups of conservative amino acids in that sense represent, e.g., the non-polar amino acids Gly, Ala, Val, Ile and Leu; the aromatic amino acids Phe, Trp and Tyr; the positively charged amino acids Lys, Arg and His; and the negatively charged amino acids Asp and Glu.

Importantly, Fc region of the antibody ADC of the present disclosure is genetically engineered such as to comprise at least one cysteine amino acid substitution at a site within the Fc region such that this engineered cysteine is available for conjugation but does not perturb immunoglobulin folding and assembly. Corresponding cysteine-substituted or cysteine-engineered antibodies have disclosed in WO2016040856A2, or Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249 and

WO2016/142049). The preferred cysteine substitution in the Fc region of the inventive antibodies as disclosed herein is D265C (according to EU numbering system) as disclosed in WO2016142049A1.

Preferably, the antibody comprises a heavy chain constant (Fc) region which comprises the amino acid substitution D265C. More specifically, the mAb1 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 39, the mAb8 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 40, and the mAb41 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 41. More preferably, said heavy chain constant (Fc) region comprises the amino acid substitutions L234A, L235A and D265C according to EU numbering system (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85). The EU numbering system may also be referred to as "EU index as in Kabat" and refers to the numbering of the human IgG1 EU antibody, which refers to the numbering of the EU antibody of Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85. Specifically, in embodiments where all three substitutions L234A, L235A and D265C are present in the Fc region of the antibody of the ADC of the invention, mAb1 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 42, mAb8 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 43, and mAb41 antibody comprises the heavy chain constant (Fc) region of SEQ ID NO: 44. More preferably, the antibody comprises (a) an mAb1-L234A-L235A-D265C heavy chain of SEQ ID NO: 42; or (b) an mAb8-L234A-L235A-D265C heavy chain of SEQ ID NO: 43; or (c) an mAb41-L234A-L235A-D265C of SEQ ID NO: 44. The use of said Fc regions comprising the substitutions L234A, L235A and D265C in the anti-GUCY2C antibodies of the ADC of the first aspect is particularly advantageous to reduce the interaction of the respective Fc regions (i.e. those comprising said mutations) with FcγRs by at least 95%, 97.5%, 99% compared to a wild-type Fc region while at the same time providing the attachment site for the drug-carrying linker to the antibody of the ADC via site-specific thiol-based conjugation. Corresponding use of such cysteine substituted Fc regions and antibodies comprising such mutations for linker-payload conjugation is, e.g., disclosed in WO2016142049A1.

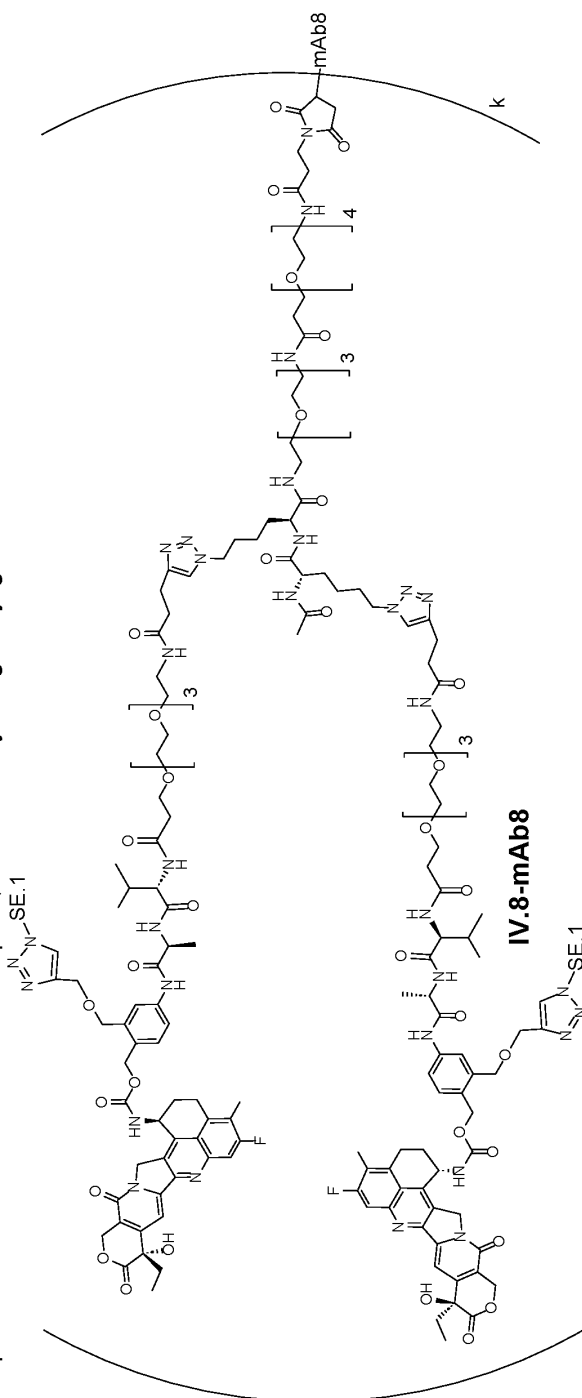
In a preferred embodiment, the mAb1 antibody comprises a light chain amino acid sequence according to SEQ ID NO: 45 and a heavy chain amino acid sequence according to SEQ ID NO: 42.

In a preferred embodiment, the mAb8 antibody comprises a light chain amino acid sequence according to SEQ ID NO: 46 and a heavy chain amino acid sequence according to SEQ ID NO: 43.

In a preferred embodiment, the mAb41 antibody comprises a light chain according to SEQ ID NO: 47 and a heavy chain according to SEQ ID NO: 44.

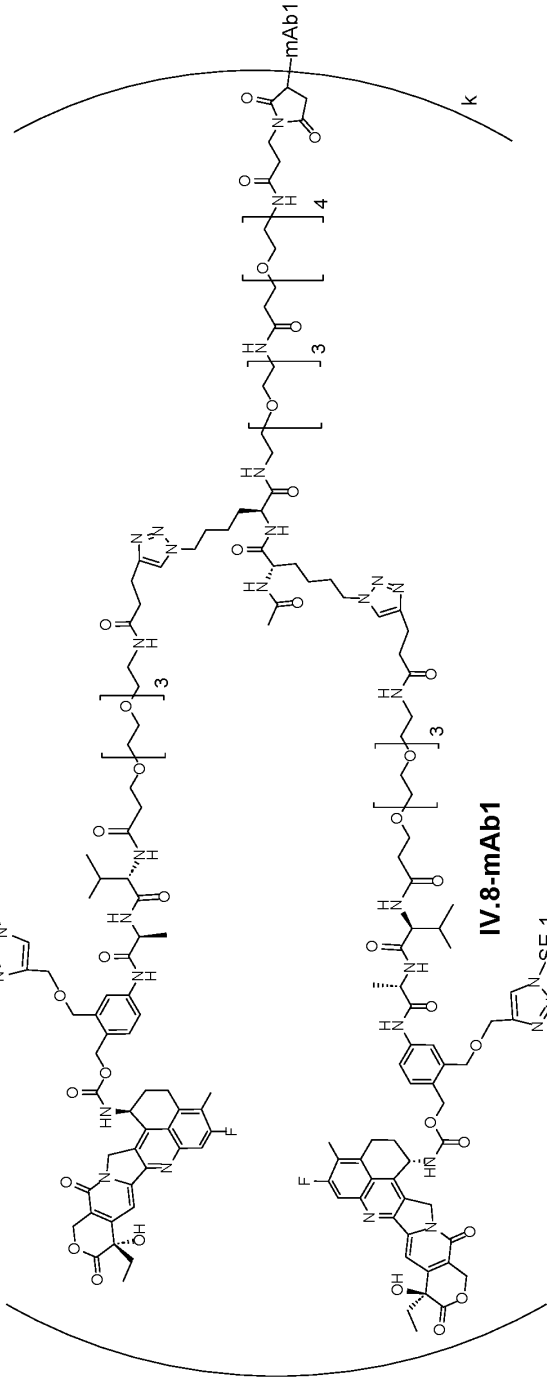
In some embodiments, the present disclosure pertains to the use of an cysteine-engineered anti-GUCY2C antibody as disclosed above comprising at least the amino acid substitution D265C (numbering according to EU numbering system) in the manufacture of an antibody-drug conjugate according to the present disclosure, preferably, the cysteine-engineered anti-GUCY2C antibody comprises the amino acid substitutions L234A, L235A and D265C (numbering according to EU numbering system). For example, the present disclosure pertains to the use of anti-GUCY2C antibodies mAb1, mAb8 and mAb41 as disclosed herein in the manufacture of an antibody-drug conjugate as disclosed herein. For example, in some embodiments, the present disclosure pertains to the use of mAb1 as disclosed herein in the manufacture of an antibody-drug conjugate by conjugating mAb1 to one of linker payloads IV.9, IV.8, IV.12, IV.18, IV.20 or IV.22. In some embodiments, the present disclosure pertains to the use of mAb8 as disclosed herein in the manufacture of an antibody-drug conjugate by conjugating mAb8 to one of linker payloads IV.9, IV.8, IV.12, IV.18, IV.20 or IV.22. In some embodiments, the present disclosure pertains to the use of mAb41 as disclosed herein in the manufacture of an antibody-drug conjugate by conjugating mAb41 to one of linker payloads IV.9, IV.8, IV.12, IV.18, IV.20 or IV.22. For the above embodiments conjugation may e.g. be done as described in the present disclosure.

In one specific embodiment of the first aspect, the antibody-drug conjugate is:



wherein k is 2.

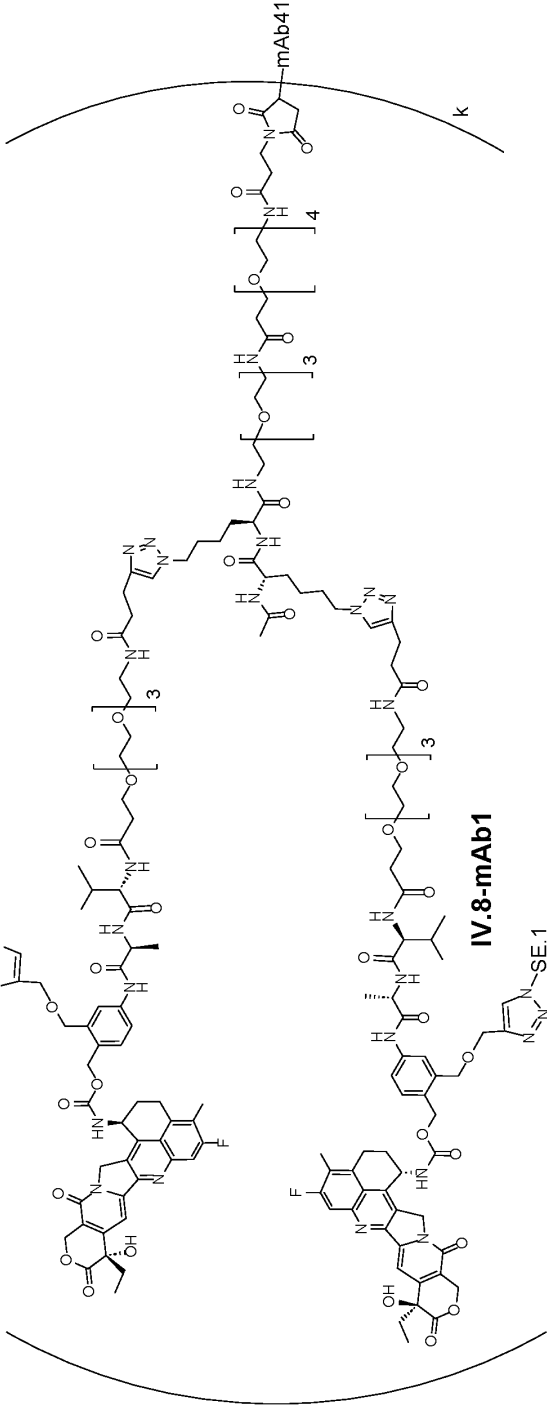
In one specific embodiment of the first aspect, the antibody-drug conjugate is:



wherein k is 2.

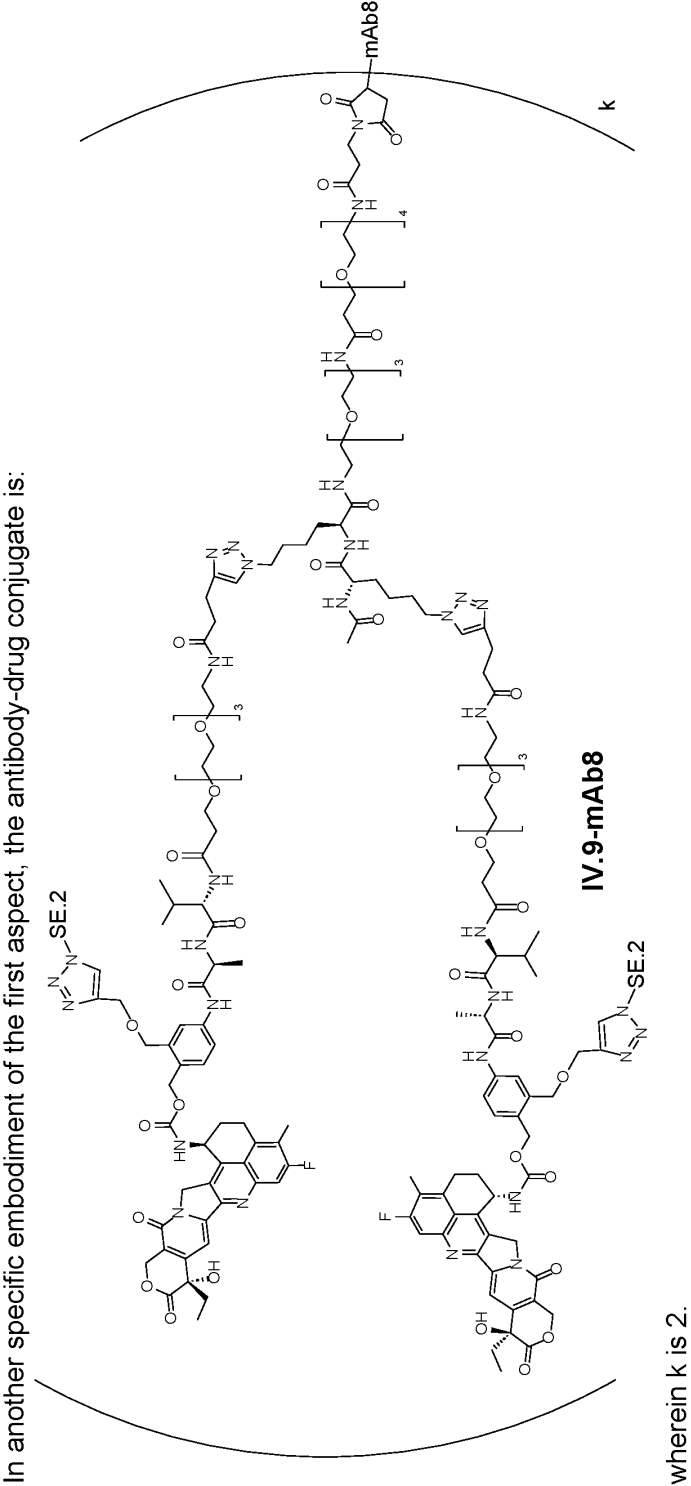
k

In one specific embodiment of the first aspect, the antibody-drug conjugate is:

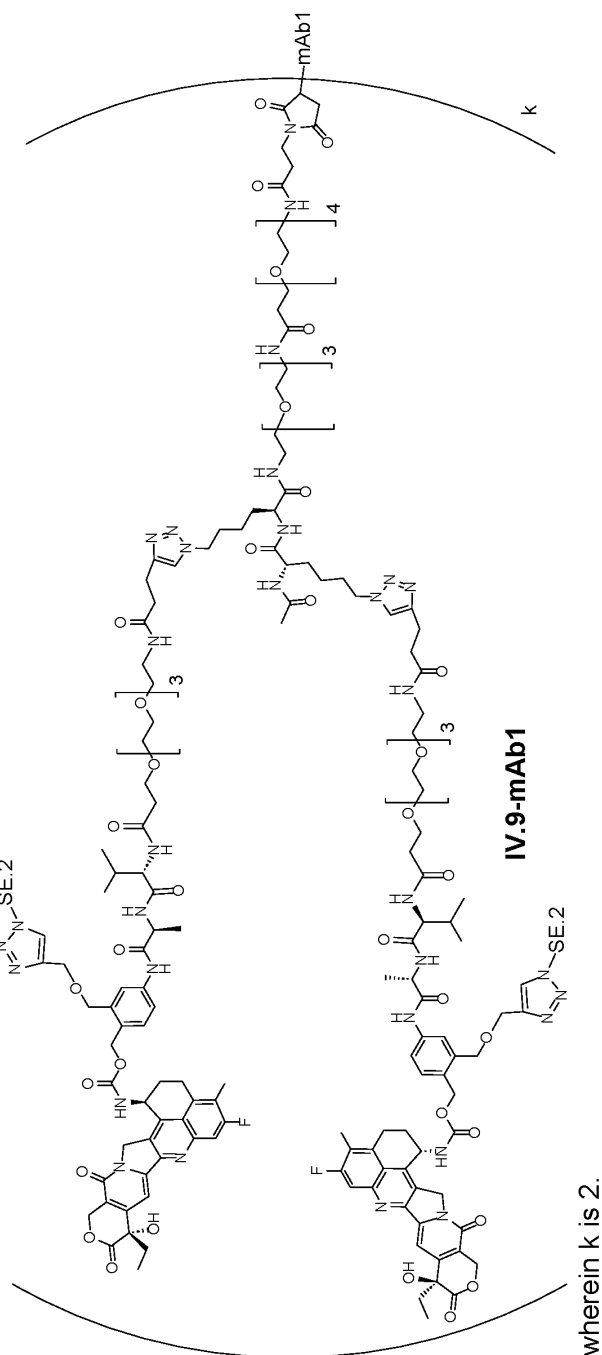


wherein k is 2.

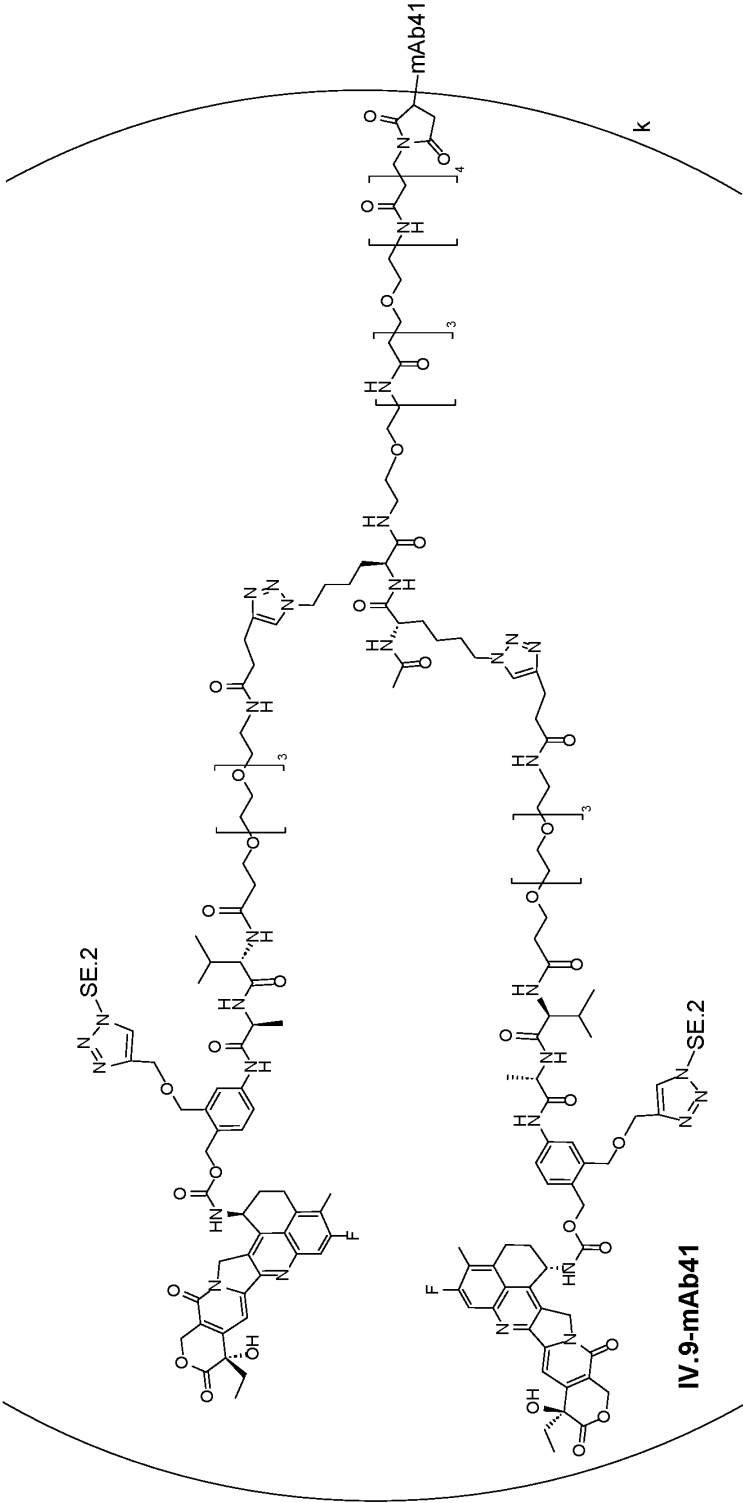
k



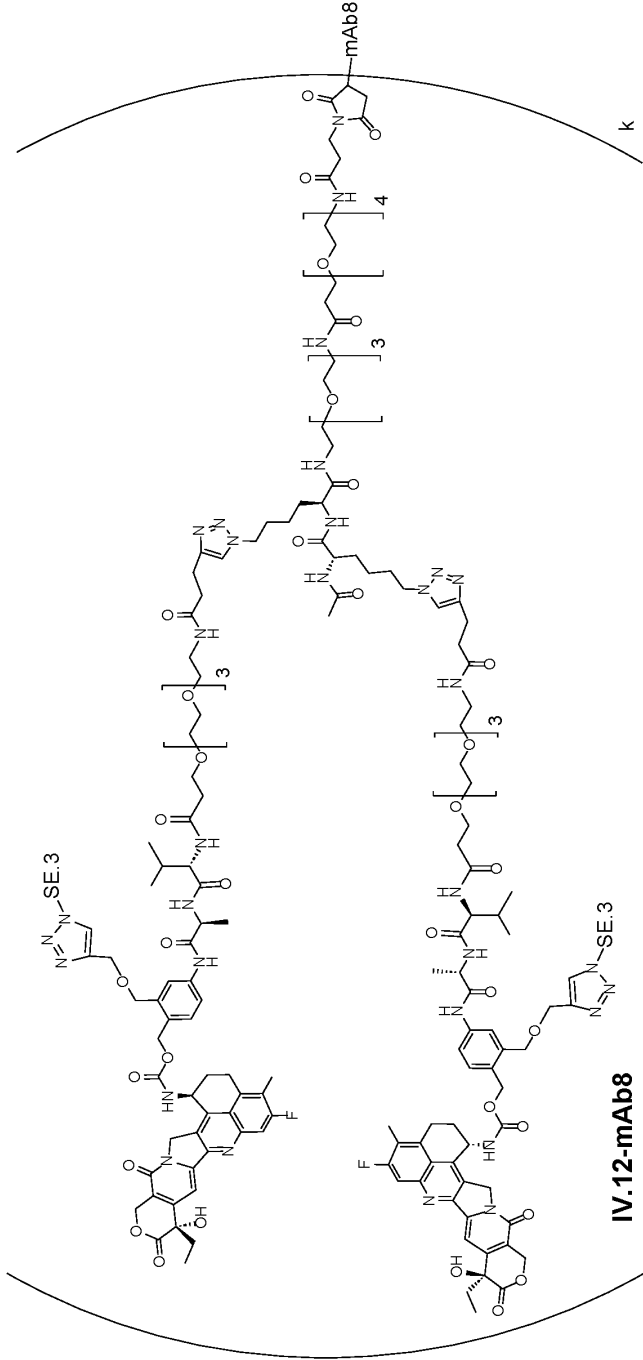
In another specific embodiment of the first aspect, the antibody-drug conjugate is:



In another specific embodiment of the first aspect, the antibody-drug conjugate is:

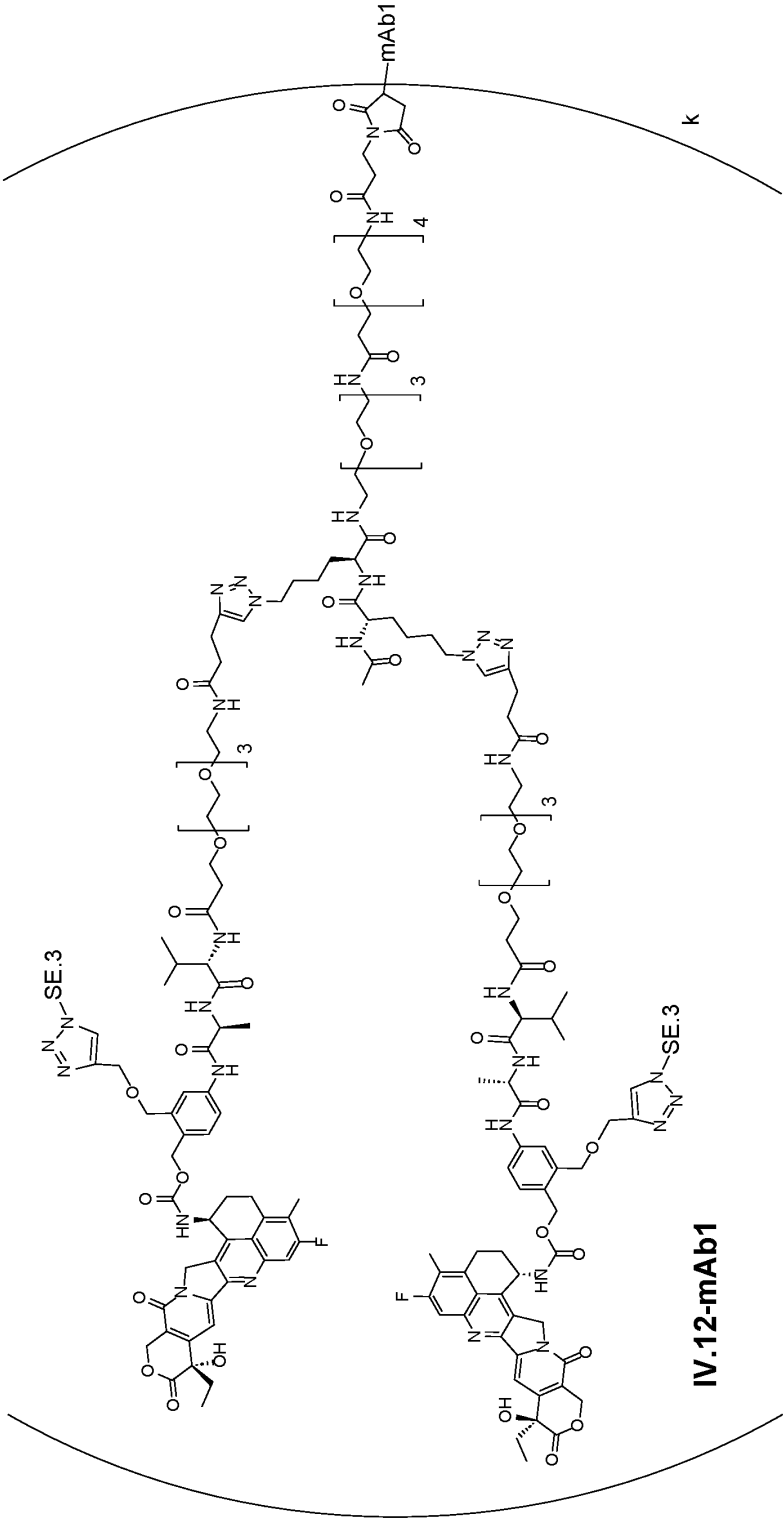


In another specific embodiment of the first aspect, the antibody-drug conjugate is:



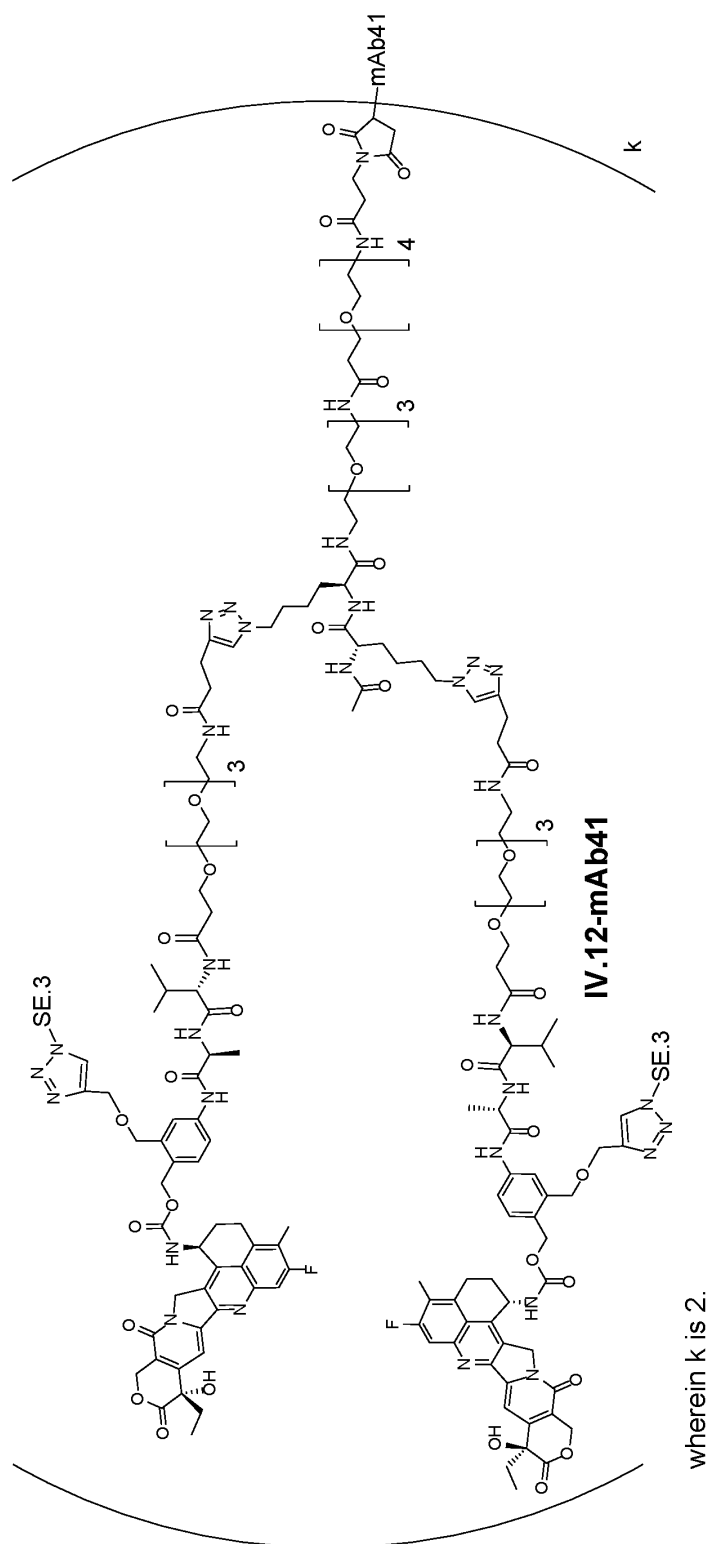
wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:



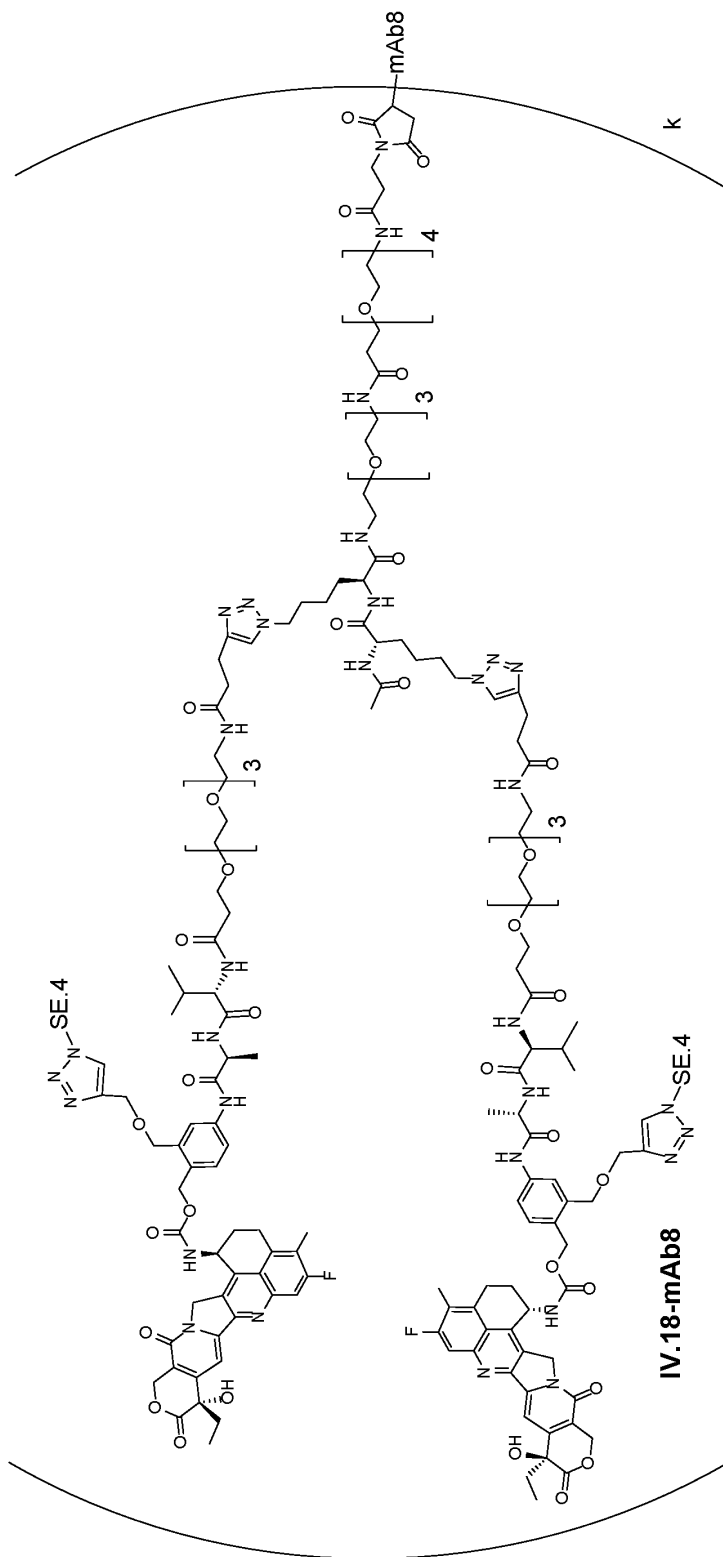
wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:



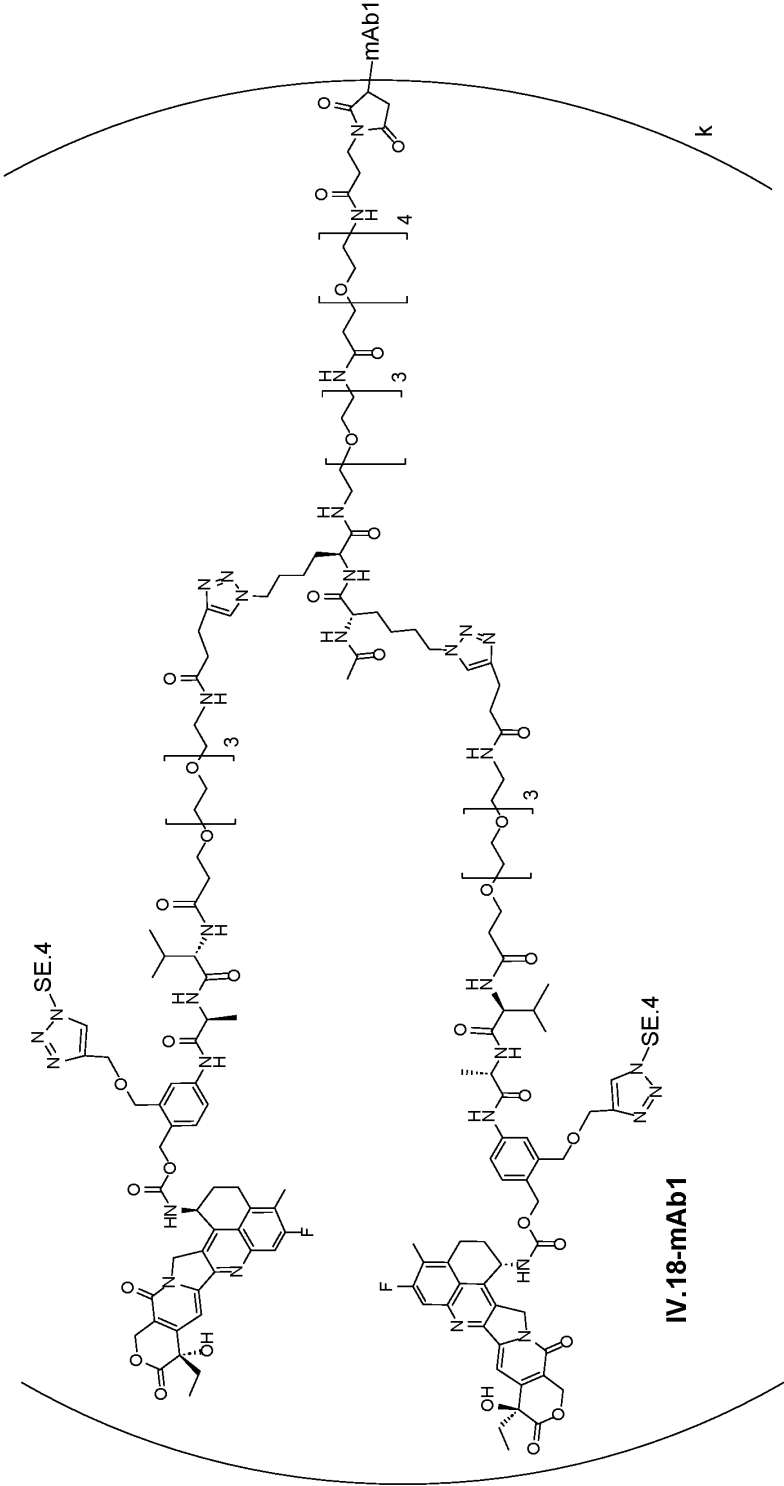
wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:



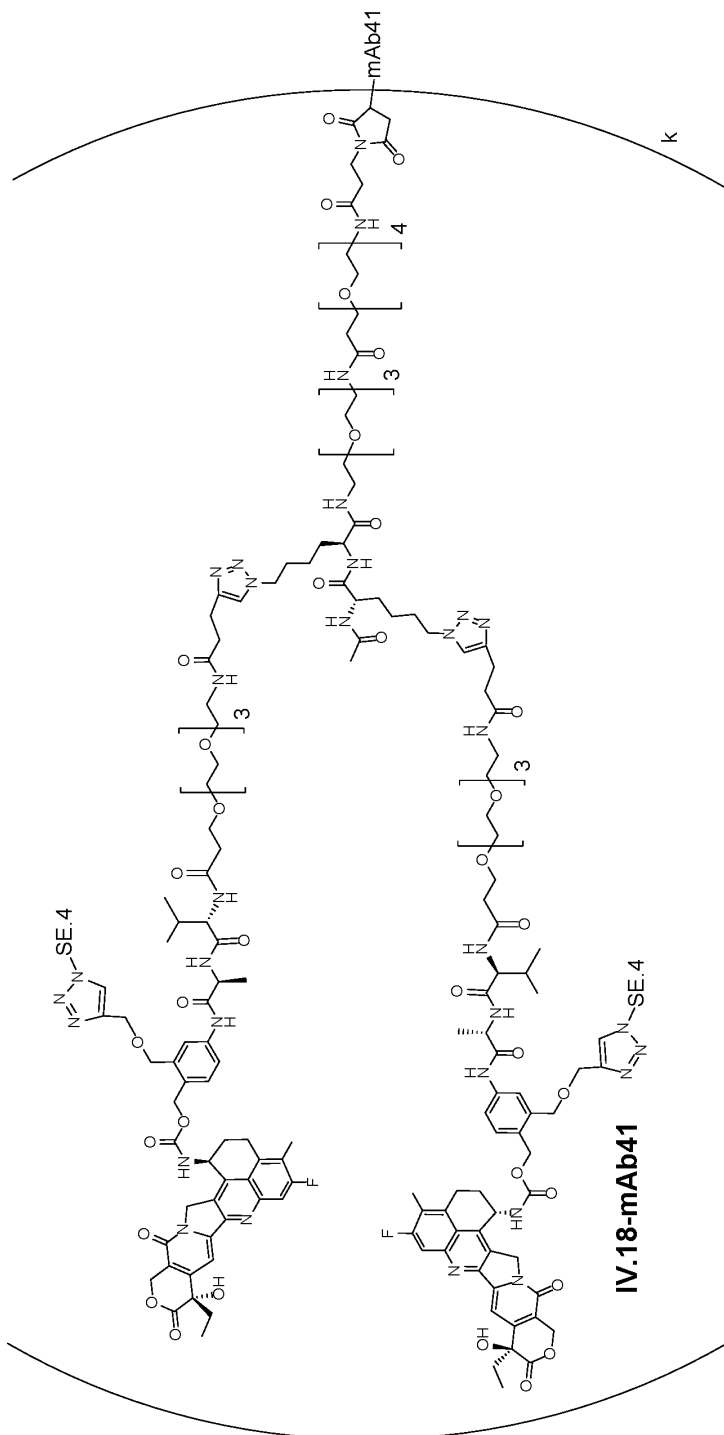
wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:

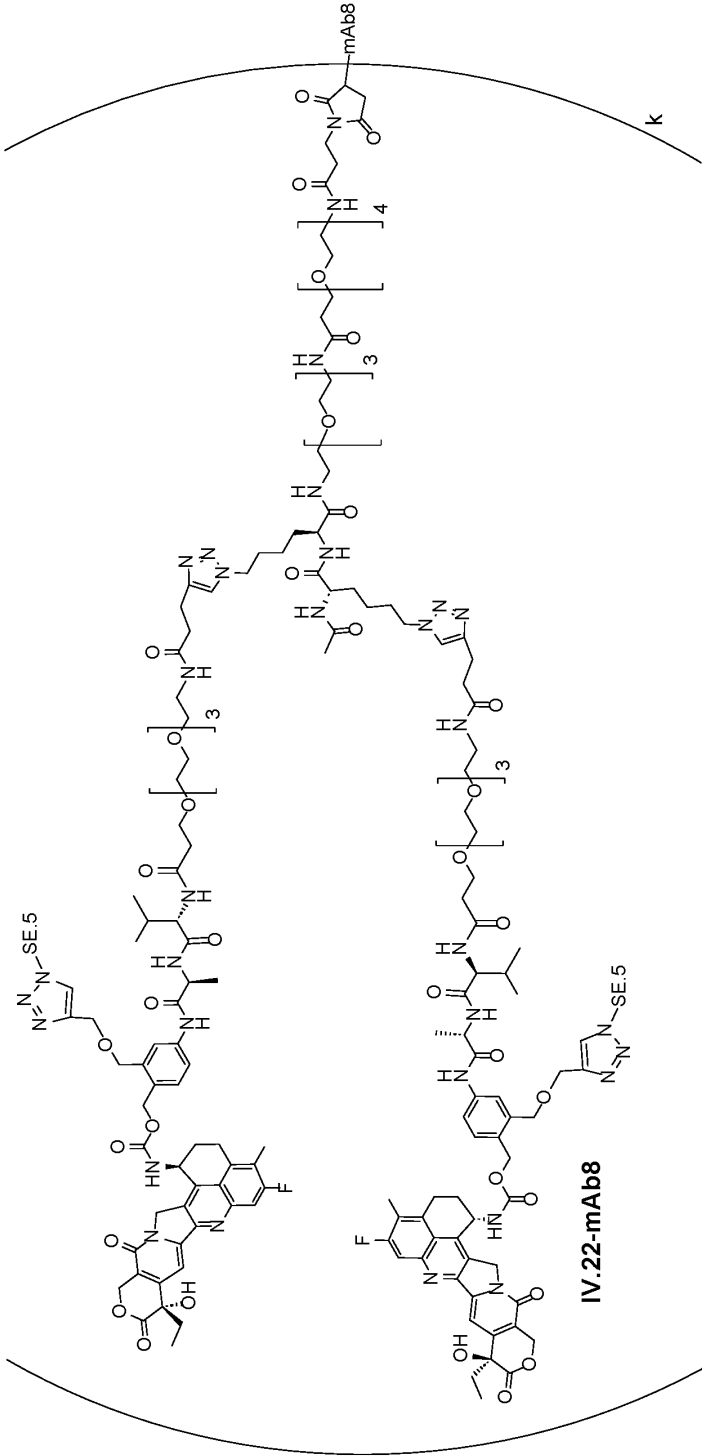


wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:

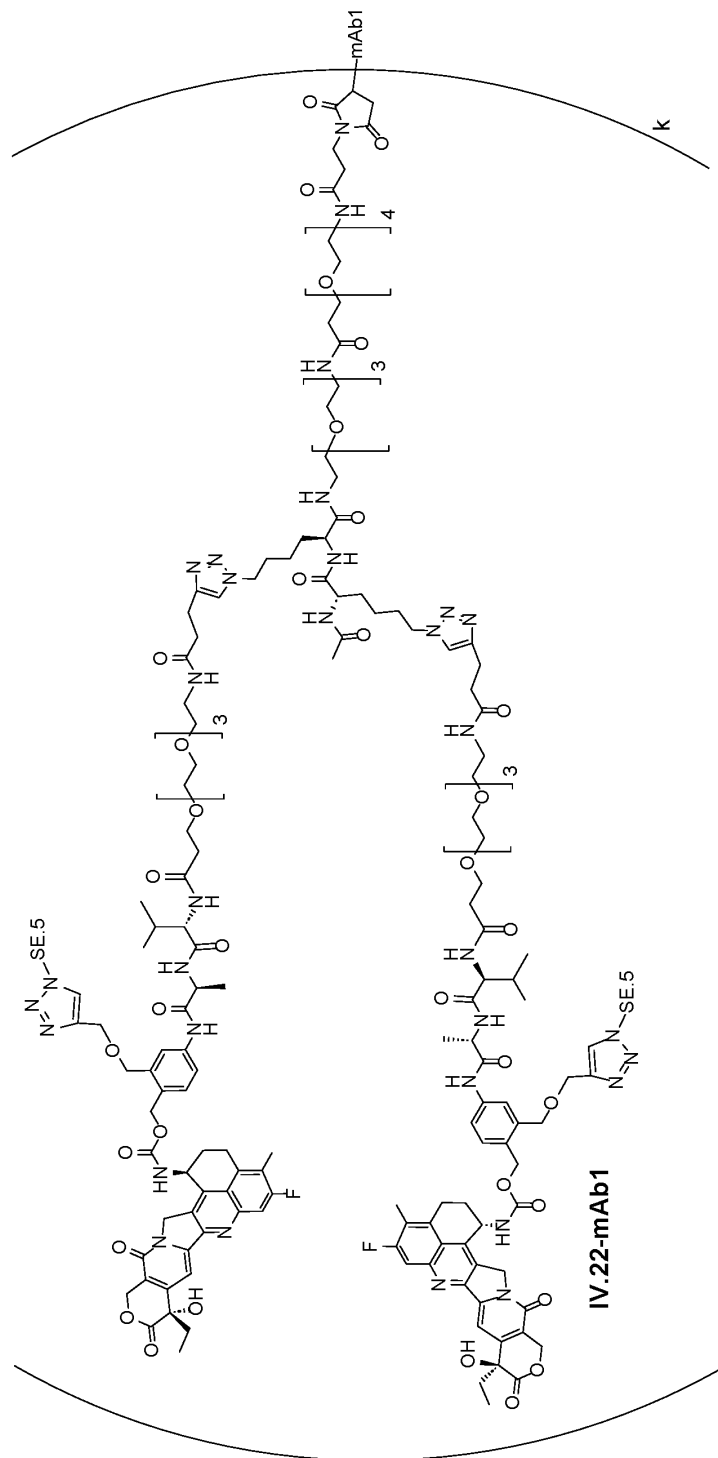


In another specific embodiment of the first aspect, the antibody-drug conjugate is:



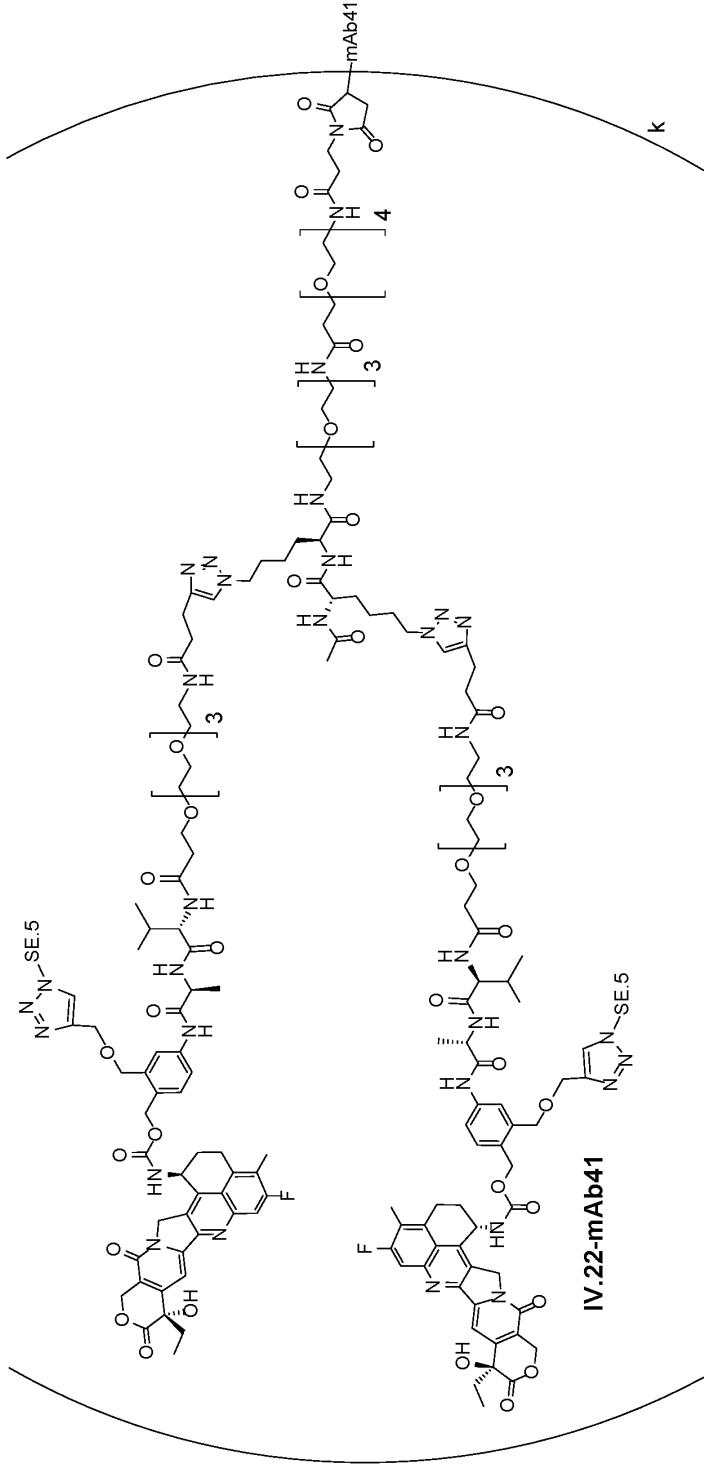
wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:



wherein k is 2.

In another specific embodiment of the first aspect, the antibody-drug conjugate is:



As indicated above, in a second aspect, the present disclosure further relates to the ADC of the first aspect for use as a medicament.

As described above, the ADC is particularly suited for use in the treatment of cancer, more preferably for the treatment of gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer, where the gastrointestinal cancer can be characterized by the expression of a target sequence or epitope of a Guanylate Cyclase 2C (GUCY2C) antigen.

Furthermore, in a third aspect, the present disclosure relates to a pharmaceutical composition comprising the antibody-drug conjugate of the first aspect.

In particular embodiments, the pharmaceutical composition is used in the form of a systemically administered medicament. This includes parenterals, which comprise among others injectables and infusions. Injectables are formulated either in the form of ampoules or as so called ready-for-use injectables, e.g. ready-to-use syringes or single-use syringes and aside from this in puncturable flasks for multiple withdrawal. The administration of injectables can be in the form of subcutaneous (s.c), intramuscular (i.m.), intravenous (i.v.) or intracutaneous (i.e.) application. In particular, it is possible to produce the respectively suitable injection formulations as a suspension of crystals, solutions, nanoparticulate or a colloid dispersed systems like, e.g. hydrosols.

Injectable formulations can further be produced as concentrates, which can be dissolved or dispersed with aqueous isotonic diluents. The infusion can also be prepared in form of isotonic solutions, fatty emulsions, liposomal formulations and micro-emulsions. Like injectables, infusion formulations can also be prepared in the form of concentrates for dilution. Injectable formulations can also be applied in the form of permanent infusions both in in-patient and ambulant therapy, e.g. by way of mini-pumps.

It is possible to add to parenteral drug formulations, for example, albumin, plasma, expander, surface-active substances, organic diluents, pH-influencing substances, complexing substances or polymeric substances, in particular as substances to influence the adsorption of the TBDCs of the present disclosure to proteins or

polymers or they can also be added with the aim to reduce the adsorption of the TBDCs of the present disclosure to materials like injection instruments or packaging-materials, for example, plastic or glass.

Adjuvants and carriers added during the production of the pharmaceutical compositions of the present disclosure formulated as parenterals are preferably aqua sterilisata (sterilized water), pH value influencing substances like, e.g. organic or inorganic acids or bases as well as salts thereof, buffering substances for adjusting pH values, substances for isotonization like e.g. sodium chloride, sodium hydrogen carbonate, glucose and fructose, tensides and surfactants, respectively, and emulsifiers like, e.g. partial esters of fatty acids of polyoxyethylene sorbitans (for example, polyethylene glycol sorbitan monolaurate, commonly referred to as Tween®) or, e.g. fatty acid esters of polyoxyethylenes (for example, polyoxyethylated castor oil surfactant, commonly referred to as Cremophor®), fatty oils like, e.g. peanut oil, soybean oil or castor oil, synthetic esters of fatty acids like, e.g. ethyl oleate, isopropyl myristate and neutral oil (for example, Miglyol® or a mixture of medium-chain triglycerides) as well as polymeric adjuvants like, e.g. gelatine, dextran, polyvinylpyrrolidone, additives which increase the solubility of organic solvents like, e.g. propylene glycol, ethanol, N,N-dimethylacetamide, propylene glycol or complex forming substances like, e.g. citrate and urea, preservatives like, e.g. benzoic acid hydroxypropyl ester and methyl ester, benzyl alcohol, antioxidants like e.g. sodium sulfite and stabilizers like e.g. EDTA.

When formulating the pharmaceutical compositions of the present disclosure as suspensions in a preferred embodiment thickening agents to prevent the setting of the TBDCs of the present disclosure or, tensides and polyelectrolytes to assure the resuspendability of sediments and/or complex forming agents like, for example, EDTA are added. It is also possible to achieve complexes of the active ingredient with various polymers. Examples of such polymers are polyethylene glycol, polystyrene, carboxymethyl cellulose, Pluronics® or polyethylene glycol sorbitol fatty acid ester. The TBDCs of the present disclosure can also be incorporated in liquid formulations in the form of inclusion compounds e.g. with cyclodextrins. In particular embodiments dispersing agents can be added as further adjuvants. For the production of lyophilisates scaffolding agents like mannite, dextran, saccharose, human albumin, lactose, PVP or varieties of gelatine can be used.

In light of the above, it will be appreciated that the second and third aspects of the present disclosure also encompass methods of treating cancer, preferably of treating gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer, wherein the method comprises administering a therapeutically effective amount of the antibody-drug conjugate of the first aspect or of the pharmaceutical composition of the third aspect to a patient in need thereof. Similarly, as the second aspect of the present disclosure also encompasses use of the antibody-drug conjugate of the first aspect in the manufacture of a medicament for the treatment of cancer, preferably for the treatment of gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer.

Again, in such embodiments, the gastrointestinal cancer is typically characterized by the expression of a target sequence or epitope of a Guanylate Cyclase 2C (GUCY2C) antigen.

Sequences

Table 1: Amino acid sequences of the present disclosure

SEQ ID NO	Sequence	Description
1	RASQSISSYLN	mAb1 CDRL1
2	AASSLQS	mAb1 CDRL2
3	QQSYSTPWT	mAb1 CDRL3
4	SSAMH	mAb1 CDRH1
5	LINPGNGNTKYSQKFQG	mAb1 CDRH2
6	AYFRGLGFDI	mAb1 CDRH3
7	QQSYSTPLT	mAb8 CDRL3
8	TGTSSDVGSYNLVS	mAb41 CDRL1
9	EGSKRPS	mAb41 CDRL2
10	SYVPRSSLV	mAb41 CDRL3
11	TYTIN	mAb41 CDRH1
12	RIIPVLGIANYAQKFQG	mAb41 CDRH2
13	DTPRLRSSYYMDV	mAb41 CDRH3
14	DIQMTQSPSSLSASAGDRVITC	mAb1 FR _L 1
15	WYQQKPGKAPKLLIY	mAb1 FR _L 2
16	GVPSRFSGSGSGTDFTLAISSLQPEDFATYYC	mAb1 FR _L 3
17	FGQGTKVDIK	mAb1 FR _L 4
18	QVQLVQSGAEVKKPGASVKVSCKASGYIFI	mAb1 FR _H 1
19	WVRQAPGQRLEWMG	mAb1 FR _H 2
20	RVTITRDTASTAYMELSSLRSEDTAVYYCAR	mAb1 FR _H 3
21	WGQGTLVTVSS	mAb1 FR _H 4

22	DIRMTQSPSSLSASAGDRVITIC	mAb8 FR _L 1
23	GVPSRFSGSGSGTDFTLTISLQPEDFATYYC	mAb8 FR _L 3
24	FGGGTKLEIK	mAb8 FR _L 4
25	QSALTQPASVSGSPGQSITISC	mAb41 FR _L 1
26	WYQQHPGKAPKLMY	mAb41 FR _L 2
27	GVSNRFSASKSGNTASLTISGLQTEDEADYYC	mAb41 FR _L 3
28	FGGGTKLTVL	mAb41 FR _L 4
29	QVQLVQSGAEVKKPGSSVKVSKASGGTFS	mAb41 FR _H 1
30	WVRQAPGQGLEWMG	mAb41 FR _H 2
31	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR	mAb41 FR _H 3
32	DIQMTQSPSSLSASAGDRVITICRASQSISSYL NWKYQQKPGKAPKLLIYAASSLQSGVPSRFSGS GSGTDFTLAISLQPEDFATYYCQQ SYSTPWTFGQGTKVDIK	mAb1 V _L
33	QVQLVQSGAEVKKPGASVKVSKASGYIFISS AMHWVRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTSASTAYMELSSLRSEDTAVYY CARAYFRGLGFDIWGQGTLLTVSS	mAb1 V _H
34	DIRMTQSPSSLSASAGDRVITICRASQSISSYL NWKYQQKPGKAPKLLIYAASSLQSGVPSRFSGS GSGTDFTLTISLQPEDFATYYCQQSYSTPLTF GGGKLEIK	mAb8 V _L
35	QVQLVQSGAEVKKPGASVKVSKASGYIFISS AMHWVRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTSASTAYMELSSLRSEDTAVYY CARAYFRGLGFDIWGQGTLLTVSS	mAb8 V _H
36	QSALTQPASVSGSPGQSITISCTGTSSDVGSYN LVSWYQQHPGKAPKLMYEGSKRPSGVSNRF SASKSGNTASLTISGLQTEDEADYYCSSYVPRS SLVFGGGTKLTVL	mAb41 V _L
37	QVQLVQSGAEVKKPGSSVKVSKASGGTFST YTINWVRQAPGQGLEWMGRIIPVLGIANAYAK FQGRVTITADKSTSTAYMELSSLRSEDTAVYYC ARDTPRLRSSYMDVWGQGTLLTVSS	mAb41 V _H
38	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTCTPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPGK	HC (wild type)
39	QVQLVQSGAEVKKPGASVKVSKASGYIFISS AMHWVRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTSASTAYMELSSLRSEDTAVYY CARAYFRGLGFDIWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS	mAb1 heavy chain (HC) comprising the mutation D265C (according to EU numbering system)

	WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVCVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	
40	QVQLVQSGAEVKKPGASVKVSCASGYIFISS AMHWWRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTASTAYMELSSLRSEDTAVYY CARAYFRGLGFDIWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVCVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	mAb8 HC comprising the mutation D265C (according to EU numbering system)
41	QVQLVQSGAEVKKPGSSVKVSCASGGTFST YTINWWRQAPGQGLEWMGRIIPVLGIANAYAK FQGRVTITADKSTSTAYMELSSLRSEDTAVYYC ARDTPRLRSSYMDVWGQGTLLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVCVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK	mAb41 HC comprising the mutation D265C (numbering according to EU numbering system)
42	QVQLVQSGAEVKKPGASVKVSCASGYIFISS AMHWWRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTASTAYMELSSLRSEDTAVYY CARAYFRGLGFDIWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVCVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLN	mAb1 HC comprising the mutations L234A, L235A and D265C (numbering according to EU numbering system)

	GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK	
43	QVQLVQSGAEVKKPGASVKVSKASGYIFISS AMHWVRQAPGQRLEWMGLINPGNGNTKYSQ KFQGRVTITRDTSASTAYMELSSLRSEDNAVYY CARAYFRGLGFDIWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLM ISRTPEVTCVVCVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK	mAb8 HC comprising the mutations L234A, L235A and D265C (numbering according to EU numbering system)
44	QVQLVQSGAEVKKPGSSVKVSKASGGTFST YTINWVRQAPGQGLEWMGRIIPVLGIANYAQK FQGRVTITADKSTSTAYMELSSLRSEDNAVYYC ARDTPRLRSSYMDVWGQGTLLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVCVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKMABLALADC	mAb41 HC comprising the mutations L234A, L235A and D265C (numbering according to EU numbering system)
45	QSALTQPASVSGSPGQSITISCTGTSSDVGSYN LVSWYQQHPGKAPKLMIEGSKRPSGVSNRF SASKSGNTASLTISGLQTEDEADYYCSSYVPRS SLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTVAWKADSSPVKAGV ETTPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS	mAb41 light chain (LC)
46	DIRMTQSPSSLSASAGDRVITICRASQSISSYL NWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS GSGTDFTLTISLQPEDFATYYCQQSYSTPLTF GGGKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC	mAb8 light chain (LC)

47	DIQMTQSPSSLSASAGDRVITCRASQSISSYL NWWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS GSGTDFTLAISLQPEDFATYYCQQSYSTPWT FGQGTKVDIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC	mAb1 light chain (LC)
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EXAMPLES

The present disclosure is further described by the following non-limiting Examples.

General information

While the present disclosure has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the present disclosure is not limited to the disclosed embodiments.

All amino acid sequences disclosed herein are shown from N-terminus to C-terminus; all nucleic acid sequences disclosed herein are shown 5'→3'.

Chemicals and solvents were obtained from commercial suppliers such as ABCR, Fisher Scientific, Merck Chemicals, Carl Roth, Sigma-Aldrich, VWR International, Biozol Diagnostika, TCI Deutschland, IRIS Biotech, AnalytiChem, Hycultec, Hölzel Diagnostika, Bachem, Thermo Fisher, Activate Scientific, AxisPharm, Biosynth or BLD Pharmatech and were used without further purification. The water used for reactions, workups and purifications was purified by an ELGA Purelab flex 2 pure water system. TLC was performed with silica gel 60-coated polyester sheets (Macherey-Nagel) and spots visualised by irradiation ($\lambda = 254$ nm), or ninhydrin, molybdate or KMnO_4 staining solutions.

General experimental methods

Analytical HPLC

Analytical RP-HPLC was carried out on a VWR-Hitachi Chromaster system equipped with a diode array detector 5430, autosampler 5260, quaternary pump 5160, column oven 6310 and a Luna® C18 (2) 5 μm 100 Å 250 x 4.6 mm, Kinetex® EVO-C18 5 μm 100 Å 250 x 4.6 mm and ACE C18-PFP 5 μm 100 Å 250 x 4.6 mm at 25 °C with a flowrate between 1 - 1.4 ml/min using linear gradient elution of acetonitrile in water. The specified parameters are stated below.

Method A

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	22 °C		
Run Time	22 min		

Method B

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	0.5	70	30
	15.5	50	50
	16.0	0	100
	19.0	0	100
	19.5	95	5
	22.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	22 °C		
Run Time	22 min		

Method C

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	246, 210 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method D

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method E

Column	Avantor ACE C18-PFP, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	2.0	70	30
	26.0	40	60
	26.5	0	100
	28.0	0	100
	28.5	95	5
	30.0	95	5
Wavelengths	210, 380 nm		
Flow Rate	1 mL/min		
Column Temperature	25 °C		
Run Time	30 min		

Method F

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	2.0	70	30
	26.0	40	60
	26.5	0	100
	28.0	0	100
	28.5	95	5
	30.0	95	5
Wavelengths	210, 380 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	30 min		

Method G

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: Water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	14.8	50	50
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	190, 210 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method H

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	14.8	50	50
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.00	95	5
Wavelengths	210, 246 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method I

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	210, 305 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method J

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	26.0	15	85
	26.5	0	100
	28.0	0	100
	28.5	95	5
	30	95	5
Wavelengths	210, 380 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	30 min		

Method K

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	14.8	70	30
	15.0	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	210, 230 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method L

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	25.0	40	60
	25.2	0	100
	27.8	0	100
	28.0	95	5
	30.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	30 min		

Method M

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	15.0	80	20
	15.2	0	100
	18.0	0	100
	18.5	95	5
	22.0	95	5
Wavelengths	205, 220 nm		
Flow Rate	1.4 mL/min		
Column Temperature	25 °C		
Run Time	22 min		

Method N

Column	Phenomenex Luna C18(2), 100 Å, 250 x 4.6 mm, 5 or 10 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	0.5	80	20
	15.5	60	40
	16.0	0	100
	19.0	0	100
	19.5	95	5
	22.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	22 °C		
Run Time	22 min		

Method O

Column	Phenomenex Kinetex EVO-18, 100 Å, 250 x 4.6 mm, 5 µm		
Mobile Phase	A: 0.05 % TFA in water B: Acetonitrile		
Gradient	Time [min]	A [%]	B [%]
	0.0	95	5
	0.5	80	20
	15.5	60	40
	16.0	0	100
	19.0	0	100
	19.5	95	5
	22.0	95	5
Wavelengths	210, 260 nm		
Flow Rate	1.4 mL/min		
Column Temperature	22 °C		
Run Time	22 min		

NMR spectroscopy

¹H and spectra were recorded at 500 MHz at room temperature and were referenced to trimethylsilane (TMS). Chemical shifts (δ) are reported in parts per million (ppm) from low field to high field. Coupling constants (*J*) are reported in Hertz (Hz) and abbreviations indicating multiplicity are used as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet.

Mass spectrometry (MS)

MS measurements were carried out on an Advion expression® CMS. Samples were submitted by direct injection with a syringe. For ionization, an electrospray ionization source (ESI) or atmospheric pressure ionization source (APCI) was used. Analyses were performed in positive ion mode. For ESI, the capillary temperature was set at 250°C. The source gas temperatures were set at 250 and 300°C, respectively with a flow of 4 and 5 L/min, respectively. Nebulizing gas flow was set to 0.5 L/min. The electrospray voltage was set at 3.5 kV. For APCI, the capillary temperature was set at 250°C, source gas temperature at 350°C and APCI corona discharge at 5 μ A was used.

Preparative RP-HPLC purification

Preparative RP-HPLC purification was carried out on an Agilent 1260 Infinity II system equipped with a binary pump, multisampler, VWD, fraction collector and column organizer. As columns, Phenomenx Luna® C18 (2) 10 μ m 100 Å 250 x 21.2 mm, Kinetex® EVO-C18 5 μ m 100 Å 250 x 21.2 mm and ACE C18-PFP 5 μ m 100 Å 250 x 21.2 mm with flow rates between 24 - 30 ml/min using linear gradient elution of acetonitrile in water (+0.05 % TFA).

Flash chromatography

Purification by flash chromatography on silica was performed using a Teledyne ISCO CombiFlash NextGen 300+ equipped with UV and ELSD detection and RediSep® single-use normal-phase silica columns with mesh between 230 and 400.

General Procedure 1 (GP1): CuAAC click reaction for attachment of solubility enhancer

To a stirred solution of azide (0.9-1.5 equiv.) and alkyne (1 equiv.) in DMF was added DMF:water (5:1 or 10:1) containing tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amin)

(TBTA, 0.125-0.625 equiv.) or tris(3-hydroxypropyltriazolymethyl)amine (THPTA, 0.125-2 equiv.), copper(II) sulfate pentahydrate (0.1-1 equiv.) and Na-ascorbate (0.25-3.0 equiv.). The resulting reaction mixture was allowed to stir at room temperature until completion. Upon completion, the reaction mixture was purified directly without any pretreatment by prep HPLC to yield the product after lyophilization.

General Procedure 2 (GP2): CuAAC click reaction on polyazide scaffold

To a stirred solution of polyazide (1 equiv.) and alkyne (1.05-1.25 equiv. per azide moiety) in DMF was added water containing Tris(3-hydroxypropyltriazolymethyl)amine (THPTA, 0.63-1.35 equiv. per azide moiety), copper(II) sulfate pentahydrate (0.525-0.65 equiv. per azide moiety) and Na-ascorbate (1-2.15 equiv. per azide moiety). The resulting ratio of DMF/water was 5:1 or 10:1. The resulting reaction mixture was allowed to stir at room temperature until completion. Upon completion, the reaction mixture was purified directly without any pretreatment by prep HPLC to yield the multimeric linker-payload after lyophilization.

General Procedure 3 (GP3): Attachment of intermediate 3.1 or 3.2

Fmoc-protected linker-payload was treated with diethylamine (Et_2N , 20-24 equiv.) in DMF at room temperature and the reaction progress followed by RP-HPLC. The solvent was removed *in vacuo* and the residue co-evaporated once with DMF. Then, the residue was dissolved in DMF under argon and a solution of **intermediate 3.1 or 3.2** (1.1-1.5 equiv.) in DMF was added followed by *N*-ethyldiisopropylamine (DIPEA, 1.4-3 equiv.). The solution was stirred at room temperature and the progress followed by RP-HPLC. Upon completion, the solvent was evaporated, and the crude product purified by RP-HPLC.

General Procedure 4 (GP4): Attachment of maleimide

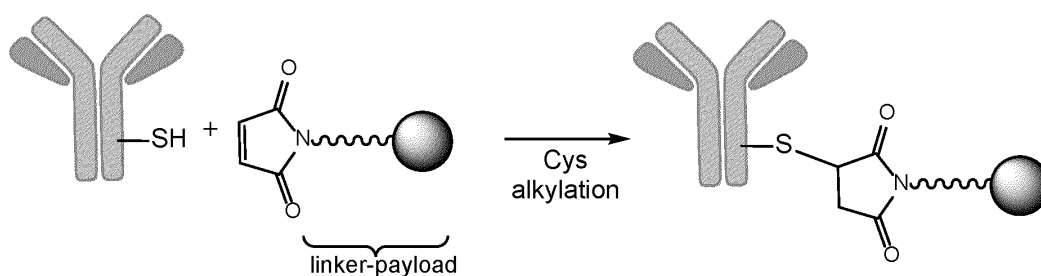
To a stirred solution of multimeric linker-payload in dry DMF was added Mal-PEG(4)-NHS ester (1-4 equiv.) and DIPEA (1-4 equiv.). The reaction mixture was stirred at room temperature for 30 min. Upon completion, the reaction mixture was purified directly without any pretreatment by prep HPLC to yield the final compound after lyophilization.

General Procedure 5 (GP5): Attachment of PEG-azide to Mono-6-amino-6-deoxy-cyclodextrin

Azido-PEG-NHS ester (1 equiv.) was dissolved in water or DMF and a solution of mono-6-amino-6-deoxy-cyclodextrin (1 equiv.) in water or DMF was added. In case DMF was used as solvent, DIPEA (1.5-2 equiv.) was added if necessary. The resulting reaction mixture was allowed to stir at room temperature until completion and monitored by RP-HPLC. If necessary, another portion of mono-6-amino-6-deoxy-cyclodextrin (0.2 equiv.) was added. Upon completion, the reaction mixture was freeze dried and used as crude without further purification in case water was used as solvent. In case DMF was used as solvent, the product was isolated by precipitation from MTBE. In case of mono-6-amino-6-deoxy-beta-cyclodextrin use, commercially available mono-6-amino-6-deoxy-beta-cyclodextrin was employed. In case of mono-6-amino-6-deoxy-gamma-cyclodextrin use, the material was synthesized from gamma-cyclodextrin according to Tang, W., Ng, S.-C. *Nat. Protoc.* 2008, 3, 691–697.

ADC generation - conjugation of linker payloads to antibodies

Antibodies were conjugated to the drug-carrying linker conjugates by means of the so-called Thiomab technology. In this approach, the conjugation takes place by coupling a terminal thiol-reactive moiety such as a maleimide residue of the drug-carrying linker (“linker-payload”) to the free SH group of a genetically-engineered cysteine residue in the Fc region of the antibody, as shown in the following reaction scheme:



The principles of this conjugation method are disclosed in Junutula *et al.* (2008), the content of which is incorporated herein by reference.

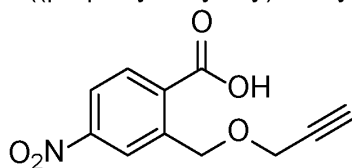
The anti-GUCY2C antibodies used in the present experiments comprise a D265C substitution in both Fc domains, to provide a cysteine residue that has a free SH

group. The respective technology is disclosed in WO2016/142049 A1, the content of which is incorporated herein by reference.

EXAMPLE 1 – Synthesis of modular linker compounds

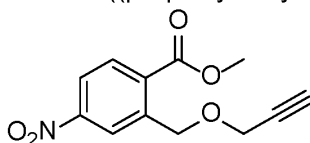
Example 1.1- Syntheses of Linker Intermediates

Step 1: Synthesis of 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid



To a solution of Methyl-2-(bromomethyl)-4-nitrobenzoate (CAS-Nr. 133446-99-8, 2 g, 7.30 mmol, 1 equiv.) in 10 ml acetonitrile were added prop-2-yn-ol (2.16 ml, 36.5 mmol, 5 equiv.) and Cs_2CO_3 (5.96 g, 18.3 mmol, 2.5 equiv.) at room temperature. The mixture was stirred at 80 °C for 3 h and the progress monitored by RP-HPLC. The reaction mixture was filtered. The filtrate was diluted with ethyl acetate (20 ml) and washed with water (10 ml) and extracted with NaHCO_3 (2x 10 ml). For precipitation, the combined aqueous layers were acidified to pH 2 by using 2 M HCl (50 ml). After filtration, the solid obtained was taken up in 1,4-dioxane and lyophilized overnight to yield 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid as pale red solid (1.44 g, 83 %). ^1H NMR (500 MHz, DMSO) δ 8.36 (d, J = 2.4 Hz, 1H), 8.22 (dd, J = 8.6, 2.5 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H), 4.94 (s, 2H), 4.36 (d, J = 2.4 Hz, 2H), 3.53 (t, J = 2.4 Hz, 1H). HPLC (method A): t_R = 11.3 min.

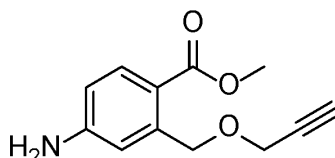
Step 2: Synthesis of Methyl- 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate



To a solution of 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoic acid (1.44 g, 6.12 mmol, 1 equiv.) in 30 ml dry methanol was added SOCl_2 (3.56 ml, 49 mmol, 8 equiv.) at 0°C. The mixture was stirred at 70°C for 3.5 h and the progress monitored by RP-HPLC. Upon completion, the solvent and the volatiles were removed under reduced pressure. The obtained residue was taken up in 1,4-dioxane and lyophilized overnight to yield methyl- 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate (1.52 g, 99%) as pale brown solid. ^1H NMR (500 MHz, DMSO) δ 8.37 (dd, J = 2.3, 1.1 Hz, 1H), 8.28 – 8.22

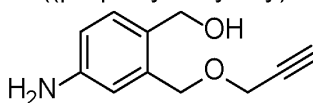
(m, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 4.92 (d, $J = 0.8$ Hz, 2H), 4.34 (d, $J = 2.4$ Hz, 2H), 3.90 (s, 3H), 3.49 (t, $J = 2.4$ Hz, 1H). HPLC (method A): $t_R = 13.1$ min.

Step 3: Synthesis of Synthesis of methyl 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate



To a stirred suspension of methyl- 4-nitro-2-((prop-2-yn-1-yloxy)methyl)benzoate (1.52 g, 6.1 mmol, 1 equiv.) in a mixture of EtOH (15 ml) and water (7.5 ml) was added iron powder (2.73 g, 48.8 mmol, 8 equiv.) and NH_4Cl (2.61 g, 48.8 mmol, 8 equiv.) at room temperature. The resulting mixture was allowed to stir at 80°C for 4 h and the progress monitored by RP-HPLC. After total consumption of the starting material, the reaction was cooled to rt and filtered through celite and washed with ethyl acetate (15 ml). The filtrate was washed with sat. sodium bicarbonate solution, water and brine (each 15 ml). The aqueous layers were extracted with ethyl acetate (15 ml). The combined organic layers were dried over MgSO_4 . After filtration the solvent was removed under reduced pressure to obtain 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate as red solid (1.29 g, 96 %) after drying *in vacuo*. ^1H NMR (500 MHz, CDCl_3) δ 7.87 (d, $J = 8.5$ Hz, 1H), 6.99 (dt, $J = 2.2, 1.0$ Hz, 1H), 6.59 (dd, $J = 8.5, 2.5$ Hz, 1H), 5.00 (s, 2H), 4.31 (d, $J = 2.4$ Hz, 2H), 3.84 (s, 3H), 2.48 (t, $J = 2.4$ Hz, 1H). HPLC (method A): $t_R = 10.5$ min.

Step 4: Synthesis of (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol



To a cooled solution (0°C) of dry THF (13 ml) was added LiAlH_4 (1 M in THF, 12.01 mmol, 12.01 ml, 2.5 equiv.) slowly. A solution of 4-amino-2-((prop-2-yn-1-yloxy)methyl)benzoate (1.06 g, 4.8 mmol, 1 equiv.) in dry THF (10 ml) was added dropwise at 0°C . After complete addition, the resulting mixture was allowed to stir overnight at rt. The reaction progress was monitored by RP-HPLC. The reaction mixture was poured into a vigorous stirred 1 M potassium sodium tartrate solution (120 ml) and stirred for another 30 min at rt. The reaction mixture was extracted with ethyl acetate (3x 30 ml). The combined organic layers were washed with water and

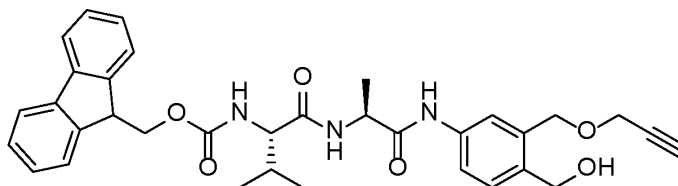
brine (each 30 ml). The organic layer was dried over MgSO_4 . The solvent was removed under reduced pressure to obtain crude product. Purification by ISCO SiO_2 flash chromatography (0-100 % ethyl acetate in hexane) yielded (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol (0.65 g, 71 %). ^1H NMR (500 MHz, DMSO) δ 6.99 (d, J = 8.1 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.45 (dd, J = 8.0, 2.4 Hz, 1H), 4.93 (s, 2H), 4.62 – 4.53 (m, 1H), 4.47 (s, 2H), 4.35 (d, J = 5.4 Hz, 2H), 4.15 (d, J = 2.4 Hz, 2H), 3.42 (t, J = 2.4 Hz, 1H). HPLC (method A): t_R = 4.8 min. MS (ESI): found m/z = 174.2 $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, calcd. m/z = 174.1

Step 5: Synthesis Fmoc-Val-Ala-OH

The synthesis of Fmoc-Val-Ala-OH was performed as disclosed in WO 2017/149077.

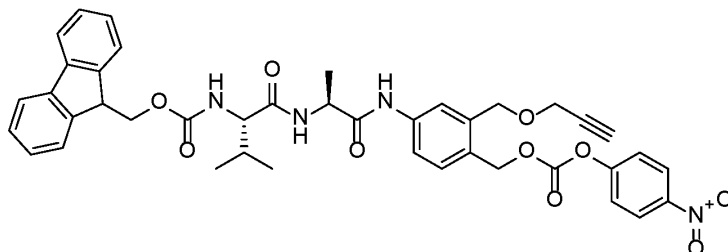
Example 1.2 - Syntheses of Linker-Payload Intermediates

Step 6: Synthesis of **intermediate 1.1**



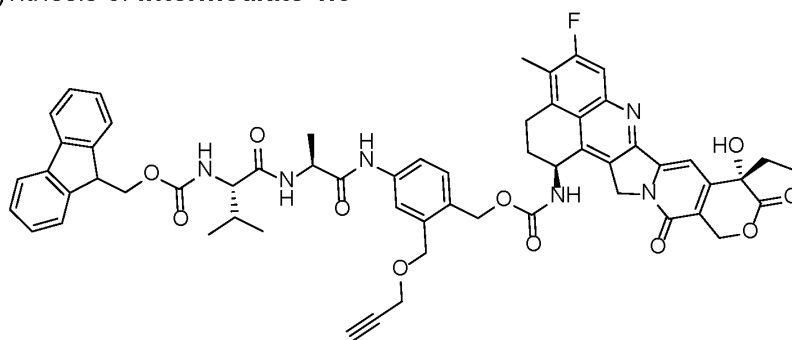
To a stirred solution of Fmoc-Val-Ala-OH (1.43g, 3.41 mmol, 1 equiv.) and (4-amino-2-((prop-2-yn-1-yloxy)methyl)phenyl)methanol (0.65 g, 3.41 mmol, 1 equiv.) in dry THF (20 ml) was added N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 1.05 g, 4.1 mmol, 1.2 equiv.) at room temperature. The resulting mixture was stirred at room temperature in the absence of light for 5 d. The solvent was removed under reduced pressure. The resulting crude intermediate was purified by flash chromatography on silica (gradient from 0-5 % MeOH in DCM) to yield **intermediate 1.1** as colorless solid (1.25 g, 63 %). HPLC (method A): t_R = 12.9 min. MS (ESI): found m/z = 601.2 $[\text{M}+\text{NH}_4]^+$, calcd. m/z = 601.3

Step 7: Synthesis of **intermediate 1.2**

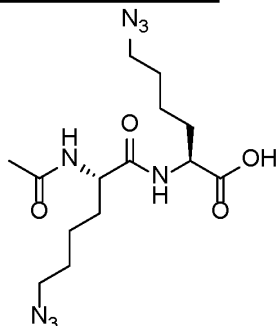


To a stirred solution of **intermediate 1.1** (0.72 g, 1.24 mmol) in dry DMF (15 ml) were added bis-4-nitrophenyl-carbonate (0.75 g, 2.48 mmol, 2 equiv.) and *N*-ethyldiisopropylamine (DIPEA, 3.1 mmol, 0.53 ml, 2.5 equiv.) under an argon atmosphere. The reaction was allowed to stir at room temperature for 4 h. After completion, the solvent was removed under reduced pressure. The crude intermediate was purified by flash chromatography on silica (gradient from 0-100 % ethylacetate in n-hexane) to yield **intermediate 1.2** (0.75 g, 81 %) as pale yellow solid after lyophilization from 1,4-dioxane:water (4:1). HPLC (method **C**): t_R = 13.4 min. MS (ESI): found m/z = 771.2 $[M+Na]^+$, calcd. m/z = 771.3

Step 8: Synthesis of **Intermediate 1.3**



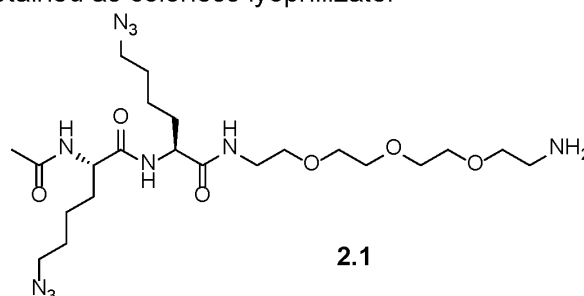
To a stirring suspension of exatecan mesylate (0.58g, 1.1 mmol, 1 equiv.) in dry DMF (20 ml) was added a freshly prepared solution of **intermediate 1.2** (0.82 g; 1.1 mmol, 1 equiv.) and 1-hydroxybenzotriazole (HOBt, 0.042 g, 0.275 mmol, 0.25 equiv.) in dry DMF (20 ml). DIPEA was added to the resulting mixture (0.28 g, 2.16 mmol, 0.37 ml, 2 equiv.) and 2,6-lutidine (141 mmol, 16.3 ml, 129 equiv.). The reaction mixture was stirred at room temperature overnight and the progress monitored by RP-HPLC. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica (gradient from 0-5 % MeOH in DCM) and lyophilization from 1,4-dioxane:water (4:1) yielded intermediate **1.3** (1.08 g, 95 %) as gray solid. HPLC (method **D**): t_R = 13.0 min. MS (ESI): found m/z = 1045.3 $[M+H]^+$, calcd. m/z = 1045.4

Example 1.3 - Synthesis of intermediate 2.1**Ac-Lys(N₃)-Lys(N₃)-OH**

2-chlorotrityl resin (4 g) were swollen for 10 min in abs. DCM (20 mL). The suspension was filtered, a solution of Fmoc-Lys(N₃)-OH (1.578 g, 4 mmol, 1 equiv.), DIPEA (3.48 mL, 20 mmol, 5 equiv.) in abs. DCM (20 mL) added and shaken for 2 h at room temperature. The suspension was filtered, the resin washed with DMF (3 x 1 min) and the capping solution (20 mL, MeOH/DIPEA/DCM 1/1/8) was added and shaken for 10 min at room temperature. The suspension was filtered, and the capping procedure repeated once. The resin was washed with DMF (3 x 1 min) and DCM (3 x 1 min) and the resin dried *in vacuo*. The loading of the resin was determined as described in *J. Pept. Sci.* **2017**, 23, 757-762.

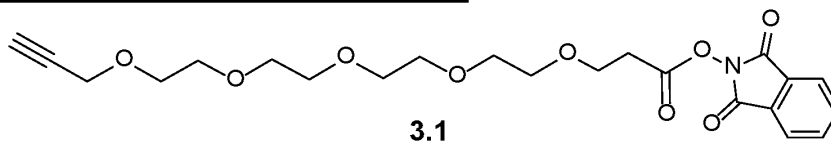
For the next step, the resin was swollen in DMF for 10 min. The suspension was filtered and a Fmoc-deprotection solution of 20 % piperidine in DMF added to the resin. The suspension was incubated for 20 min, filtered and the deprotection procedure repeated once. Then, the resin was washed with DMF (3 x 1 min) and DCM (3 x 1 min). The resin was swollen in DMF for 10 min, filtered and a solution of Fmoc-Lys(N₃)-OH (3 equiv.), TBTU (3 equiv.) and DIPEA (6 equiv.) in DMF added. The suspension was shaken for 2 h at room temperature. The resin was washed with DMF (3 x 1 min) and DCM (3 x 1 min) and the Fmoc-group removed with 20 % piperidine as described above. For acetylation, the resin was swollen in DMF for 10 min, the suspension filtered and a solution of acetic anhydride (3 equiv.) and pyridine (3 equiv.) in DMF added. The suspension was incubated for 2 h at room temperature, filtered, the resin washed with DMF (3 x 1 min) and DCM (3 x 1 min) and dried *in vacuo*.

For cleavage, the resin was treated with a solution of hexafluoroisopropanol in DCM (1:4, 20 mL) for 15 min. The solution is collected, and the resin treated again with hexafluoroisopropanol in DCM (1:4, 20 mL). The resin is washed with hexafluoroisopropanol in DCM (1:4) and the combined filtrates concentrated *in vacuo*. The resulting crude peptide was purified by RP-HPLC and **Ac-Lys(N₃)-Lys(N₃)-OH** (731 mg, 50 %) obtained as colorless lyophilizate.



Ac-Lys(N₃)-Lys(N₃)-OH (731 mg, 1.98 mmol, 1 equiv.) and HOBt (910 mg, 5.94 mmol, 3 equiv.) were dissolved in DMF (8 mL). DIC (920 μ L, 5.9 mmol, 3 equiv.) was added, the solution stirred for 5 min at room temperature after which a solution of Boc-Amino-PEG(3)-Amine (CAS-Nr. 101187-40-0, 868 mg, 2.97 mmol, 1.5 equiv.) in DMF (4 mL) was added followed by DIPEA (1.04 mL, 5.98 mmol, 3 equiv.). The reaction was stirred at room temperature and followed by RP-HPLC. After 20 h, the solution was concentrated and the residue dissolved in ethyl acetate. The organic layer was washed with 0.2 M citric acid (1 x 50 mL), sodium bicarbonate solution (3 x 50 mL) and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* giving 1.428 g of crude product. Purification by RP-HPLC followed by freeze drying in the presence of HCl (1 equiv.) for two times afforded **intermediate 2.1** (705 mg, 66 %) as HCl-salt. HPLC (method H): t_R = 11.2 min. MS (ESI): found m/z = 543.3 $[M+H]^+$, calcd. m/z = 543.3 $[M+H]^+$.

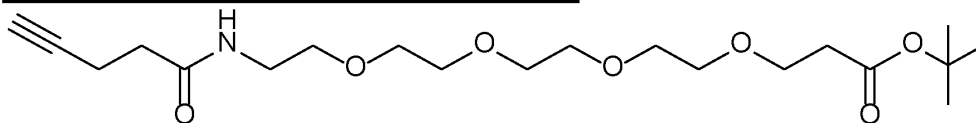
Example 1.4- Synthesis of intermediate 3.1



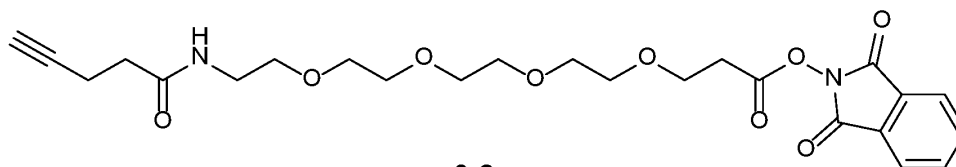
EDC x HCl (485.8 mg, 2.53 mmol, 1.3 equiv.) and DIPEA (1.09 mL, 6.24 mmol, 3.2 equiv.) were added to a solution of Propargyl-PEG(5)-acid (CAS-Nr. 1245823-51-1, 593.1 mg, 1.94 mmol, 1 equiv.) in abs. THF (8.9 mL). A solution of *N*-hydroxyphthalimide (413.4 mg, 2.53 mmol, 1.3 equiv.) in abs. THF (8.9 mL) was

added dropwise for 10 minutes. The reaction was stirred at room temperature and monitored by TLC and RP-HPLC. After 21 h, the solution was diluted with ethyl acetate (60 mL) and the organic layer washed with 1N HCl (30 mL), sodium bicarbonate solution (30 mL) and brine (30 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting crude product (724.7 mg) was purified by flash chromatography on silica using a gradient from n-hexane to ethyl acetate. **Intermediate 3.1** (457.3 mg, 52 %) was obtained as yellowish oil. $C_{22}H_{27}NO_9$. 1H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.89 (dd, J = 5.5, 3.1 Hz, 2H), 7.80 (dd, J = 5.5, 3.1 Hz, 2H), 4.21 (d, J = 2.3 Hz, 2H), 3.89 (t, J = 6.4 Hz, 2H), 3.73 – 3.63 (m, 17H), 2.97 (t, J = 6.4 Hz, 2H), 2.44 (t, J = 2.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 167.65, 161.73, 134.73, 128.82, 123.92, 79.61, 74.48, 70.69, 70.58, 70.54, 70.53, 70.49, 70.46, 70.33, 69.04, 65.76, 58.33, 32.14. HPLC (method I): t_R = 11.7 min. MS (ESI): found m/z = 472.2 $[M+Na]^+$, calcd. m/z = 472.2 $[M+Na]^+$.

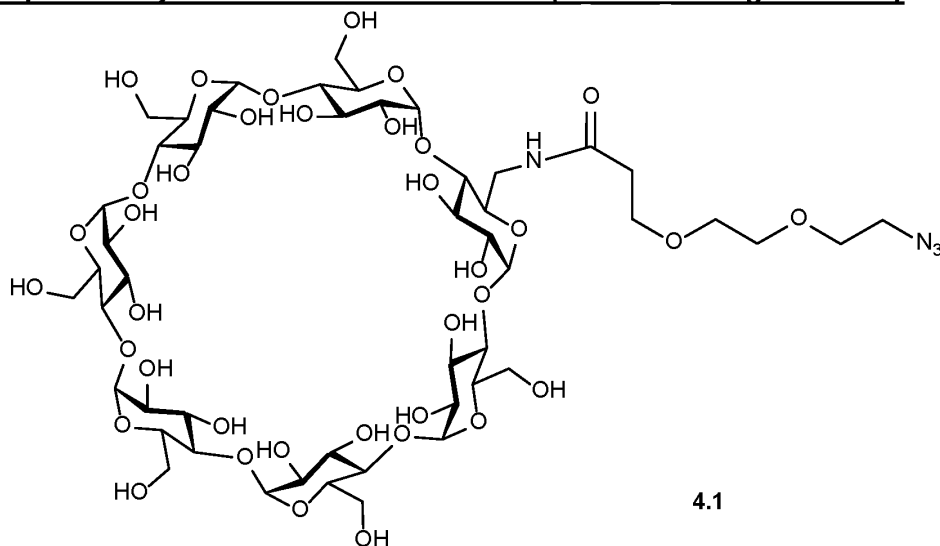
Example 1.5 - Synthesis of intermediate 3.2



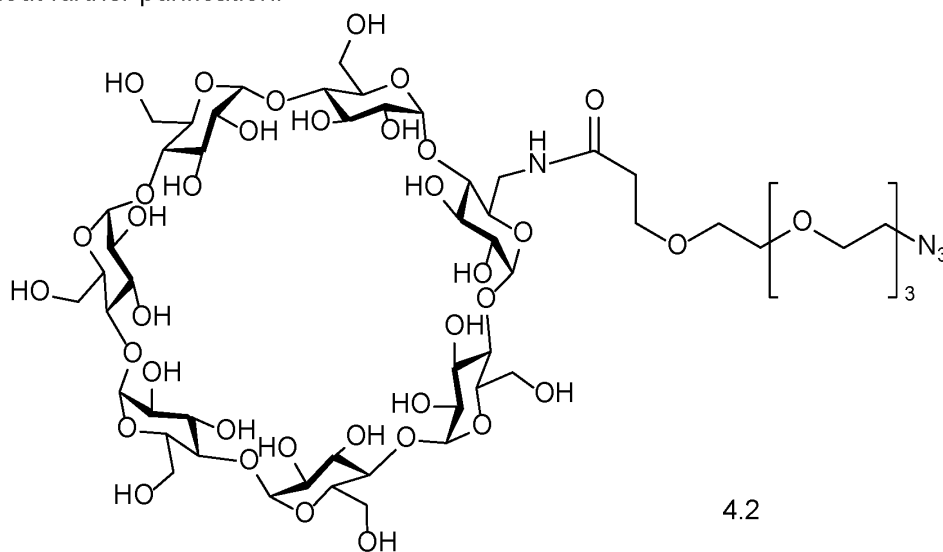
To a solution of pent-4-ynoic acid (2 g, 20.4 mmol, 1.05 equiv.) in DMF (40 mL) were added HATU (11.64 g, 30.6 mmol, 1.6 equiv.) and DIPEA (10.7 mL, 61.2 mmol) at 5 °C and the resulting solution stirred for 10 min at 5 °C. Then, a solution of *tert*-butyl 1-amino-3,6,9,12-tetraoxapentadecan-15-oate (6.24 g, 19.4 mmol, 1 equiv.) in DMF (10 mL) was added dropwise and the resulting solution allowed to warm to room temperature. After 2 h, the solution was concentrated *in vacuo* and the residue taken up in ethyl acetate (300 mL). The organic layer was washed with 0.2 M citric acid (150 mL), saturated sodium bicarbonate solution (150 mL) and brine (150 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Purification by flash chromatography on silica using a gradient from hexane to acetone afforded *tert*-butyl 1-(pent-4-ynamido)-3,6,9,12-tetraoxapentadecan-15-oate (8.20 g, quant.). $C_{20}H_{35}NO_7$. HPLC (method A): t_R = 10.6 min. MS (ESI): found m/z = 424.4 $[M+Na]^+$, calcd. m/z = 424.2 $[M+Na]^+$.

**3.2**

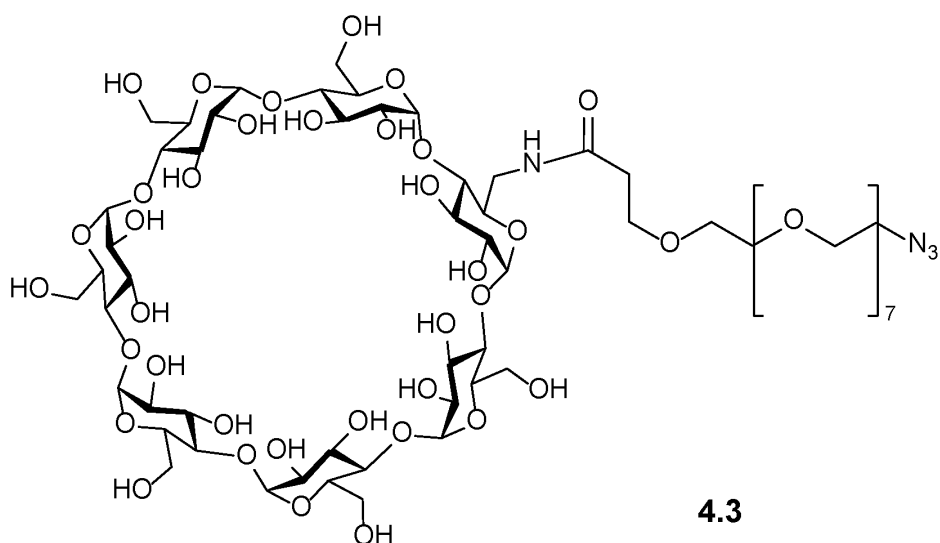
tert-Butyl 1-(pent-4-ynamido)-3,6,9,12-tetraoxapentadecan-15-oate (8.20 g, max. 19.4 mmol, 1 equiv.) was dissolved in TFA (15.6 mL) and stirred for 1 h at 300 mbar. TFA was removed *in vacuo* and the residue co-evaporated twice with toluene. The residue obtained was dissolved in THF (abs., 50 mL) and *N*-Ethyl-*N'*-carbodiimide hydrochloride (4.66 g, 24.3 mmol, 1.25 equiv.) as well as DIPEA (11 mL, 63 mmol, 3.25 mmol) were added. Then, a solution of *N*-hydroxyphthalimide (3.96 g, 24.3 mmol, 1.25 equiv.) in THF (abs., 50 mL) was added dropwise and the resulting reddish solution stirred overnight at room temperature. Ethyl acetate (400 mL) was added, and the organic layer washed with 2N HCl (100 mL), saturated sodium bicarbonate solution (100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Purification by flash chromatography on silica using a gradient from dichloromethane to acetone afforded **intermediate 3.2** (4.96 g, 52 %) as yellowish oil. C₂₄H₃₀N₂O₉. ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 8.01 – 7.89 (m, 4H), 7.87 (s, 1H), 3.77 (t, *J* = 6.0 Hz, 2H), 3.63 – 3.46 (m, 12H), 3.40 (t, *J* = 5.9 Hz, 2H), 3.20 (q, *J* = 5.8 Hz, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.70 (t, *J* = 2.6 Hz, 1H), 2.39 – 2.31 (m, 2H), 2.31 – 2.23 (m, 2H). ¹³C NMR (126 MHz, DMSO-d₆): δ (ppm) = 170.18, 168.21, 161.61, 135.39, 128.10, 123.85, 83.65, 71.00, 69.73, 69.68, 69.62, 69.60, 69.50, 69.01, 65.18, 38.50, 34.02, 31.55, 14.09. HPLC (method **A**): *t_R* = 10.5 min. MS (ESI): found *m/z* = 491.4 [M+H]⁺, calcd. *m/z* = 491.2 [M+H]⁺.

Example 1.6 - Synthesis of intermediates 4.x (N_3 -PEG_n-beta/gamma-CD)

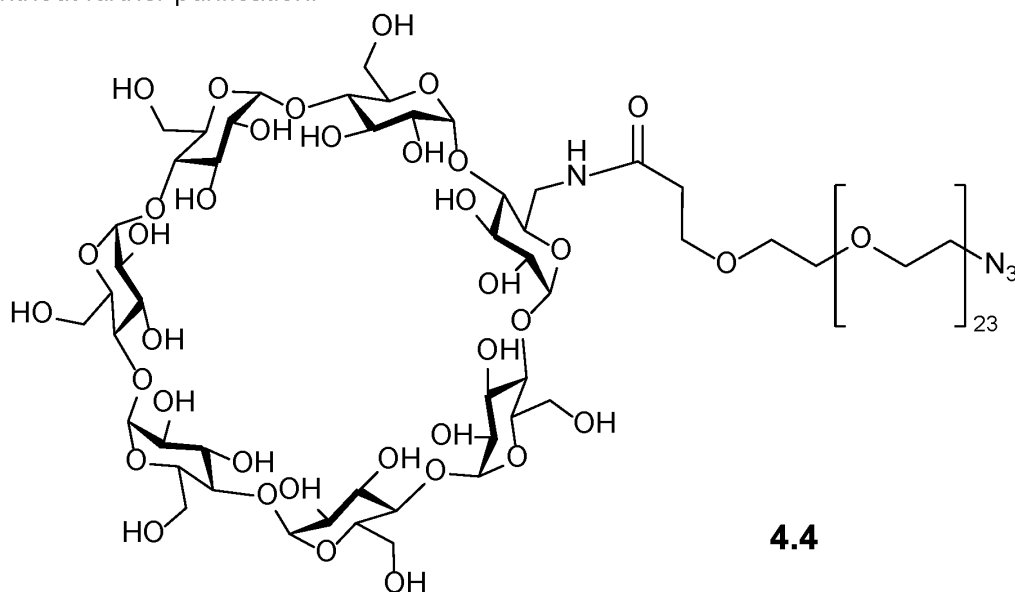
Intermediate 4.1 was synthesized according to GP5 using azido-PEG(2)-NHS ester and 6-amino-6-deoxy-beta-cyclodextrin. The obtained crude product was used without further purification.



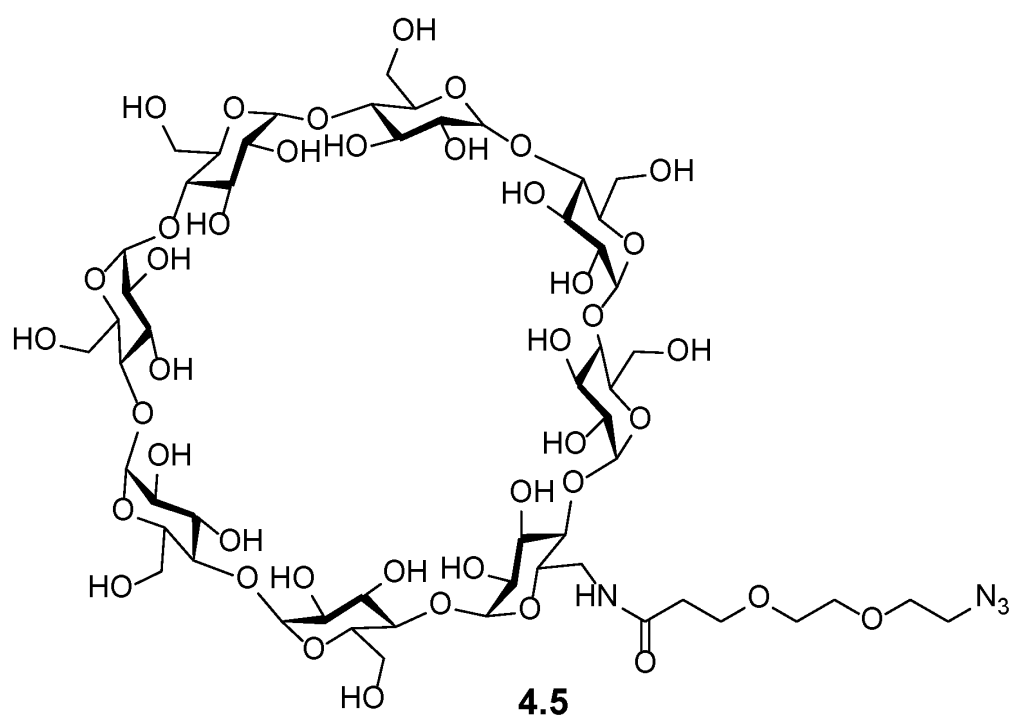
Intermediate 4.2 was synthesized according to GP5 using azido-PEG(4)-NHS ester and 6-amino-6-deoxy-beta-cyclodextrin. The obtained crude product was used without further purification.

**4.3**

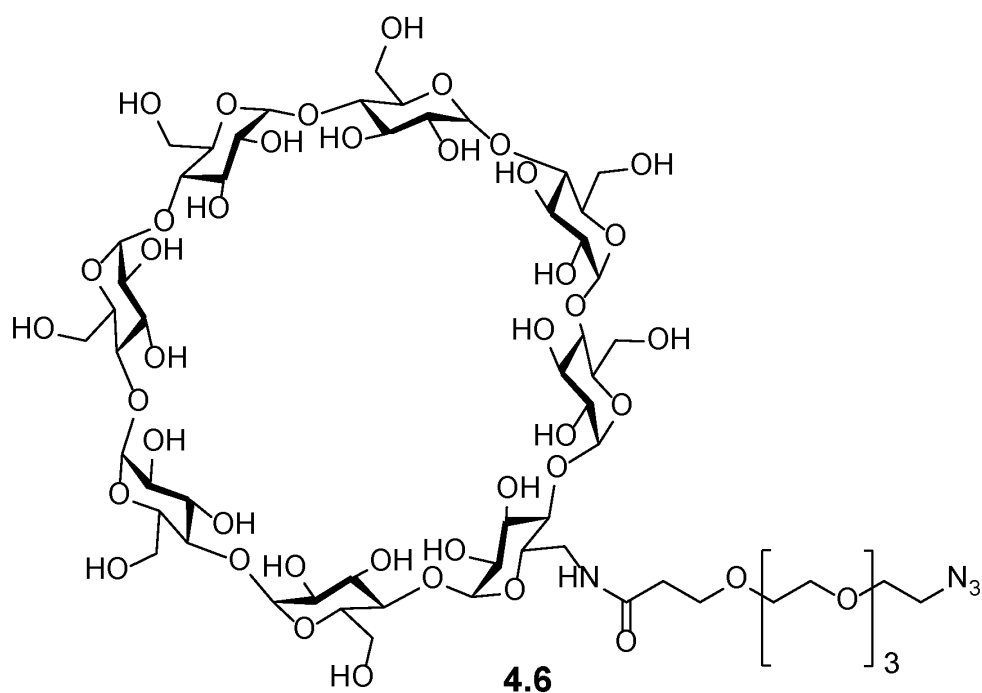
Intermediate 4.3 was synthesized according to GP5 using azido-PEG(8)-NHS ester and 6-amino-6-deoxy-beta-cyclodextrin. The obtained crude product was used without further purification.

**4.4**

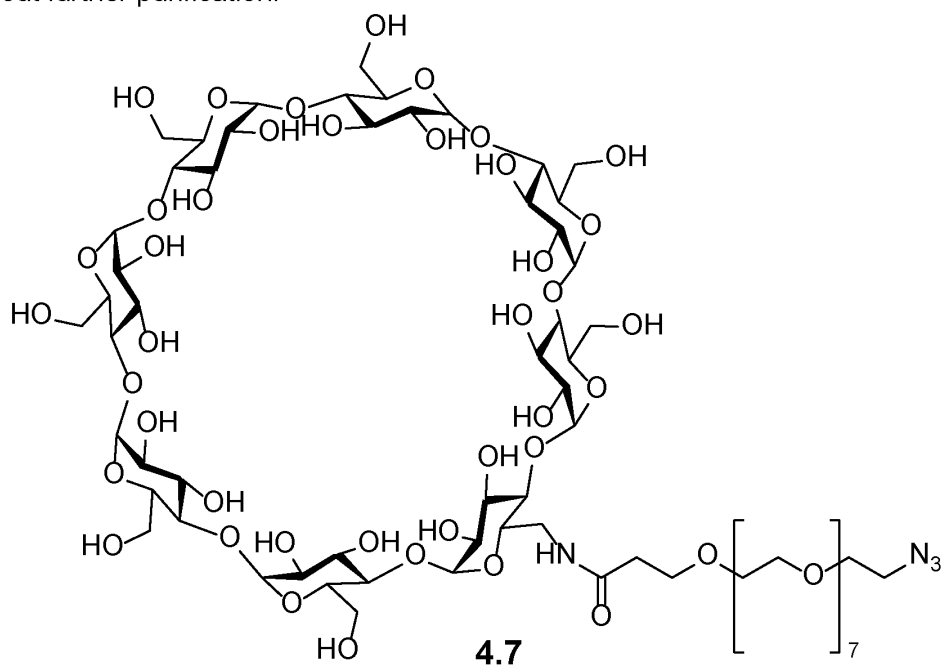
Intermediate 4.4 was synthesized according to GP5 using azido-PEG(24)-NHS ester and 6-amino-6-deoxy-beta-cyclodextrin. The obtained crude product was used without further purification.



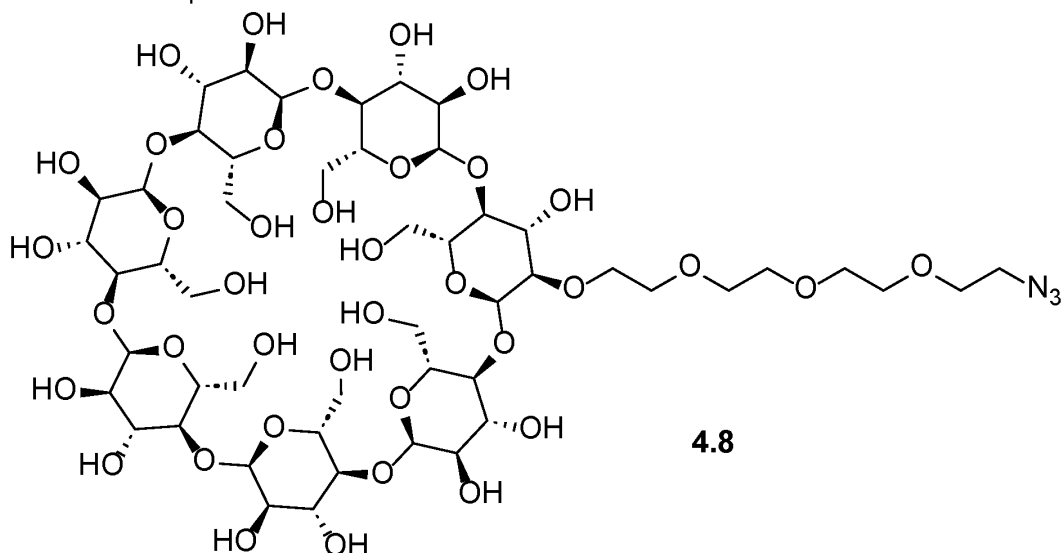
Intermediate 4.5 was synthesized according to GP5 using azido-PEG(2)-NHS ester and 6-amino-6-deoxy- γ -cyclodextrin. The obtained crude product was used without further purification.



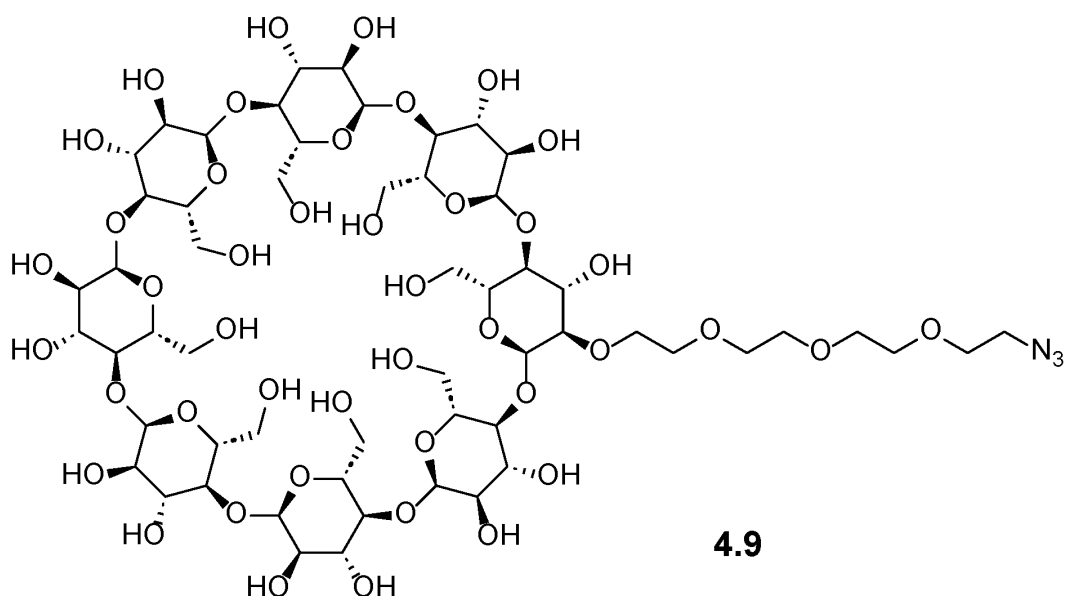
Intermediate 4.6 was synthesized according to GP5 using azido-PEG(4)-NHS ester and 6-amino-6-deoxy-γ-cyclodextrin. The obtained crude product was used without further purification.



Intermediate 4.7 was synthesized according to GP5 using azido-PEG(8)-NHS ester and 6-amino-6-deoxy- γ -cyclodextrin. The obtained crude product was used without further purification.

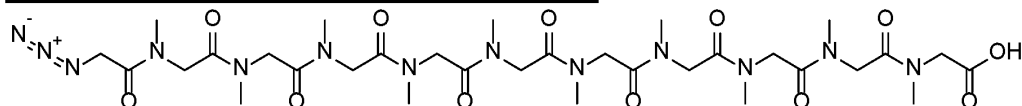


Beta-cyclodextrin (2.000 g, 1.762 mmol) was co-evaporated twice with 30 ml dry DMSO (abs.) and re-dissolved in 30 ml DMSO. Lithium hydride (21 mg, 2.643 mmol, 1.5 equiv.) was added and the suspension was stirred under Argon atmosphere for 15 h. Subsequently, 1-azido-2-{2-[2-(2-bromoethoxy)ethoxy]ethoxy}ethane (497 mg, 1.762 mmol, 1.0 equiv.) dissolved in 2 ml DMSO (abs.) was added, followed by Lithium iodide (24 mg 176 μ mol, 0.1 equiv.) and the mixture was stirred at 55°C for 7.5 h and then at room temperature. After TLC (acetonitrile/water/conc. ammonia 6:3:1) indicated no further conversion, the mixture was concentrated to approximately 6 ml by *in vacuo* and the remaining was added dropwise to 160 ml ice cooled acetone. The resulted precipitate was isolated by centrifugation, resuspended in 120 ml cold acetone by sonification and centrifuged again. After drying *in vacuo*, the solid was taken up in 5 ml water, insoluble beta-cyclodextrin removed by filtration and the filtrate was freeze-dried. The crude product (2.263 g) was acidified to pH 2 with trifluoroacetic acid and purified by prep. RP-HPLC to result in 532 mg (23 %) pure **intermediate 4.8** as colorless lyophilisate. MS (ESI): found m/z = 1336.8 $[M+H]^+$, calcd. m/z = : 1336.5 $[M+H]^+$.



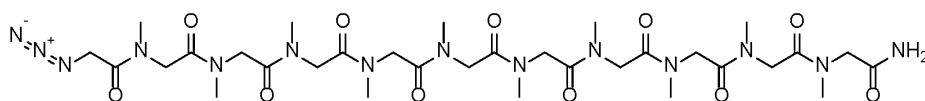
Intermediate 4.9 was synthesized according to **intermediate 4.8** using gamma-cyclodextrin. The target product was obtained in 22% yield as a colorless lyophilisate. MS (ESI): found m/z = 1498.9 [M+H]⁺, calcd. m/z = 1498.5 [M+H]⁺.

Example 1.7 - Synthesis of intermediates 5.x



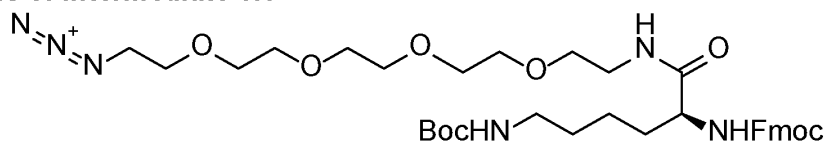
5.1

Intermediate 5.1 was synthesized according to WO2019081455 and WO2022207699 using Wang resin loaded with Fmoc-Sar, Fmoc-Sar-Sar-OH, Fmoc-Sar-OH and azidoacetic acid for *N*-terminal capping. Fmoc-deprotection was performed with 20 % piperidine in DMF for 15 min (twice). Amino acid coupling was performed using amino acid (3 equiv.), HATU (2.9 equiv.) and DIPEA (6 equiv.) in DMF. Cleavage from resin was performed using neat TFA (10 mL/g resin) followed by precipitation from ice-cold methyl *tert*-butyl ether and purification by RP-HPLC. **Intermediate 5.1** (166.7 mg, 41 %) was obtained as colourless solid. C₃₂H₅₃N₁₃O₁₂. HPLC (method H): t_R = 7.4 min. MS (ESI): found m/z = 834.2 [M+Na]⁺, calcd. m/z = 834.4 [M+Na]⁺.



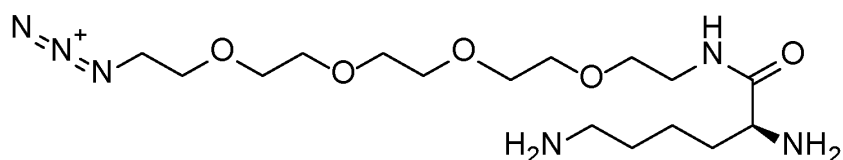
5.2

Intermediate 5.2 was synthesized according to WO2019081455 and WO2022207699 using NovaGel Rink-Amide resin loaded with Fmoc-Sar, Fmoc-Sar-Sar-OH, Fmoc-Sar-OH and azidoacetic acid for *N*-terminal capping. Fmoc-deprotection was performed with 20 % piperidine in DMF for 15 min (twice). Amino acid coupling was performed using amino acid (3 equiv.), HATU (2.9 equiv.) and DIPEA (6 equiv.) in DMF. Cleavage from resin was performed using TFA/TIS 95/5 (10 mL/g resin) followed by precipitation from ice-cold methyl *tert*-butyl ether and purification by RP-HPLC. **Intermediate 5.2** (83.6 mg, 54 %) was obtained as colourless solid. C₃₂H₅₄N₁₄O₁₁. HPLC (method K): *t_R* = 7.1 min. MS (ESI): found *m/z* = 811.5 [M+H]⁺, calcd. *m/z* = 811.4 [M+H]⁺.

Example 1.8 - Synthesis of intermediates 6.x**Synthesis of intermediate 6.1****Fmoc-Lys(Boc)-NH-PEG4-N₃**

To a solution of Fmoc-Lys(Boc)-OH (3.056 g, 6.5 mmol, 1 equiv.) in DMF (30 mL) were added TBTU (2.467 g, 7.8 mmol, 1.2 equiv) and DIPEA (1.33 mL, 7.8 mmol, 1.2 equiv.) and the resulting solution mixed for 1 min. Then, the pre-activated amino acid solution was added to a solution of 14-azido-3,6,9,12-tetraoxatetradecan-1-amine (1.680 g, 6.5 mmol, 1 equiv.) in DMF (10 mL) and the resulting solution stirred for 2 h at room temperature. Water (5 mL) was added, and the solution evaporated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL) and the organic layer washed with aqueous 0.2 M citric acid solution (50 mL), aqueous saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate and evaporated *in vacuo*. Purification by flash chromatography on silica using a gradient from MTBE to acetone afforded **Fmoc-Lys(Boc)-NH-PEG4-N₃** (3.545 g, 76 %) as colourless, sticky solid. C₃₆H₅₂N₆O₉. ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 7.88 (dt, *J* = 7.7, 0.9 Hz, 2H), 7.82 (t, *J* = 5.7 Hz, 1H), 7.76 – 7.68 (m,

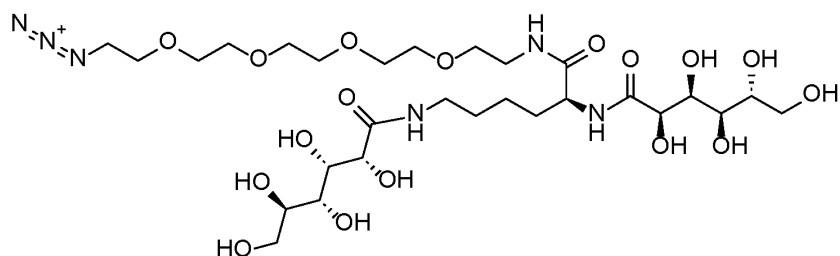
2H), 7.42 (td, $J = 7.5, 1.0$ Hz, 2H), 7.33 (tt, $J = 7.4, 1.2$ Hz, 3H), 6.68 (s, 1H), 4.30 – 4.18 (m, 3H), 3.93 (td, $J = 8.6, 5.2$ Hz, 1H), 3.62 – 3.56 (m, 2H), 3.55 – 3.48 (m, 12H), 3.41 (t, $J = 6.0$ Hz, 2H), 3.37 (dd, $J = 5.6, 4.4$ Hz, 2H), 3.21 (ddt, $J = 19.6, 13.6, 7.6$ Hz, 2H), 2.89 (q, $J = 10.3, 8.3$ Hz, 2H), 1.69 – 1.43 (m, 2H), 1.37 (s, 9H), 1.24 (s, 4H). ^{13}C NMR (126 MHz, DMSO- d_6): δ (ppm) = 171.86, 155.78, 155.45, 143.79, 143.70, 140.60, 127.49, 126.92, 125.17, 119.95, 77.21, 69.71, 69.69, 69.67, 69.63, 69.60, 69.48, 69.11, 68.88, 65.50, 54.56, 49.93, 46.62, 38.47, 31.63, 29.08, 28.16, 22.69. HPLC (method A): $t_R = 14.3$ min. MS (ESI): found $m/z = 735.4$ $[\text{M}+\text{Na}]^+$, calcd. $m/z = 735.4$ $[\text{M}+\text{Na}]^+$.



Lys-NH-PEG4-N₃

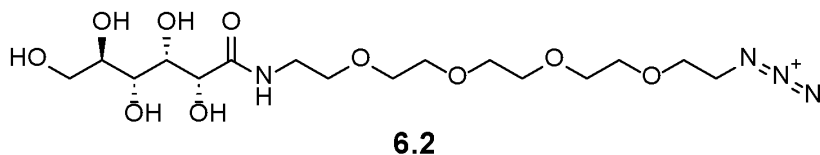
To a solution Fmoc-Lys(Boc)-NH-PEG4-N₃ (2.00 g, 2.8 mmol, 1 equiv.) in DMF (20 mL) was added diethylamine (5.00 mL, 56.1 mmol, 17 equiv.) and the resulting solution stirred for 30 min at 600 mbar at room temperature. The solvent was removed *in vacuo* and purification by flash chromatography on silica using a gradient from dichloromethane to dichloromethane/MeOH/conc. ammonia (9:1:0.1) afforded the Fmoc-deprotected intermediate H-Lys(Boc)-NH-PEG4-N₃ (1.182 g, 86 %) as colourless oil. Next, to a solution of H-Lys(Boc)-NH-PEG4-N₃ (1.163 g, 2.4 mmol, 1 equiv.) in 1,4-dioxane (20 mL) was added 1M HCl (11.85 mL, 11.8 mmol, 5 equiv.) and stirred for 10 min at 600 mbar at room temperature. The solvent was removed *in vacuo*, the residue dissolved in 1M HCl (11.85 mL, 11.8 mmol, 5 equiv.) and stirred for 10 min at 600 mbar at room temperature. The solvent was removed *in vacuo* and additionally two times co-evaporated with 1,4-dioxane. **Lys-NH-PEG4-N₃** (1.119 g, quant.) was obtained as highly viscous, colorless oil. C₁₆H₃₄N₆O₅. ^1H NMR (500 MHz, DMSO- d_6): δ (ppm) = 8.67 (t, $J = 5.6$ Hz, 1H), 8.32 (s, 3H), 8.11 (s, 3H), 3.78 – 3.72 (m, 1H), 3.61 – 3.58 (m, 2H), 3.59 – 3.47 (m, 12H), 3.45 (t, $J = 5.9$ Hz, 2H), 3.38 (dd, $J = 5.5, 4.4$ Hz, 2H), 3.28 (d, $J = 5.9$ Hz, 1H), 3.23 (dq, $J = 13.7, 5.8$ Hz, 1H), 2.73 (d, $J = 8.7$ Hz, 2H), 1.73 (q, $J = 7.3$ Hz, 2H), 1.59 (qd, $J = 8.5, 7.8, 6.0$ Hz, 2H), 1.42 – 1.33 (m, 2H). ^{13}C NMR (126 MHz, DMSO- d_6): δ (ppm) = 168.43, 69.74, 69.70, 69.66, 69.62, 69.49, 69.13, 68.63, 51.78, 49.97, 38.66, 38.11, 30.18, 26.08, 20.97. HPLC

(method H): t_R = 6.9 min. MS (ESI): found m/z = 391.3 $[M+H]^+$, calcd. m/z = 391.3 $[M+H]^+$.

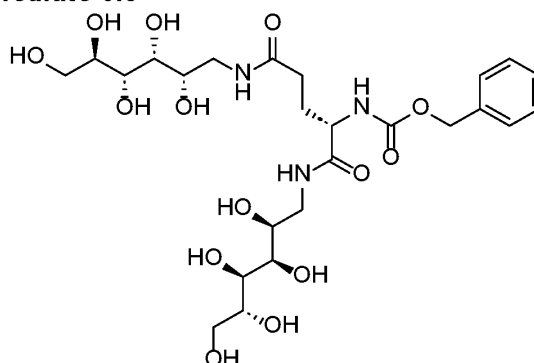


6.1

To a solution of Lys-NH-PEG4-N₃ (500 mg, 1.1 mmol, 1 equiv.) in DMF (10 mL) were added D-Glucono-1,5-lactone (481 mg, 2.7 mmol, 2.5 equiv.) and DIPEA (917 μ L, 5.4 mmol, 5.0 equiv.) and the resulting solution stirred for 15 h at room temperature followed by 1 h at 80°C. After 16.5 h reaction time in total, D-Glucono-1,5-lactone (481 mg, 2.7 mmol, 2.5 equiv.) and DIPEA (917 μ L, 5.4 mmol, 5.0 equiv.). After 21 h reaction time in total, the solvent was removed *in vacuo* and the residue co-evaporated with a mixture of methanol (10 mL) and DIPEA (917 μ L) followed by co-evaporation with methanol (10 mL). The crude product was precipitated from ice-cold MTBE. Purification by preparative HPLC afforded **intermediate 6.1** (294 mg, 36 %) as colorless, glass-like solid. C₂₈H₅₄N₆O₁₇. ¹H NMR (500 MHz, DMSO-d₆): δ (ppm) = 7.90 (t, J = 5.7 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.53 (t, J = 5.9 Hz, 1H), 4.41 (s, 10H), 4.22 (td, J = 8.4, 5.0 Hz, 2H), 4.06 (d, J = 3.5 Hz, 1H), 3.97 (d, J = 3.7 Hz, 1H), 3.93 – 3.86 (m, 3H), 3.62 – 3.56 (m, 4H), 3.56 – 3.43 (m, 14H), 3.43 – 3.35 (m, 4H), 3.20 (qd, J = 6.0, 3.5 Hz, 4H), 3.05 (dp, J = 13.9, 6.9 Hz, 2H), 1.73 – 1.64 (m, 1H), 1.56 (tdd, J = 13.5, 9.4, 5.3 Hz, 1H), 1.45 – 1.33 (m, 2H), 1.23 (s, 2H). ¹³C NMR (126 MHz, DMSO-d₆): δ (ppm) = 172.28, 172.17, 171.28, 73.50, 73.32, 72.36, 72.16, 71.46, 70.42, 70.10, 69.72, 69.64, 69.50, 69.14, 68.75, 63.29, 63.24, 51.99, 49.97, 38.52, 38.08, 31.75, 28.77, 22.46. HPLC (method M): t_R = 13.5 min. MS (ESI): found m/z = 747.4 $[M+H]^+$, calcd. m/z = 747.4 $[M+H]^+$.

Synthesis of intermediate 6.2

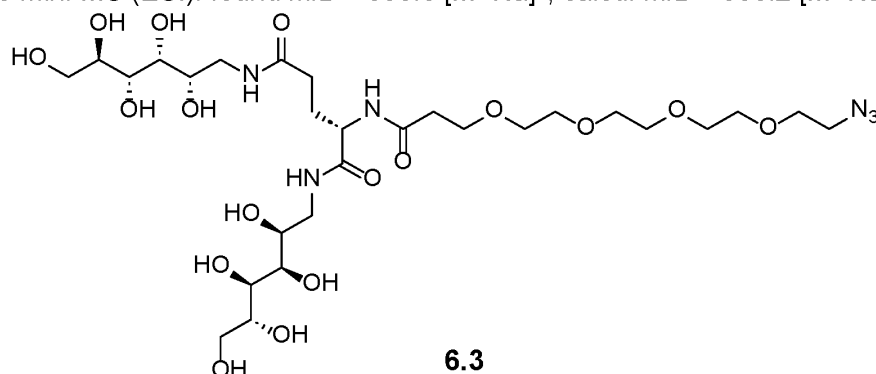
14-Azido-3,6,9,12-tetraoxatetradecan-1-amine (262 mg, 1.0 mmol, 1 equiv.) was dissolved in DMF (2 mL). D-Glucono-1,5-lactone (214 mg, 1.2 mmol, 1.2 equiv.) was added as solid followed by the addition of DIPEA (348 μ L, 2.0 mmol, 2 equiv.) and the resulting solution stirred for 2h at 110°C. After cooling to room temperature, the solvent was evaporated *in vacuo*. Purification by flash chromatography on silica using a gradient from dichloromethane to dichloromethane/methanol/water (5:1:0.1) afforded intermediate **6.1** (318 mg, 72 %). $C_{16}H_{32}N_4O_{10}$. HPLC (method M): t_R = 13.7 min. MS (ESI): found m/z = 441.3 $[M+H]^+$, calcd. m/z = 441.2 $[M+H]^+$.

Synthesis of intermediate 6.3**Z-L-Glu(NHDG)-NHDG**

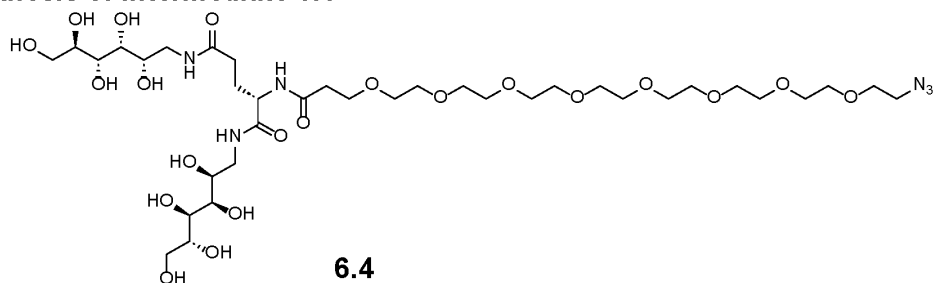
To a solution of Z-L-Glu-OH (2.50 g, 8.9 mmol, 1 equiv.) in THF (abs., 50 mL) was added *N*-hydroxy-succinimid (2.32 g, 20.2 mmol, 2.3 equiv.) and the solution cooled to 0°C. Then, *N,N'*-dicyclohexylcarbodiimide (3.85 g, 18.7 mmol, 2.1 equiv.) was added and the resulting suspension was stirred 2 h at 0°C followed by stirring overnight at room temperature. For work-up, the resulting urea was removed by filtration and the oily residue was dissolved in ethyl acetate (75 mL). The organic layer was washed with saturated aqueous sodium bicarbonate solution, brine and water, dried over magnesium sulfate and the solvent removed *in vacuo*. After treatment with

diethyl ether, Z-L-Glu(Osu)-Osu (3.68 g, 87 %) was obtained as intermediate, which was used without further purification.

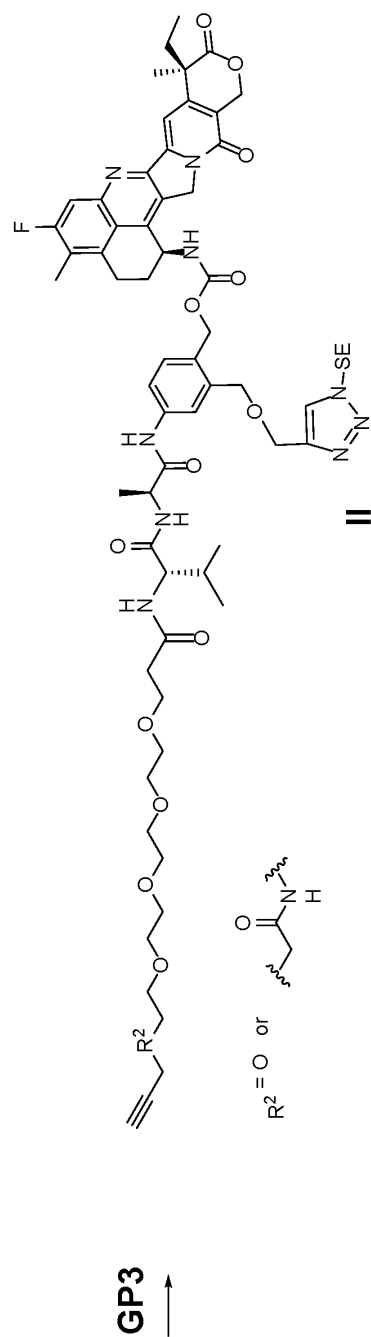
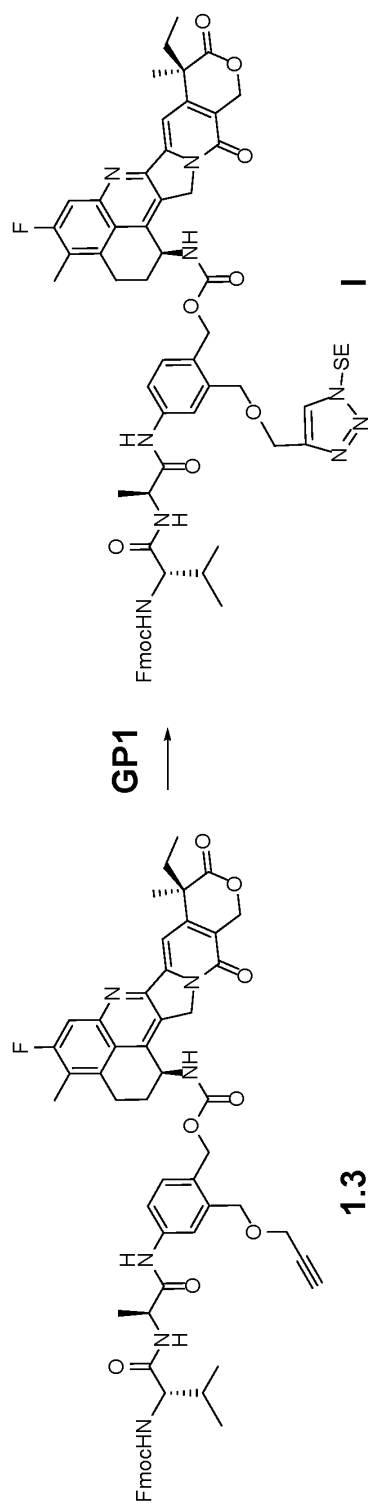
Next, to a solution of Z-L-Glu(Osu)-Osu (1.00 g, 2.1 mmol, 1 equiv.) in DMF (abs., 40 mL) was added D-glucamine (762.23 mg, 4.2 mmol, 2 equiv.) and the resulting suspension stirred for 3 h at 65°C. As the reaction progressed, the initial suspension became a solution. The solution was removed *in vacuo* and purification by RP-HPLC afforded **Z-L-Glu(NHDG)-NHDG** (933.9 mg, 73 %). C₂₅H₄₁N₃O₁₄. HPLC (method H): *t_R* = 8.0 min. MS (ESI): found *m/z* = 630.3 [M+Na]⁺, calcd. *m/z* = 630.2 [M+Na]⁺.

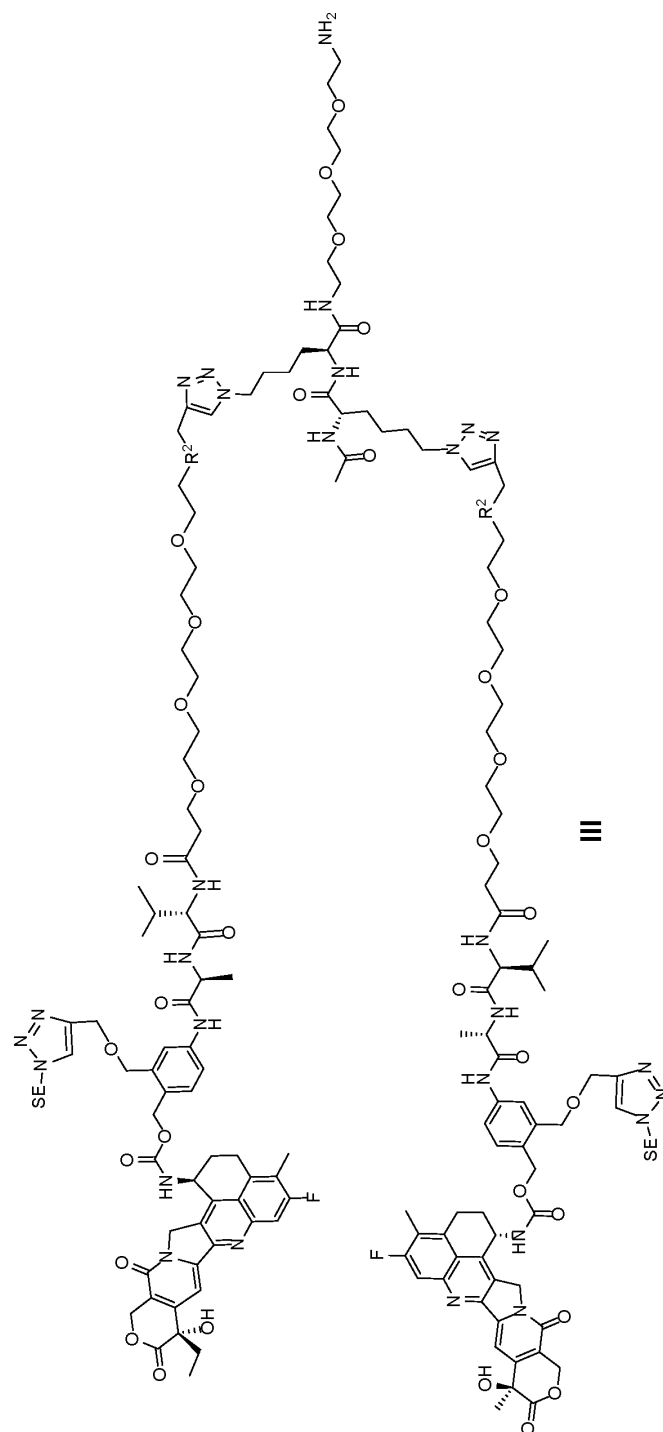


To a solution of **Z-L-Glu(NHDG)-NHDG** (924.9 mg, 1.5 mmol) in methanol (180 mL) was added Pd/C (10%, 161 mg) under Argon. The atmosphere was changed from argon to hydrogen, and the resulting suspension was stirred for 3.5 h at room temperature. The catalyst was removed by filtration through Celite and the solvent of the resulting filtrate was removed *in vacuo* affording H-L-Glu(NHDG)-NHDG (728.7 mg, 1.5 mmol, quant.). Next, H-L-Glu(NHDG)-NHDG (100.0 mg, 211.2 μmol, 1.5 equiv.) was dissolved in DMF (abs., 2 mL) and added to a solution of azido-PEG4-NHS ester (CAS-Nr. 944251-24-5, 54.68 mg, 140.8 μmol, 1 equiv.) under an atmosphere of argon. DIPEA (73.57 μL, 422.4 μmol, 3 equiv.) was added and the resulting solution stirred for 1.5h at room temperature. The solvent was removed *in vacuo* and purification by RP-HPLC afforded **intermediate 6.3** (82.74 mg, 79 %). C₂₈H₅₄N₆O₁₇. HPLC (method A): *t_R* = 5.9 min. MS (ESI): found *m/z* = 747.2 [M+H]⁺, calcd. *m/z* = 747.4 [M+H]⁺.

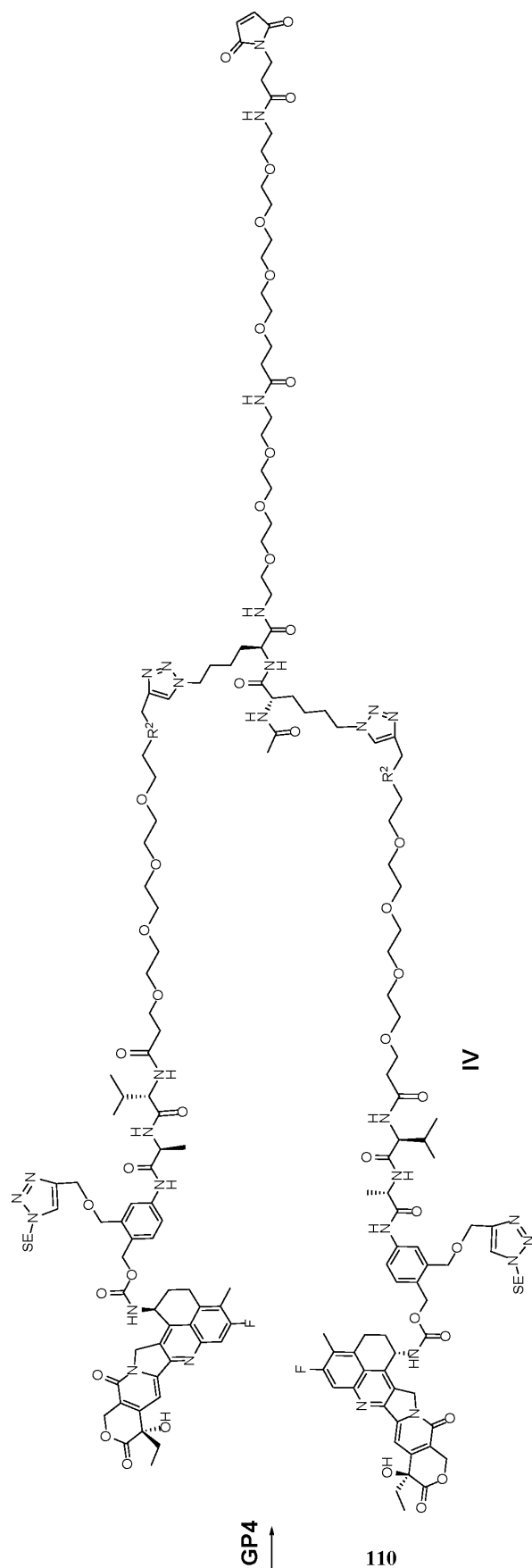
Synthesis of intermediate 6.4**6.4**

Intermediate 6.4 (91.9 mg, 71 %) was synthesized as described for intermediate 6.3 but using azido-PEG8-NHS ester (CAS-Nr. 1204834-00-3) instead of azido-PEG4-NHS ester. C₃₆H₇₀N₆O₂₁. HPLC (method A): t_R = 6.7 min. MS (ESI): found m/z = 923.6 [M+H]⁺, calcd. m/z = 923.5 [M+H]⁺.

Example 2 - Synthesis of multimeric linker exatecans

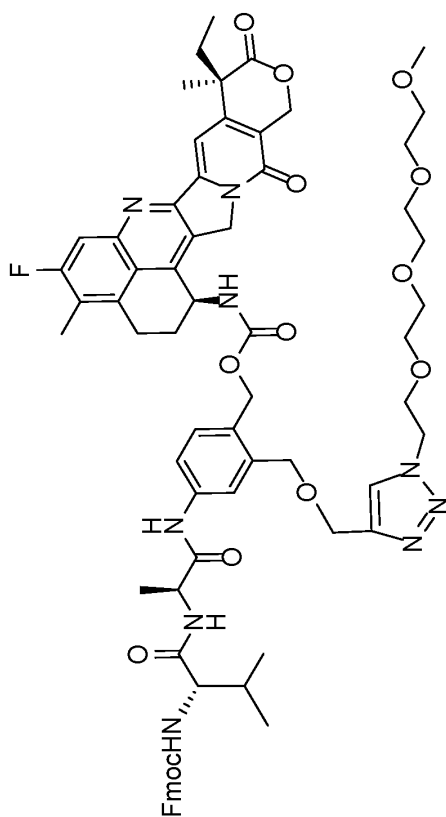


GP2 ↑



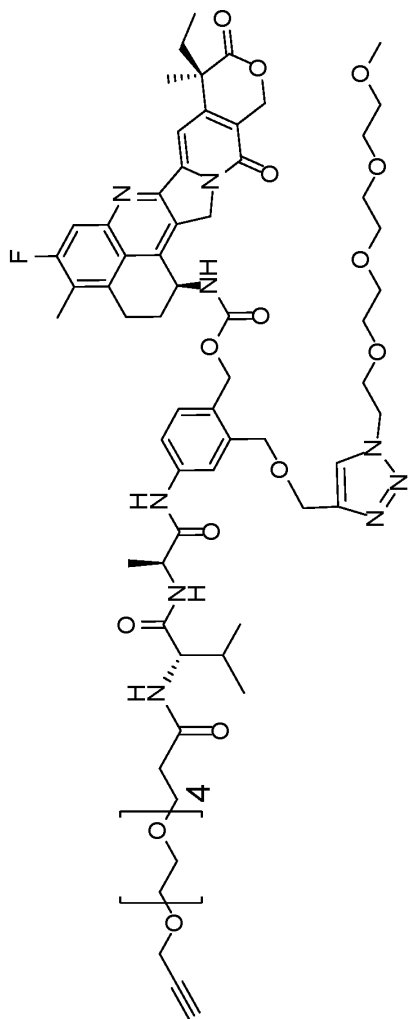
Example 3 - Synthesis of Compound IV.1

Synthesis of I.1



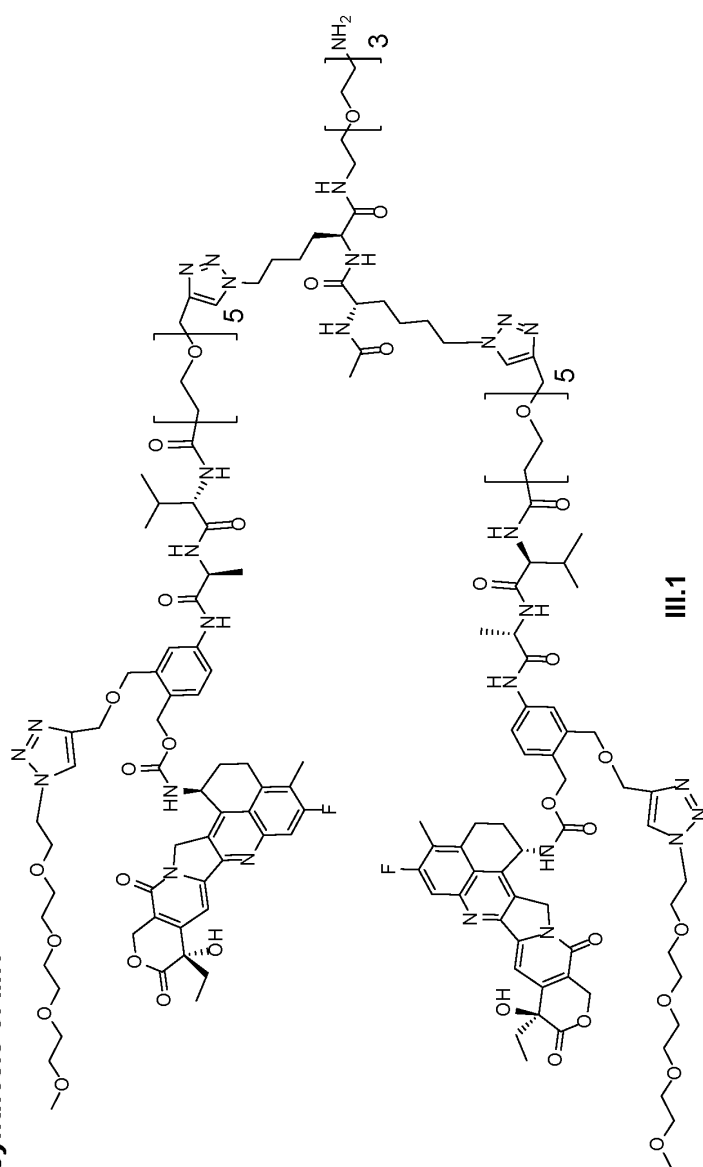
Intermediate **I.1** was synthesized according to GP1 using m-PEG(4)-azide (40.4 mg, 173 μmol , 1.2 equiv.), intermediate **1.3** (150 mg, 144 μmol , 1.0 equiv.), copper(II) sulfate pentahydrate (3.6 mg, 14.4 μmol , 0.1 equiv.), tris(benzyltriazolylmethyl)amine (9.6 mg, 18 μmol , 0.125 equiv.) and sodium ascorbate (7.1 mg, 36 μmol , 0.25 equiv.) in DMF/water (1.65 mL, 10:1). Purification by RP-HPLC afforded intermediate **I.1** (137 mg, 74 %) as yellowish solid. HPLC (method D): $t_R = 11.8$ min. MS (ESI): found $m/z = 1278.5$ $[\text{M}+\text{H}]^+$, calcd. $m/z = 1278.5$ $[\text{M}+\text{H}]^+$.

Synthesis of **II.1**



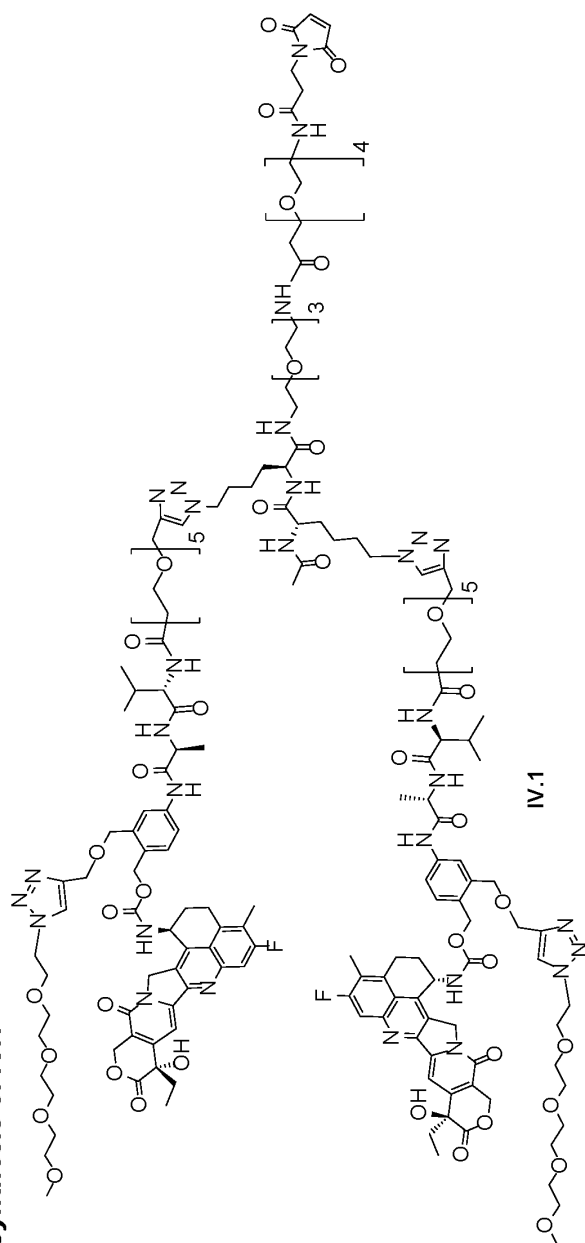
Intermediate **II.1** was synthesized according to GP3 using 222 μL diethylamine (2.14 mmol, 20 equiv.) in 1.37 mL DMF for Fmoc-deprotection and intermediate **I.1** (137 mg, 107 μmol , 1 equiv.), **intermediate 3.1** (53.0 mg, 118 μmol , 1.1 equiv.), diisopropylethylamine (56 μL , 321 μmol , 3 equiv.) in DMF (1 mL) for the coupling reaction. Purification by RP-HPLC afforded intermediate **II.1** (101 mg, 70 %) as yellowish solid. HPLC (method A): $t_R = 11.1$ min. MS (ESI): found $m/z = 1342.5$ $[\text{M}+\text{H}]^+$, calcd. $m/z = 1342.6$ $[\text{M}+\text{H}]^+$.

Synthesis of **III.1**

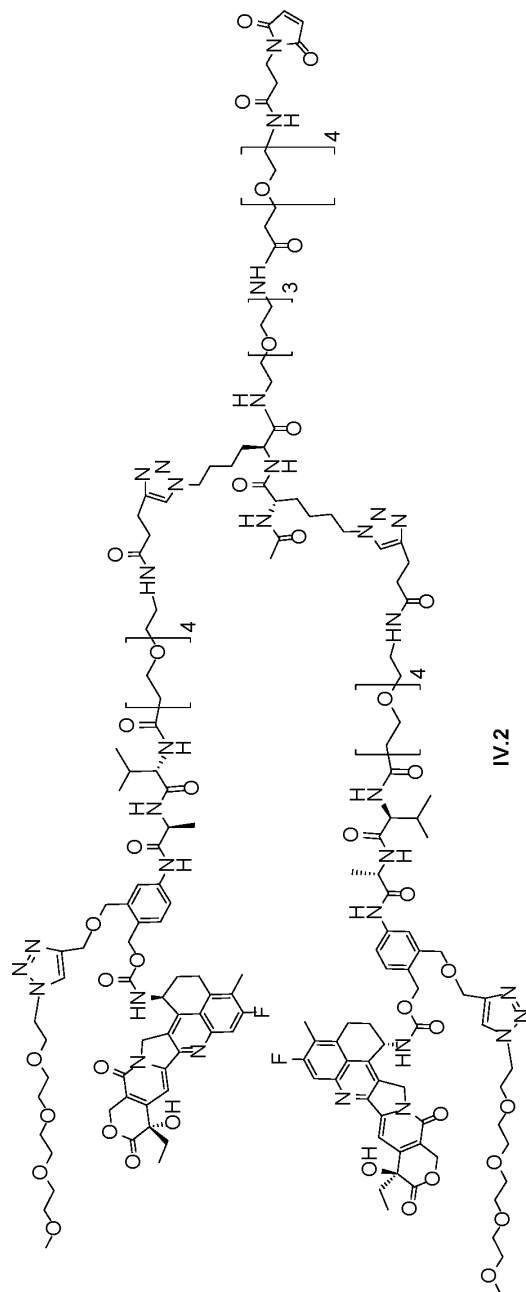


Intermediate **III.1** was synthesized according to GP2 using **intermediate 2.1** (3.3 mg, 5.0 μmol , 1 equiv.), intermediate **II.1** (17.3 mg, 12.9 μmol , 2.5 equiv.), copper(II) sulfate pentahydrate (1.6 mg, 6.5 μmol , 1.25 equiv.), Tris(3-hydroxypropyltriazolylmethyl)amine (5.6 mg, 12.9 μmol , 2.5 equiv.) and sodium ascorbate (4.2 mg, 21.4 μmol , 4.3 equiv.) in DMF/water (0.55 mL, 10:1). Purification by RP-HPLC afforded intermediate **III.1** (22.4 mg, quant.) as yellowish solid. HPLC (method J): t_R = 15.0 min. MS (ESI): found m/z = 1614.17 $[\text{M}+2\text{H}]^{2+}$, calcd. m/z = 1613.8 $[\text{M}+2\text{H}]^{2+}$.

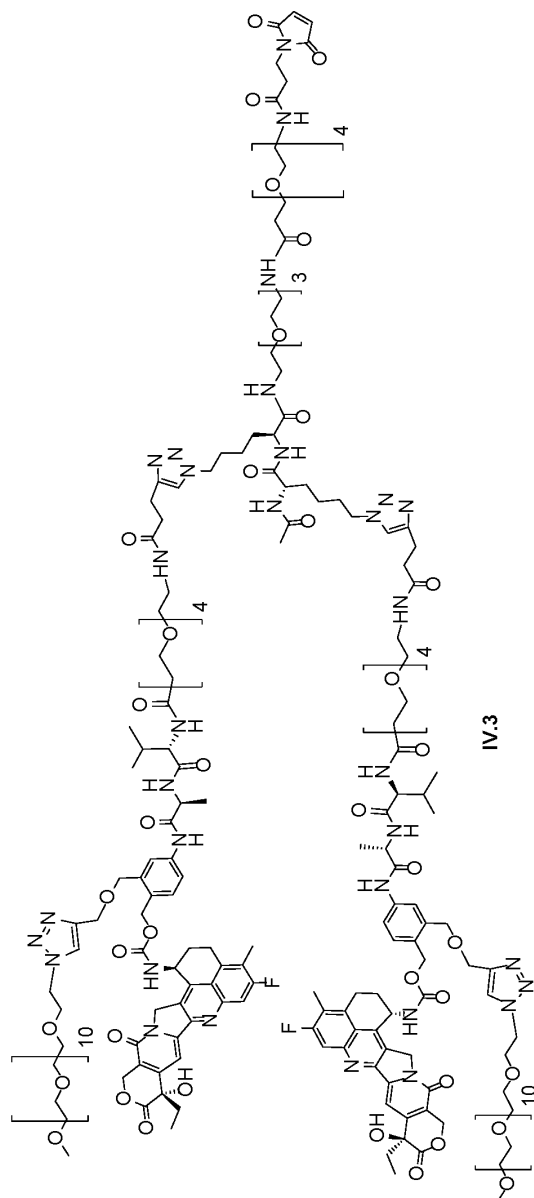
Synthesis of IV.1



Compound **IV.1** was synthesized according to GP4 using the TFA-salt of intermediate **III.1** (16.3 mg, 4.9 μmol , 1 equiv.), *N*-[15-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl]-2,5-dihydro-2,5-dioxo-1*H*-pyrrole-1-propanamide = Mal-PEG4-NHS-ester (10.8 mg, 21.0 μmol , 4.3 equiv.) and DIPEA (3.3 μL , 19.0 μmol , 3.9 equiv.) in DMF (652 μL). Purification by RP-HPLC afforded compound **IV.1** (7.4 mg, 42 %) as yellowish solid. $\text{C}_{174}\text{H}_{244}\text{F}_2\text{N}_{30}\text{O}_{52}$. HPLC (method J): t_R = 16.7 min. MS (ESI): found m/z = 1209.6 $[\text{M}+3\text{H}]^{3+}$, calcd. m/z = 1208.9 $[\text{M}+3\text{H}]^{3+}$.

Example 4 - Synthesis of Compound IV.2**IV.2**

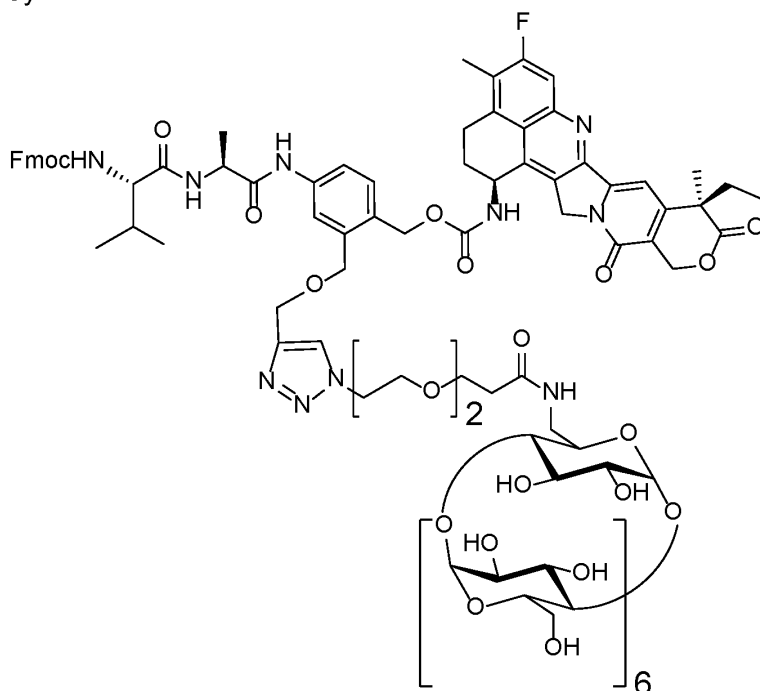
Compound **IV.2** was synthesized as described for compound IV.1 but using **intermediate 3.2**. Compound **IV.2** (30.76 mg) was obtained as yellowish solid. $C_{178}H_{250}F_2N_{32}O_{52}$. HPLC (method J): $t_R = 16.4$ min. MS (ESI): found $m/z = 1236.8$ $[M+3H]^{3+}$, calcd. $m/z = 1236.3$ $[M+3H]^{3+}$.

Example 5 - Synthesis of Compound IV.3

Compound **IV.3** was synthesized as described for compound **IV.1** but using m-PEG(12)-azide instead of m-PEG(4)-azide and **intermediate 3.2** instead of **intermediate 3.1**. Compound **IV.3** (5.07 mg) was obtained as yellowish solid. $C_{210}H_{314}F_2N_{32}O_{68}$. HPLC (method J): $t_R = 16.4$ min. MS (ESI): found $m/z = 1471.7 [M+3H]^{3+}$, calcd. $m/z = 1471.1 [M+3H]^{3+}$.

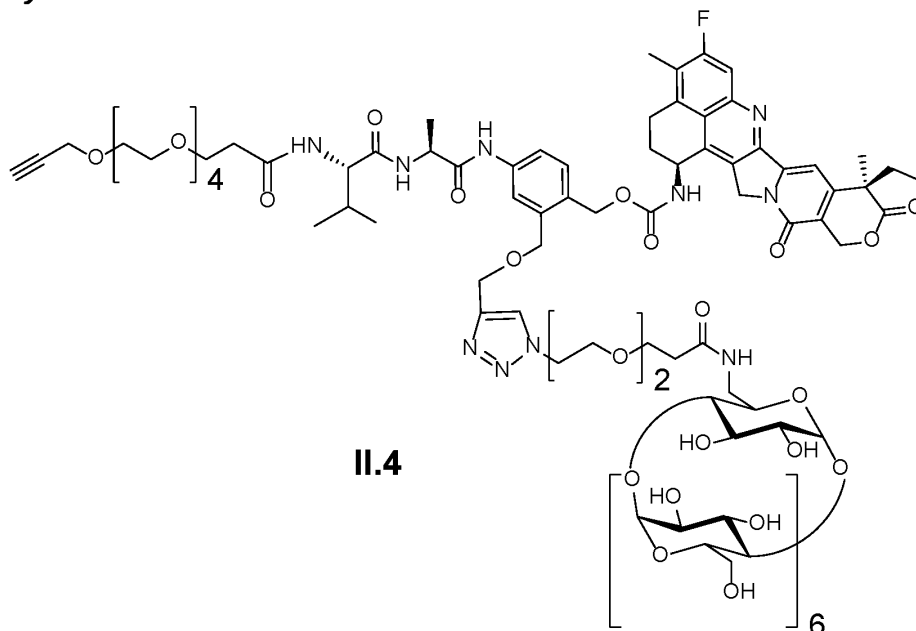
Example 6 - Synthesis of Compound IV.4

Synthesis of **1.4**

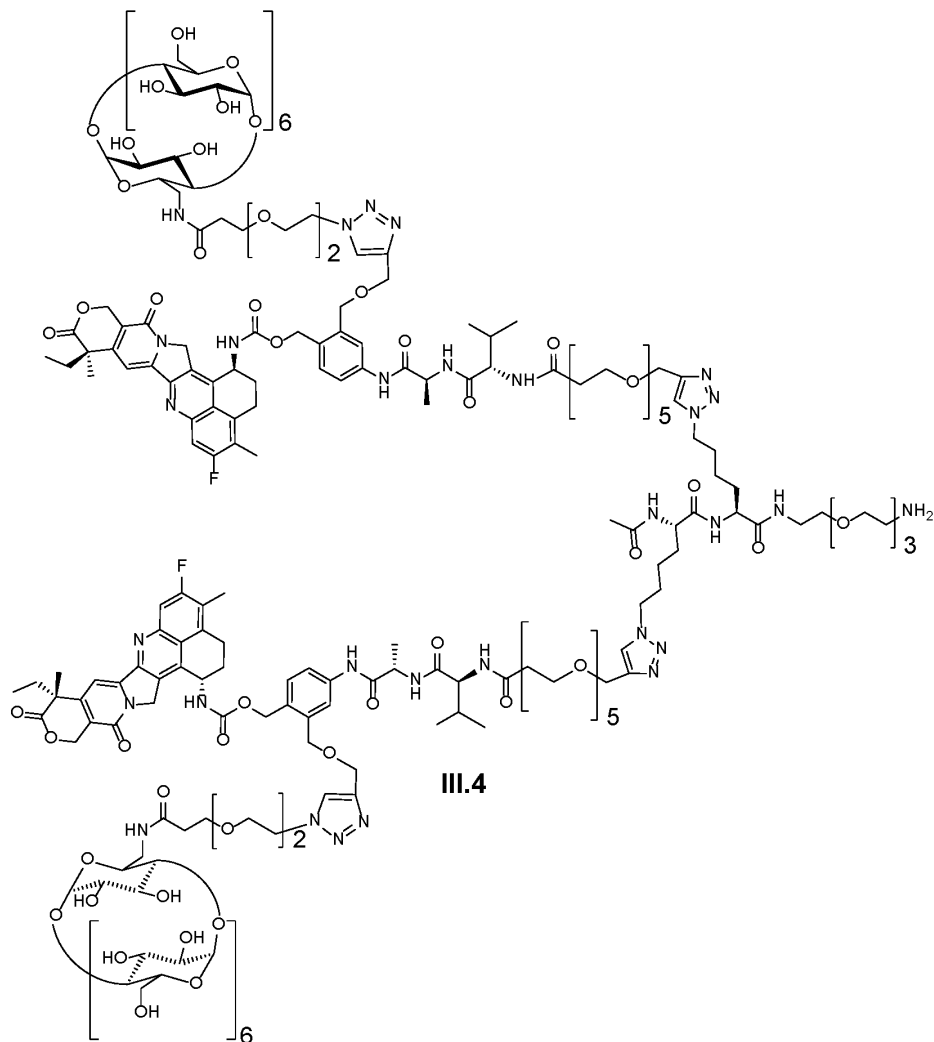


1.4

Intermediate **1.4** was synthesized according to GP1 using intermediate **4.1** (22.4 μ mol, 1 equiv.), intermediate **1.3** (26.16 mg, 25.0 μ mol, 1.1 equiv.), copper(II) sulfate pentahydrate (1.23 mg, 4.9 μ mol, 0.2 equiv.), tris(benzyltriazolylmethyl)amine (3.17 mg, 6.0 μ mol, 0.26 equiv.) and sodium ascorbate (4.33 mg, 11.9 μ mol, 0.5 equiv.) in DMF/water (0.28 mL, 10:1). Purification by RP-HPLC afforded intermediate **1.4** (31.51 mg, 60 %) as yellowish solid. C₁₀₈H₁₃₉FN₁₀O₄₈. HPLC (method D): t_R = 10.7 min. MS (ESI): found m/z = 1182.5 [M+2H]²⁺, calcd. m/z = 1182.4 [M+2H]²⁺.

Synthesis of II.4

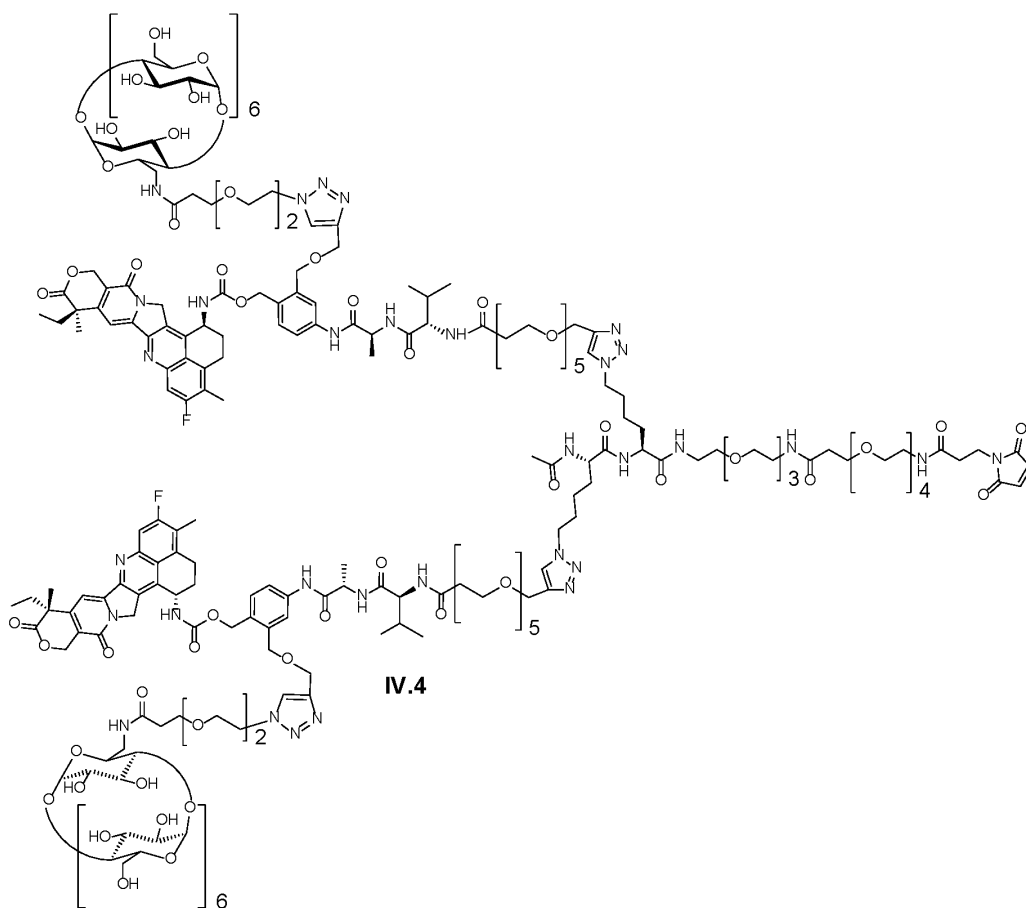
Intermediate **II.4** was synthesized according to GP3 using intermediate **I.4** (31.51 mg, 13.3 μmol , 1 equiv.), 33 μL diethylamine (319.9 μmol , 24 equiv.) in 1.6 mL DMF for Fmoc-deprotection and **intermediate 3.1** (8.98 mg, 20.0 μmol , 1.5 equiv.), diisopropylethylamine (6.8 μL , 40.0 μmol , 3 equiv.) in DMF (2.1 mL) for the coupling reaction. Purification by RP-HPLC afforded intermediate **II.4** (15.51 mg, 50 %) as yellowish solid. $\text{C}_{107}\text{H}_{151}\text{FN}_{10}\text{O}_{52}$. HPLC (method D): t_{R} = 9.1 min. MS (ESI): found m/z = 1214.5 $[\text{M}+2\text{H}]^{2+}$, calcd. m/z = 1214.5 $[\text{M}+2\text{H}]^{2+}$.

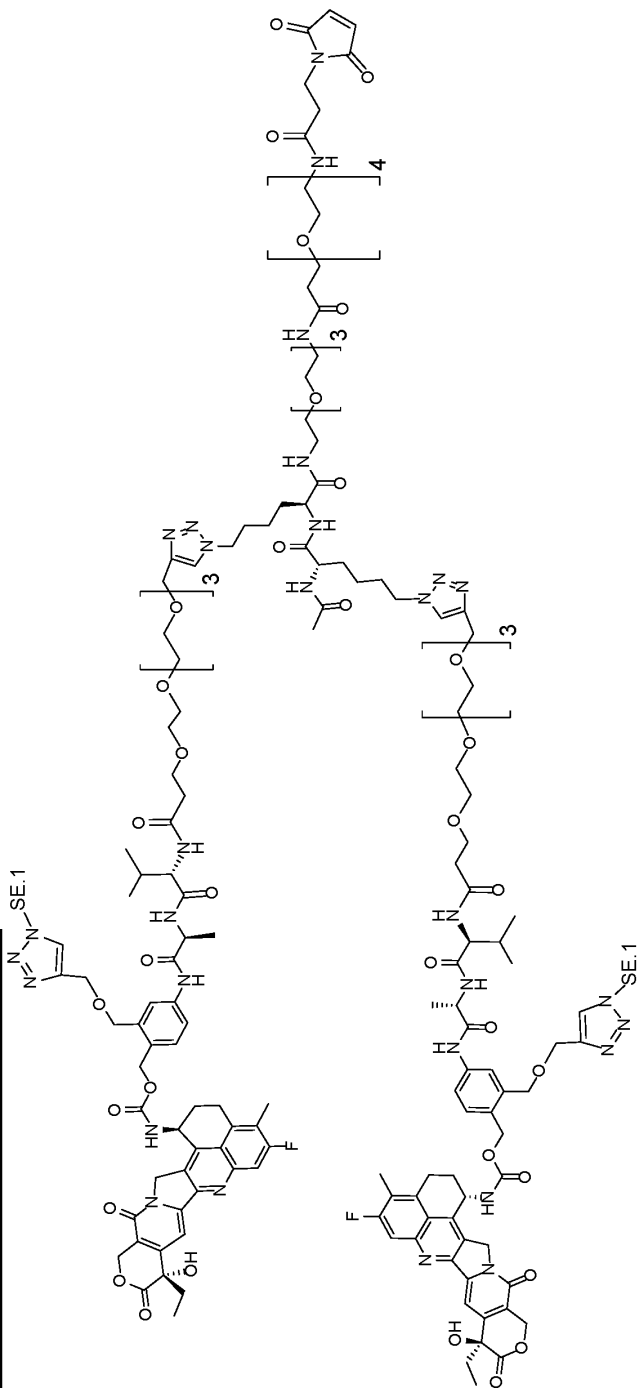
Synthesis of III.4

Intermediate **III.4** was synthesized according to GP2 using **intermediate 2.1** (1.54 mg, 2.6 μmol , 1 equiv.), **intermediate II.4** (15.51 mg, 6.4 μmol , 2.5 equiv), copper(II) sulfate pentahydrate (0.87 mg, 3.5 μmol , 1.3 equiv.), Tris(3-hydroxypropyltriazolylmethyl)amine (2.83 mg, 6.5 μmol , 2.5 equiv.) and sodium ascorbate (1.97 mg, 9.9 μmol , 3.8 equiv.) in DMF/water (0.12 mL, 5:1). Purification by RP-HPLC afforded intermediate **III.4** (9.61 mg, 70 %) as yellowish solid. $\text{C}_{236}\text{H}_{344}\text{F}_2\text{N}_{30}\text{O}_{110}$. HPLC (method E): $t_R = 9.2$ min. MS (ESI): found $m/z = 1800.6$ $[\text{M}+3\text{H}]^{3+}$, calcd. $m/z = 1799.7$ $[\text{M}+3\text{H}]^{3+}$.

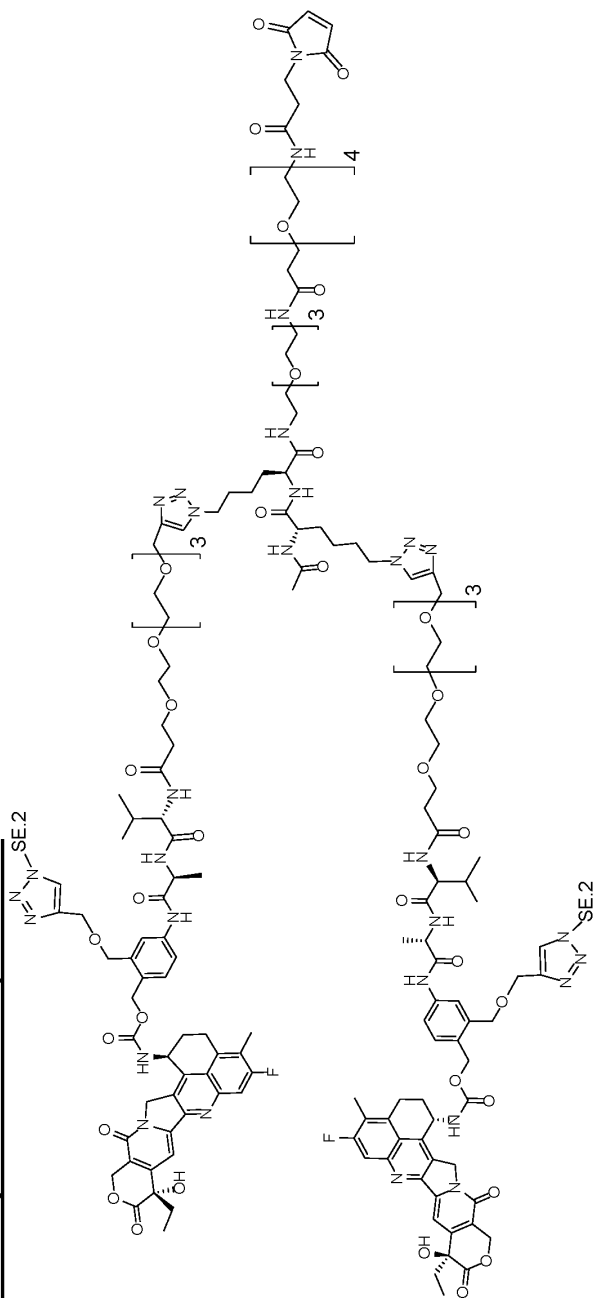
Synthesis of IV.4

Compound **IV.4** was synthesized according to GP4 using the TFA-salt of intermediate **III.4** (9.61 mg, 1.74 μmol , 1 equiv.), *N*-[15-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl]-2,5-dihydro-2,5-dioxo-1*H*-pyrrole-1-propanamide = Mal-PEG4-NHS-ester (1.89 mg, 3.68 μmol , 2.1 equiv.) and DIPEA (0.59 μL , 3.48 μmol , 2 equiv.) in DMF (0.4 mL). Purification by RP-HPLC afforded compound **IV.4** (6.95 mg, 69 %) as yellowish solid. $\text{C}_{254}\text{H}_{370}\text{F}_2\text{N}_{32}\text{O}_{118}$. HPLC (method J): t_R = 12.9 min. MS (ESI): found m/z = 1455.1 $[\text{M}+3\text{H}+\text{Na}]^{4+}$, calcd. m/z = 1455.7 $[\text{M}+3\text{H}+\text{Na}]^{4+}$.

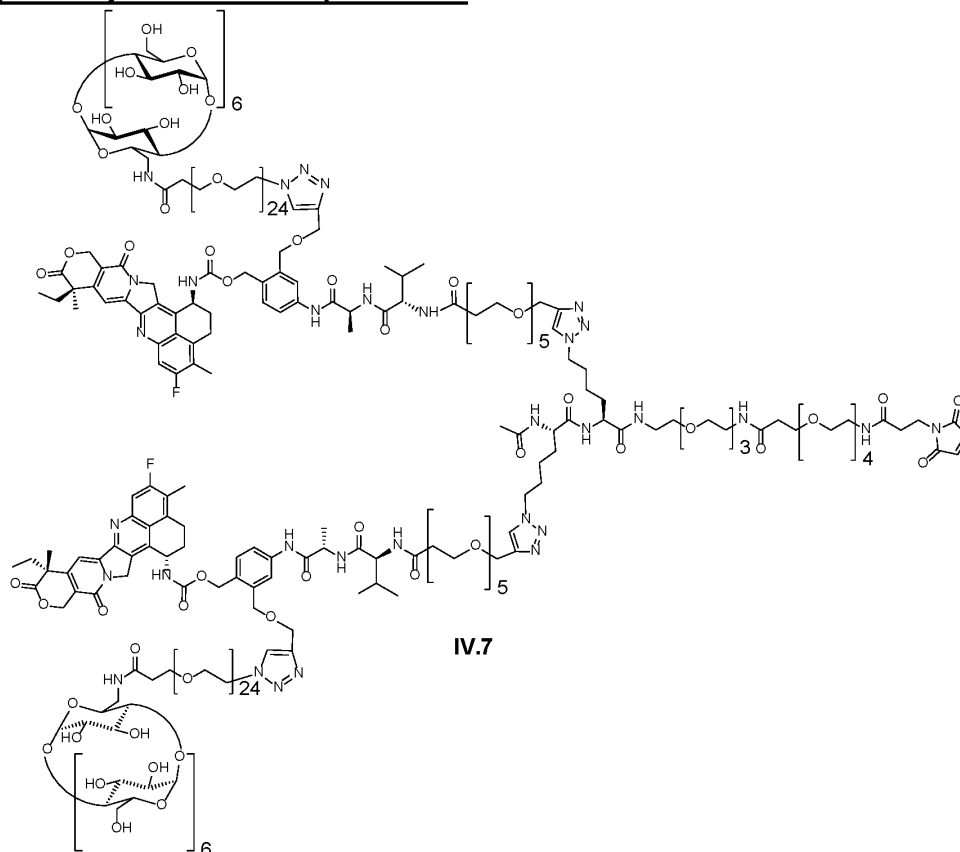


Example 7 - Synthesis of Compound IV.5**IV.5**

Compound **IV.5** was synthesized as described for compound **IV.4** but using **intermediate 4.2**. Compound **IV.5** (25.55 mg, 68 %) was obtained as yellowish solid. $C_{262}H_{386}F_2N_{32}O_{122}$. HPLC (method J): $t_R = 13.1$ min. MS (ESI): found $m/z = 1494.3$ $[M+4H]^{4+}$, calcd. $m/z = 1493.6$ $[M+4H]^{4+}$.

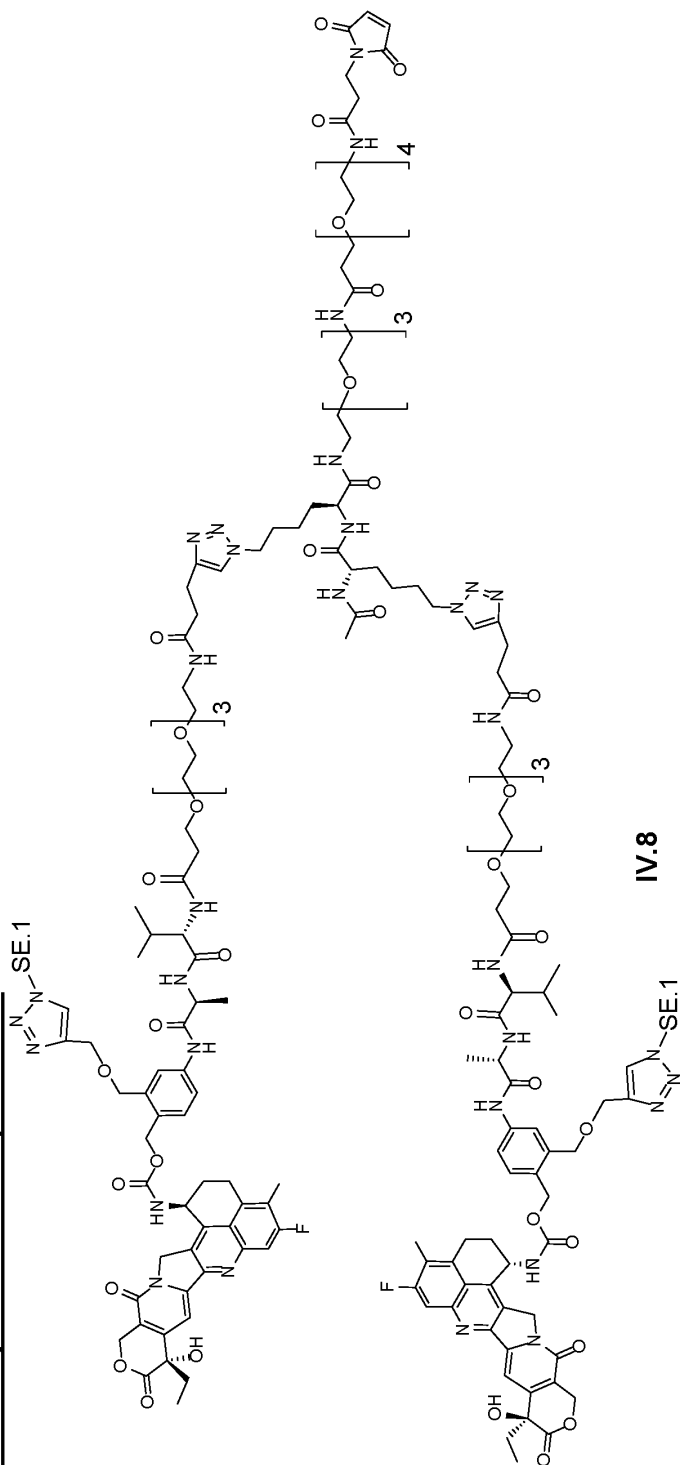
Example 8 - Synthesis of Compound IV.6**IV.6**

Compound **IV.6** was synthesized as described for compound **IV.4** but using **intermediate 4.3**. Compound **IV.6** (6.70 mg, 83 %) was obtained as yellowish solid. $C_{278}H_{418}F_2N_{32}O_{130}$. HPLC (method J): $t_R = 13.3$ min. MS (ESI): found $m/z = 1582.3$ $[M+4H]^4+$, calcd. $m/z = 1581.7$ $[M+4H]^4+$.

Example 9 - Synthesis of Compound IV.7

Compound **IV.7** was synthesized as described for compound **IV.4** but using **intermediate 4.4**. Compound **IV.7** (10.41 mg, 51 %) was obtained as yellowish solid. $C_{342}H_{546}F_2N_{32}O_{162}$. HPLC (method J): $t_R = 14.0$ min. MS (ESI): found $m/z = 1934.3$ $[M+4H]^{4+}$, calcd. $m/z = 1933.9$ $[M+4H]^{4+}$.

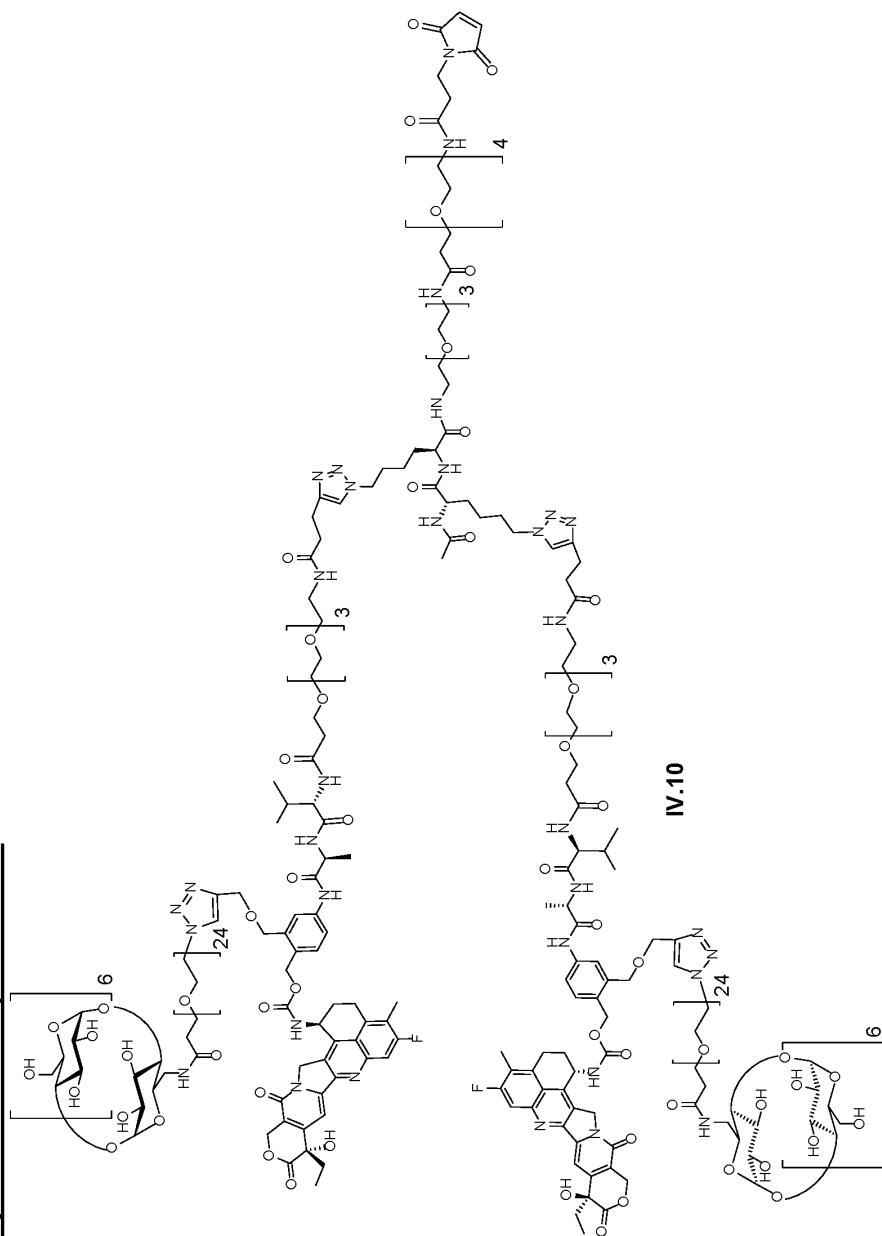
Example 10 - Synthesis of Compound IV.8



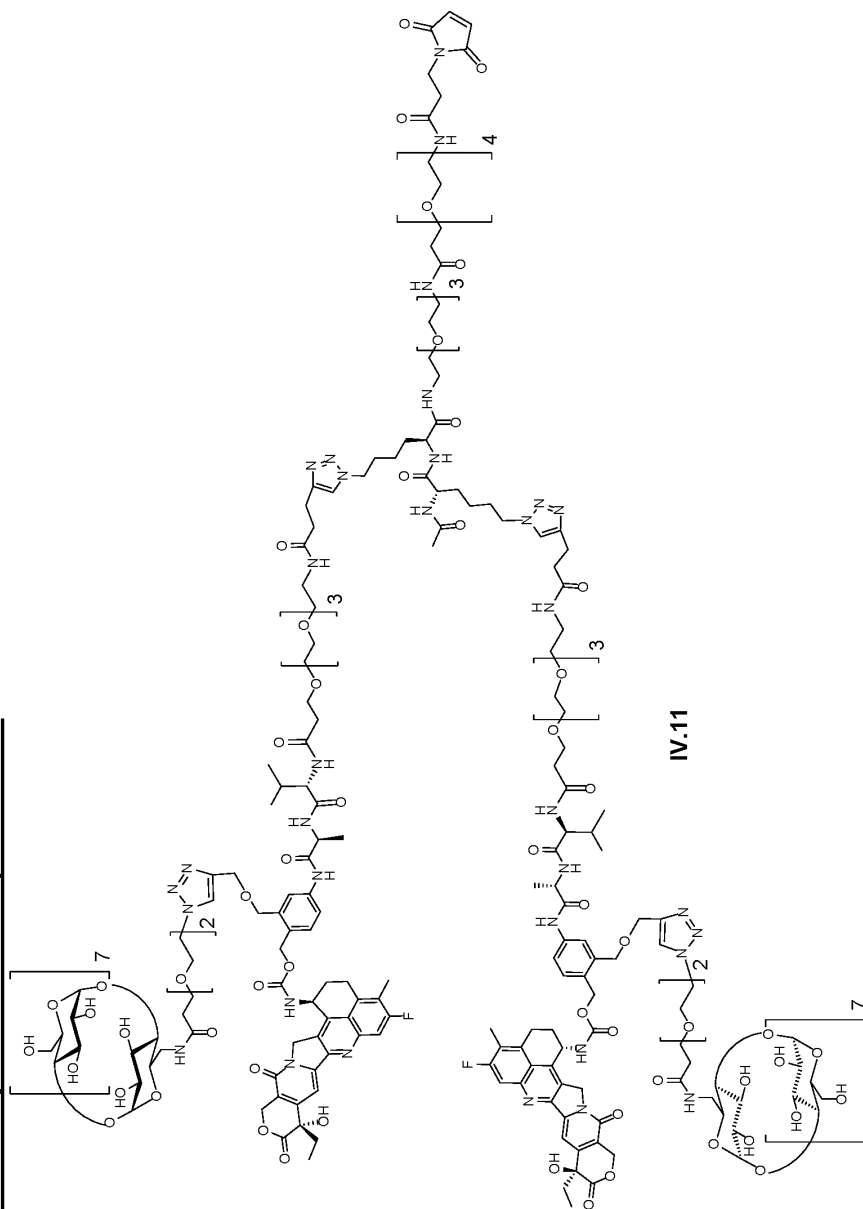
Compound **IV.8** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.2**. Compound **IV.8** (19.3 mg, 35 %) was obtained as yellowish solid. C₂₈₆H₃₉₂F₂N₃₄O₁₂₂. HPLC (method J): t_R = 12.9 min. MS (ESI): found *m/z* = 1514.6 [M+4H]⁴⁺, calcd. *m/z* = 1514.1 [M+4H]⁴⁺.

[illegible]

Compound **IV.9** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.3**. Compound **IV.9** (8.87 mg, 49 %) was obtained as yellowish solid. $C_{282}H_{424}F_{2}N_{34}O_{130}$. HPLC (method J): $t_R = 13.2$ min. MS (ESI): found $m/z = 1602.7$ $[M+4H]^{4+}$, calcd. $m/z = 1602.2$ $[M+4H]^{4+}$.

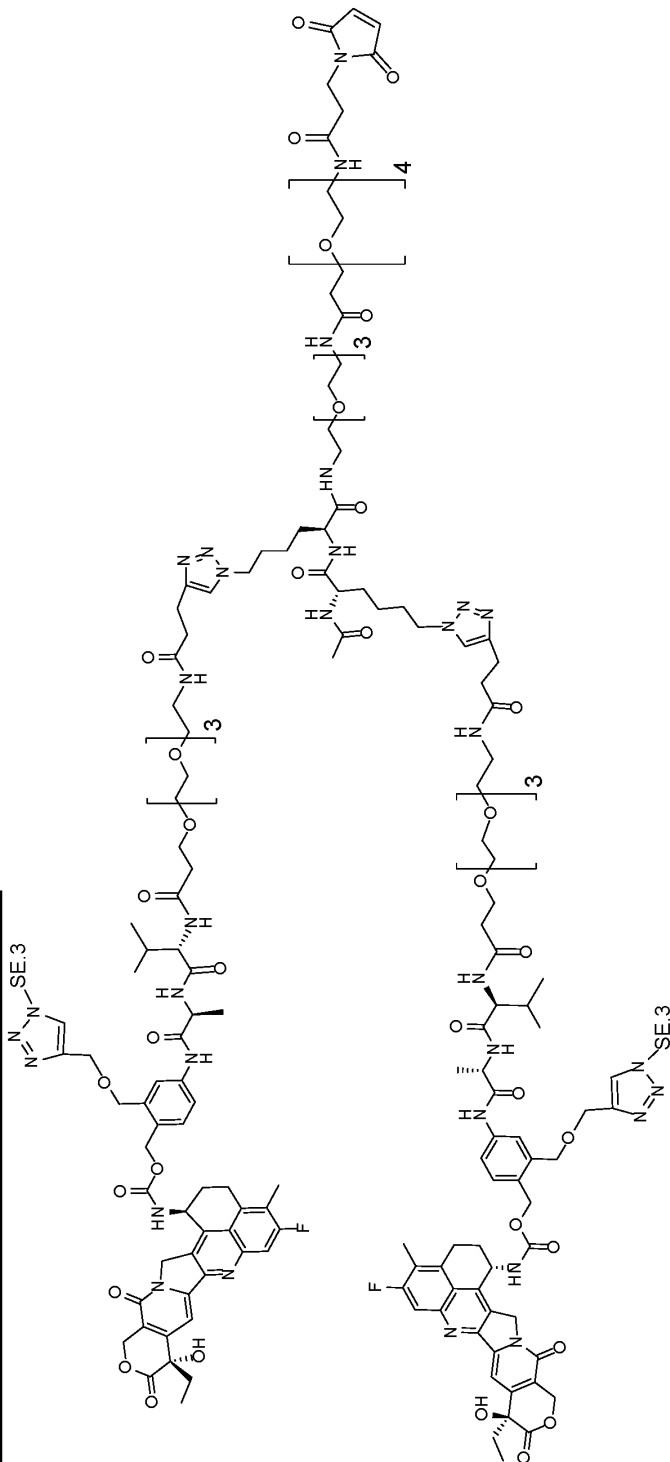
Example 12 - Synthesis of Compound IV.10

Compound **IV.10** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.4**. Compound **IV.10** (12.26 mg, 49 %) was obtained as yellowish solid. $C_{346}H_{552}F_2N_{34}O_{162}$. HPLC (method J): $t_R = 13.9$ min. MS (ESI): found $m/z = 1304.3$ $[M+6H]^{6+}$, calcd. $m/z = 1303.3$ $[M+6H]^{6+}$.

Example 13 - Synthesis of Compound IV.11

Compound **IV.11** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.5**. Compound **IV.11** (5.69 mg, 56 %) was obtained as yellowish solid. $C_{270}H_{396}F_2N_{34}O_{128}$. HPLC (method J): $t_R = 12.6$ min. MS (ESI): found $m/z = 1551.8 [M+4H]^{4+}$, calcd. $m/z = 1551.1 [M+4H]^{4+}$.

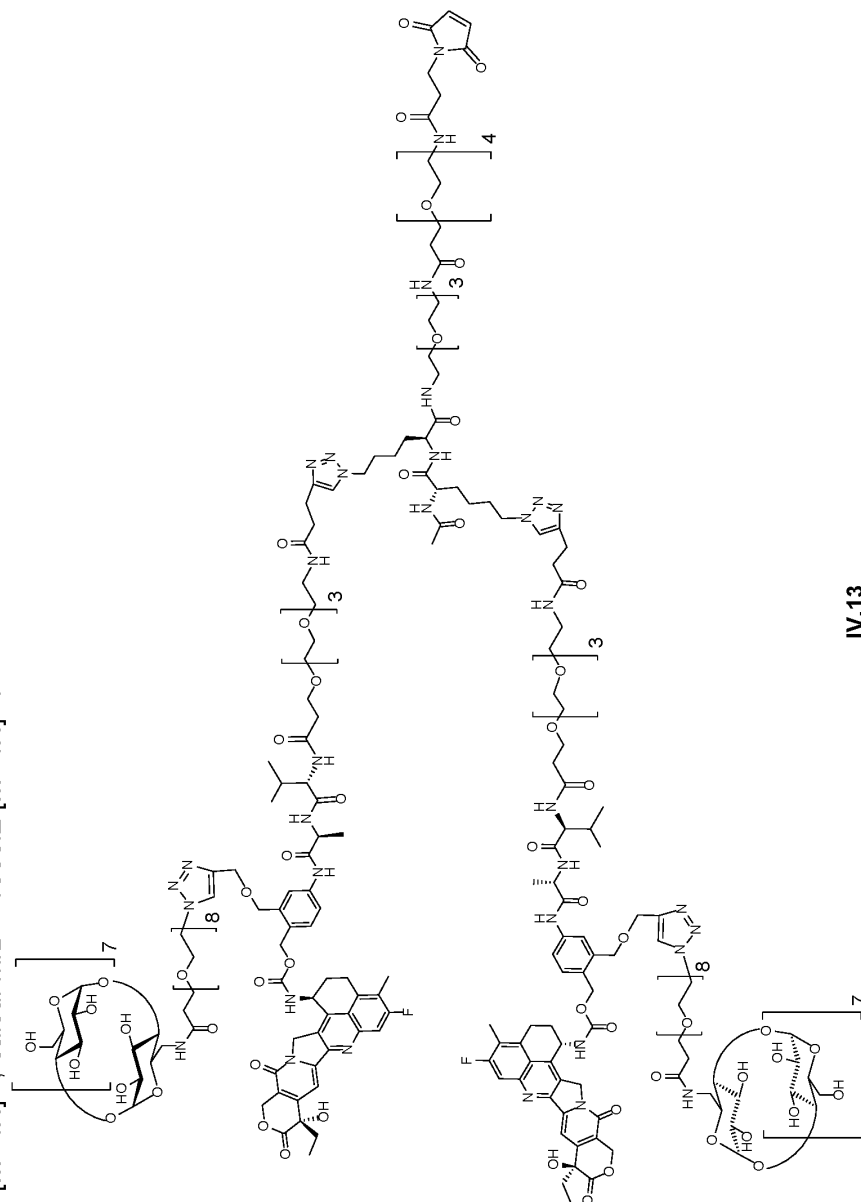
Example 14 - Synthesis of Compound IV.12

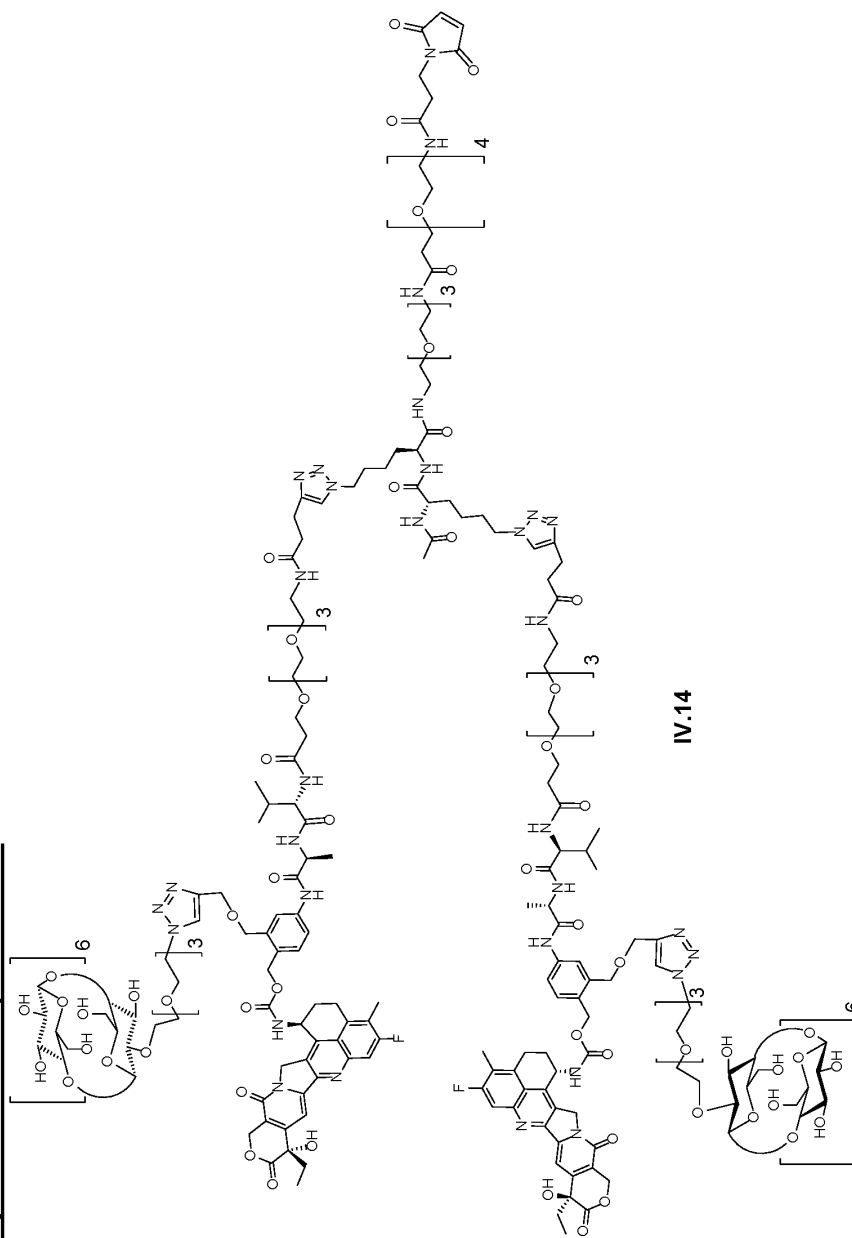


Compound **IV.12** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.6**. Compound **IV.12** (43.17 mg, 59 %) was obtained as yellowish solid. $C_{278}H_{412}F_2N_{34}O_{132}$. HPLC (method J): $t_R = 12.4$ min. MS (ESI): found $m/z = 1596.8$ $[M+4H]^{4+}$, calcd. $m/z = 1595.67$ $[M+4H]^{4+}$.

Example 15 - Synthesis of Compound IV.13

Compound **IV.13** was synthesized as described for compound **IV.4** but using intermediates **3.2** and **4.7**. Compound **IV.13** (29.51 mg, 59 %) was obtained as yellowish solid. $C_{294}H_{444}F_2N_{34}O_{140}$. HPLC (method J): $t_R = 12.7$ min. MS (ESI): found $m/z = 1684.5$ $[M+4H]^{4+}$, calcd. $m/z = 1683.2$ $[M+4H]^{4+}$.

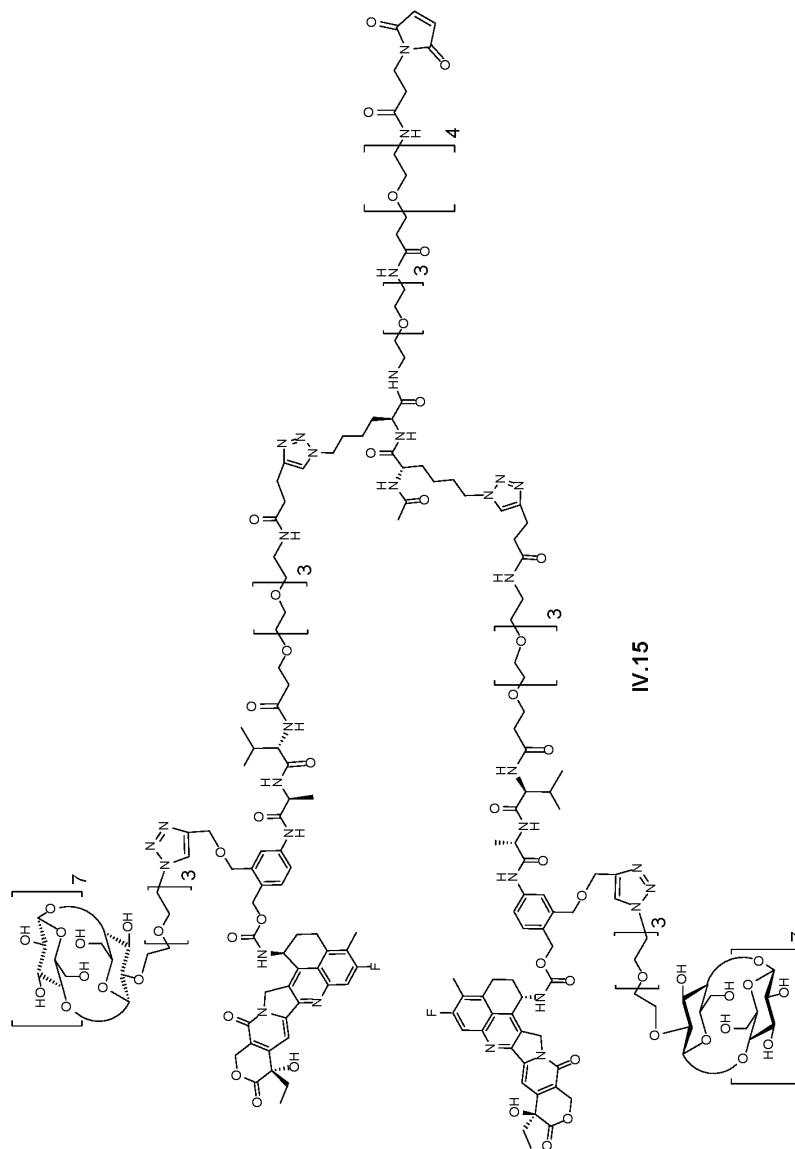
**IV.13**

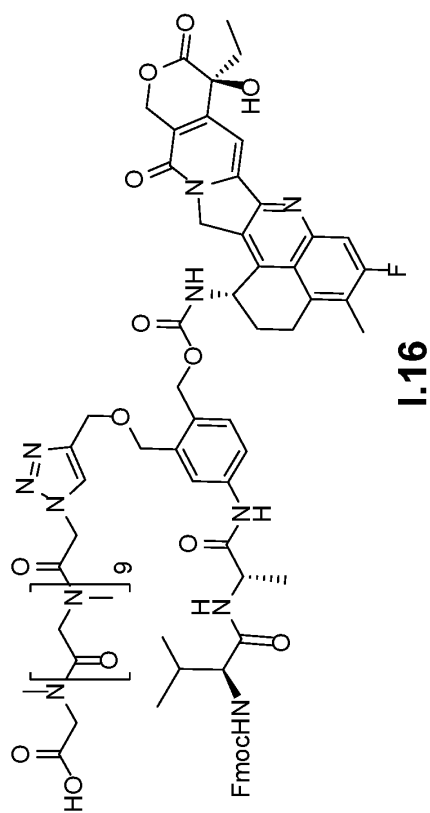
Example 16 - Synthesis of Compound IV.14

Compound **IV.14** was synthesized as described for compound **IV.4** but using intermediates **3.2** and **4.8**. Compound **IV.14** (61.21 mg, 81 %) was obtained as yellowish solid. $C_{260}H_{382}F_2N_{32}O_{120}$. HPLC (method J): $t_R = 12.7$ min. MS (ESI): found $m/z = 1479.8$ $[M+4H]^{4+}$, calcd. $m/z = 1478.6$ $[M+4H]^{4+}$.

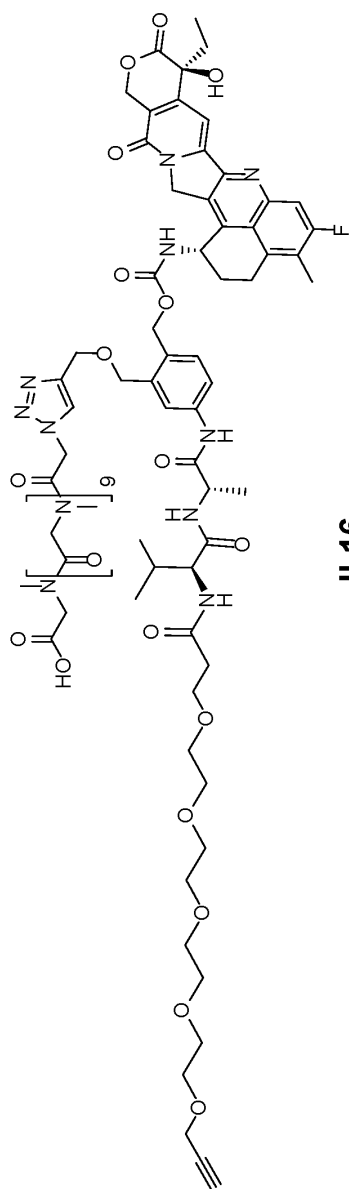
Example 17 - Synthesis of Compound IV.15

Compound **IV.15** was synthesized as described for compound **IV.4** but using **intermediates 3.2** and **4.9**. Compound **IV.15** (29.68 mg, 68 %) was obtained as yellowish solid. $C_{272}H_{402}F_2N_{32}O_{130}$. HPLC (method J): $t_R = 12.5$ min. MS (ESI): found $m/z = 1560.1$ $[M+4H]^{4+}$, calcd. $m/z = 1559.7$ $[M+4H]^{4+}$.



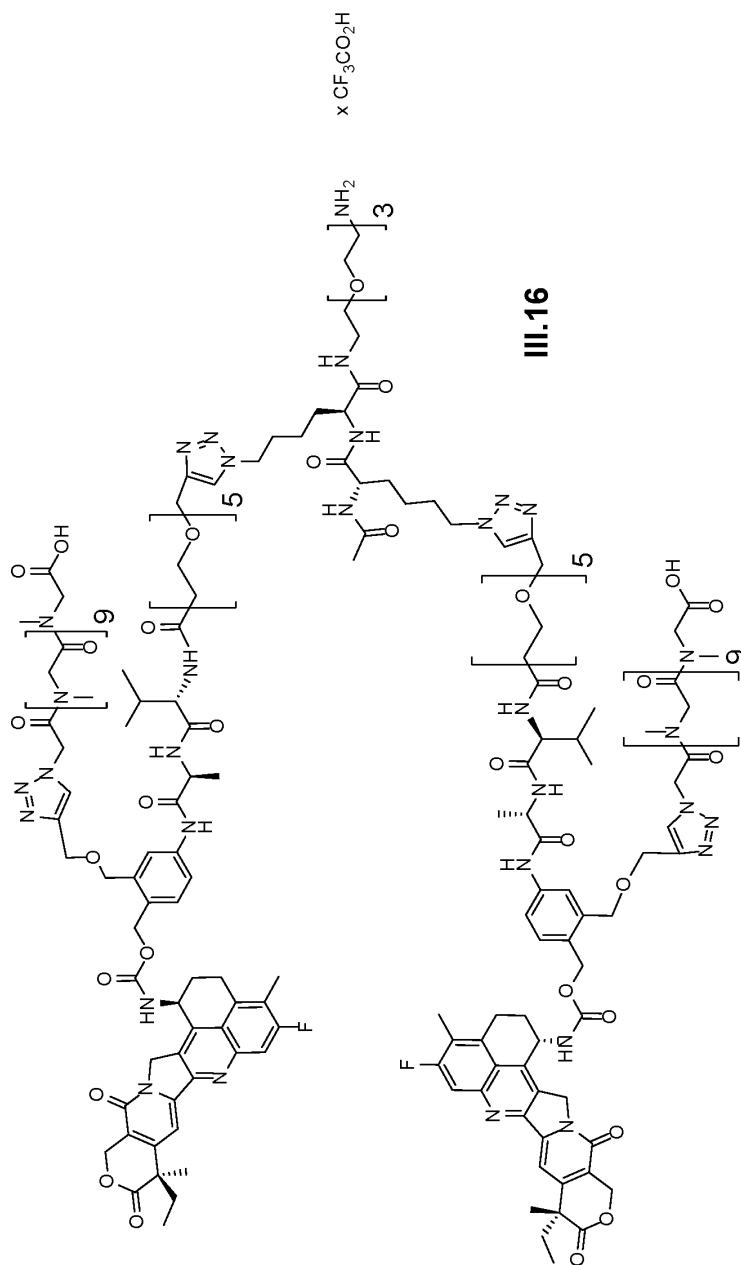
Example 18 - Synthesis of Compound IV.16Synthesis of **I.16**

Intermediate **I.16** was synthesized according to GP1 using intermediate **5.1** (30 mg, 37.0 μmol , 1 equiv.), intermediate **1.3** (42.5 mg, 40.7 μmol , 1.1 equiv.), copper(II) sulfate pentahydrate (1.85 mg, 7.4 μmol , 0.2 equiv.), tris(benzyltriazolylmethyl)amine (4.91 mg, 9.25 μmol , 0.25 equiv.) and sodium ascorbate (3.66 mg, 18.5 μmol , 0.5 equiv.) in DMF/water (0.33 mL, 10:1). Purification by RP-HPLC afforded intermediate **I.16** (51.7 mg, 74 %) as yellowish solid. $\text{C}_{91}\text{H}_{110}\text{FN}_{19}\text{O}_{23}$. HPLC (method A): $t_R = 11.1$ min. MS (ESI): found $m/z = 1855.1$ $[\text{M}-\text{H}]^-$; calcd. $m/z = 1854.8$ $[\text{M}-\text{H}]^-$.

Synthesis of **II.16****II.16**

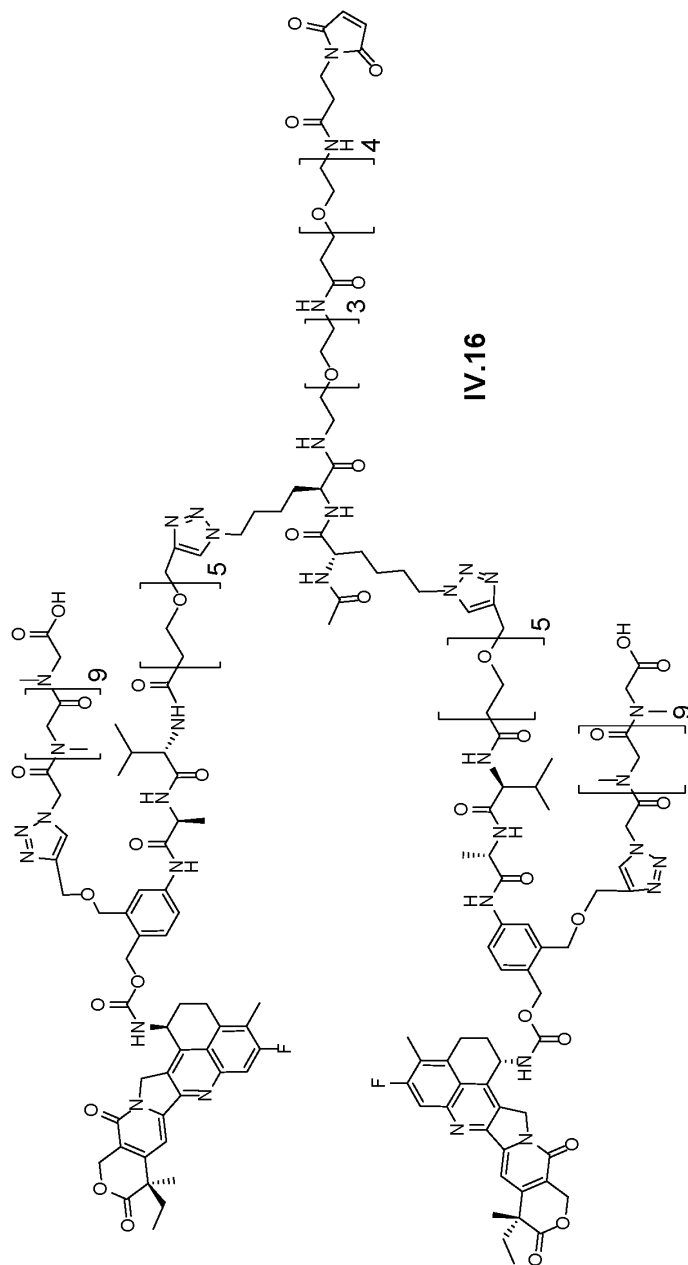
Intermediate **II.16** was synthesized according to GP3 using intermediate **I.16** (51.7 mg, 27.8 μmol , 1 equiv.), diethylamine (68.7 μL , 667.0 μmol , 24 equiv.) in 2.5 mL DMF for Fmoc-deprotection and **intermediate 3.1** (18.7 mg, 41.7 μmol , 1.5 equiv.), diisopropylethylamine (14.2 μL , 83.4 μmol , 3 equiv.) in DMF (3.44 mL) for the coupling reaction. Purification by RP-HPLC afforded intermediate **II.16** (43.8 mg, 82 %) as yellowish solid. $\text{C}_{90}\text{H}_{122}\text{FN}_{19}\text{O}_{27}$. HPLC (method A): t_{R} = 9.4 min. MS (ESI): found m/z = 961.6 $[\text{M}+2\text{H}]^{2+}$, calcd. m/z = 960.9 $[\text{M}+2\text{H}]^{2+}$.

Synthesis of III.16

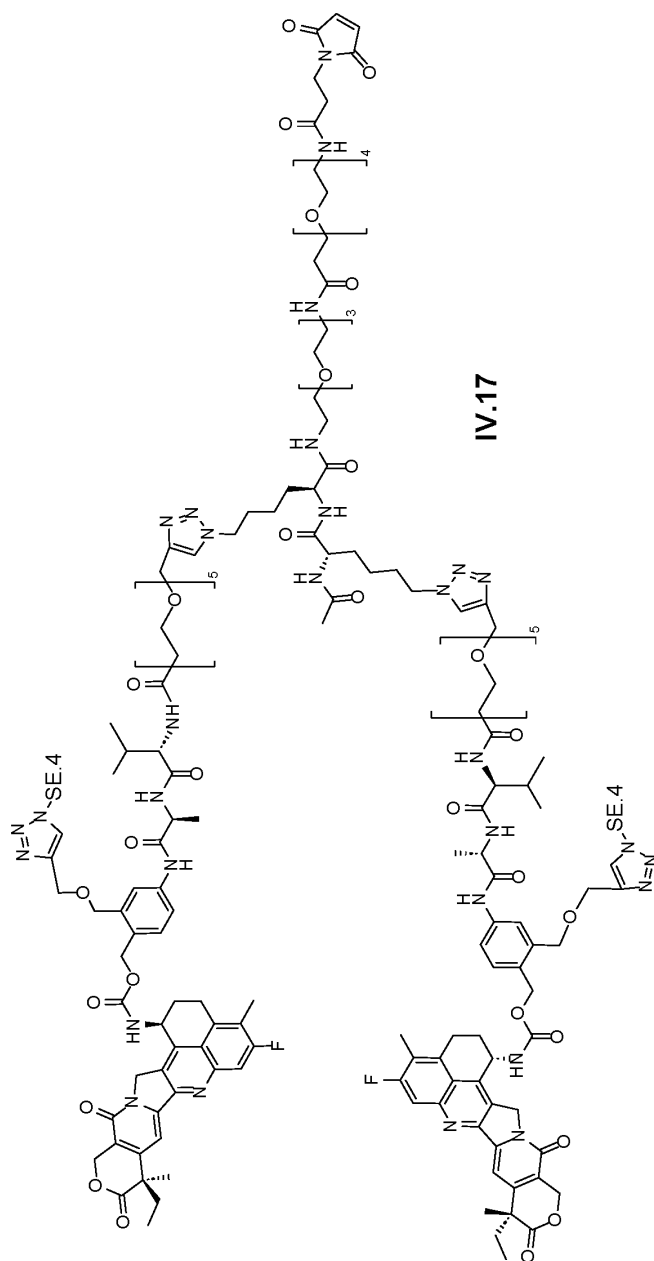


Intermediate **III.16** was synthesized according to GP2 using **intermediate 2.1** (2.67 mg, 4.6 μmol , 1 equiv.), intermediate **II.16** (20.4 mg, 10.6 μmol , 2.3 equiv), copper(II) sulfate pentahydrate (1.44 mg, 5.8 μmol , 1.25 equiv.), Tris(3-hydroxypropyltriazolylmethyl)amine (5.02 mg, 11.6 μmol , 2.5 equiv.) and sodium ascorbate (3.43 mg, 17.3 μmol , 3.8 equiv.) in DMF/water (0.44 mL, 10:1). Purification by RP-HPLC afforded intermediate **III.16** (9.2 mg, 46 %) as yellowish solid. $\text{C}_{202}\text{H}_{286}\text{F}_{2}\text{N}_{48}\text{O}_{60}$. HPLC (method L): $t_{\text{R}} = 16.8$ min. MS (ESI): found $m/z = 1462.5$ $[\text{M}+3\text{H}]^{3+}$, calcd. $m/z = 1461.7$ $[\text{M}+3\text{H}]^{3+}$.

Synthesis of IV.16

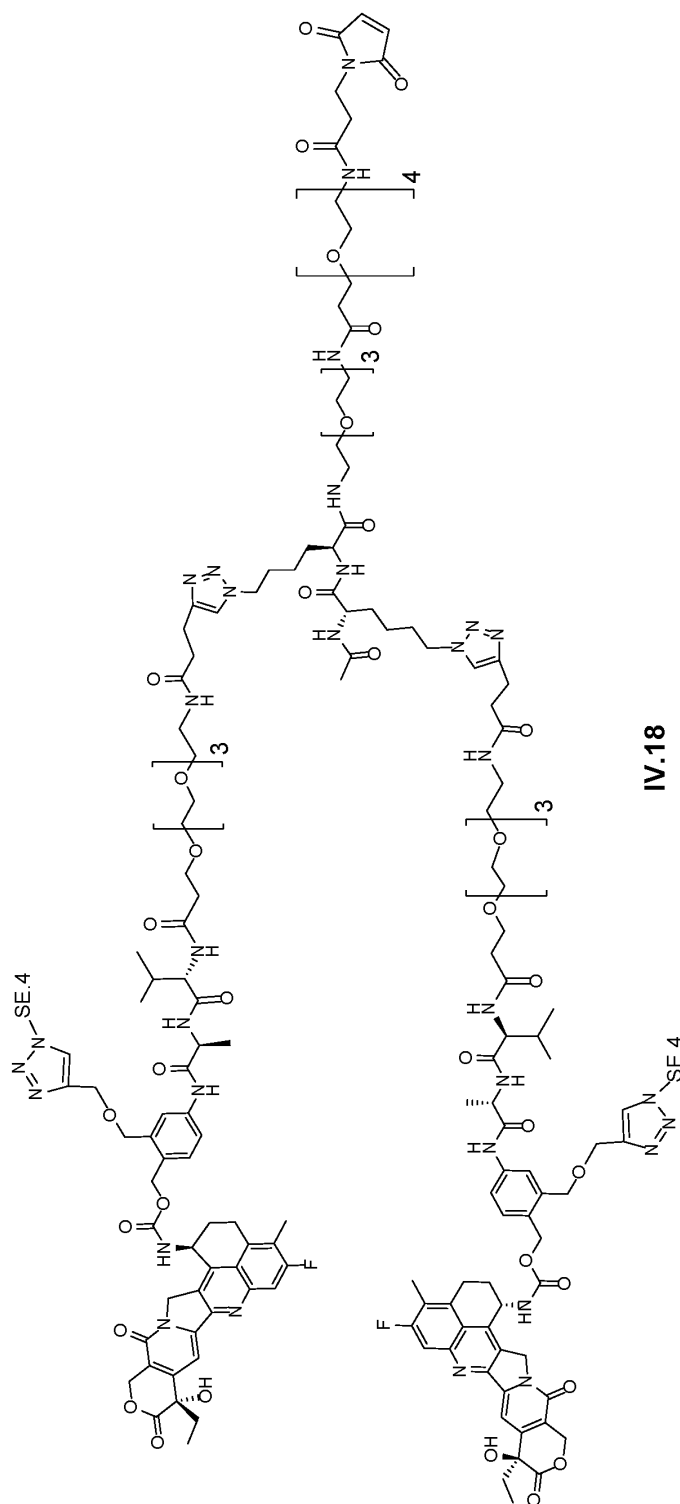


Compound **IV.16** was synthesized according to GP4 using the TFA-salt of intermediate **III.16** (7.4 mg, 1.26 μ mol, 1 equiv.), *N*-[15-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl]-2,5-dihydro-2,5-dioxo-1-*H*-pyrrole-1-propanamide = Mal-PEG4-NHS-ester (0.72 mg, 1.39 μ mol, 1.1 equiv.) and DIPEA (0.22 μ L, 1.26 μ mol, 1 equiv.) in DMF (0.3 mL). Purification by RP-HPLC afforded compound **IV.16** (3.85 mg, 61 %) as yellowish solid. C₂₂₀H₃₁₂F₂N₅O₆₈. HPLC (method J): *t*_R = 14.1 min. MS (ESI): found *m/z* = 1595.3 [M+3H]³⁺, calcd. *m/z* = 1594.4 [M+3H]³⁺.

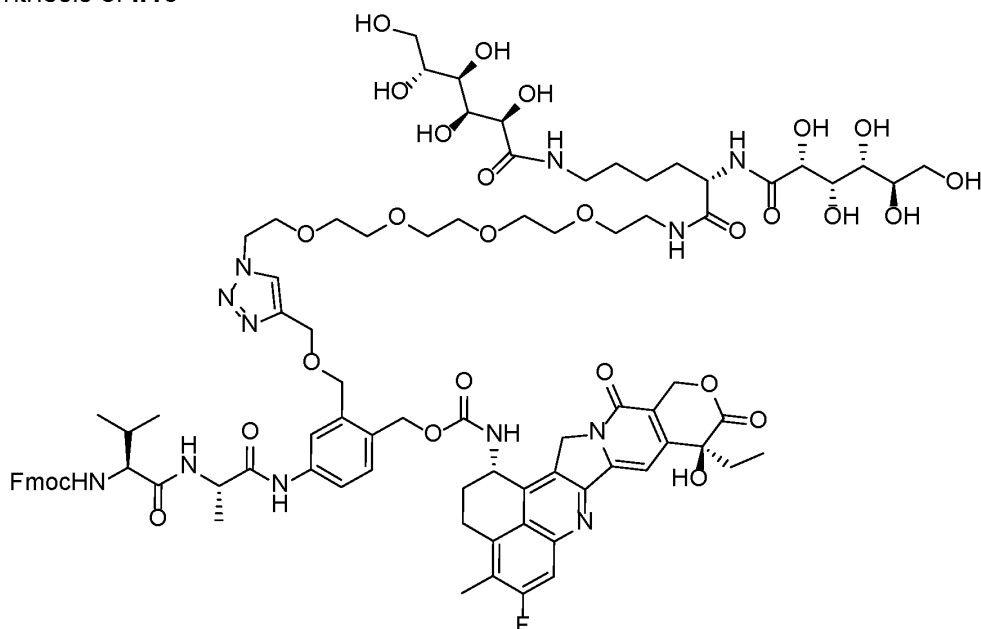
Example 19 - Synthesis of Compound IV.17

Compound **IV.17** was synthesized as described for compound **IV.16** but using intermediate **5.2**. Compound **IV.17** (23.5 mg, 75 %) was obtained as yellowish solid. C₂₂₀H₃₁₄F₂N₅₂O₆₆. HPLC (method J): t_R = 13.8 min. MS (ESI): found m/z = 1594.2 [M+3H]³⁺, calcd. m/z = 1593.8 [M+3H]³⁺.

Example 20 - Synthesis of Compound IV.18

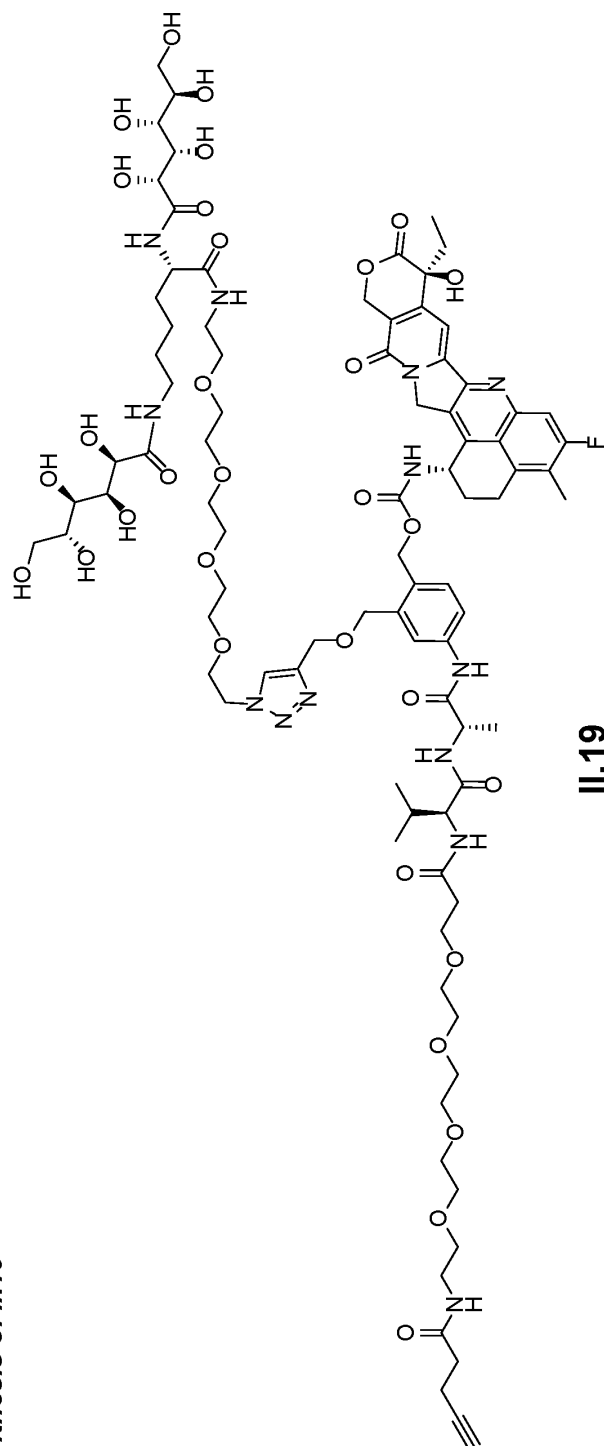


Compound **IV.18** was synthesized as described for compound **IV.16** but using **intermediates 3.2** and **5.2**. Compound **IV.18** (15.9 mg, 57 %) was obtained as yellowish solid. $C_{22}H_{32}O_7F_2N_5O_{66}$. HPLC (method J): $t_R = 12.9$ min. MS (ESI): found $m/z = 1621.8$ $[M+3H]^{3+}$, calcd. $m/z = 1621.1$ $[M+3H]^{3+}$.

Example 21 - Synthesis of Compound IV.19**Synthesis of I.19****I.19**

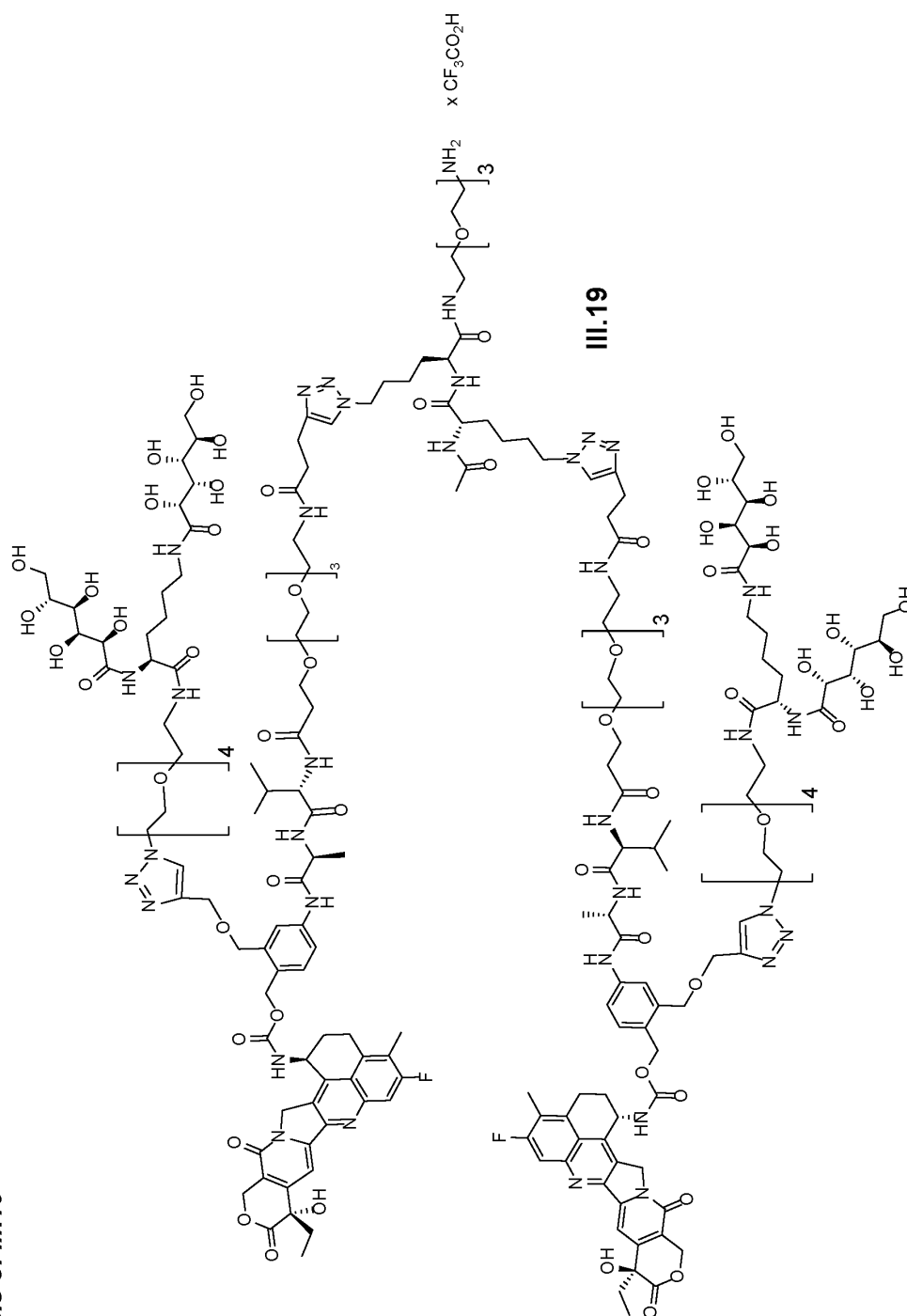
Intermediate **I.19** was synthesized according to GP1 using intermediate **6.1** (64.31 mg, 86.11 μmol , 1 equiv.), intermediate **1.3** (60.0 mg, 57.4 μmol , 1.0 equiv.), copper(II) sulfate pentahydrate (14.3 mg, 57.4 μmol , 1 equiv.), THPTA (50.08 mg, 115.2 μmol , 2 equiv.) and sodium ascorbate (34.38 mg, 173.5 μmol , 3 equiv.) in DMF/water (1.72 mL, 2:1). Purification by RP-HPLC afforded intermediate **I.17** (76.18 mg, 74 %) as yellowish solid. $\text{C}_{37}\text{H}_{111}\text{FN}_{12}\text{O}_{28}$. HPLC (method B): t_R = 16.8 min. MS (ESI): found m/z = 896.7 $[\text{M}+2\text{H}]^{2+}$, calcd. m/z = 896.4 $[\text{M}+2\text{H}]^{2+}$.

Synthesis of 11.19



Intermediate **II.19** was synthesized according to GP3 using intermediate **I.19** (76.18 mg, 42.51 μmol , 1 equiv.), diethylamine (105 μL , 1020 μmol , 24 equiv.) in 5 mL DMF (abs.) for Fmoc-deprotection and intermediate **3.2** (31.28 mg, 63.8 μmol , 1.5 equiv.), diisopropylethylamine (21.7 μL , 127.5 μmol , 3 equiv.) in DMF (1 mL) for the coupling reaction. Purification by RP-HPLC afforded intermediate **II.19** (38.97 mg, 48 %) as yellowish solid. $\text{C}_{88}\text{H}_{126}\text{FN}_{13}\text{O}_{32}$. HPLC (method N): t_{R} = 16.1 min. MS (ESI): found m/z = 949.6 $[\text{M}+2\text{H}]^{2+}$, calcd. m/z = 948.9 $[\text{M}+2\text{H}]^{2+}$.

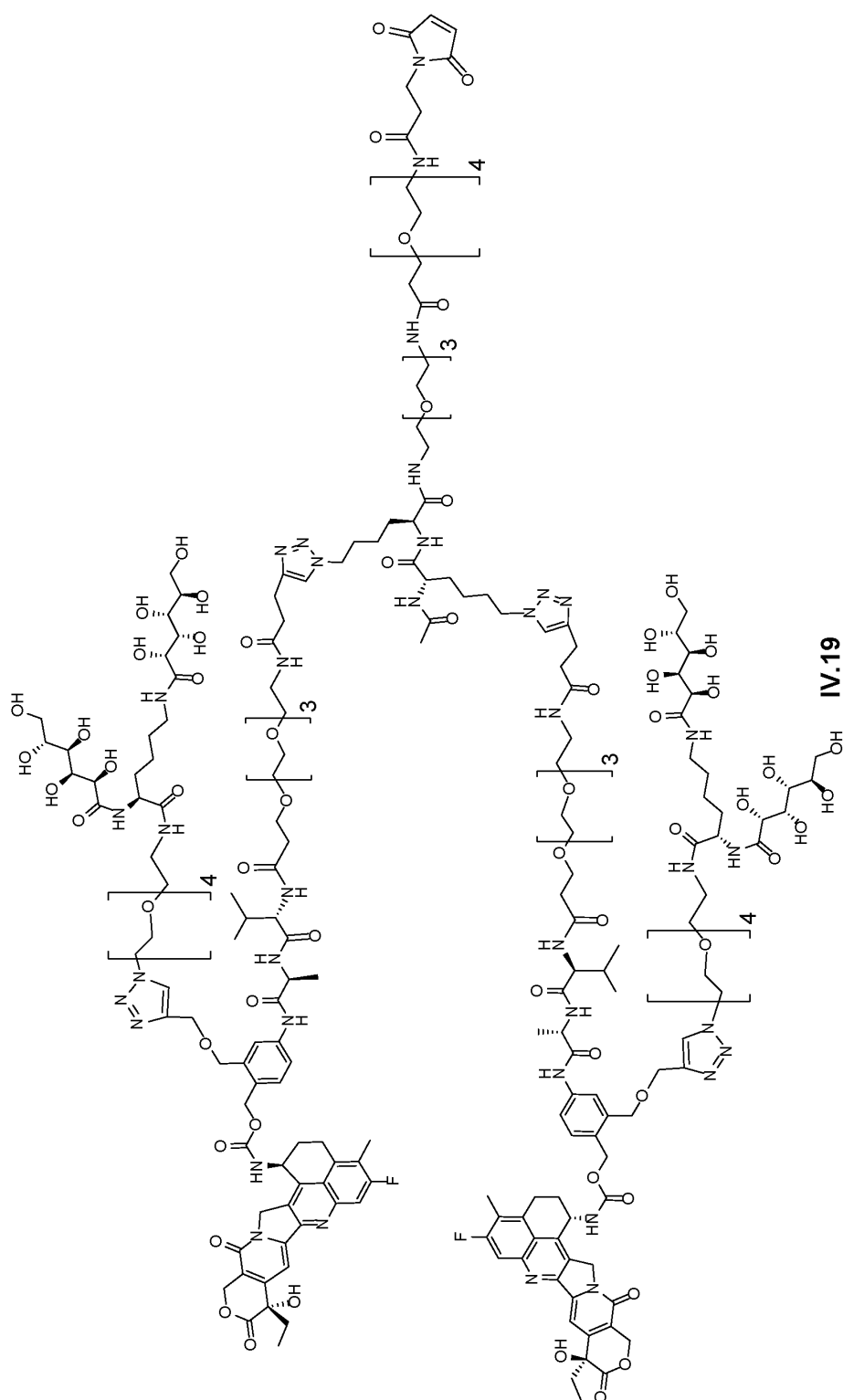
Synthesis of III.19



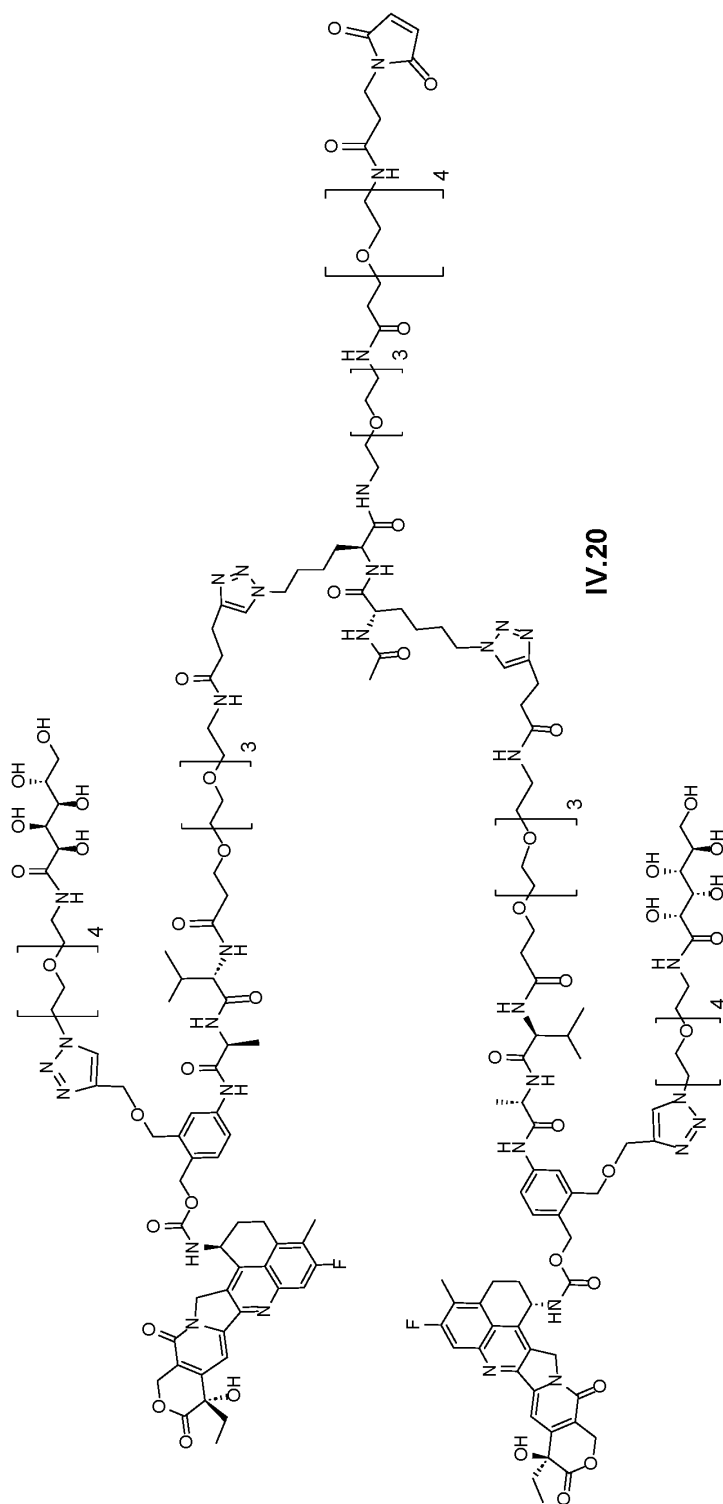
Intermediate **III.19** was synthesized according to GP2 using **intermediate 2.1** (4.96 mg, 8.6 μmol , 1 equiv.), intermediate **II.19** (38.97 mg, 20.5 μmol , 2.4 equiv), copper(II) sulfate pentahydrate (2.57 mg, 10.3 μmol , 1.2 equiv.), Tris(3-hydroxypropyltriazolylmethyl)amine (8.95 mg, 20.6 μmol , 2.4 equiv.) and sodium ascorbate (6.10 mg, 30.8 μmol , 3.6 equiv.) in DMF/water (1.13 mL, 10:1). After 3 h, the same catalyst amount was added again and the solution stirred for additional 40 min at room temperature. Purification by RP-HPLC afforded intermediate **III.19** (29.03 mg, 76 %) as yellowish solid. $\text{C}_{198}\text{H}_{294}\text{F}_2\text{N}_{36}\text{O}_{70}$. HPLC (method O): t_{R} = 12.3 min. MS (ESI): found m/z = 1446.6 $[\text{M}+3\text{H}]^{3+}$, calcd. m/z = 1245.7 $[\text{M}+3\text{H}]^{3+}$.

Synthesis of IV.19

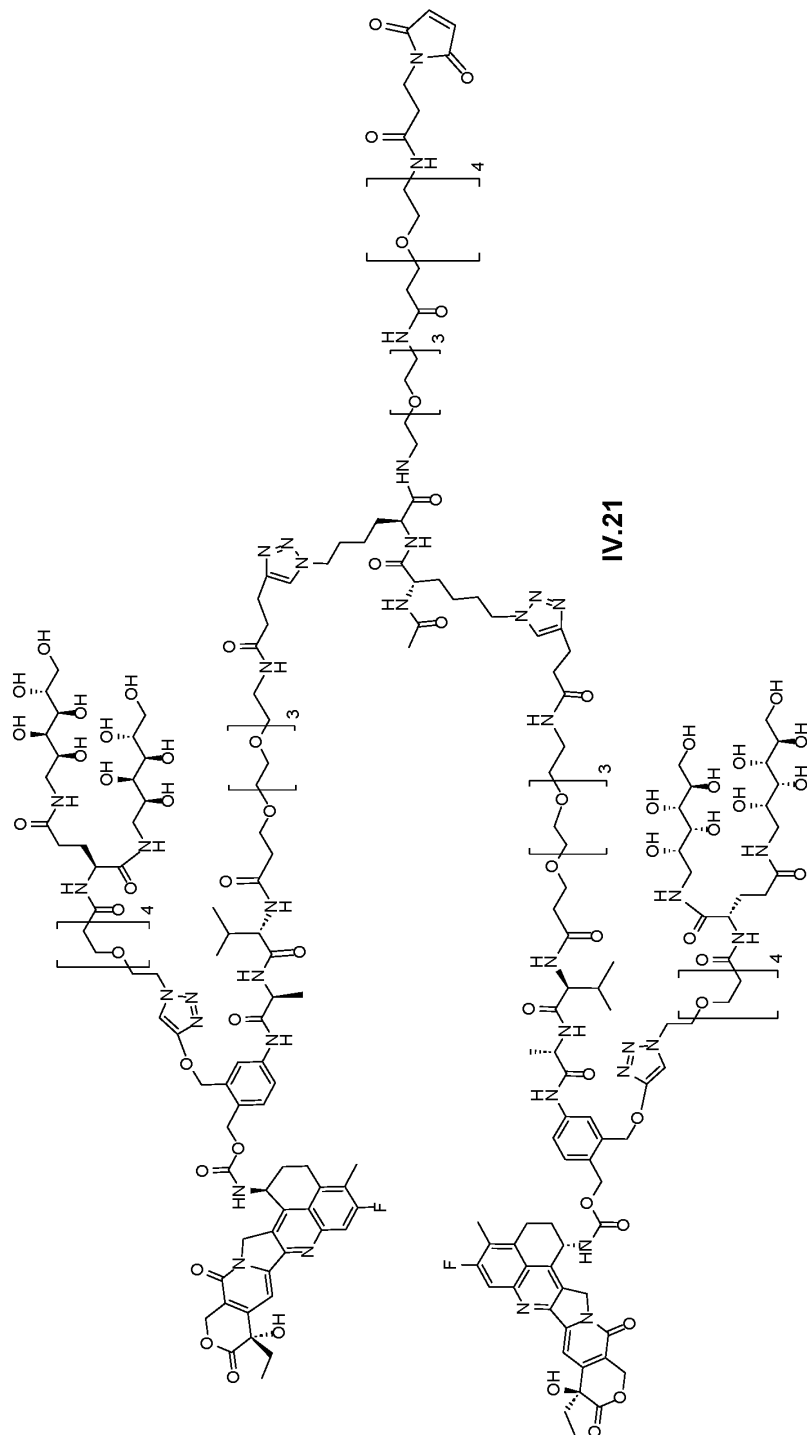
Compound **IV.19** was synthesized according to GP4 using the TFA-salt of intermediate **III.19** (29.03 mg, 6.5 μmol , 1 equiv.), N -[15-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl]-2,5-dihydro-2,5-dioxo-1-*H*-pyrrole-1-propanamide = Mal-PEG4-NHS-ester (13.40 mg, 26.1 μmol , 4 equiv.) and DIPEA (4.43 μL , 26.1 μmol , 4 equiv.) in DMF (0.78 mL). Upon completion after 1.5 h, the product was precipitated from ice-cold MTBE acidified with TFA (6 equiv.) and purification by RP-HPLC afforded compound **IV.17** (21.48 mg, 70 %) as yellowish solid. $\text{C}_{216}\text{H}_{320}\text{F}_2\text{N}_{38}\text{O}_{78}$. HPLC (method J): t_{R} = 13.3 min. MS (ESI): found m/z = 1578.7 $[\text{M}+3\text{H}]^{3+}$, calcd. m/z = 1578.4 $[\text{M}+3\text{H}]^{3+}$.



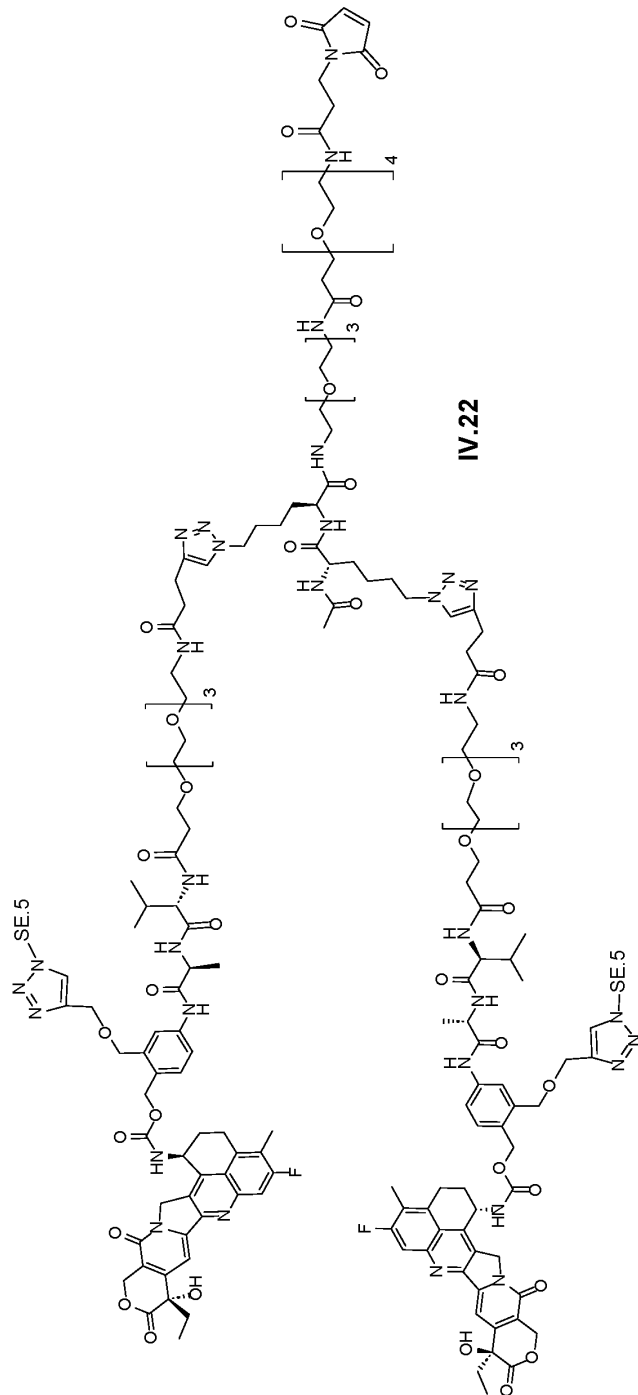
Example 22 - Synthesis of IV.20



Compound **IV.20** was synthesized as described for compound **IV.19** but using **intermediate 6.2**. Compound **IV.20** (14.34 mg, 31 %) was obtained as yellowish solid. $C_{192}H_{276}F_2N_{34}O_{64}$. HPLC (method J): $t_R = 13.5$ min. MS (ESI): found $m/z = 1374.9$ $[M+3H]^{3+}$, calcd. $m/z = 1374.3$ $[M+3H]^{3+}$.

Example 23 - Synthesis of IV.21

Compound **IV.21** was synthesized as described for compound **IV.19** but using **intermediate 6.3**. Compound **IV.21** (17.83 mg, 64 %) was obtained as yellowish solid. $C_{216}H_{320}F_2N_{38}O_{78}$. HPLC (method J): $t_R = 13.1$ min. MS (ESI): found $m/z = 1579.1$ $[M+3H]^{3+}$, calcd. $m/z = 1578.4$ $[M+3H]^{3+}$.

Example 24 - Synthesis of IV.22

Compound **IV.22** was synthesized as described for compound **IV.19** but using **intermediate 6.4**. Compound **IV.22** (27.9 mg, 55 %) was obtained as yellowish solid. $C_{232}H_{352}F_2N_{38}O_{86}$. HPLC (method J): $t_R = 13.4$ min. MS (ESI): found $m/z = 1697.0$ $[M+3H]^{3+}$, calcd. $m/z = 1695.8$ $[M+3H]^{3+}$.

Example 25 - In vitro cytotoxicity assays using 2D and 3D cell cultures

Cytotoxicity of anti-GUCY2C ADCs with modular linker compounds of the present disclosure was assessed by using *in vitro* cytotoxicity assay with target-positive HEK293-GUCY2C and target-negative HEKwt cells based on 2D and 3D cell culture.

At the start of the 2D cytotoxicity assay, 2×10^3 target-positive or target-negative cells were seeded per well in 96 Well plates and were incubated at 37°C and 5% CO₂. Indicated ADCs were added 24h later at various concentrations ranging from 1×10^{-6} M to 1.28×10^{-12} M to the adherent cells before the plates were incubated for additional 4 days at 37°C and 5% CO₂. Cytotoxicity was assessed by using Cell Proliferation ELISA, BrdU (colorimetric) of Roche.

At the start of the 3D cytotoxicity assay, 2×10^3 target-positive or target-negative cells were seeded per well in ultra-low adhesion (ULA) 96-Well plates and were incubated at 37°C and 5% CO₂. Spheroids were treated after 48h with the indicated ADCs at various concentrations ranging from 1×10^{-6} M to 1.28×10^{-12} M before the plates were incubated for a further 5 days at 37°C and 5% CO₂. Cytotoxicity was assessed by using CellTiter-Glo® 2.0 Cell Viability Assay of Promega.

Anti-GCC ADCs that were used as controls comprised the linker-payloads (IV.11), (IV.17) and (IV.19) which do not form part of the present invention.

Example 26 - Study design in vivo animal models

Female NOD SCID mice were inoculated subcutaneously with 5×10^6 GCC-overexpressing human embryonic kidney HEK293-GUCY2C-(HDP)-2B3 cells in 200 µL RPMI medium containing 50% Matrigel without phenol red per animal into their right flanks. Once a mean tumor volume of approximately 150 mm³ was reached, animals were allocated to control or treatment groups according to tumor size. On the same day (day 0) or the day after (day 1), the animals were treated with either a single intravenous dose of vehicle control or anti-GCC ADC conjugated to the respective modular linker compound of the invention, indicated as ("IV.x"), or once a week for three weeks. The tumor volume was measured twice per week by caliper and body weights were determined in parallel. Clinical signs and survival were monitored daily.

The animals were sacrificed, and necropsy was performed when one or more termination criteria arose or at study termination. The indicated dosing of the respective ADCs corresponds to the dose of total ADC in mg/kg as indicated in each case.

Many modifications and other embodiments of the present disclosure set forth herein will come to mind to the one skilled in the art to which the present disclosure pertains having the benefit of the teachings presented in the foregoing description and the associated drawings. Therefore, it is to be understood that the present disclosure is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

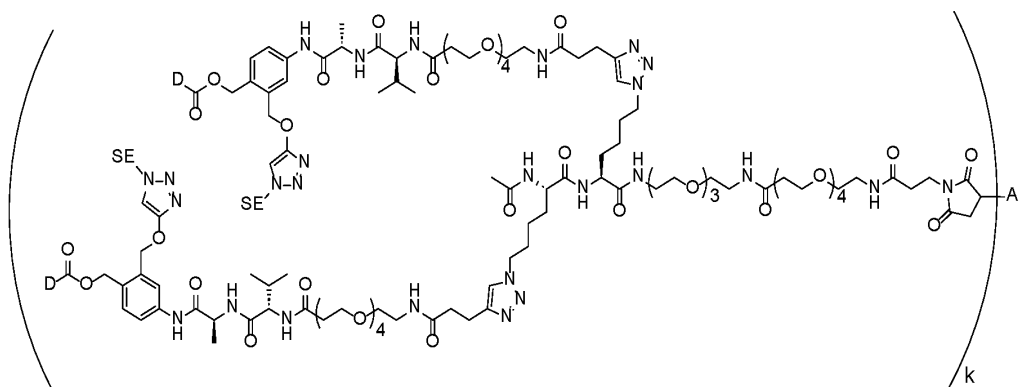
Example 27 – In vitro bystander assay

In an *in vitro* bystander assay, target-negative HEKwt cells, stained with CellTracer FarRed, and GUCY2C-expressing HEK-GUCY2C cells, stained with CFDA, were co-cultured and treated with anti-GCC ADCs. After 72 h, bystander killing of compounds was assessed by analyzing apoptotic (7AAD+) target-negative HEKwt cells.

All three anti-GCC ADCs showed strong bystander killing activity while no effect was observed on HEKwt cells without target-expressing HEK-GUCY2C cells (see fig. 9).

CLAIMS

1. An antibody-drug conjugate (ADC) of Formula (I)



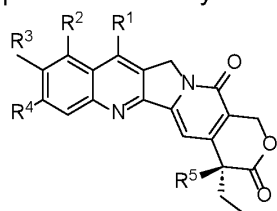
Formula (I)

or a pharmaceutically acceptable salt or solvate thereof; wherein:

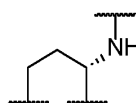
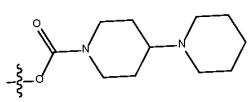
k is an integer from 2 to 4;

Ab is an anti-GUCY2C antibody;

D is a pharmaceutically active substance selected from



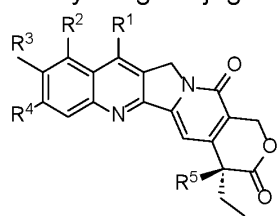
, wherein:

- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH; or
- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H and R⁵ is –O–; or

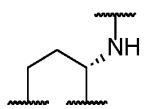
- R¹ is –H, R² is –NO₂, R³ –H, and R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₂NHCH(CH₃)₂, R² is –H, R³ –H, and R⁴ is –H and R⁵ is –O–; or
- R¹ is –CH₂CH₃, R² is –H, R³ –OH, and R⁴ is –H and R⁵ is –O–;

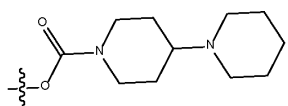
SE is a solubility enhancing group and comprises an alpha cyclodextrin, a beta-cyclodextrin, a gamma-cyclodextrin, a polysarcosine, a PEG, or a polyalcohol made from carbohydrates.

2. The antibody-drug conjugate according to claim 1, wherein D is

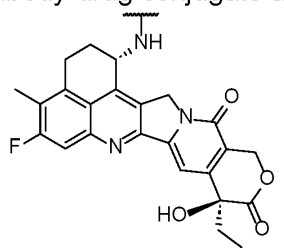


, wherein:

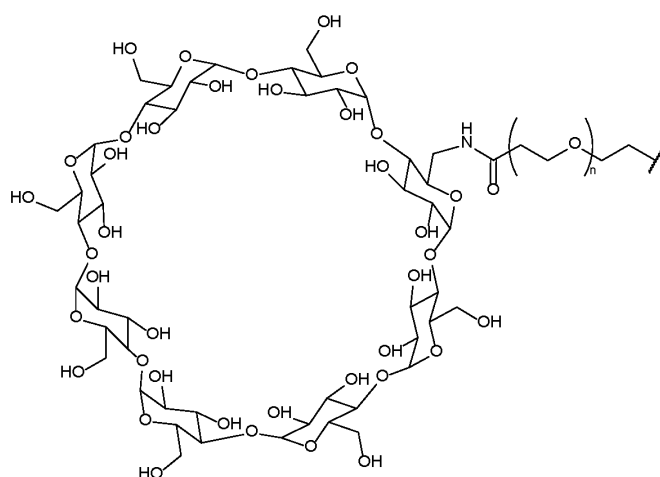
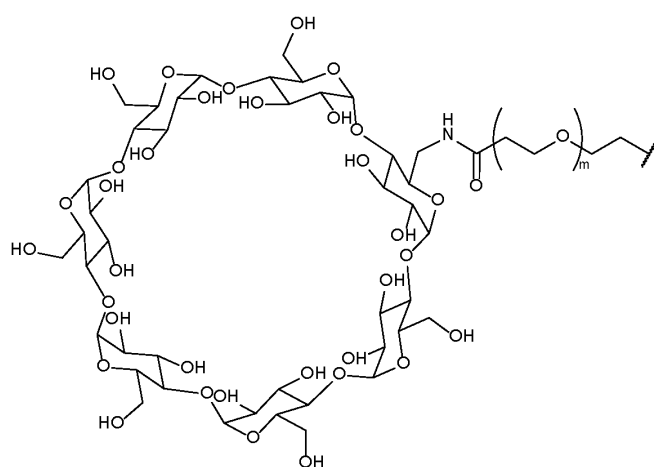
- R² and R¹ together form , R³ is –CH₃, R⁴ is –F and R⁵ is –OH; or
- R¹ is –H, R² is –CH₂N(CH₃)₂, R³ –OH, R⁴ is –H and R⁵ is –O–; or

- R¹ is –CH₂CH₃, R² is –H, R³ is , and R⁴ is –H and R⁵ is –O–.

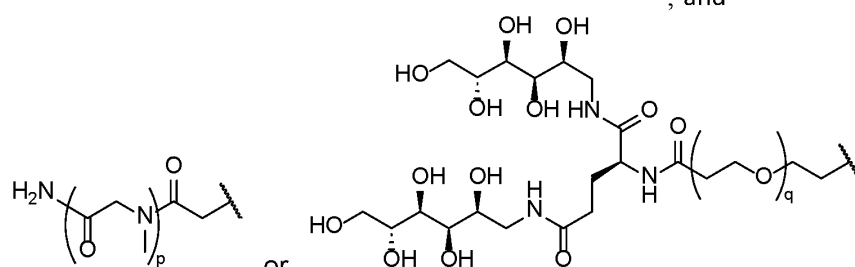
3. The antibody-drug conjugate according to claim 1 or claim 2, wherein D is



4. The antibody-drug conjugate according to any one of claims 1 to 3, wherein SE is selected from the group consisting of:



, and



wherein

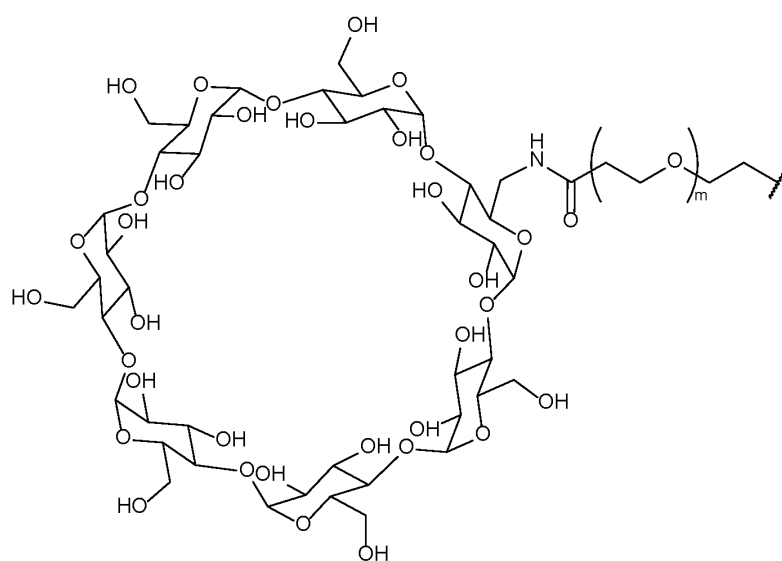
m is 2 to 12;

n is 2 to 12;

p is 1 to 20;

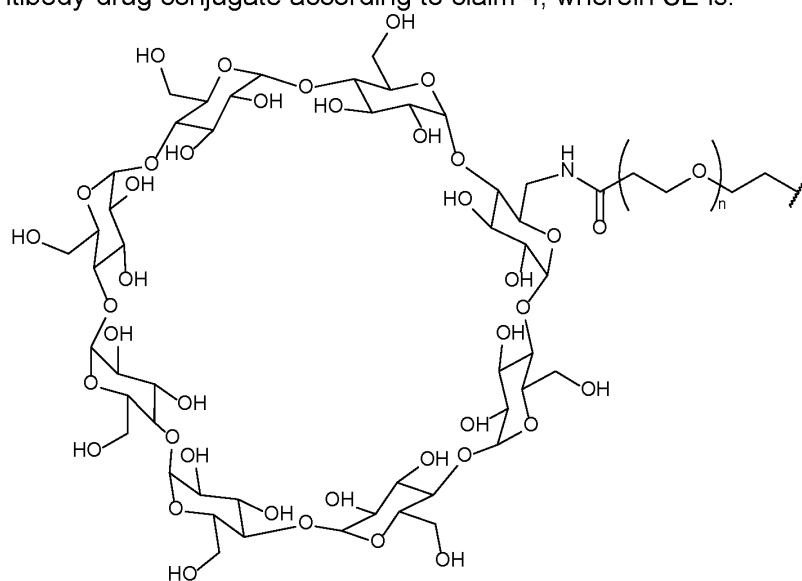
q is 2 to 12.

5. The antibody-drug conjugate according to claim 4, wherein SE is:



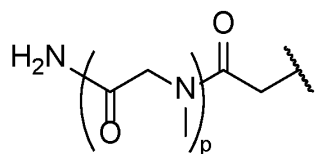
and m is 4 or 8.

6. The antibody-drug conjugate according to claim 4, wherein SE is:



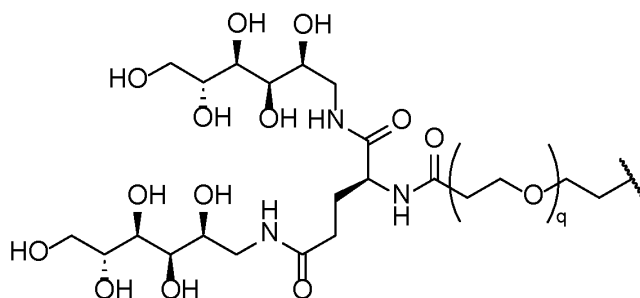
and n is 4.

7. The antibody-drug conjugate according to claim 4, wherein SE is:



and p is 10.

8. The antibody-drug conjugate according to claim 4, wherein SE is:



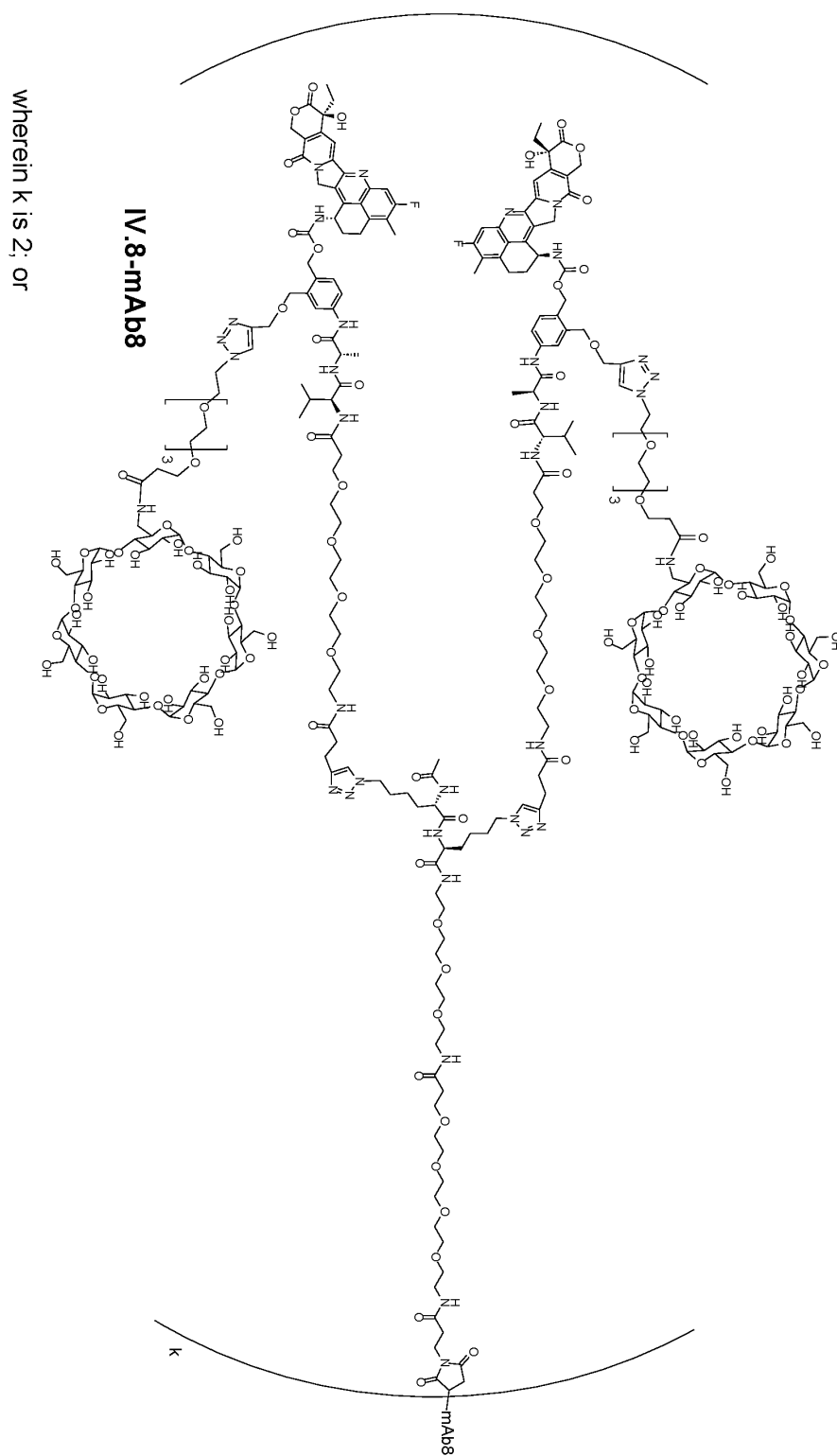
and q is 8.

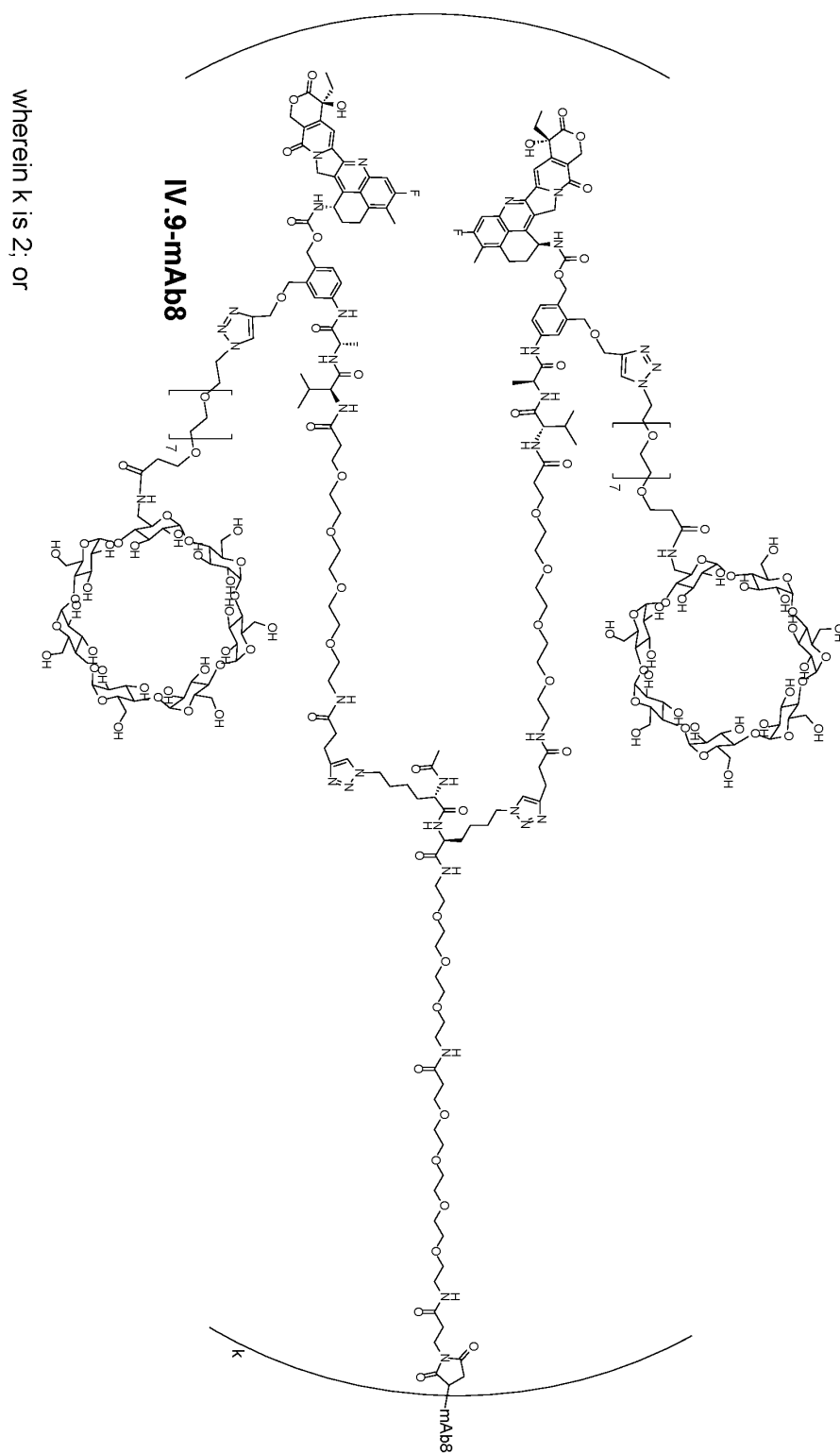
9. The antibody-drug conjugate according to any one of claims 1 to 8, wherein the antibody specifically recognizes and binds a target sequence or epitope of an antigen, preferably the antibody is one of:
- a monoclonal antibody;
 - a functional antibody fragment or antibody derivative such as a single-chain variable fragment (scFv), an antigen-binding fragment such as a Fab fragment, a F(ab'), fragment, a F(ab')₂ fragment or a bi- or tri-specific antibody construct,
 - a Diabody,
 - a Camelid Antibody,
 - a Domain Antibody,
 - a Nanobody,
 - a bivalent homodimer with two chains consisting of scFvs,
 - a shark antibody,
 - an antibody consisting of new-world primate framework sequences plus non-new world primate complementarity-determining regions (CDR) or
 - a dimerized construct comprising a constant heavy chain 3 (CH₃) domain, a variable light chain (V_L) and a variable heavy chain (V_H).

10. The antibody-drug conjugate according to any one of claims 1 to 9, wherein the antibody is a monoclonal antibody that recognizes and binds a target sequence or epitope of Guanylate Cyclase 2C (GUCY2C), preferably human or cynomolgus Guanylate Cyclase 2C, more preferably human Guanylate Cyclase 2C.
11. The antibody-drug conjugate according to any one of claims 1 to 10, wherein the antibody comprises an antigen binding region comprising:
 - an mAb1 light chain variable region (mAb1 V_L) complementarity-determining region (CDR_L) sequences CDR_L1 of SEQ ID NO: 1, CDR_L2 of SEQ ID NO: 2 and CDR_L3 of SEQ ID NO: 3 and
 - an mAb1 heavy chain variable region (mAb1 V_H) CDR_H1 of SEQ ID NO: 4, CDR_H2 of SEQ ID NO: 5 and CDR_H3 of SEQ ID NO: 6.
12. The antibody-drug conjugate according to any one of claims 1 to 9, wherein the antibody comprises an antigen binding region comprising:
 - an mAb8 light chain variable region (mAb8 V_L) complementarity-determining region (CDR_L) sequences CDR_L1 of SEQ ID NO: 1, CDR_L2 of SEQ ID NO: 2 and CDR_L3 of SEQ ID NO: 7 and
 - an mAb8 heavy chain variable region (mAb8 V_H) CDR_H1 of SEQ ID NO: 4, CDR_H2 of SEQ ID NO: 5 and CDR_H3 of SEQ ID NO: 6.
13. The antibody-drug conjugate according to any one of claims 1 to 10, wherein the antibody comprises an antigen binding region comprising:
 - an mAb41 light chain variable region (mAb41 V_L) complementarity-determining region (CDR_L) sequences CDR_L1 of SEQ ID NO: 8, CDR_L2 of SEQ ID NO: 9 and CDR_L3 of SEQ ID NO: 10 and
 - an mAb41 heavy chain variable region (mAb41 V_H) CDR_H1 of SEQ ID NO: 11, CDR_H2 of SEQ ID NO: 12 and CDR_H3 of SEQ ID NO: 13.
14. The antibody-drug conjugate according to claim 11, wherein the antibody comprises an antigen binding region comprising:
 - the mAb1 V_L comprises a framework region 1 (FR_L1) of SEQ ID NO: 14, CDR_L1 of SEQ ID NO: 1, a FR_L2 of SEQ ID NO: 15, CDR_L2 of SEQ ID NO:

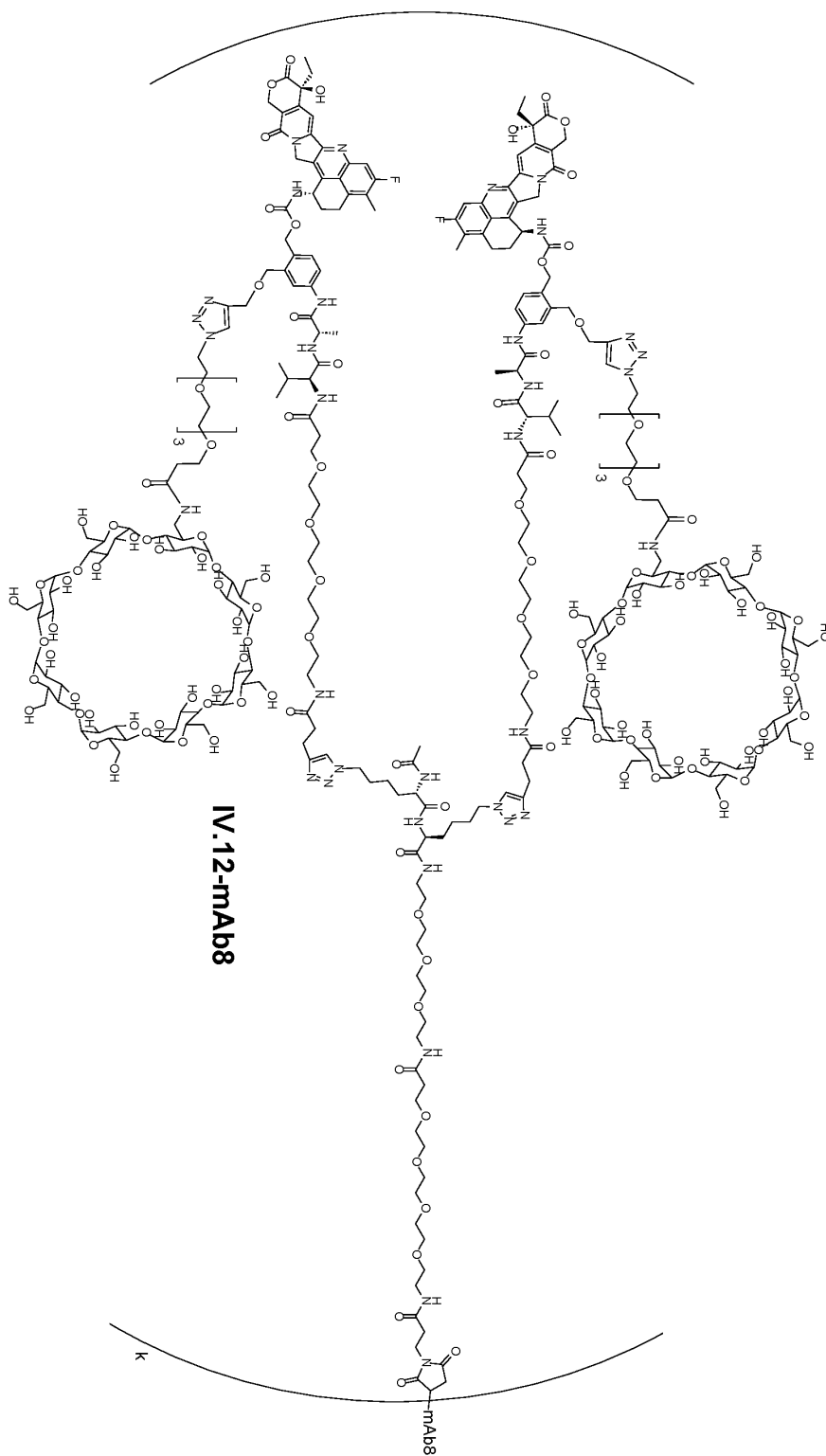
- 2, a FR_L3 of SEQ ID NO: 16, CDR_L3 of SEQ ID NO: 3 and a FR_L4 of SEQ ID NO: 17; and
- the mAb1 V_H comprises a framework region 1 (FR_H1) of SEQ ID NO: 18, CDR_H1 of SEQ ID NO: 4, a FR_H2 of SEQ ID NO: 19, CDR_H2 of SEQ ID NO: 5, a FR_H3 of SEQ ID NO: 20, CDR_H3 of SEQ ID NO: 6 and a FR_H4 of SEQ ID NO: 21.
15. The antibody-drug conjugate according to claim 12, wherein the antibody comprises an antigen binding region comprising:
- the mAb8 V_L comprises a framework region 1 (FR_L1) of SEQ ID NO: 22, CDR_L1 of SEQ ID NO: 1, a FR_L2 of SEQ ID NO: 15, CDR_L2 of SEQ ID NO: 2, a FR_L3 of SEQ ID NO: 23, CDR_L3 of SEQ ID NO: 7 and a FR_L4 of SEQ ID NO: 24; and
 - the mAb8 V_H comprises a framework region 1 (FR_H1) of SEQ ID NO: 18, CDR_H1 of SEQ ID NO: 4, a FR_H2 of SEQ ID NO: 19, CDR_H2 of SEQ ID NO: 5, a FR_H3 of SEQ ID NO: 20, CDR_H3 of SEQ ID NO: 6 and a FR_H4 of SEQ ID NO: 21.
16. The antibody-drug conjugate according to claim 13, wherein the antibody comprises an antigen binding region comprising:
- the mAb41 V_L comprises a framework region 1 (FR_L1) of SEQ ID NO: 25, CDR_L1 of SEQ ID NO: 8, a FR_L2 of SEQ ID NO: 26, CDR_L2 of SEQ ID NO: 9, a FR_L3 of SEQ ID NO: 27, CDR_L3 of SEQ ID NO: 10 and a FR_L4 of SEQ ID NO: 28; and
 - the mAb41 V_H comprises a framework region 1 (FR_H1) of SEQ ID NO: 29, CDR_H1 of SEQ ID NO: 11, a FR_H2 of SEQ ID NO: 30, CDR_H2 of SEQ ID NO: 12, a FR_H3 of SEQ ID NO: 31, CDR_H3 of SEQ ID NO: 13 and a FR_H4 of SEQ ID NO: 21.
17. The antibody-drug conjugate according to claim 14, wherein the antibody comprises an antigen binding region comprising:
- the mAb1 V_L of SEQ ID NO: 32 and
 - the mAb1 V_H of SEQ ID NO: 33.

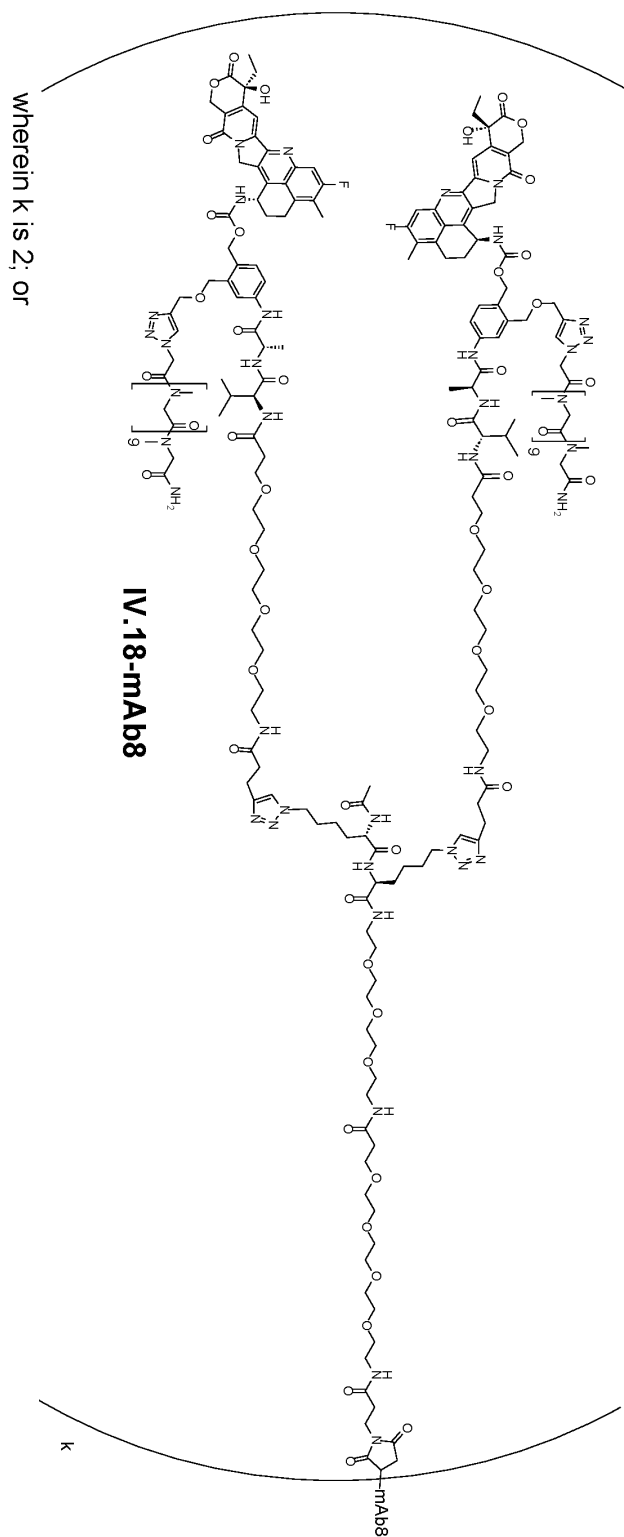
18. The antibody-drug conjugate according to claim 15, wherein the antibody comprises an antigen binding region comprising:
 - the mAb8 V_L of SEQ ID NO: 34 and
 - the mAb8 V_H of SEQ ID NO: 35.
19. The antibody-drug conjugate according to claim 16, wherein the antibody comprises an antigen binding region comprising:
 - the mAb41 V_L of SEQ ID NO: 36 and
 - the mAb41 V_H of SEQ ID NO: 37.
20. The antibody-drug conjugate according to any one of claims 1 to 19, wherein the antibody is: (a) a humanized or human antibody; and/or (b) an IgG type antibody, preferably an IgG1 antibody; and/or (c) a recombinant antibody.
21. The antibody according to claim 20, wherein the antibody comprises a heavy chain constant (Fc) region which comprises the amino acid substitution D265C, preferably said heavy chain constant (Fc) region comprises the amino acid substitutions L234A, L235A and D265C (according to EU numbering system).
22. The antibody according to claim 21, wherein the antibody comprises
 - an mAb1-D265C heavy chain of SEQ ID NO: 39; or
 - an mAb8-D265C heavy chain of SEQ ID NO: 40; or
 - an mAb41-D265C of SEQ ID NO: 41.
23. The antibody according to claim 21, wherein the antibody comprises
 - an mAb1-L234A-L235A-D265C heavy chain of SEQ ID NO: 42; or
 - an mAb8-L234A-L235A-D265C heavy chain of SEQ ID NO: 43; or
 - an mAb41-L234A-L235A-D265C of SEQ ID NO: 44.
24. The antibody-drug conjugate according to any one of claims 1 to 23, wherein the antibody-drug conjugate is:

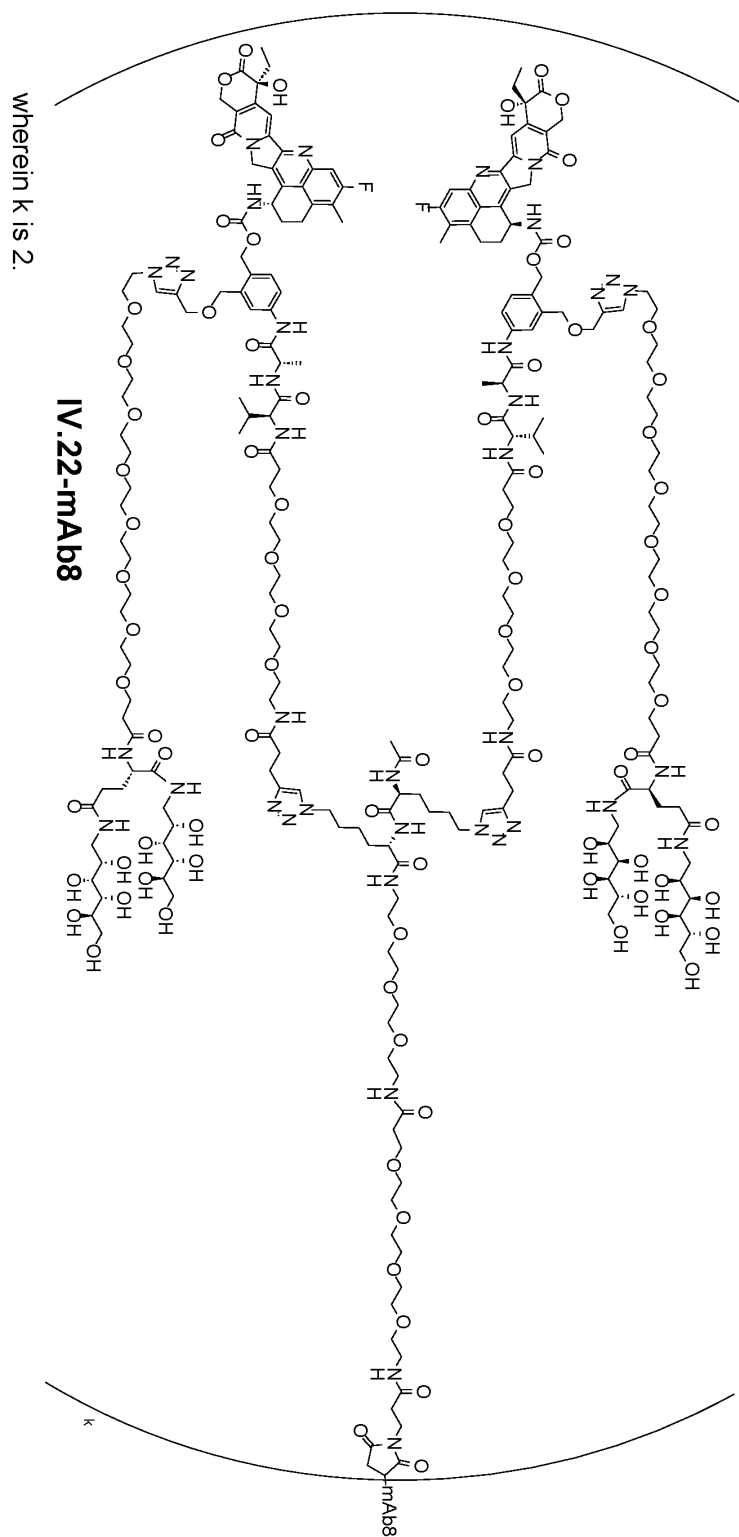




wherein k is 2, or







25. The antibody-drug conjugate according to any one of claims 1 to 24 for use as a medicament, preferably for use in the treatment of cancer, more preferably for the treatment of gastrointestinal cancer, such as colorectal cancer (CRC), metastatic colorectal cancer (mCRC) or pancreatic cancer.

26. A pharmaceutical composition comprising the antibody-drug conjugate or pharmaceutically acceptable salt thereof according to any one of claims 1 to 24.

27. A method of treating cancer, wherein the method comprises administering the antibody-drug conjugate or pharmaceutically acceptable salt thereof according to any one of claims 1 to 24 to a subject in need thereof.

28. The method of claim 27, wherein the cancer is a gastrointestinal cancer.

29. The method of claim 28, wherein the cancer is selected from the group consisting of colorectal cancer (CRC), metastatic colorectal cancer (mCRC) and pancreatic cancer.

30. The method of any one of claims 26-29, further comprising administering to the subject ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), and/or atezolizumab (anti-PD-L1).