

Macrocyclic deaza-purinones for the treatment of viral infections

This invention relates to macrocyclic deaza-purinones derivatives, processes for their preparation, pharmaceutical compositions, and their use in treating viral infections.

The present invention relates to the use of macrocyclic deaza-purinones derivatives in the treatment of viral infections, immune or inflammatory disorders, whereby the modulation, or agonism, of toll-like-receptors (TLRs) is involved. Toll-Like Receptors are primary transmembrane proteins characterized by an extracellular leucine rich domain and a cytoplasmic extension that contains a conserved region. The innate immune system can recognize pathogen-associated molecular patterns via these TLRs expressed on the cell surface of certain types of immune cells. Recognition of foreign pathogens activates the production of cytokines and upregulation of co-stimulatory molecules on phagocytes. This leads to the modulation of T cell behaviour.

It has been estimated that most mammalian species have between ten and fifteen types of Toll-like receptors. Thirteen TLRs (named TLR1 to TLR13) have been identified in humans and mice together, and equivalent forms of many of these have been found in other mammalian species. However, equivalents of certain TLR found in humans are not present in all mammals. For example, a gene coding for a protein analogous to TLR10 in humans is present in mice, but appears to have been damaged at some point in the past by a retrovirus. On the other hand, mice express TLRs 11, 12, and 13, none of which are represented in humans. Other mammals may express TLRs which are not found in humans. Other non-mammalian species may have TLRs distinct from mammals, as demonstrated by TLR14, which is found in the Takifugu pufferfish. This may complicate the process of using experimental animals as models of human innate immunity.

For detailed reviews on toll-like receptors see the following journal articles. Hoffmann, J.A., Nature, 426, p33-38, 2003; Akira, S., Takeda, K., and Kaisho, T., Annual Rev. Immunology, 21, p335-376, 2003; Ulevitch, R. J., Nature Reviews: Immunology, 4, p512-520, 2004.

Compounds indicating activity on Toll-Like receptors have been previously described such as purine derivatives in WO 2006/117670, adenine derivatives in WO 98/01448 and WO 99/28321, and pyrimidines in WO 2009/067081.

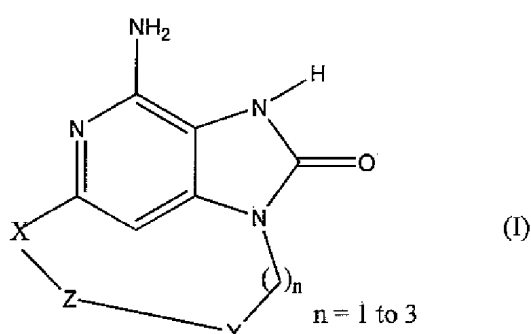
However, there exists a strong need for novel Toll-Like receptor modulators having preferred selectivity, higher potency, higher metabolic stability, and an improved safety profile compared to the compounds of the prior art.

In the treatment of certain viral infections, regular injections of interferon (IFN-alfa) can be administered, as is the case for hepatitis C virus (HCV). For more information see Fried et al. Peginterferon-alfa plus ribavirin for chronic hepatitis C virus infection, *N Engl J Med* 2002; 347:

975-82. Orally available small molecule IFN inducers offer the potential advantages of reduced immunogenicity and convenience of administration. Thus, novel IFN inducers are potentially effective new class of drugs for treating virus infections. An example of a small molecule IFN inducer having antiviral effect see De Clercq, E.; Descamps, J.; De Somer, P. *Science* 1978, 200, 563-565.

IFN- α is also given in combination with other drugs in the treatment of certain types of cancer (Eur. J. Cancer 46, 2849-57, and Cancer Res. 1992, 52, 1056). TLR 7/8 agonists are also of interest as vaccine adjuvants because of their ability to induce pronounced Th1 response (Hum. Vaccines 2010, 6, 322-335, and Hum. Vaccines 2009, 5, 381-394).

In accordance with the present disclosure, in one aspect, a compound of formula (I) is provided



and pharmaceutically accepted salts thereof, wherein

X is oxygen, nitrogen or sulfur

Y represents an aromatic ring or heterocyclic ring comprising at least a nitrogen, optionally substituted by one or more substituents independently selected from C_{1-6} alkyl, C_{1-4} alkoxy, trifluoromethyl or halogen,

Z represents C_{1-10} saturated or unsaturated alkyl optionally substituted by an alkyl or alkylhydroxyl;

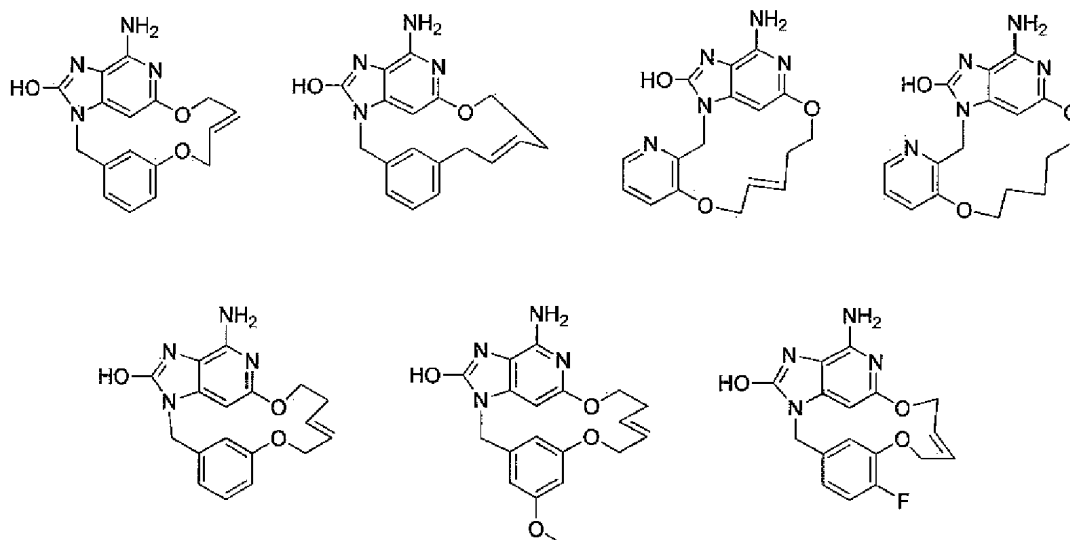
or Z represents C_{1-6} alkyl -NH-C(O)- C_{1-6} alkyl-, C_{1-6} alkyl-NH- or C_{1-6} alkyl -NH-C(O)- C_{1-6} alkyl -O-;

or Z represents C_{1-10} alkyl -O- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl ,

or Z represents C_{1-6} alkyl-O- C_{1-6} alkyl- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl

or Z represents C_{1-6} alkyl-O- C_{1-6} alkyl-O- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl.

Preferred compounds having one of the following formula's according to the disclosure were selected from the group of:



In another aspect, there is provided a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof together with one or more pharmaceutically acceptable excipients, diluents or carriers.

- 5 In another aspect, there is provided a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof or a pharmaceutical composition above mentioned for use as a medicament.

- In another aspect, there is provided a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof or a pharmaceutical composition above mentioned for use in
10 the treatment of a disorder in which the modulation of TLR7 is involved.

The term “alkyl” refers to a straight-chain or branched-chain mostly saturated (but in specific compounds according to the invention being unsaturated) aliphatic hydrocarbon containing the specified number of carbon atoms.

The term “halogen” refers to fluorine, chlorine, bromine or iodine.

- 15 The term “alkoxy” refers to an alkyl (carbon and hydrogen chain) group singular bonded to oxygen like for instance a methoxy group or ethoxy group.

Pharmaceutically acceptable salts of the compounds of formula (I) include the acid addition and base salts thereof. Suitable acid addition salts are formed from acids which form non-toxic salts. Suitable base salts are formed from bases which form non-toxic salts.

- 20 The compounds of the invention may also exist in unsolvated and solvated forms. The term “solvate” is used herein to describe a molecular complex comprising the compound of the invention and one or more pharmaceutically acceptable solvent molecules, for example, ethanol.

The term “polymorph” refers to the ability of the compound of the invention to exist in more than one form or crystal structure.

The compounds of the invention can be present in a so-called “tautomer(s)” formation referring to isomers of organic compounds that readily interconvert by a chemical reaction called tautomerization. This reaction results in the formal migration of a hydrogen atom or proton, accompanied by a switch of a single bond and adjacent double bond.

The compounds of the present invention may be administered as crystalline or amorphous products. They may be obtained for example as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze drying, spray drying, or evaporative drying. They may be administered alone or in combination with one or more other compounds of the invention or in combination with one or more other drugs. Generally, they will be administered as a formulation in association with one or more pharmaceutically acceptable excipients. The term “excipient” is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient depends largely on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

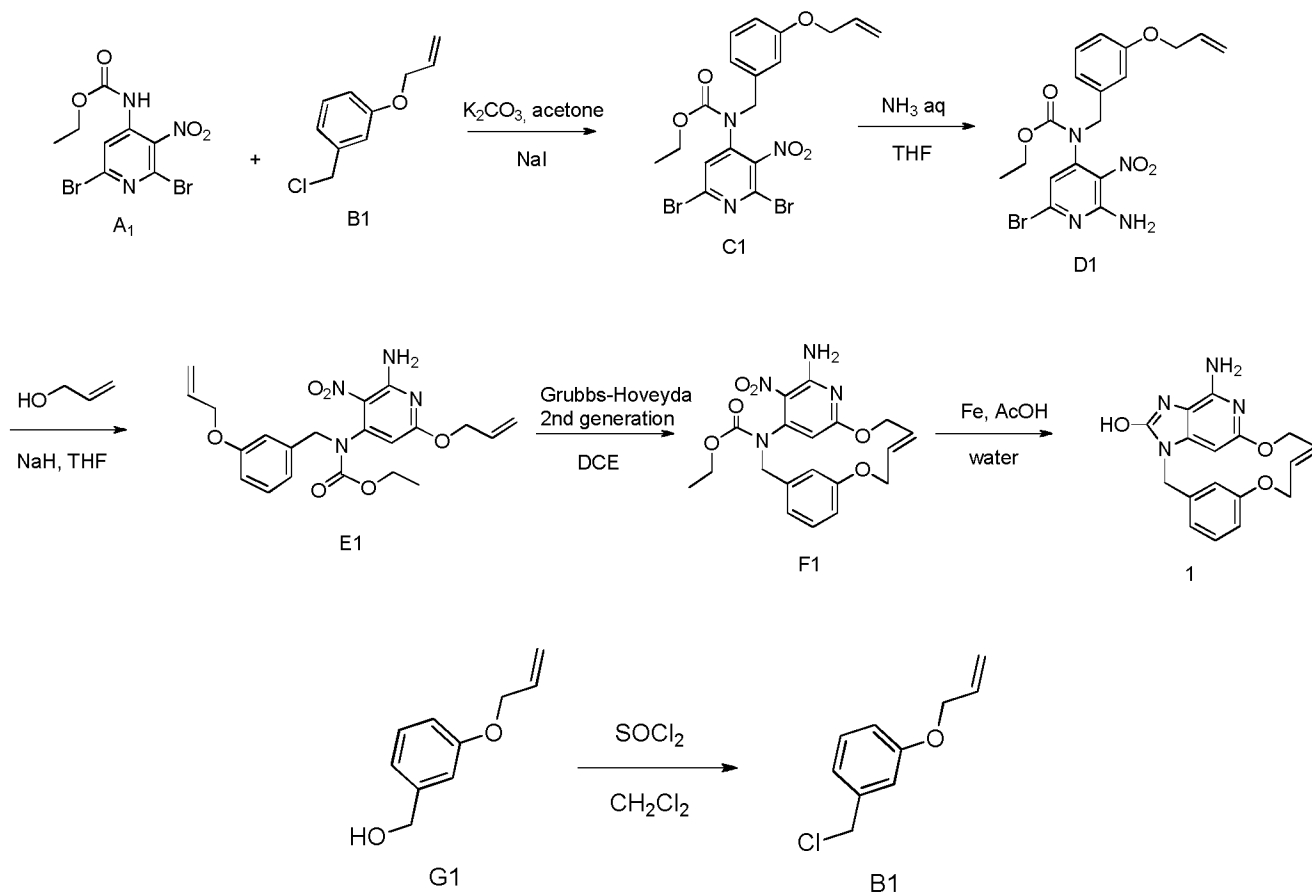
The compounds of the present invention or any subgroup thereof may be formulated into various pharmaceutical forms for administration purposes. As appropriate compositions there may be cited all compositions usually employed for systemically administering drugs. To prepare the pharmaceutical compositions of this invention, an effective amount of the particular compound, optionally in addition salt form, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirably in unitary dosage form suitable, for example, for oral, rectal, or percutaneous administration. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs, emulsions, and solutions; or solid carriers such as starches, sugars, kaolin, diluents, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are obviously employed. Also included are solid form preparations that can be converted, shortly before use, to liquid forms. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on, as an ointment. The compounds of the

present invention may also be administered via inhalation or insufflation by means of methods and formulations employed in the art for administration via this way. Thus, in general the compounds of the present invention may be administered to the lungs in the form of a solution, a suspension or a dry powder.

- 5 It is especially advantageous to formulate the aforementioned pharmaceutical compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such unit dosage forms are
10 tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, suppositories, injectable solutions or suspensions and the like, and segregated multiples thereof.

Those of skill in the treatment of infectious diseases will be able to determine the effective amount from the test results presented hereinafter. In general it is contemplated that an effective daily amount would be from 0.01 mg/kg to 50 mg/kg body weight, more preferably from 0.1
15 mg/kg to 10 mg/kg body weight. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses may be formulated as unit dosage forms, for example, containing 1 to 1000 mg, and in particular 5 to 200 mg of active ingredient per unit dosage form.

The exact dosage and frequency of administration depends on the particular compound of
20 formula (I) used, the particular condition being treated, the severity of the condition being treated, the age, weight and general physical condition of the particular patient as well as other medication the individual may be taking, as is well known to those skilled in the art. Furthermore, it is evident that the effective amount may be lowered or increased depending on the response of the treated subject and/or depending on the evaluation of the physician
25 prescribing the compounds of the instant invention. The effective amount ranges mentioned above are therefore only guidelines and are not intended to limit the scope or use of the invention to any extent.

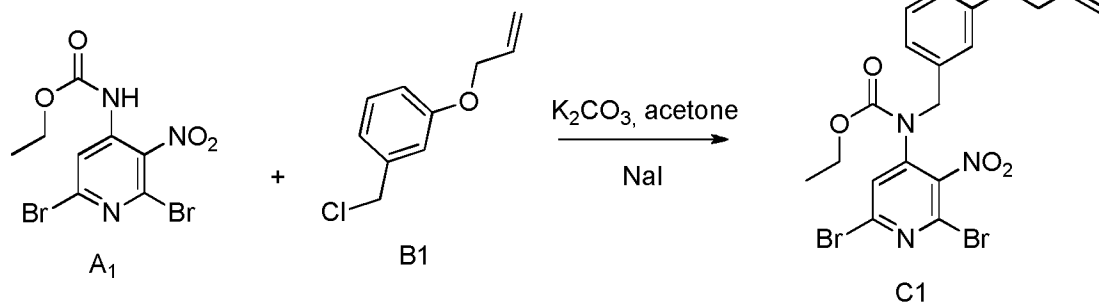
Overall Scheme in the preparation of final products: method 1

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Synthesis of intermediate B1

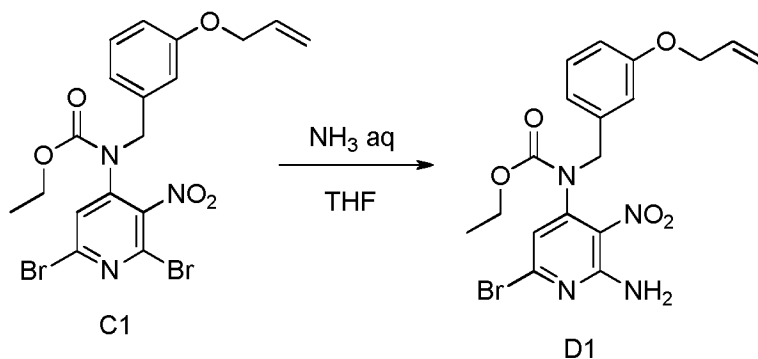
SOC₂ (80 mL; 1.11 mol) was added drop wise to a mixture of G1 (19.1 g; 111 mmol) in CH₂Cl₂ (230 mL) at rt. The mixture was stirred at rt for 16 h. The solvent was evaporated and the residue was solubilized in CH₂Cl₂ and treated with a saturated aqueous solution of NaHCO₃ until basic pH. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (twice). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo to give 20.1 g of a brown oil. The crude compound was used in the next step without further purification.

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15 **Synthesis of intermediate C1**

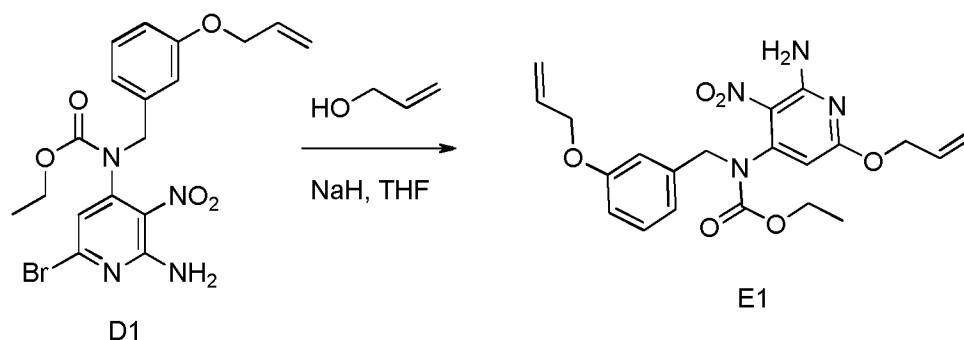
The reaction was performed on two batches in parallel (13.6 mmol and 27.1 mmol of A1).

To a solution of A1 (5.0 g; 13.6 mmol) and K_2CO_3 (3.75 g; 27.1 mmol) in acetone (100 mL) were added B1 (4.46 g; 24.4 mmol) and NaI (2.23 g; 14.9 mmol) at rt. The mixture was stirred at
 5 rt for 16 h. The mixture was filtered and the filtrate was evaporated in vacuo to give a brown oil. The two batches were combined and purified by preparative LC (Irregular SiOH 15-40 μ m, 220 g Grace, mobile phase gradient: CH_2Cl_2 /EtOAc from 100/0 to 80/20). The fractions containing product were combined and the solvent was removed in vacuo to give 18.6 g of intermediate C1 (89% yield).



Synthesis of intermediate D1

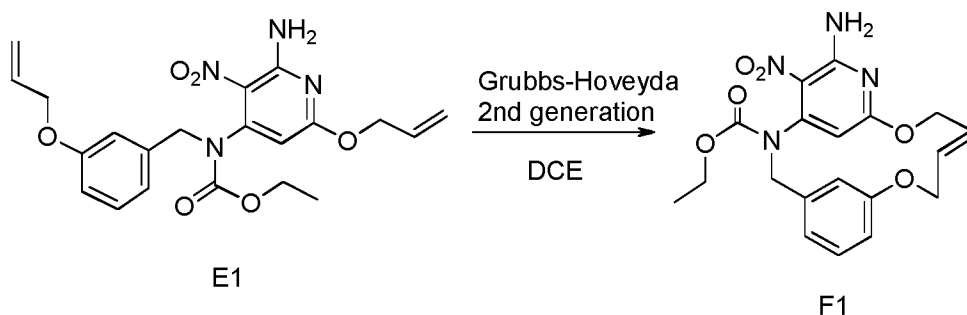
To a solution of C1 (18.6 g; 36.1 mmol) in THF (300 mL) was added an aqueous solution of NH_3 (30%) (290 mL) at rt, and the mixture was stirred at rt for 16 h. The mixture was taken up
 15 with EtOAc and saturated NaCl solution, the layers were separated and the organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give 16.7 g of a yellow-orange oil. The crude was dried under high vacuum to give 16.5 g of a sticky yellow-orange solid, which was used directly in the next step.



Synthesis of intermediate E1

NaH (60% in oil) (1.75 g; 43.7 mmol) was added portion wise to allyl alcohol (50 mL) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of D1 (5 g; 11.1 mmol) in THF (124 mL) at 0°C. The resulting mixture was then stirred at rt for 1 h and was

poured in saturated NH_4Cl aqueous solution. EtOAc and saturated NaCl aqueous solution were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give a yellow oil. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Grace, liquid injection, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50) to give 4.04 g of intermediate E1 as a yellow oil (79% yield).



Synthesis of intermediate F1

The reaction was performed in 2 batches of 850 mg and 2 batches of 1 g of E1.

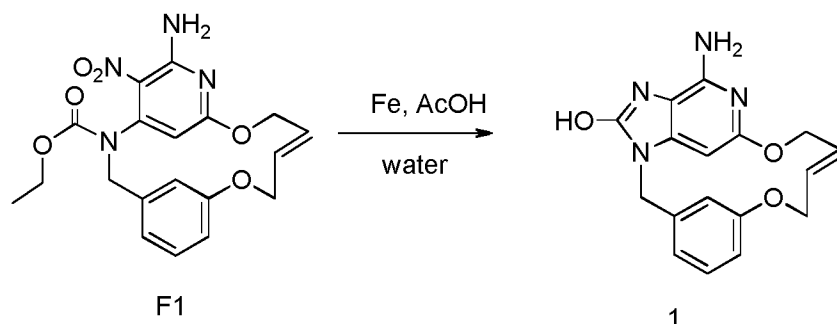
Herein is the procedure for one batch of 850 mg:

In a schlenk flask, a solution of E1 (0.85 g; 1.98 mmol) and chlorodicyclohexylborane (1M solution in hexane) (400 μL ; 400 μmol) in dichloroethane (570 mL) was stirred at 80°C under N_2 atmosphere for 1 h. Grubbs-Hoveyda catalyst 2nd generation (124 mg; 198 μmol) was added and the mixture was stirred at 120°C for 16 h. The mixture was degassed by N_2 bubbling for 10 min and further Grubbs-Hoveyda catalyst 2nd generation (124 mg; 198 μmol) and chlorodicyclohexylborane (1M solution in hexane) (400 μL ; 400 μmol) were added. The mixture was stirred at 120°C for 20 h.

The 2 batches were mixed and a ruthenium scavenger (SiliaBond DMT from SiliCycle) (10.4 g; 6.35 mmol) was added and the mixture was stirred at rt for 20 h. The reaction mixture was filtered through a pad of celite and the solvent was removed under reduced pressure to give a brown residue.

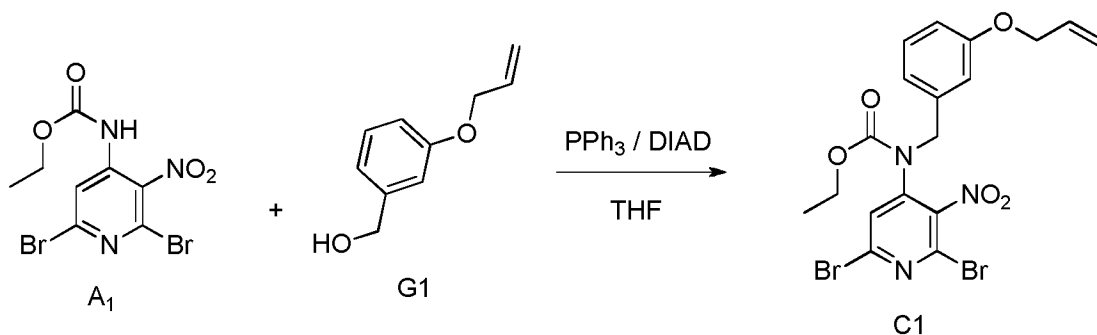
The residue was combined with the residue obtained from the two batches of 1 g of E1. The resulting brown residue was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Grace, dry loading, mobile phase gradient: from Heptane/EtOAc 100/0 to 0/100) to give 1.19 g of a brown solid. The brown solid was further purified by preparative LC (Stationary phase: irregular bare silica 40 g, mobile phase gradient: from CH_2Cl_2 /EtOAc 90/10 to 80/20) to give 705 mg of a yellow solid. The yellow solid was further purified by achiral SFC (stationary phase: Amino

6 μ m 150x21.2mm), mobile phase: Gradient from 85% CO₂, 15% MeOH to 65% CO₂, 35% MeOH) to give 660 mg of intermediate F1 as a yellow solid (19% yield, E isomer).



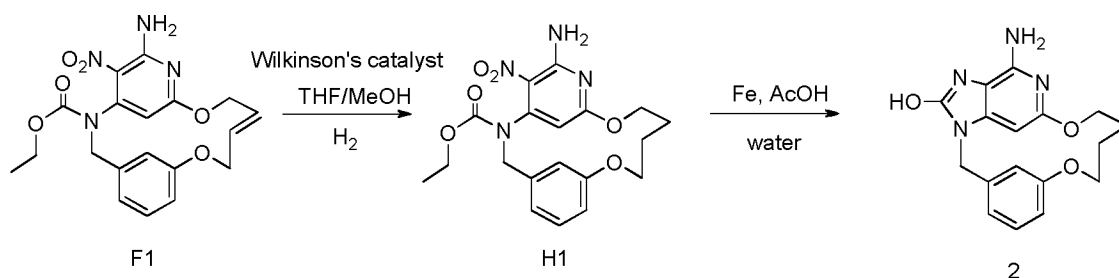
5 Synthesis of final compound 1

A mixture of F1 (570 mg; 1.42 mmol) and iron (795 mg; 14.2 mmol) in AcOH (21 mL) and water (4.2 mL) was stirred at 50°C for 2 h. The mixture was concentrated until dryness. DMF was added, the mixture was sonicated, heated and filtered through a pad of celite and the celite was rinsed with hot DMF. An iron scavenger (SiliaBond Imidazole from SiliCycle) (25.4 g; 29.5 mmol) was added to the filtrate and the mixture was stirred at rt for 16 h. The mixture was filtered through celite, the celite was rinsed with DMF and the filtrate was concentrated in vacuo to give 620 mg of a brown solid. The crude was purified by preparative LC (irregular SiOH, 15-40 μ m, 30 g Merck, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 98/2/0.2 to 85/15/1.5) to give 360 mg of final compound 1 as an off-white solid (75% yield).



Alternative synthesis of intermediate C1

At 0°C, diisopropylazodicarboxylate (DIAD) (3.0 mL, 15.0 mmol) was added drop wise to a mixture of A1 (3.70 g, 10.028 mmol), G1 (1.98 g, 12.0 mmol) and PPh₃ (3.94 g, 15.0 mmol) in THF (70 mL). The mixture was stirred at rt for 12 h. EtOAc and water were added. The layers were decanted. The organic layer was washed with water, dried over MgSO₄, filtered and the solvent was evaporated. The crude was purified by preparative LC on (Irregular SiOH 20-45 μ m 450 g Matrex), mobile phase (85% Heptane, 15% AcOEt) to give 4.5 g of intermediate C1 (87% yield).

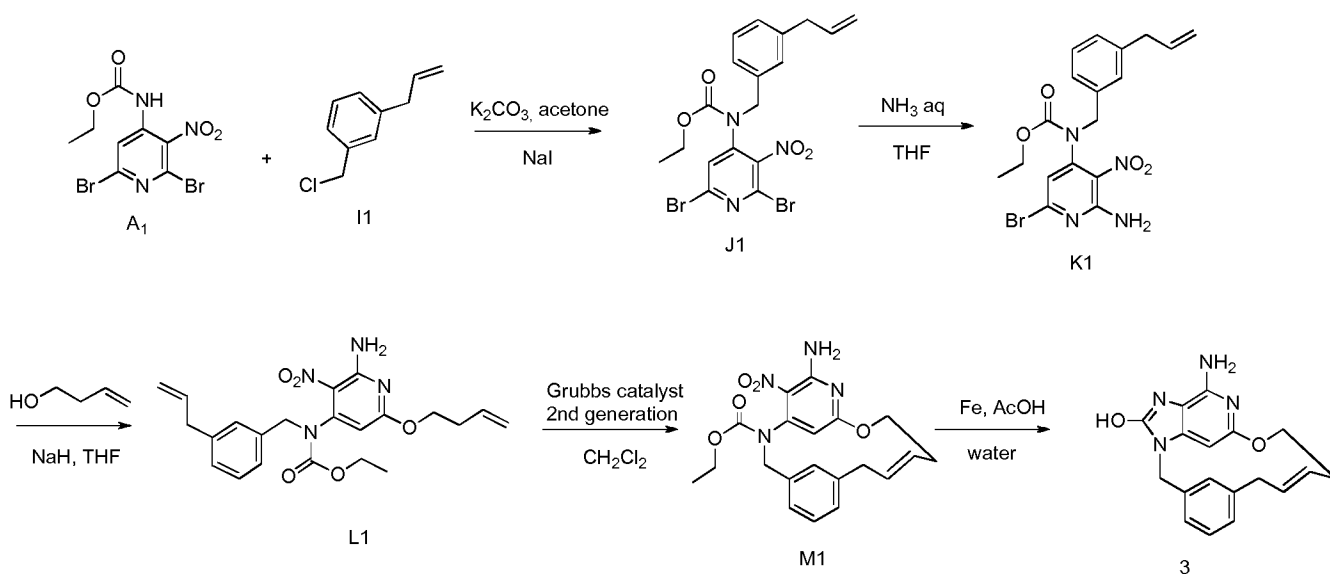
Overall Scheme in the preparation of final products: method 2**5 Synthesis of intermediate H1**

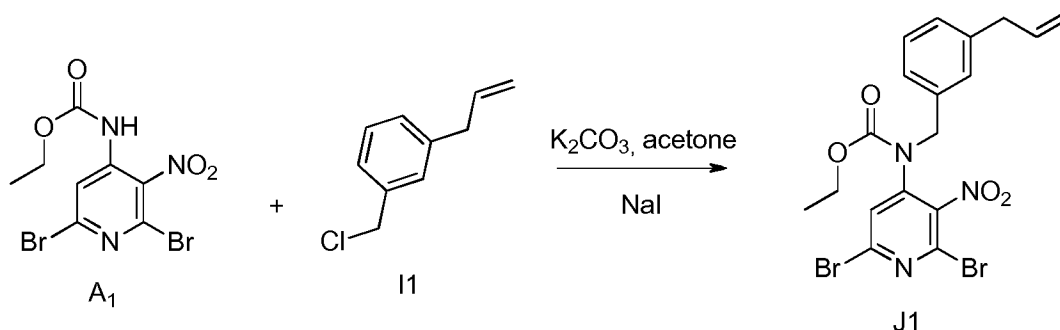
Wilkinson's catalyst (44 mg; 47.5 μmol) was added to a solution of F1 (190 mg; 475 μmol) in THF/MeOH (50/50) (50 mL) purged by N_2 bubbling for 15 min. The mixture was hydrogenated (8 bars) at rt for 16 h. The mixture was purged for 15 min and Wilkinson's catalyst (44 mg; 47.5 μmol) was further added. The reaction mixture was hydrogenated (8 bars) at rt for 4 h. The mixture was concentrated in vacuo to give a brown oil. The oil was purified by preparative LC (Irregular SiOH 15-40 μm , 12 g Grace, dry loading, mobile phase gradient: from $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ 100/0 to 80/20) to give 150 mg of intermediate H1 as a yellow solid (79% yield).

Synthesis of final compound 2

Compound 2 was obtained using the procedure to prepare compound 1 (54 mg, 44% yield).

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Overall Scheme in the preparation of final products: method 3

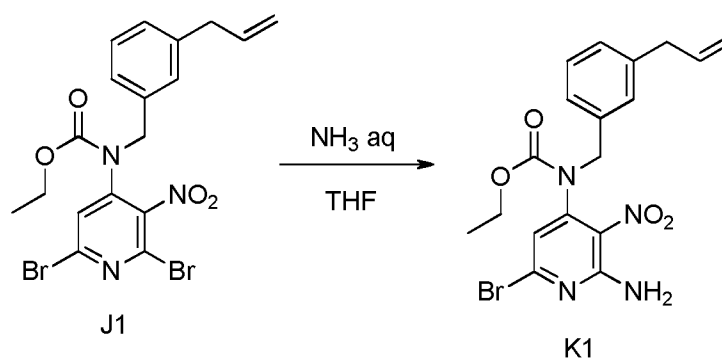


Synthesis of intermediate J1

I1 (5.9 g; 35.6 mmol) was added to a solution of A1 (7.3 g; 19.8 mmol), K₂CO₃ (5.5 g; 39.6 mmol) and NaI (3.3 g; 21.8 mmol) in acetone (145 mL). The mixture was stirred at rt for 20 h.

5 The mixture was filtered through a pad of celite and the filtrate was evaporated in vacuo to give an orange solid. The residue was taken up in CH₂Cl₂. The precipitate was filtered and the filtrate was concentrated in vacuo to give 13 g of a yellow oil. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μm, 300 g Interchim, mobile phase gradient: from Heptane/EtOAc 100/0 to 80/20). The fractions containing product were combined and the

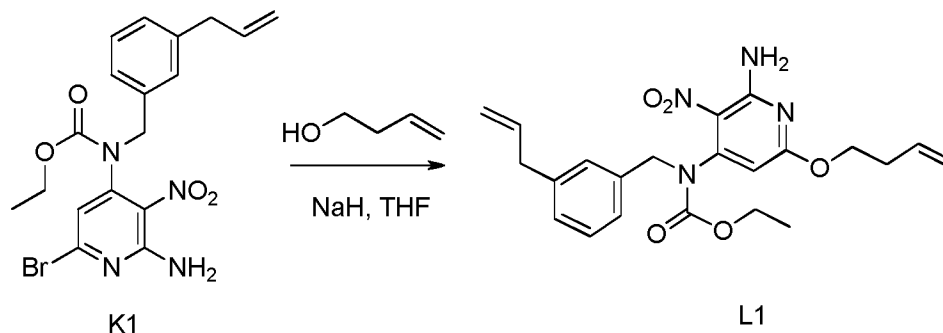
10 solvent was removed in vacuo to give 7.1 g of intermediate J1 (72% yield) as a yellow oil.



Synthesis of intermediate K1

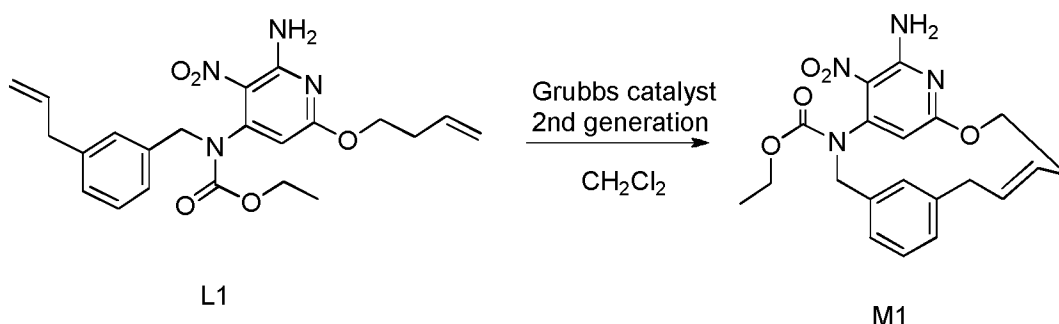
In a schlenk flask, a solution of J1 (7.1 g; 14.2 mmol) in THF (130 mL) and an aqueous solution

15 of NH₃ (30%) (130 mL) was stirred at rt for 16 h. The mixture was taken up with EtOAc and a saturated water solution of NaCl, layers were separated. The organic layer was dried over MgSO₄, filtered and concentrated in vacuo to give 6.4 g of a yellow oil (100% yield).



Synthesis of intermediate L1

NaH (2.2 g; 54.2 mmol) was added portion wise at rt and under N₂ atmosphere to 3-buten-1-ol (76 mL). The mixture was stirred at rt for 30 min before being added drop wise at 0°C to a solution of K1 (5.9 g; 13.6 mmol) in THF (150 mL). The resulting mixture was stirred at 0°C for 1 h. The mixture was poured into an aqueous saturated NH₄Cl solution. EtOAc and saturated aqueous NaCl solution were added, the layers were separated. The organic layers was dried over MgSO₄, filtered and concentrated in vacuo to give a yellow residue which was azeotropically distilled with toluene (once) to give 6.6 g of a yellow oil. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μm, 220 g Grace, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50). The fractions containing product were combined and the solvent was removed in vacuo to give 4.46 g of intermediate L1 (77% yield) as a yellow oil.



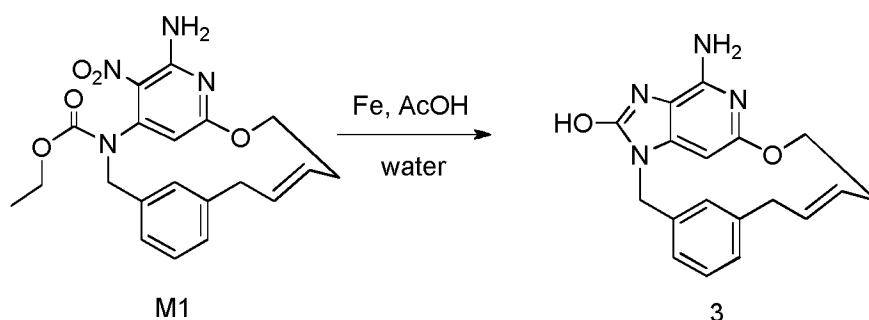
Synthesis of intermediate M1

The reaction was performed in 2 batches.

Typical procedure for one batch:

A solution of L1 (2.45 g; 5.75 mmol) in dry CH₂Cl₂ (1.7 L) was degassed by N₂ bubbling for 15 min. Grubbs catalyst 2nd generation (488 mg; 574 μmol) was added and the mixture was stirred at rt for 72 h. SiliaBond DMT (7.66 g; 4.59 mmol) was added and the mixture was stirred at rt for 16 h. The 2 batches were combined and filtered through celite. The filtrate was concentrated in vacuo to give a black solid. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μm, 150 g Merck, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50). The fractions containing product were combined and the solvent was removed in vacuo to give

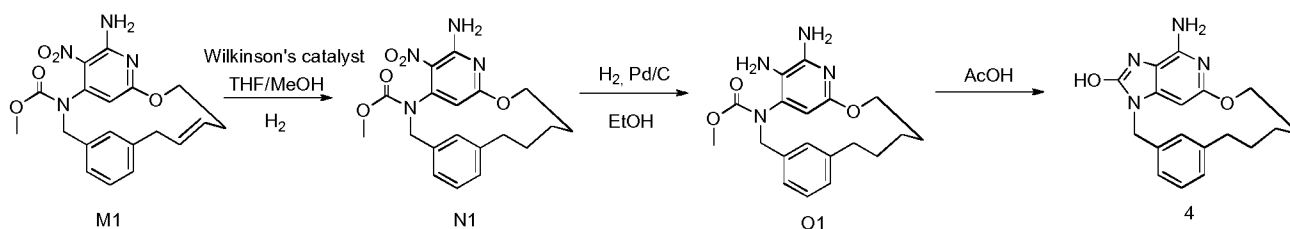
230 mg of fraction 1 and 2.3 g of fraction 2. Fraction 2 was re-purified by preparative LC (Stationary phase: irregular SiOH 40 μ m 120 g, mobile phase: Heptane/CH₂Cl₂/MeOH 55/43/2). The isolated compound was combined with fraction 1 and purified by achiral SFC (Stationary phase: Chiralpak IC 5 μ m 250x20 mm, mobile phase: 70% CO₂, 30% iPrOH) to give 1.51 g of intermediate M1 (33% yield, isomer E) as a yellow solid.



Synthesis of final compound 3

Iron (631 mg; 11.3 mmol) was added to a solution of M1 (750 mg; 1.88 mmol) in AcOH (150 mL) and water (25 mL). The mixture was stirred at 80°C for 16 h. Iron (315 mg; 5.65 mmol) was added and the mixture was stirred at 80°C for 2 h. Iron (315 mg; 5.65 mmol) was added and the mixture was stirred at 80°C for 4 h. Iron (315 mg; 5.65 mmol) was added and the mixture was stirred at 80°C for 16 h. The mixture was concentrated until dryness. DMF was added, the mixture was filtered through celite and the celite was rinsed with hot DMF. SiliaBond imidazole (48.7 g; 56.5 mmol) was added to the filtrate and the mixture was stirred at rt for 16 h. The mixture was filtered through celite, the celite was rinsed with DMF and the filtrate was concentrated in vacuo. The crude compound was purified by preparative LC (irregular SiOH, 15-40 μ m, 25 g Merck, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 98/2/0.2 to 85/15/1.5) to give 2 fractions. Fraction 1 was taken-up with EtOH and filtered to give fraction 3 and fraction 2 was taken-up with MeCN and filtered to give fraction 4. Fractions 3 and 4 were combined in EtOH, filtered and dried in vacuo to give 199 mg of final compound 3 (33% yield).

Overall Scheme in the preparation of final products: method 4



Synthesis of intermediate N1

Wilkinson's catalyst (46 mg; 50.2 μ mol) was added to a solution of M1 (200 mg; 502 μ mol) in THF/MeOH (50/50) (50 mL) purged by N₂ bubbling for 15 min. The mixture was hydrogenated (7 bars) at rt for 20 h. The mixture was purged by N₂ bubbling for 15 min, further Wilkinson's catalyst (46 mg; 50.2 μ mol) was added and the reaction mixture was hydrogenated (7 bars) at rt for 16 h. The reaction mixture was concentrated in vacuo to give a green oil. The oil was purified by preparative LC (Irregular SiOH 15-40 μ m, 25 g Merck, dry loading, mobile phase gradient: from Heptane/EtOAc 100/0 to 70/30) to give 130 mg of intermediate N1 as a yellow solid (66% yield).

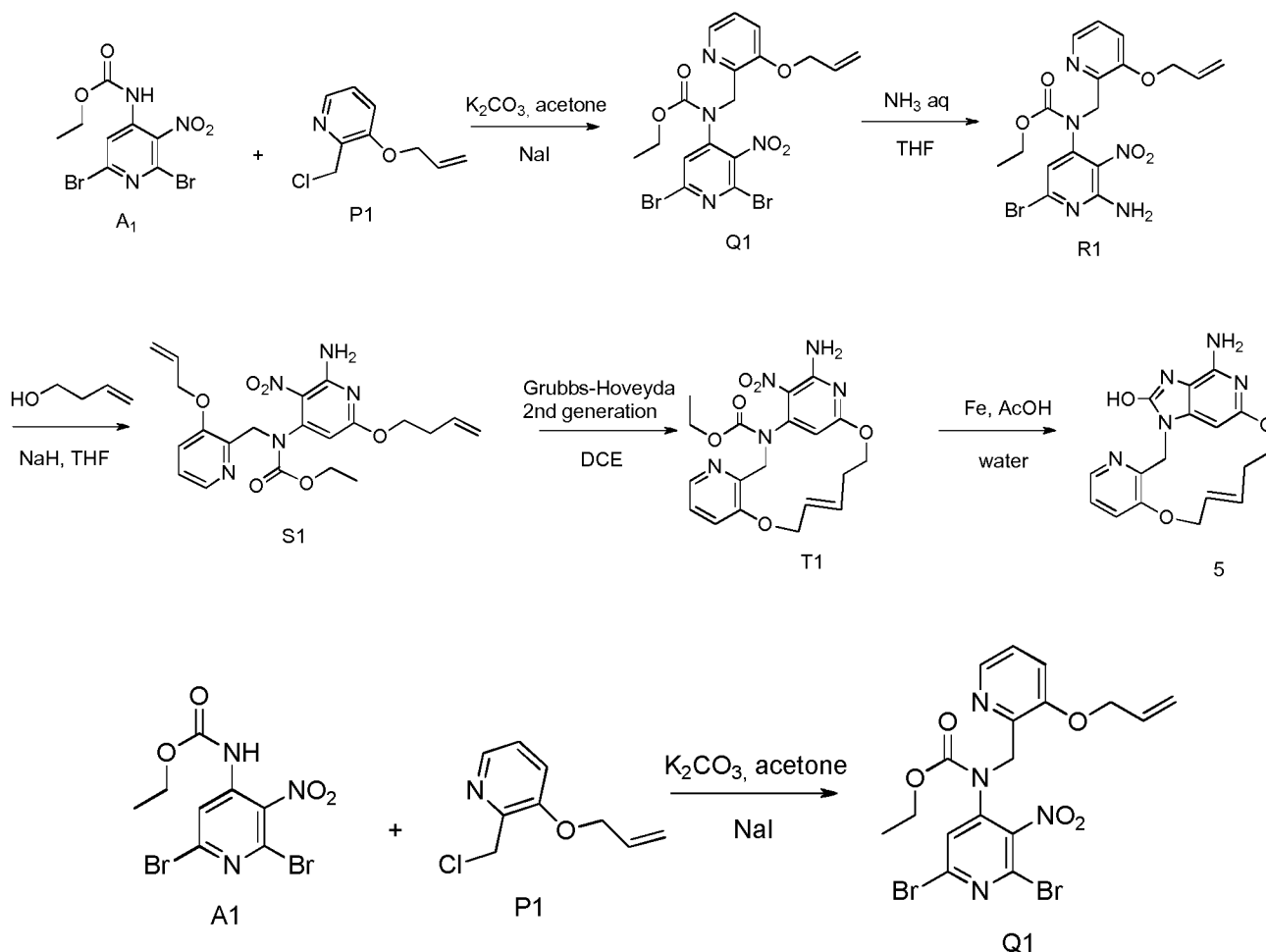
10 Synthesis of intermediate O1

In a pressure vessel reactor, N1 (110 mg; 275 μ mol) was hydrogenated in EtOH (5 mL) with Pd/C (10%) (30 mg; 28.5 μ mol) as catalyst at 40°C (3 bars) for 6 h. The catalyst was removed by filtration over celite, the celite was washed with EtOH and the filtrate was evaporated under vacuum to give 100 mg of a yellow residue (98% yield). Intermediate O1 was used in the next step without further purification.

Synthesis of final compound 4

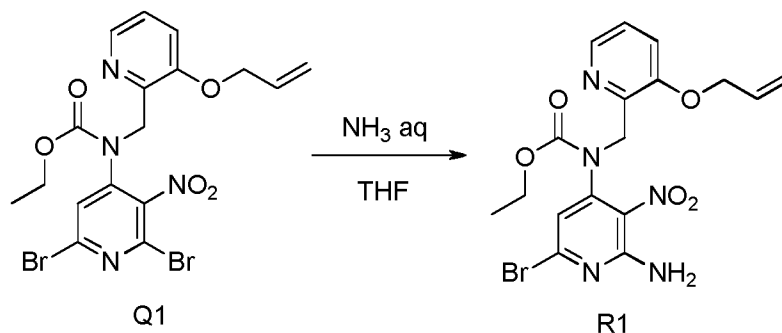
In a sealed tube, O1 (100 mg; 270 μ mol) in pure acetic acid (5 mL) was stirred at rt for 90 min. The solvent was removed under reduced pressure to give a yellow residue. The residue was taken up with CH₂Cl₂ and the solvent was removed under reduced pressure (twice) to give 87 mg of a yellow-green solid. The solid was azeotropically distilled with toluene (four times), and was then triturated and sonicated in Et₂O. The mixture was filtered off (glass frit n°5) to give 75 mg of final compound 4 (77% yield, acetate salt).

Overall Scheme in the preparation of final products: method 5



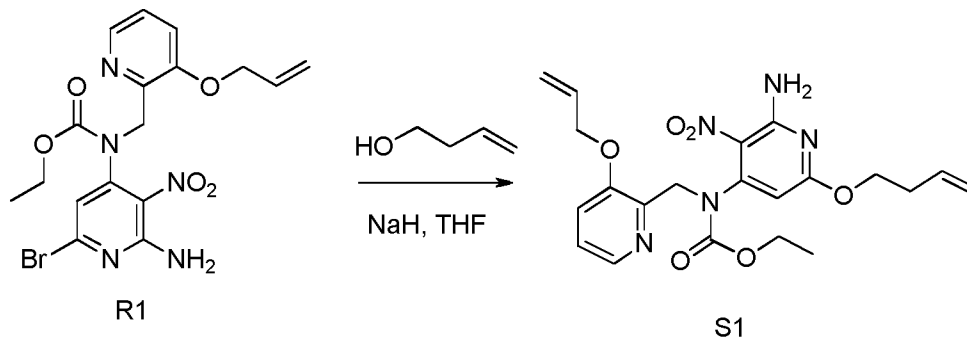
5 Synthesis of intermediate Q1

To a solution of A1 (3.52 g; 9.54 mmol) and K_2CO_3 (2.64 g; 19.1 mmol) in acetone (80 mL) was added P1 (1.93 g; 10.5 mmol) and NaI (1.57 g; 10.5 mmol) at rt. The mixture was stirred at rt for 16 h, further P1 (1.5 g; 8.17 mmol) was added and the mixture was stirred at rt for 24 h. The reaction mixture was filtered through a pad of celite and the filtrate was evaporated in vacuo to give a black residue. The residue was purified by preparative LC (irregular SiOH 15-40 μ m, 80 g Grace, dry loading, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50) to give 3.28 g of intermediate Q1 as an orange oil (67% yield).



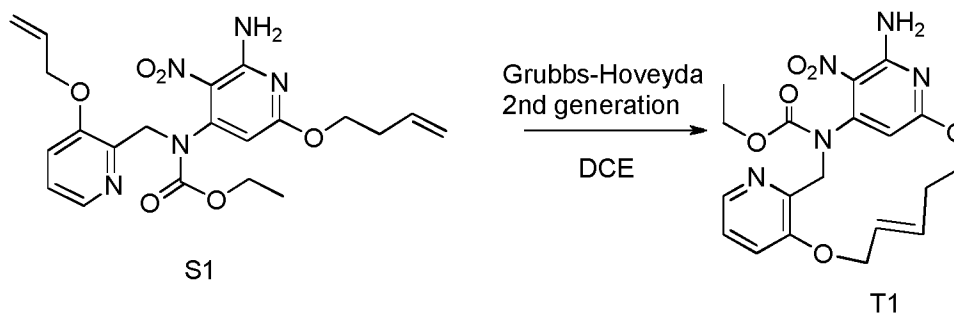
Synthesis of intermediate R1

In a schlenk flask, to a solution of Q1 (3.28 g; 6.36 mmol) in THF (52 mL), was added an aqueous solution of NH_3 (30%) (52 mL) at rt. The mixture was stirred at rt for 26 h and further aqueous solution of NH_3 (10 mL) was added and the mixture was stirred at rt for 4 h. The mixture was taken up with EtOAc and saturated aqueous solution of NaCl, the layers were separated and the organic layer was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give 2.74 g of intermediate R1 as a yellow oil (87% yield).



Synthesis of intermediate S1

NaH (60% in oil) (888 mg; 22.2 mmol) was added portion wise to 3-buten-1-ol (30 mL; 354 mmol) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of R1 (2.74 g; 5.63 mmol) in THF (62 mL) at 0°C . The resulting mixture was stirred at rt for 1 h and was poured in NH_4Cl saturated aqueous solution. EtOAc and NaCl saturated aqueous solution were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give a yellow oil. The oil was purified by preparative LC (Irregular SiOH 15-40 μm , 80 g Grace, dry loading, mobile phase gradient: from Heptane/EtOAc 100/0 to 20/80) to give 1.06 g of intermediate S1 as a yellow residue (42% yield).



Synthesis of intermediate T1

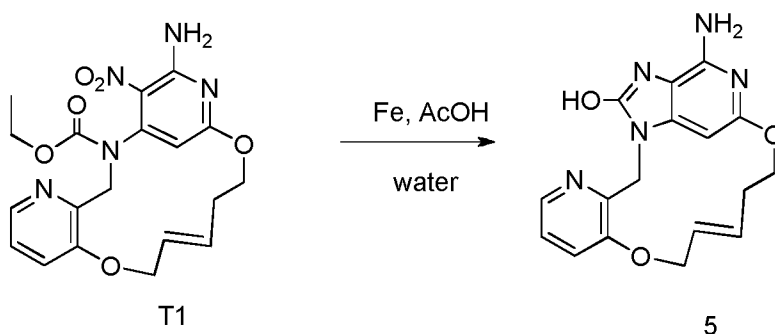
The reaction was performed in two batches of 480 mg of intermediate S1.

Herein is reported the procedure for one batch:

In a schlenk flask, a solution of S1 (480 mg; 1.08 mmol) and chlorodicyclohexylborane (1M in hexane) (216 μ L; 216 μ mol) in dry dichloroethane (300 mL) was stirred at 80°C and under N₂ atmosphere for 1 h. Grubbs-Hoveyda catalyst 2nd generation (68 mg; 108 μ mol) was added and the mixture was stirred at 120°C for 2 h.

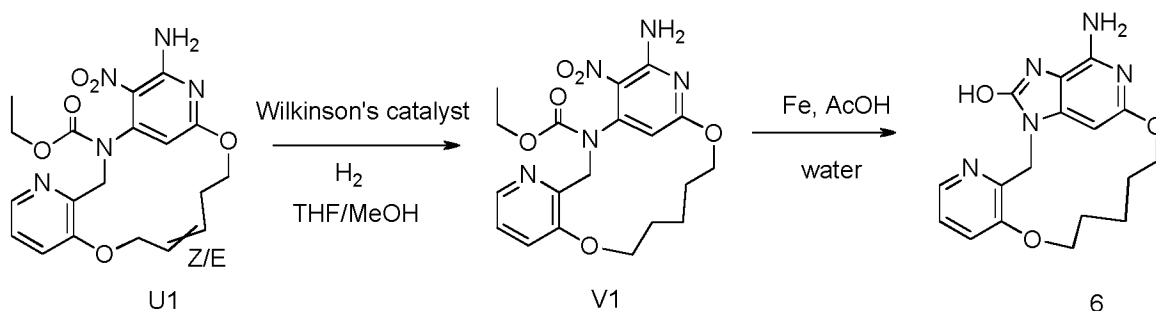
- 5 The two batches were mixed, SiliaBond DMT (2.84 g; 1.73 mmol) was added and the mixture was stirred at rt for 20 h.

The mixture was filtered through a pad of celite, the celite was washed with EtOAc and the filtrate was evaporated in vacuo to give a brown solid. The brown solid was purified by preparative LC (Irregular SiOH 15-40 μ m, 40 g Grace, dry loading, mobile phase gradient: CH₂Cl₂/EtOAc from 100/0 to 20/80) to give 610 mg of a yellow residue (mixture of E and Z isomers, intermediate U1). 310 mg of intermediate U1 was purified by Reverse phase (Stationary phase: Nucleodur-Sphinx rp 5 μ m 21x150 mm, mobile phase: Gradient from 70% formic acid 0.1%, 30% MeCN to 0% formic acid 0.1%, 100% MeCN) to give 195 mg of intermediate T1 (E isomer) as a yellow solid (22% yield).



Synthesis of final compound 5

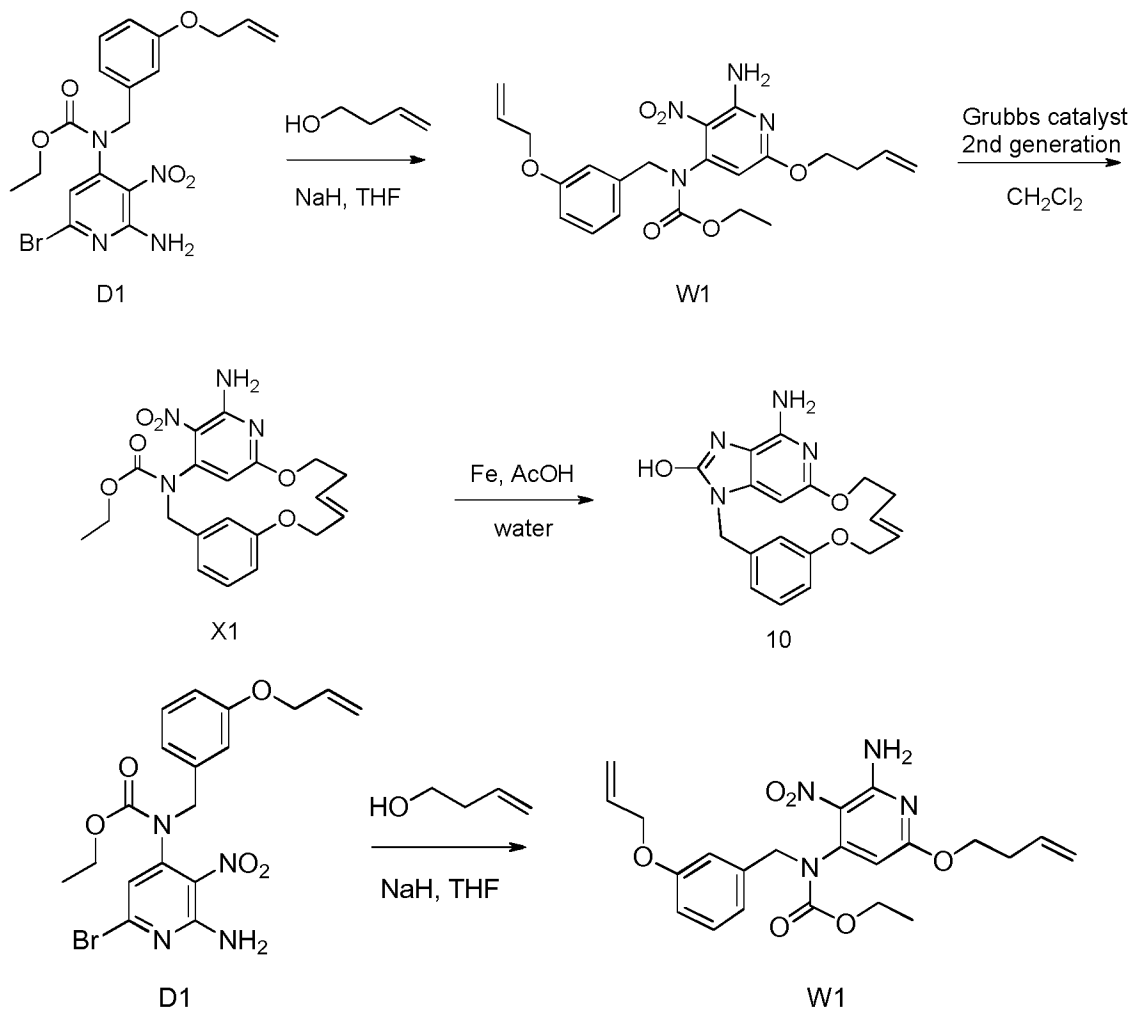
A mixture of T1 (160 mg; 385 μ mol) and iron (129 mg; 2.31 mmol) in acetic acid (21 mL) and water (2.4 mL) was stirred at 80°C for 7 h. Further iron (129 mg; 2.31 mmol) was added and the mixture was stirred at 80°C for 16 h. Further iron (129 mg; 2.31 mmol) was added and the mixture was stirred at 80°C for 3 h. The mixture was concentrated in vacuo to give a residue. The residue was diluted in DMF and filtered through a pad of celite. SiliaBond imidazole (12.7 g; 14.7 mmol) was added to the filtrate and the mixture was stirred at rt for 16 h. The mixture was filtered through a pad of celite and the filtrate was evaporated in vacuo to give a brown solid. The brown solid was purified by preparative LC (irregular SiOH 15-40 μ m, 12 g Grace, dry loading, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 97/3/0.3 to 80/20/2) to give 65 mg of an off-white solid. The solid was purified by Reverse phase (Stationary phase: X-Bridge-C18 5 μ m 30*150mm, mobile phase gradient: from H₂O(0.5%NH₄CO₃)/MeOH 70/30 to 0/100) to give 43 mg of final compound 5 as a white solid (31% yield, E isomer).

Overall Scheme in the preparation of final products: method 6**Synthesis of intermediate V1**

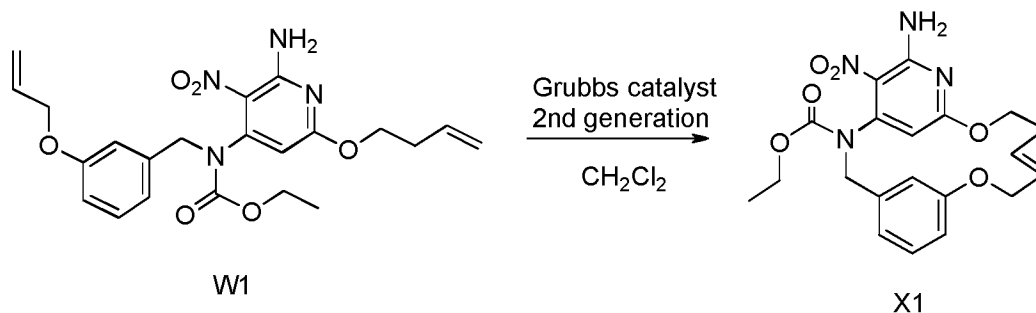
Wilkinson's catalyst (58 mg; 62.6 μ mol) was added to a solution of U1 (Z/E mixture, 260 mg; 626 μ mol) in THF/MeOH (50/50) (66 mL) purged by N₂ bubbling for 15 min. The mixture was hydrogenated (7 bars) at rt for 16 h. Further Wilkinson's catalyst (58 mg; 62.6 μ mol) was added and the mixture was hydrogenated (7 bars) at rt for 6 h. The reaction mixture was concentrated in vacuo to give a brown solid. The solid was purified by preparative LC (Irregular SiOH 15-40 μ m, 25 g Merck, dry loading, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50) to give 250 mg of intermediate V1 as a yellow oil (54% yield).

Synthesis of final compound 6

A mixture of V1 (238 mg; 359 μ mol) and iron (120 mg; 2.16 mmol) in acetic acid (20 mL) and water (2.2 mL) was stirred at 80°C for 6 h. Further iron (120 mg; 2.16 mmol) was added and the mixture was stirred at 80°C for 20 h. Further iron (120 mg; 2.16 mmol) was added and the mixture was stirred at 80°C for 5 h. The mixture was concentrated in vacuo to give a residue. The residue was diluted in DMF and filtered through a pad of celite. SiliaBond imidazole (11.1 g; 12.9 mmol) was added to the filtrate and the mixture was stirred at rt for 16 h. The mixture was filtered through a pad of celite and the filtrate was evaporated in vacuo to give a brown solid. The solid was purified by preparative LC (irregular SiOH 15-40 μ m, 12 g Grace, dry loading, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 97/3/0.3 to 80/20/2) to give 32 mg of an off-white solid. The solid was taken up with water, triturated and sonicated. The resulting suspension was filtered off (glass frit n°5) and washed with Et₂O (twice) to give 19 mg of final compound 6 as an off-white solid (15% yield).

Overall Scheme in the preparation of final products: method 7**5 Synthesis of intermediate W1**

NaH (60% in oil) (2.1 g; 52.1 mmol) was added portion wise to 3-buten-1-ol (74 mL) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of D1 (5.97 g; 13.2 mmol) in THF (150 mL) at 0°C. The resulting mixture was then stirred at rt for 2 h 30 min and was poured in aqueous saturated solution of NH_4Cl . EtOAc and saturated aqueous solution of NaCl were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give 6.77 g of a yellow oil. The crude was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Grace, liquid injection, mobile phase gradient: from Heptane/EtOAc 100/0 to 50/50) to give 5.12 g of intermediate W1 as a yellow oil (83% yield).

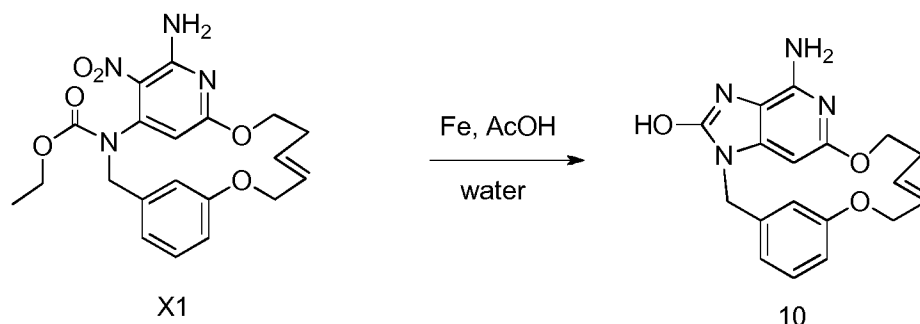


Synthesis of intermediate X1

To a solution of W1 (3 g; 6.78 mmol) in CH₂Cl₂ (1.3 L) degassed by N₂ bubbling for 15 min was added Grubbs catalyst 2nd generation (578 mg; 678 μmol) at rt. The solution was stirred at rt for 20 h. SiliaBond DMT (8.89 g; 5.42 mmol) was added and the mixture was stirred at rt for 20 h. The reaction mixture was filtered through a pad of celite and the solvent was removed under reduced pressure to give a brown residue, which was combined with another batch (0.226 mmol scale). The combined residue was taken up with MeOH, sonicated and heated to give a precipitate which was filtered off to give 3.2 g of a brown solid. The crude was purified by preparative LC (irregular SiOH, 15-40 μm, 220 g grace, liquid injection, mobile phase gradient: from CH₂Cl₂/EtOAc 100/0 to 50/50) to give 1.7 g of fraction 1 as a pale brown solid. Fraction 1 was taken up with MeOH, sonicated and heated to give a precipitate which was filtered off to give 820 mg of fraction 2 as a pale brown solid.

The filtrate was concentrated in vacuo to give 590 mg of fraction 3 as a brown residue (impure X1). Fraction 2 was purified by preparative LC (Stationary phase: Spherical bare silica 5 μm 150x30.0mm, mobile phase gradient: from Heptane/EtOAc 85/15 to 0/100) to give 435 mg of intermediate X1 as a yellow solid (E isomer, 15% yield).

Fraction 3 was purified with another batch.

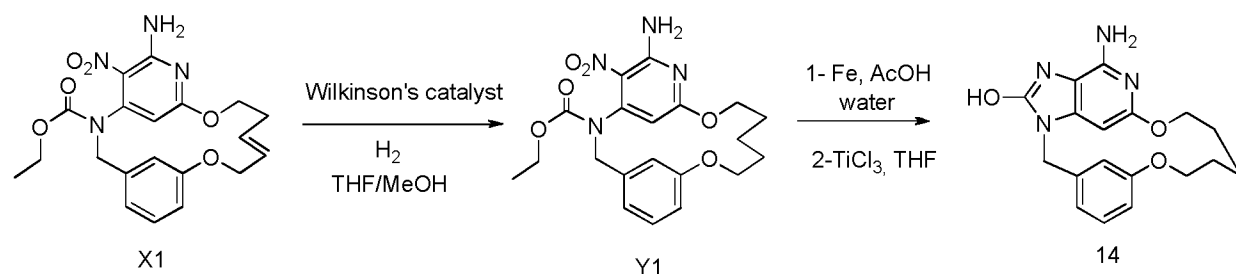


Synthesis of final compound 10

A mixture of X1 (430 mg; 1.04 mmol) and iron (579 mg; 10.4 mmol) in acetic acid (43 mL) and water (3 mL) was stirred at 50°C for 4 h. The mixture was concentrated until dryness. DMF was added. The mixture was sonicated, heated and filtered through a pad of celite and the celite was

rinsed with hot DMF. SiliaBond imidazole (17.9 g; 20.8 mmol) was added to the filtrate and the mixture was stirred at rt for 16 h. The mixture was filtered through celite, the celite was rinsed with DMF and the filtrate was concentrated in vacuo to give 670 mg of crude compound. The crude was purified by preparative LC (irregular SiOH, 15-40 μ m, 25 g Merck, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 98/2/0.2 to 85/15/1.5) to give an off-white solid. The solid was dried at 40°C under reduced pressure during 20 h to give 295 mg of final compound 10 as an off-white solid (84% yield).

Overall Scheme in the preparation of final products: method 8



Synthesis of intermediate Y1

Wilkinson's catalyst (103 mg; 111 μ mol) was added to a solution of X1 (230 mg; 0.555 mmol) in THF/MeOH (50/50) (40 mL) purged by N₂ bubbling for 15 min. The mixture was hydrogenated (8 bars) at rt for 24 h. The reaction mixture was concentrated in vacuo to give a brown residue. The solid was purified by preparative LC (irregular SiOH, 15-40 μ m, 12 g Grace, dry loading, mobile phase gradient: from CH₂Cl₂/EtOAc 100/0 to 90/10) to give 55 mg of intermediate Y1 as a yellow residue (24% yield).

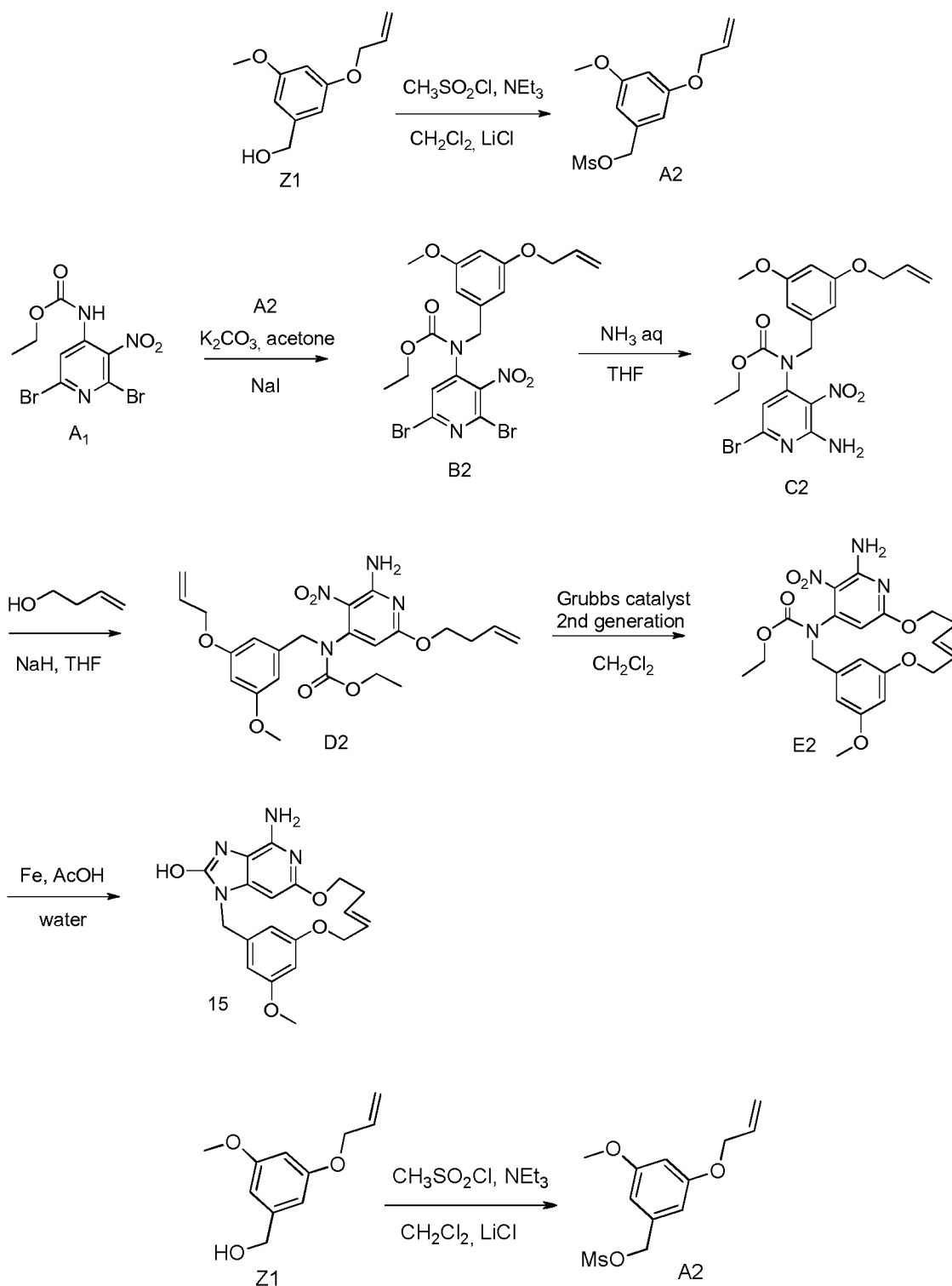
Synthesis of final compound 14

A mixture of Y1 (55 mg; 0.132 mmol) and iron (74 mg; 1.32 mmol) in acetic acid (5.5 mL) and water (0.4 mL) was stirred at 50°C for 20 h. More iron (37 mg; 0.66 mmol) was added and the mixture was stirred at 50°C for 3 h. More iron (37 mg; 0.66 mmol) was added and the mixture was stirred at 50°C for 20 h. The mixture was filtered through a pad of celite and the celite was rinsed with acetic acid. More iron (74 mg; 1.32 mmol) was added to the filtrate and the mixture was stirred at 50°C for 88 h. More iron (74 mg; 1.32 mmol) was added to the filtrate and the mixture was stirred at 80°C for 24 h. The cyclisation was not complete. The mixture was concentrated in vacuo to give a brown solid.

TiCl₃ (8.60 mL; 10.0 mmol) was added drop wise to a solution of the brown solid in C (19 mL). The mixture was stirred at rt overnight. The mixture was basified by addition of K₂CO₃ powder at 0°C. The resulting mixture was filtered through a pad of celite and the celite was washed with a solution of AcOEt/MeOH (8:2). The filtrate was concentrated in vacuo. The crude solid was

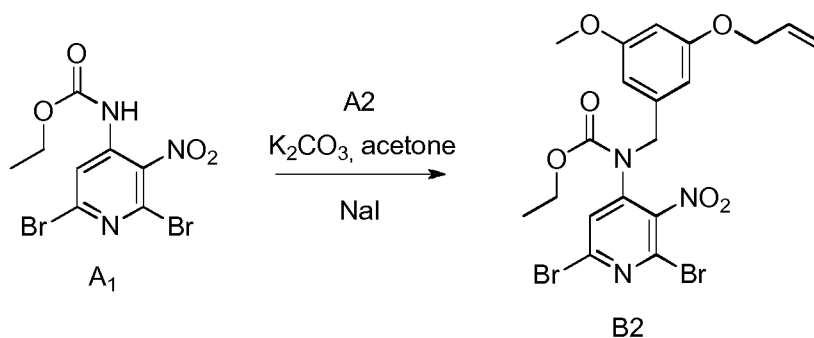
purified by preparative LC (irregular SiOH, 15-40 μm , 10 g Merck, dry loading, mobile phase gradient: from $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3\text{aq}$ 98/2/0.2 to 85/15/1.5). The fractions containing product were combined and the solvent was removed in vacuo to give 20 mg of final compound 14 (12% yield) as an off-white solid.

5

Overall Scheme in the preparation of final products: method 9

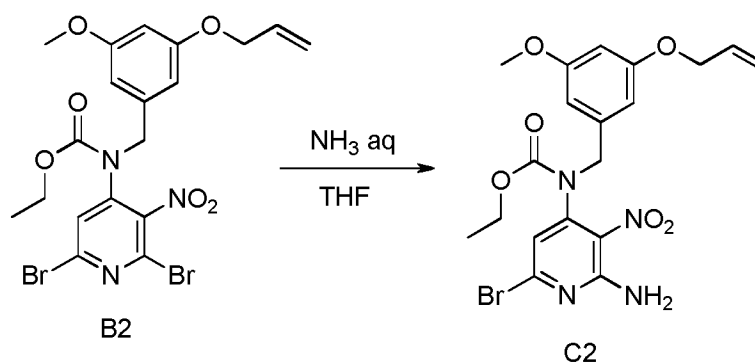
Synthesis of intermediate A2

Methanesulfonyl chloride (8.4 mL; 108 mmol) was added to a solution of Z1 (14 g; 72.1 mmol), NEt₃ (20 mL; 144 mmol) and LiCl (4.6 g; 108 mmol) in dry CH₂Cl₂ (980 mL). The mixture was stirred at rt for 1 h 30. Water was added and the layers were separated. The organic layer was washed with water (once), dried over MgSO₄, filtered and concentrated in vacuo to give 18.8 g of A2 (96%) as a green oil.



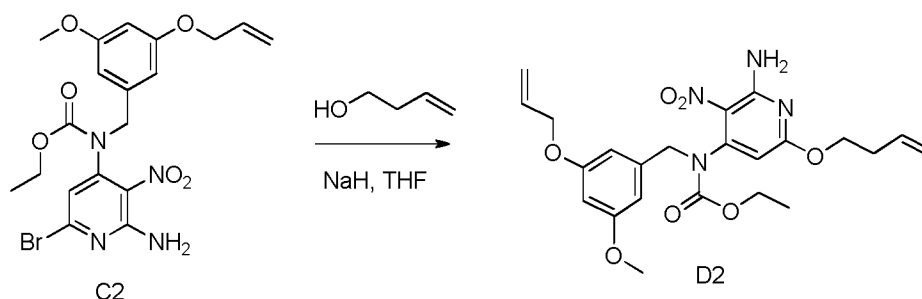
Synthesis of intermediate B2

Intermediate B2 was obtained using the procedure described for intermediate C1 (78% yield as a yellow oil).



Synthesis of intermediate C2

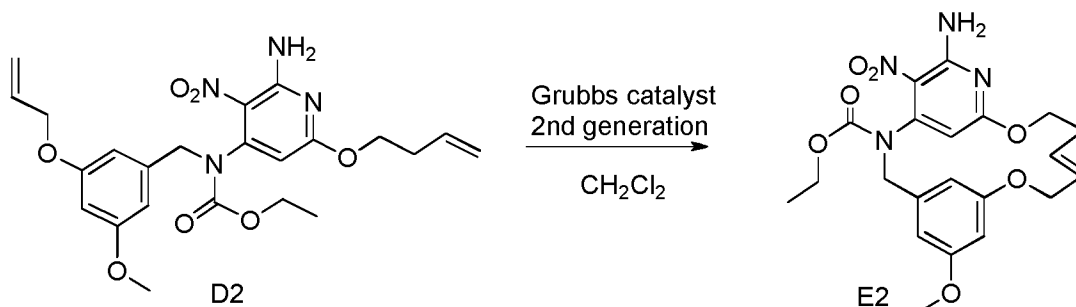
Intermediate C2 was obtained using the procedure described for intermediate D1 (quantitative yield as a yellow oil).



Synthesis of intermediate D2

Intermediate D2 was obtained using the procedure described for intermediate W1 (64% yield as a yellow solid).

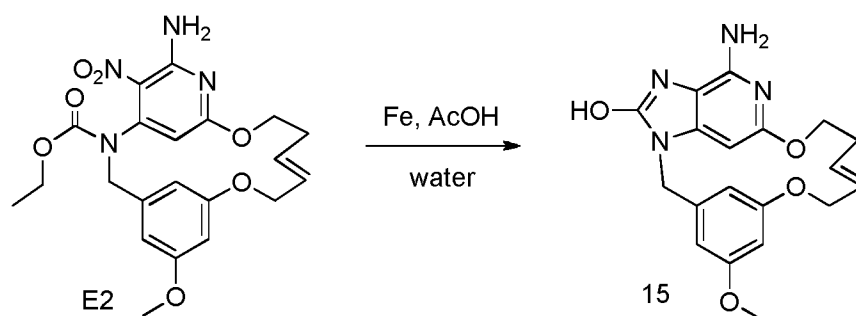
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Synthesis of intermediate E2

A solution of D2 (1 g; 2.12 mmol) in CH_2Cl_2 (400 mL) was degassed by N_2 bubbling for 15 min. Grubbs catalyst 2nd generation (181 mg; 212 μmol) was added and the mixture was stirred at rt for 16 h. SiliaBond DMT (2.78 g; 1.69 mmol) was added and the mixture was stirred at rt for 16 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give 1.11 g of a brown oil. The crude was purified by preparative LC (Irregular SiOH 15-40 μm , 50 g Merck, mobile phase gradient: from $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ 100/0 to 90/10). The fractions containing product were combined and the solvent was removed in vacuo to give 386 mg of intermediate E2 (41%, isomer E (96.2%) + isomer Z (3.8%)) as a yellow foam.

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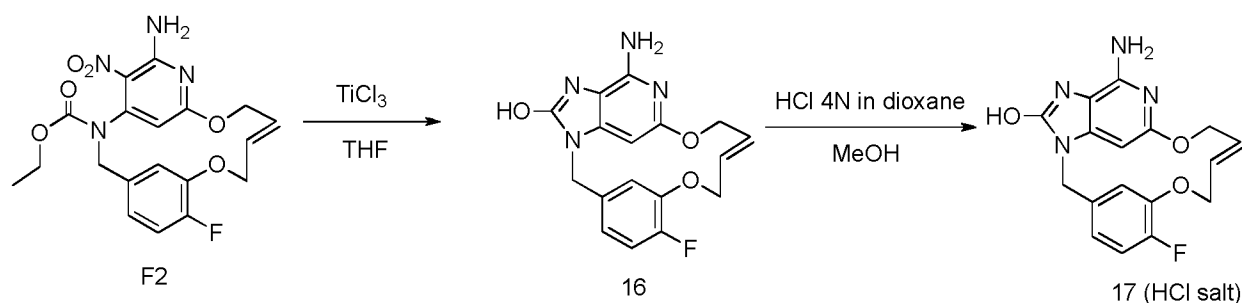
Synthesis of final compound 15

Iron (291 mg; 5.21 mmol) was added to a solution of E2 (386 mg; 0.869 mmol) in acetic acid (36 mL) and water (3 mL). The mixture was stirred at 80°C for 6 h. Iron (146 mg; 2.61 mmol) was added and the mixture was stirred at 80°C for 16 h. Iron (146 mg; 2.61 mmol) was added again and the mixture was stirred at 80°C for 5 h. The mixture was concentrated until dryness. DMF was added, the mixture was filtered through celite and the celite was rinsed with DMF. Siliabond Imidazole (18 g; 20.9 mmol) was added to the filtrate and the mixture was stirred at rt for 72 h.

20

The mixture was filtered through celite, the celite was rinsed with DMF and the filtrate was concentrated in vacuo to give 428 mg of a brown solid. The solid was taken up in CH₃CN leading to precipitation. The precipitate was filtered to give 267 mg of a brown solid. The solid was purified by preparative LC (Irregular SiOH 15-40 μm, 10 g Merck, dry loading, mobile phase gradient: from CH₂Cl₂/MeOH/NH₃aq 95/5/0.5 to 85/15/1.5). The fractions containing product were combined and the solvent was removed in vacuo to give 124 mg of an off-white solid. The solid was purified by Reverse phase (Stationary phase: Sunfire-C18 5 μm 19x150 mm, mobile phase gradient: from CH₃CN/H₂O (formic acid 0.1%) 5/95 to 50/50) to give 72 mg of final compound 15 (23% yield) as a white solid.

Overall Scheme in the preparation of final products: method 10



Synthesis of intermediate F2

Intermediate F2 was obtained with the procedures described for intermediate F1 (E isomer).

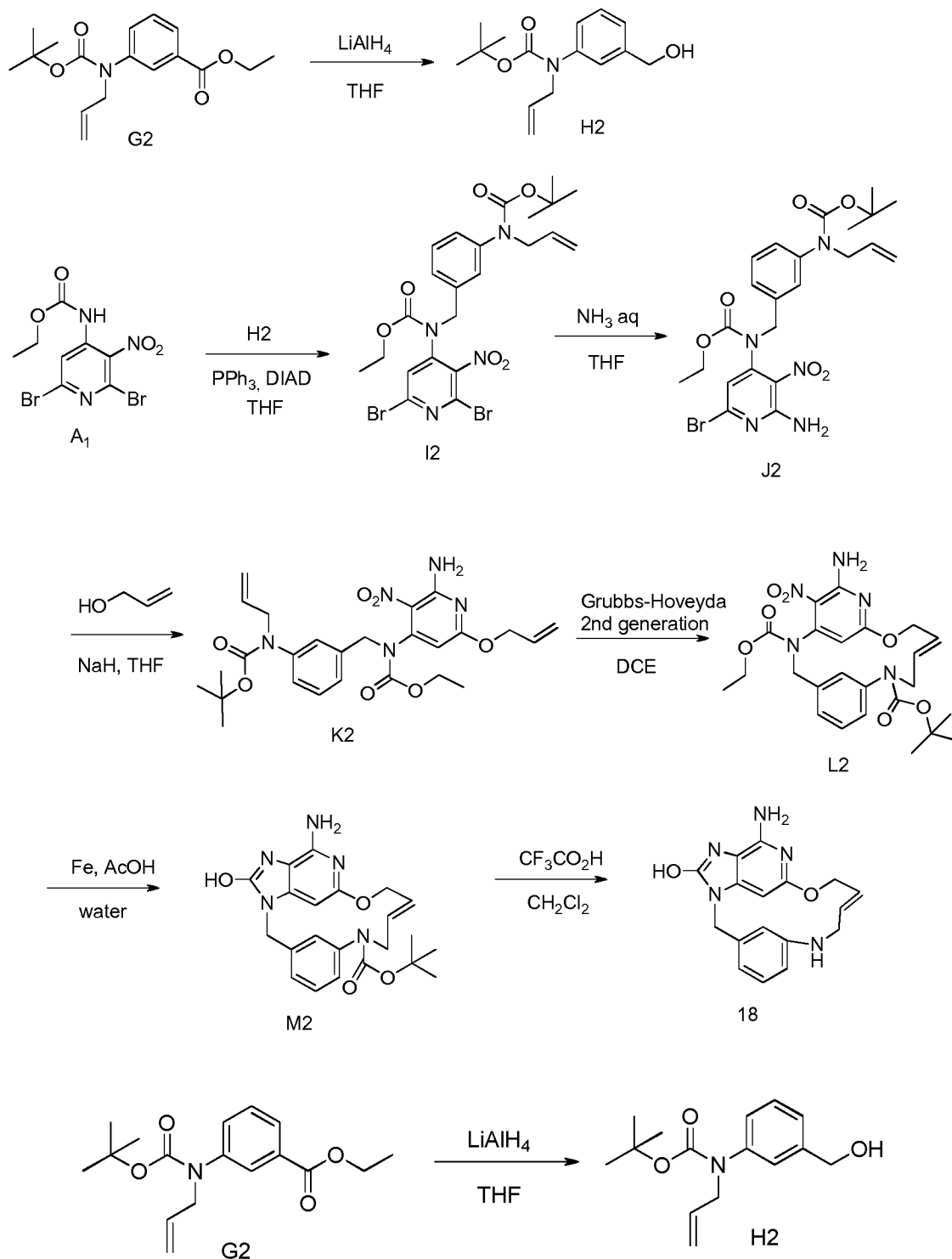
Synthesis of final compound 16

At rt, TiCl₃ (12.3 mL; 14.341 mmol) was added drop wise to a mixture of F2 (300 mg; 0.717 mmol) in THF (30 mL). The mixture was stirred at rt for 2 hours. The mixture was cooled down to 0°C and basified with K₂CO₃ powder. The resulting muddy mixture was filtered through a pad of celite and the celite was washed with a solution of AcOEt/CH₃OH 8/2. The filtrate was partially evaporated to give 175 mg of final compound 16 after filtration of a white solid and drying under vacuum pressure at 85°C (71% yield).

Synthesis of final compound 17

The hydrochloride salt was prepared with 10 eq of HCl 4N in dioxane, which was added to the suspension of compound 16 (100 mg; 0.292 mmol) in CH₃OH (10 mL). The precipitate was stirred for 3 h, filtered and dried under vacuum at 90°C overnight. The solid was solubilized in MeOH/CH₂Cl₂ 50/50, CH₃CN was added and the solvent was evaporated up to precipitation of a white solid, which was filtered and dried under vacuum at 90°C to give 47 mg of final compound 17 as an HCl salt (0.93 HCl, 0.51 H₂O ; 42% yield).

Overall Scheme in the preparation of final products: method 11



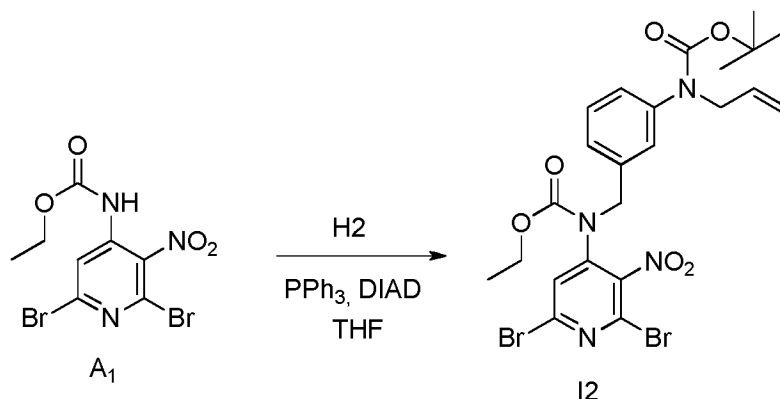
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Synthesis of intermediate H2

At -20°C under a N_2 flow, G2 (22.0 g; 72.04 mmol) in THF (100 mL) was added drop wise to a suspension of LiAlH_4 (3.28 g; 86.45 mmol) in THF (120 mL). The mixture was stirred at 0°C for 1 h. 3.5 mL of water was added dropwise, followed by 3.5 mL of NaOH 3N and 10 mL of water.

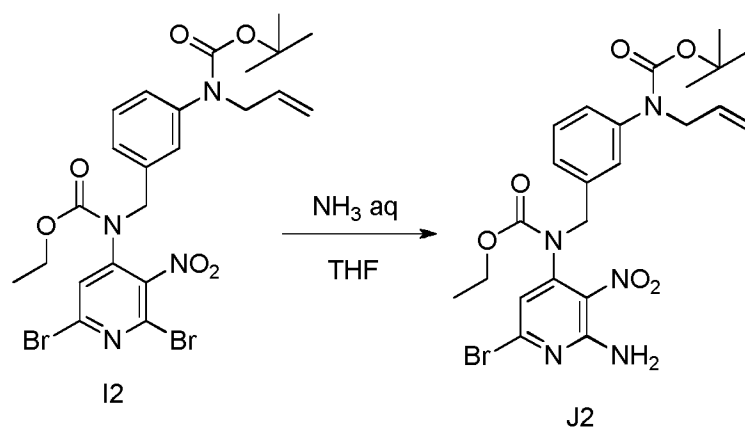
10 The resulting mixture was filtered through a pad of celite and the celite was washed with EtOAc.

The filtrate was concentrated under reduced pressure to give 19 g of intermediate H2 as a yellow oil (quantitative yield).



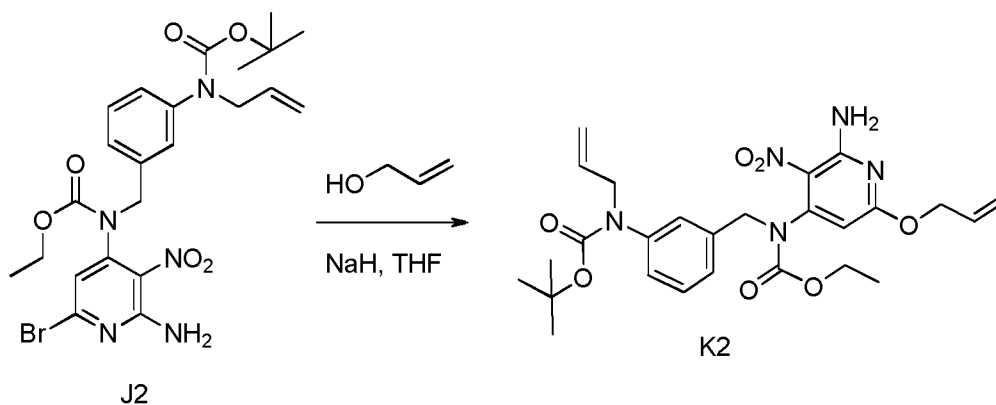
Synthesis of intermediate I2

- 5 At 0°C, diisopropylazodicarboxylate (4.0 mL; 20.32 mmol) was added drop wise to a mixture of A1 (5.0 g; 13.55 mmol), H2 (4.28 g; 16.26 mmol) and PPh₃ (5.33 g; 20.327 mmol) in THF (100 mL). The mixture was stirred at rt for 12 h. EtOAc and water were added. The layers were decanted. The organic layer was washed with water, dried over MgSO₄, filtered and the solvent was evaporated. The crude compound was dissolved in Heptane/EtOAc 80/20, the precipitate was filtered off (mainly POPh₃) and the filtrate was purified by chromatography. Purification was carried out by flash chromatography over silica gel (15-40 μm, 220 g, Heptane/EtOAc 80/20) The pure fractions were collected and evaporated to dryness to give 8.2 g of intermediate I2 (99% yield).



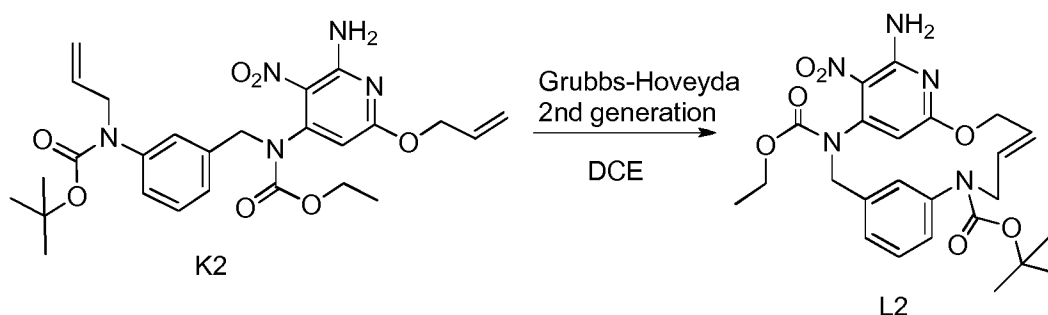
15 Synthesis of intermediate J2

- I2 (8.2 g; 13.349 mmol) was stirred in NH₄OH (100 mL) and THF (100 mL) at rt for 24 h. The mixture was half evaporated under reduced pressure. The residue was taken up with EtOAc. The organic layer was washed with water, dried over MgSO₄, filtered and the solvent was evaporated to give 8.15 g of intermediate J2 (quantitative yield). The crude compound was used directly in the next reaction step.



Synthesis of intermediate K2

Under a N₂ flow, NaH (60% in oil) (1.15 g; 28.64 mmol) was added portion wise to allyl alcohol (35 mL) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of J2 (4.0 g; 7.26 mmol) in THF (80 mL) at 0°C. The resulting mixture was then stirred at rt for 2 h 30 min and was poured in a saturated solution of NH₄Cl. EtOAc and a saturated aqueous solution of NaCl were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO₄, filtered and the solvent was removed under reduced pressure to give 4.7 g of a yellow oil. Purification was carried out by flash chromatography over silica gel (15-40 μm, 80 g, CH₂Cl₂/Heptane 65/35). The pure fractions were collected and evaporated to dryness to give 2.65 g of intermediate K2 (69% yield).



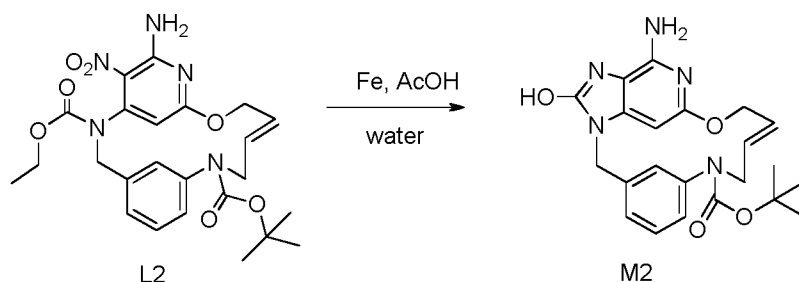
Synthesis of intermediate L2

Prior to the reaction, the dichloroethane was degassed by bubbling N₂ through.

In a Slenck tube, a solution of K2 (1.3 g; 2.464 mmol) and chlorodicyclohexylborane (1 M in hexane) (493 μL; 0.493 mmol) in dichloroethane (600 mL) was stirred at 80°C under N₂ for 1 h. Grubbs-Hoveyda catalyst 2nd generation (609 mg; 0.493 mmol) was added and the mixture was stirred at 120°C for 16 h. Siliabond DMT (2.98 g; 1.82 mmol) was added and the mixture was stirred at rt for 16 h. The reaction mixture was filtered through celite and the filtrate was evaporated in vacuo to give 1.6 g which was combined with another reaction (2.46 mmol scale) before purification (total weight to purify 3.2 g). Purification was carried out by flash

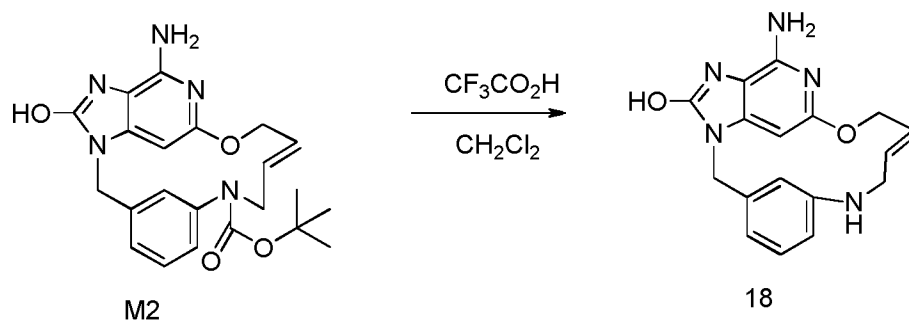
chromatography over silica gel (15-40 μm , 80 g, $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$: 99.5/0.5). The pure fractions were collected and evaporated to dryness to give 0.99 g of F1 (E/Z mixture of expected compound, 40% yield) and 0.65 g of F2 (starting material K2).

F1 was further purified by achiral SFC (Stationary phase: NH_2 5 μm 150*30 mm), Mobile phase: 92% CO_2 , 8% MeOH) to give 664 mg of intermediate L2 (E isomer, 27% yield).



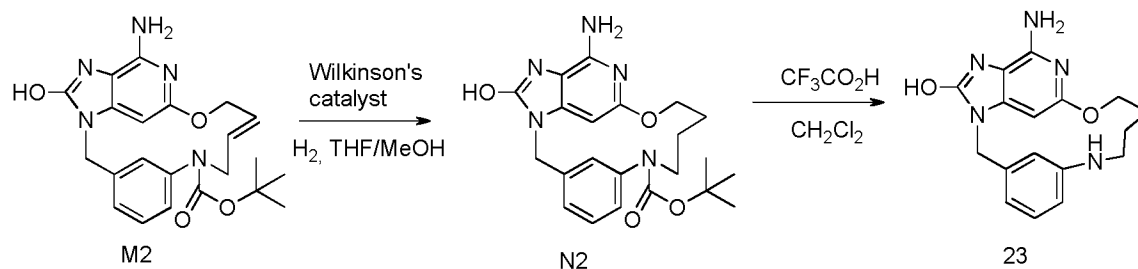
Synthesis of intermediate M2

Iron (1.45 g; 26.025 mmol) was added to a mixture of L2 (0.65 g; 1.301 mmol) in acetic acid (15 mL) and water (1.5 mL). The mixture was stirred at 50°C for 3 h, and then filtered through celite with $\text{CH}_2\text{Cl}_2/\text{MeOH}$. The filtrate was concentrated under reduced pressure. The compound was purified by flash chromatography over silica gel column (15-40 μm ; 80 g, eluent $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ 96/4/0.5) to give 640 mg. A second purification was carried out by flash chromatography over silica gel (15-40 μm , 40 g, $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$: 97/3/0.2). The pure fractions were collected and evaporated to dryness to give 240 mg of intermediate M2 (38% yield).



Synthesis of final compound 18

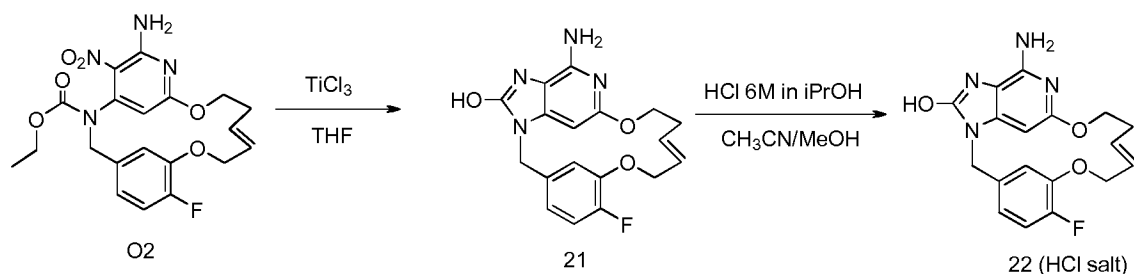
At 0°C, $\text{CF}_3\text{CO}_2\text{H}$ (0.455 mL) was added drop wise to a mixture of M2 (100 mg, 0.236 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at rt overnight, and then basified with a 10% solution of K_2CO_3 in water. The precipitate was filtered off, washed with water and CH_3CN , and finally dried under vacuum to afford 35 mg of final compound 18 (E isomer, 46% yield).

Overall Scheme in the preparation of final products: method 12**Synthesis of intermediate N2**

- 5 A mixture of M2 (140 mg, 0.331 mmol) in THF/CH₃OH (50/50) (30 mL) was hydrogenated under a 10 Bars pressure with Wilkinson's catalyst (61.2 mg, 0.0661 mmol) for 72 h. Siliabond DMT (441 mg, 0.264 mmol) was added and the mixture was stirred at rt for 18 h. The mixture was filtered through a pad of celite and the celite was washed with CH₂Cl₂/CH₃OH 95/5. The filtrate was concentrated under reduced pressure. Purification was carried out by flash chromatography over silica gel (15-40 μm, 10 g, CH₂Cl₂/CH₃OH/NH₄OH: 97/3/0.1). The pure fractions were collected and evaporated to dryness to give 62 mg of intermediate N2 (44% yield) used as such in the next step.

Synthesis of final compound 23

- 15 At 0°C, CF₃CO₂H (0.281 mL, 3.643 mmol) was added drop wise to a mixture of N2 (62 mg, 0.146 mmol) in CH₂Cl₂ (1 mL). The mixture was stirred at rt overnight. The mixture was basified with a 10% solution of K₂CO₃ in water. The mixture was extracted twice with CH₂Cl₂ and CH₃OH (80/20). The organic layer was dried over MgSO₄, filtered and the solvent was evaporated. The crude compound was taken up with DMF, 2 g of SiO₂ 60-200 μm was added and the resulting suspension was evaporated until dryness. This residue was put on the top of a chromatography column (solid deposit). Purification was carried out by flash chromatography over silica gel (15-40 μm, 25 g, CH₂Cl₂/CH₃OH/NH₄OH: 95/5/0.5). The pure fractions were collected and evaporated to dryness to give 20 mg. The fraction was taken up with CH₃CN, the precipitate was filtered off and dried under vacuum to afford 18 mg of final compound 23 (38% yield).

Overall Scheme in the preparation of final products: method 13**Synthesis of intermediate O2**

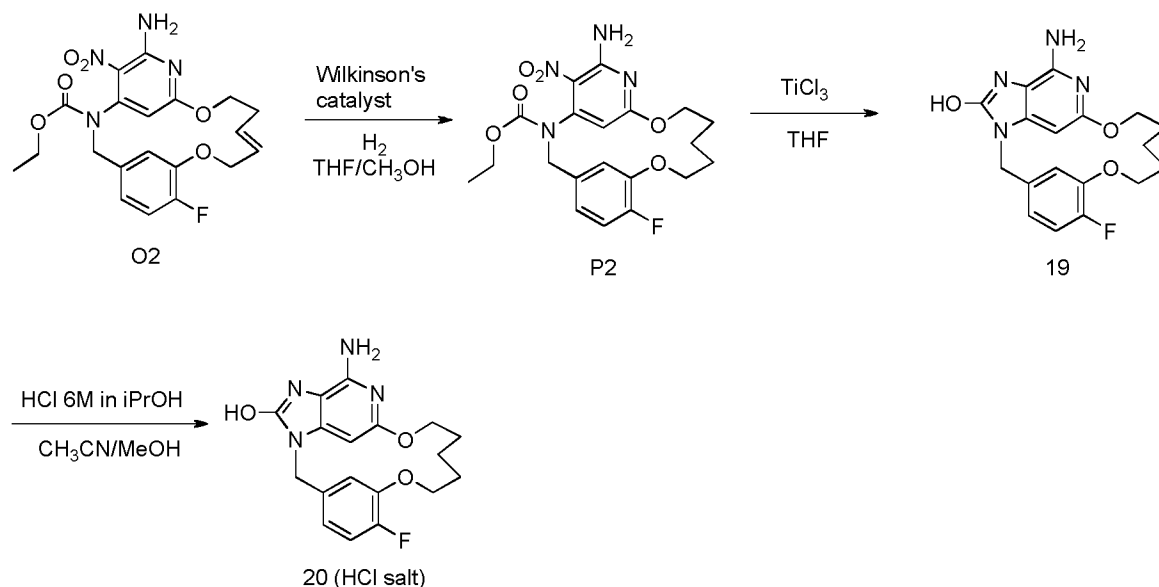
Intermediate O2 was obtained with the procedures described for intermediate X1 (E isomer).

5 Synthesis of final compound 21

At rt, TiCl_3 (51.5 mL; 60.128 mmol) was added drop wise to a mixture of O2 (1.3 g; 3.006 mmol) in THF (130 mL). The mixture was stirred at rt for 2 h. The mixture was cooled down to 0°C and then basified with K_2CO_3 powder. The resulting muddy mixture was filtered through a pad of celite and the celite was washed with a solution of $\text{AcOEt}/\text{CH}_3\text{OH}$ 8/2. The filtrate was partially evaporated to give 380 mg of final compound 21 (35% yield) after filtration of a white solid and drying under vacuum at 85°C .

Synthesis of final compound 22

Compound 21 (118 mg, 0.331 mmol) in CH_3OH (2 mL) + CH_3CN (2 mL) was cooled down to 10°C . HCl (6M in isopropanol) (0.16 mL, 0.993 mmol) was added drop wise and the mixture was stirred at rt for 1 h. The precipitate was filtered off, washed with Et_2O and dried under vacuum to give 109 mg of final compound 22 as an HCl salt (0.76 HCl 0.81 H_2O , 83% yield).

Overall Scheme in the preparation of final products: method 14**Synthesis of intermediate P2**

- 5 A mixture of O2 (320 mg; 0.74 mmol), Wilkinson's catalyst (137 mg; 0.148 mmol) in THF/CH₃OH (50/50) (45 mL) was hydrogenated under 10 bars pressure at rt for 20 h. Solvent was evaporated under vacuum. The crude compound was purified by flash chromatography over silica gel column (15-40 μ m; 24 g) in Heptane/AcOEt 80/20 to give 310 mg of intermediate P2 (96% yield).

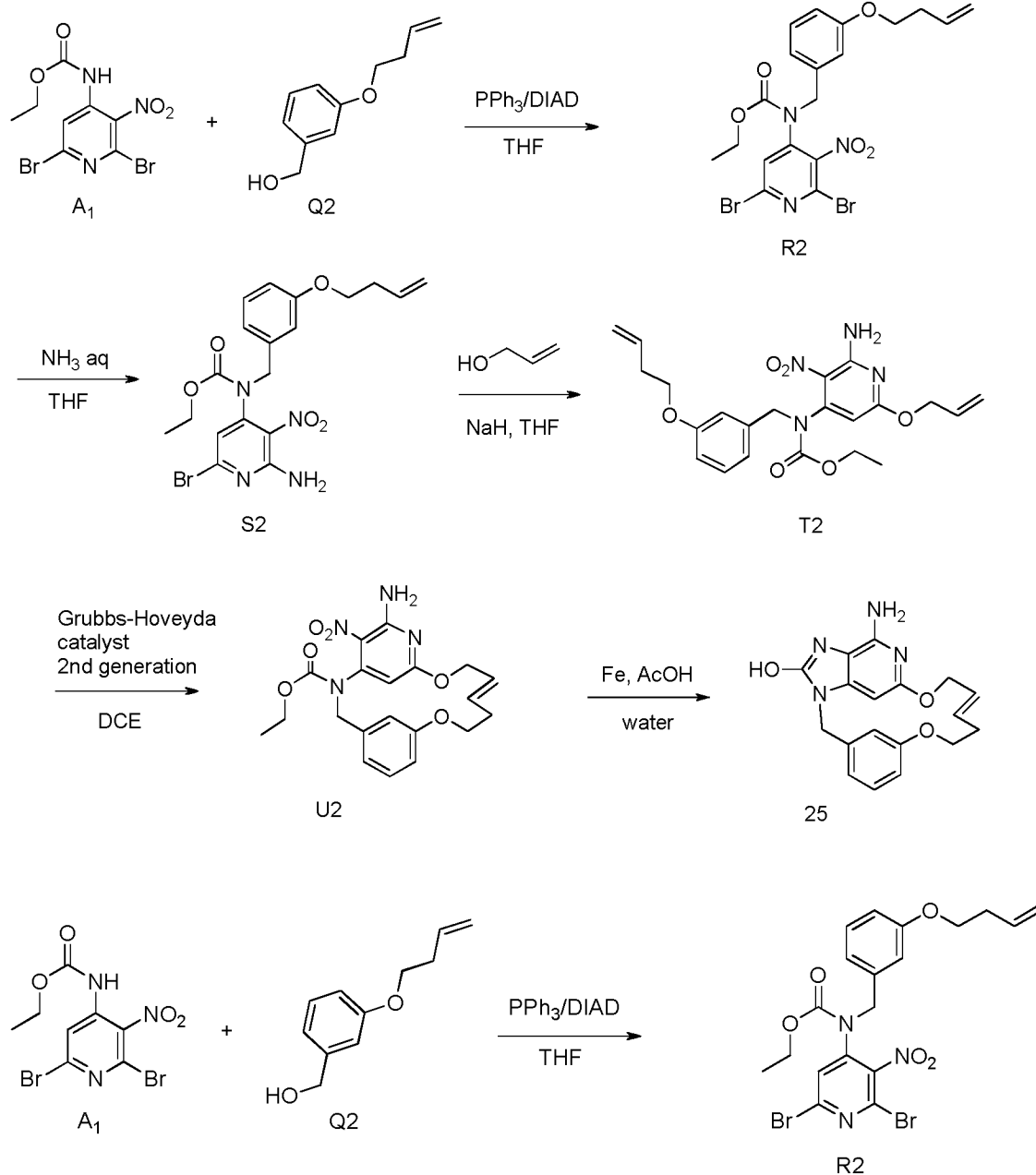
10 Synthesis of final compound 19

- At rt, TiCl₃ (9.5 mL; 11.049 mmol) was added drop wise to a mixture of P2 (0.24 g; 0.552 mmol) in THF (25 mL). The mixture was stirred at rt for 2 h. The mixture was cooled down to 0°C and then basified with K₂CO₃ powder. The resulting muddy mixture was filtered through a pad of celite and the celite was washed with a solution of AcOEt/CH₃OH 8/2. The filtrate was partially evaporated to give 100 mg of final compound 19 (50% yield) after filtration of a white solid and drying under vacuum at 85°C.

Synthesis of final compound 20

- Compound 19 (58 mg; 0.162 mmol) in CH₃OH (2 mL) + CH₃CN (4 mL) was cooled down to 5°C. HCl (6M in isopropanol) (81 μ L; 0.486 mmol) was added drop wise and the mixture was stirred at rt for 1 h. The precipitate was filtered off, washed with diisopropylether and dried under vacuum at 90°C to give 57 mg of final compound 20 as an HCl salt (0.88 HCl 0.04 H₂O, 89% yield).

Overall Scheme in the preparation of final products: method 15

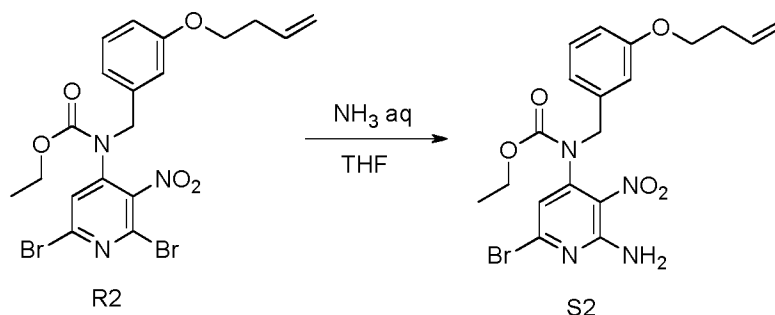


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Synthesis of intermediate R2

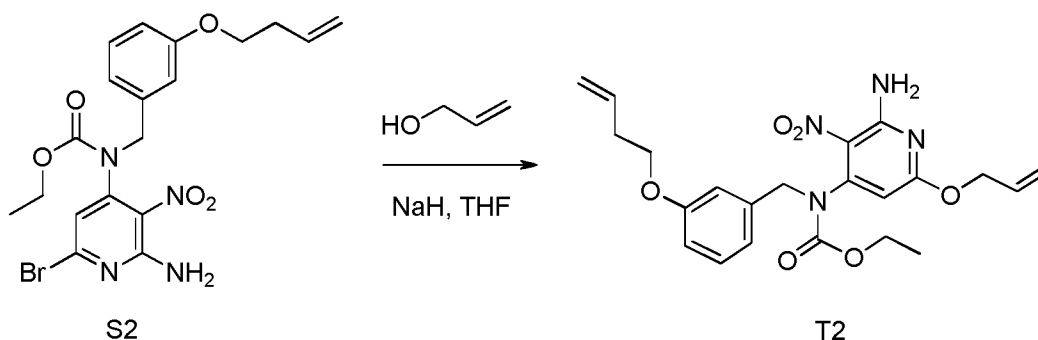
At 0°C, diisopropylazodicarboxylate (3.8 mL; 19.107 mmol) was added drop wise to a mixture of A1 (4.7 g; 12.738 mmol), Q2 (2.27 g; 12.738 mmol) and PPh₃ (5 g; 19.107 mmol) in THF (100 mL). The mixture was stirred at rt for 12 h. EtOAc and water were added. The layers were decanted. The organic layer was washed with water, dried over MgSO₄, filtered and the solvent was evaporated. The crude compound was purified by column chromatography over silicagel (15-40 μm; 220 g) in Heptane/AcOEt 85/15 to 5.3 g of intermediate R2 (79% yield).

10



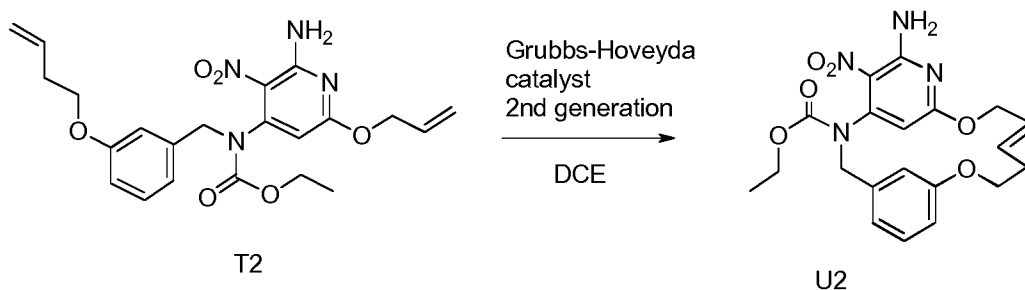
Synthesis of intermediate S2

R2 (5.3 g; 10.015 mmol) was stirred in THF (80 mL) and NH_4OH (80 mL) at rt for 24 h. The mixture was concentrated under reduced pressure. The residue was taken up with CH_2Cl_2 , the precipitate (mineral) was filtered off and the filtrate was concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel (15-40 μm ; 220 g) in Heptane/AcOEt 85/15 to give 3.65 g of intermediate S2 (78% yield).



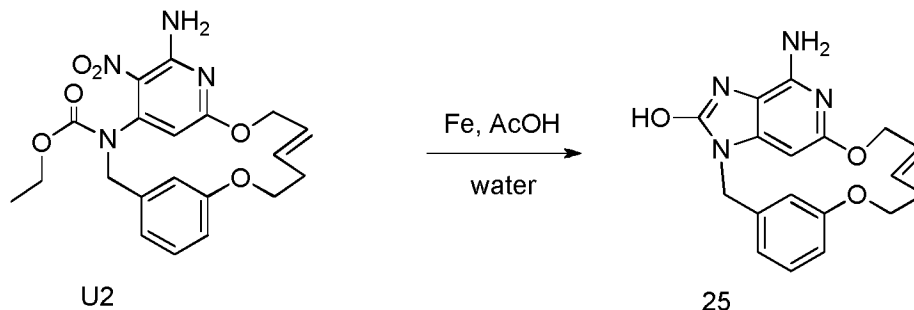
Synthesis of intermediate T2

NaH (1.35 g; 33.88 mmol) was added portion wise to allyl alcohol (41 mL) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of S2 (4 g; 8.597 mmol) in THF (100 mL) at 0°C . The resulting mixture was then stirred at rt for 2 h 30 min and was poured in an saturated aqueous solution of NH_4Cl . EtOAc and a saturated aqueous solution of NaCl were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give a yellow oil. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Grace, liquid injection, mobile phase gradient: Heptane/EtOAc 85/15) to give 3.2 g of intermediate T2 as a yellow oil (84% yield).



Synthesis of intermediate U2

A solution of T2 (1 g; 2.26 mmol) and chlorodicyclohexylborane (1M in hexane) (904 μ L; 904.013 μ mol) in dry dichloroethane (540 mL) was stirred at 80°C and under N₂ atmosphere for 1 h. The mixture was degassed by N₂ bubbling for 15 min, Grubbs-Hoveyda catalyst 2nd generation (141.6 mg; 226 μ mol) was added, the mixture was degassed again by N₂ bubbling for 15 min and then stirred at 120°C for 16 h. 0.25 eq of catalyst were added again and mixture was stirred at 120°C for 16 h. Siliabond DMT (5.9 g; 3.616 mmol) was added and the mixture was stirred rt for 16 h. The mixture was filtered through a pad of celite and the filtrate was concentrated under vacuum to give a black oil. The crude compound was purified by preparative LC (Irregular SiOH 15-40 μ m, 80 g Merck, mobile phase: CH₂Cl₂/AcOEt 97/3). The fractions containing product were combined and the solvent was removed under vacuum to give 335 mg of intermediate U2 (E isomer, 36% yield).

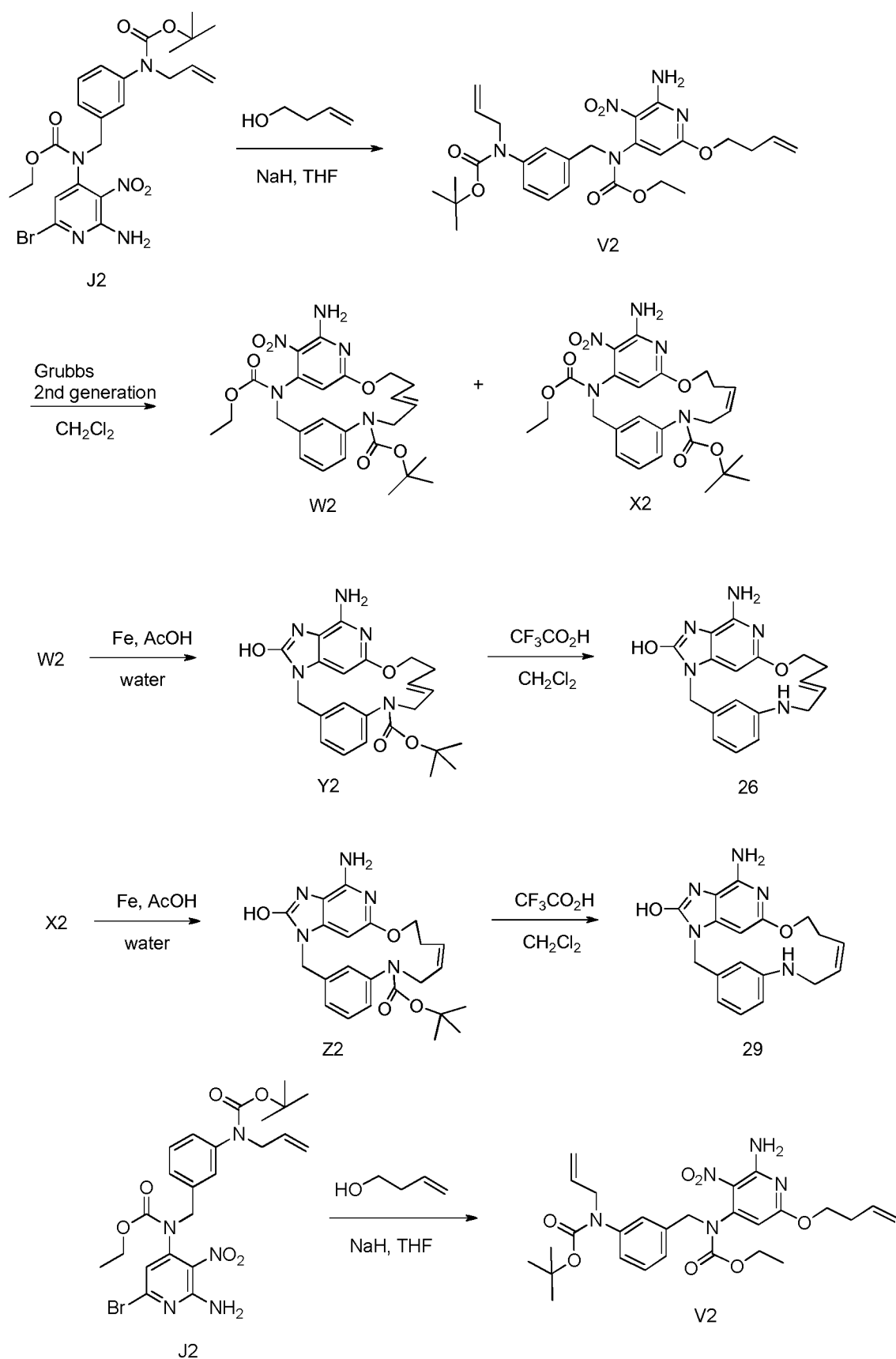


Synthesis of final compound 25

Iron (0.45 g; 8.084 mmol) was added to a mixture of U2 (0.335 g; 0.808 mmol) in acetic acid (24 mL) + water (5 mL). The mixture was stirred vigorously at 50°C for 5 h.

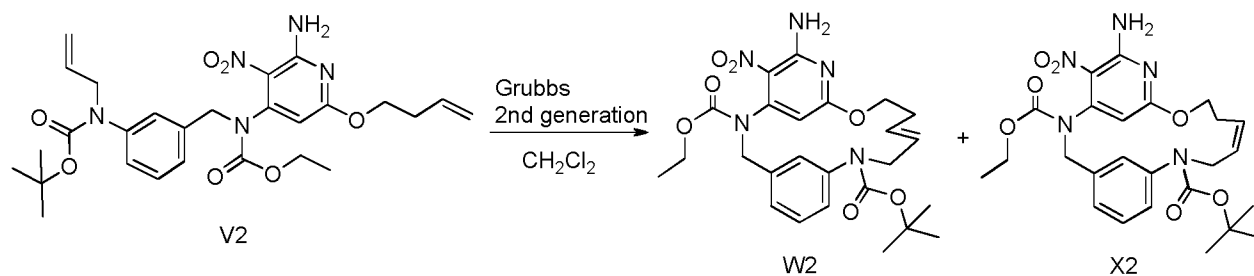
CH₂Cl₂ was added and the reaction mixture was filtered through a pad of celite, and then washed with acetic acid. The solvent was removed under reduced pressure. The crude was purified by chromatography over silicagel column (SiO₂ 15-40 μ m, 25 g) in CH₂Cl₂/CH₃OH/NH₄OH 96/4/0.5 to give 154 mg of final compound 25 (56% yield). The compound was crystallized in CH₃OH, filtered and dried under vacuum at 90°C to give 70 mg (25% yield).

Overall Scheme in the preparation of final products: method 16



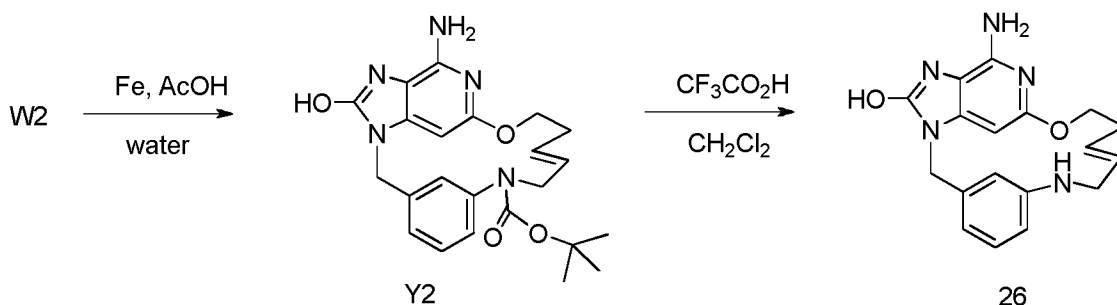
Synthesis of intermediate V2

Intermediate V2 was synthesized using the procedure described for intermediate K2 with 3-butenol as starting material (3.9 g, 44% yield).



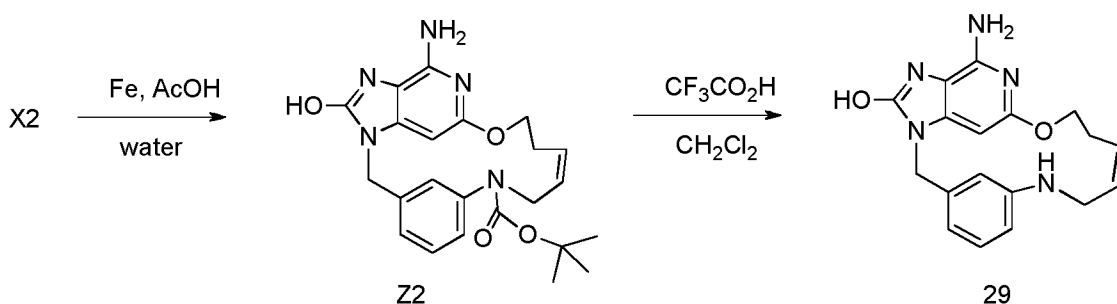
Synthesis of intermediates W2 and X2

- 5 Grubbs catalyst 2nd generation (236 mg, 0.277 mmol) was added to a mixture of V2 (1.5 g, 2.77 mmol) in dry CH₂Cl₂ (400 mL). The mixture was stirred at rt under a N₂ flow for 24 h. Siliabond DMT (3.6 g, 2.216 mmol) was added and the mixture was stirred at rt for 12 h. The mixture was filtered through celite, the celite was washed with CH₂Cl₂ and the filtrate was evaporated. Purification was carried out by flash chromatography over silica gel (15-40 μm, 80 g, CH₂Cl₂/CH₃OH: 99.5/0.5) pure fractions were collected and evaporated to dryness to give 0.98 g of a mixture of W2 and X2. The two isomers were separated by achiral SFC (Stationary phase: CHIRALPAK IC 5 μm 250x20 mm), Mobile phase: 70% CO₂, 30% CH₃OH) to give 0.805 g of intermediate W2 (E isomer, 57% yield) and 0.14 g of intermediate X2 (Z isomer, 10% yield).



15 Synthesis of final compound 26

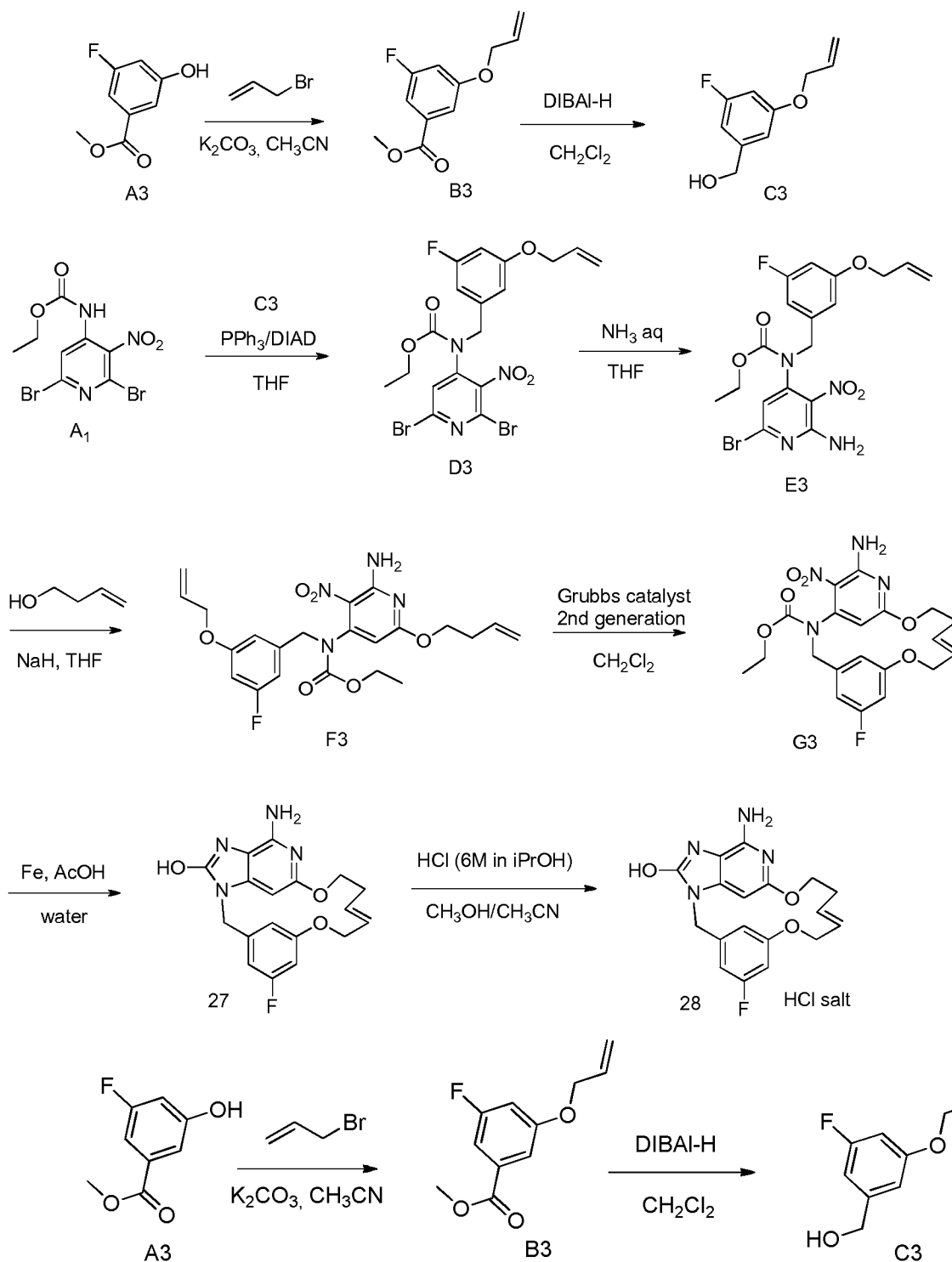
Final compound 26 was synthesized with the procedures described for final compound 18 (1st step: Y2, 0.68 g, 99% yield; 2nd step: 52 mg, 27% yield).



Synthesis of final compound 29

Final compound 29 was synthesized with the procedures described for final compound 18 (1st step: Z2, 0.12 g, 100% yield; 2nd step: 8 mg, 9% yield).

Overall Scheme in the preparation of final products: method 17

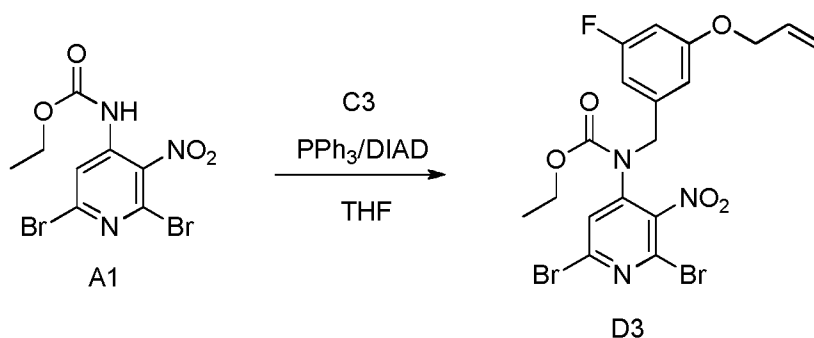


Synthesis of intermediate B3

Allyl bromide (13 mL, 0.15 mmol) was added drop wise to a mixture of A3 (23 g, 0.135 mmol) and K_2CO_3 (28 g, 0.2 mmol) in CH_3CN (460 mL). The mixture was stirred at reflux for 4 h, and then concentrated under reduced pressure. The residue was taken up with water and was extracted with EtOAc. The organic layers were combined, washed with water, dried over $MgSO_4$, filtered and the solvent was evaporated. The crude compound (27 g, 95% yield) was used directly in the next reaction step.

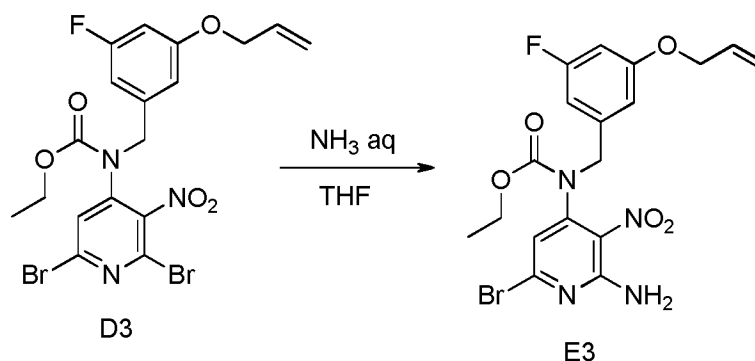
Synthesis of intermediate C3

Under N_2 , DIBAL-H (1.2 M in toluene) (97 mL; 116.5 mmol) was added to a solution of B3 (9.8 g; 46.6 mmol) in dry CH_2Cl_2 (250 mL) at $0^\circ C$. The reaction mixture was stirred at $0^\circ C$ for 1 h, and then 1 h at rt. Water was added, The organic layer was separated from the aqueous layer, dried over $MgSO_4$, filtered and concentrated under vacuum to give 8.4 g of intermediate C3 (99% yield). The crude compound was used directly in the next reaction step.



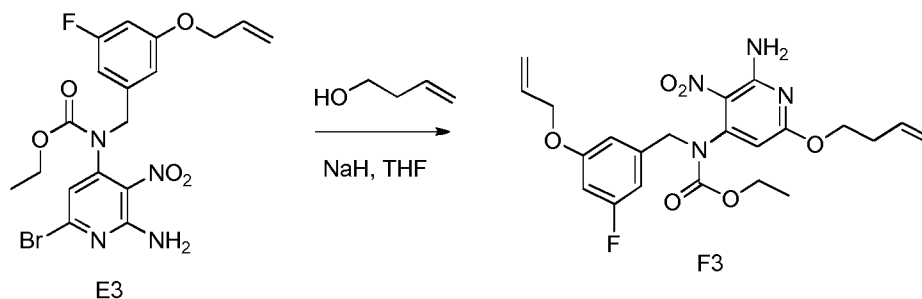
Synthesis of intermediate D3

Intermediate D3 was synthesized using the procedure described for intermediate R2 with C3 as starting material (1.9 g, 88% yield).



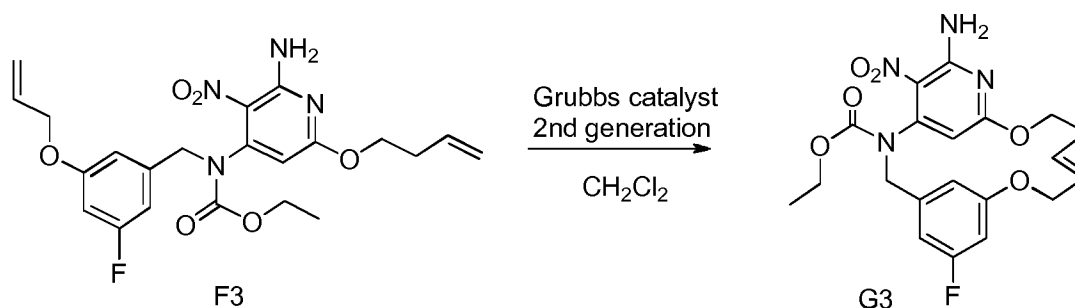
Synthesis of intermediate E3

Intermediate E3 was synthesized using the procedure described for intermediate S2 with D3 as starting material (1.8 g, 93% yield).



Synthesis of intermediate F3

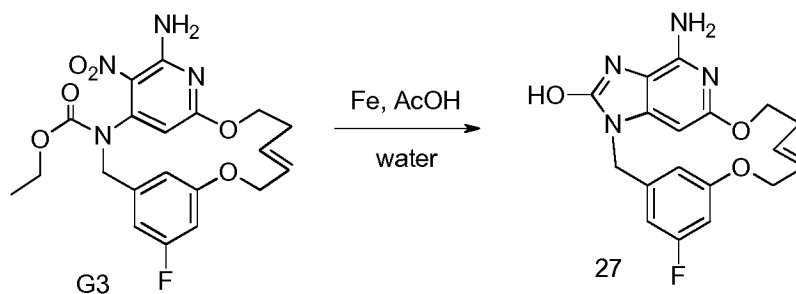
Intermediate F3 was synthesized using the procedure described for intermediate W1 with E3 as starting material (0.65 g, 66% yield).



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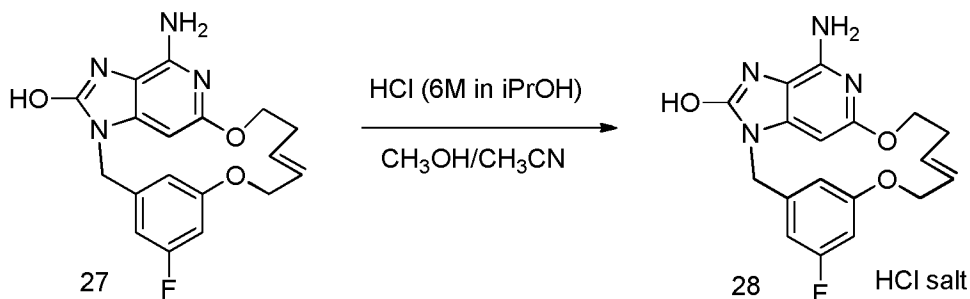
Synthesis of intermediate G3

Intermediate G3 was synthesized using the procedure described for intermediate X1 with intermediate F3 as starting material (E isomer, 520 mg, 19% yield).



10 Synthesis of final compound 27

Final compound 27 was synthesized using the procedure described for final compound 10 with intermediate G3 as starting material (174 mg, 42% yield).

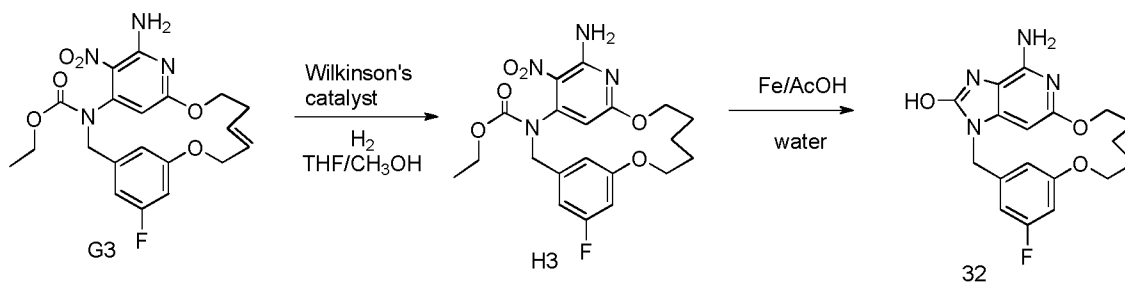


Synthesis of final compound 28

Final compound 28 was synthesized using the procedure described for final compound 20 with compound 27 as starting material (1.01 HCl 0.89 H₂O, 95 mg, 69% yield).

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Overall Scheme in the preparation of final products: method 18



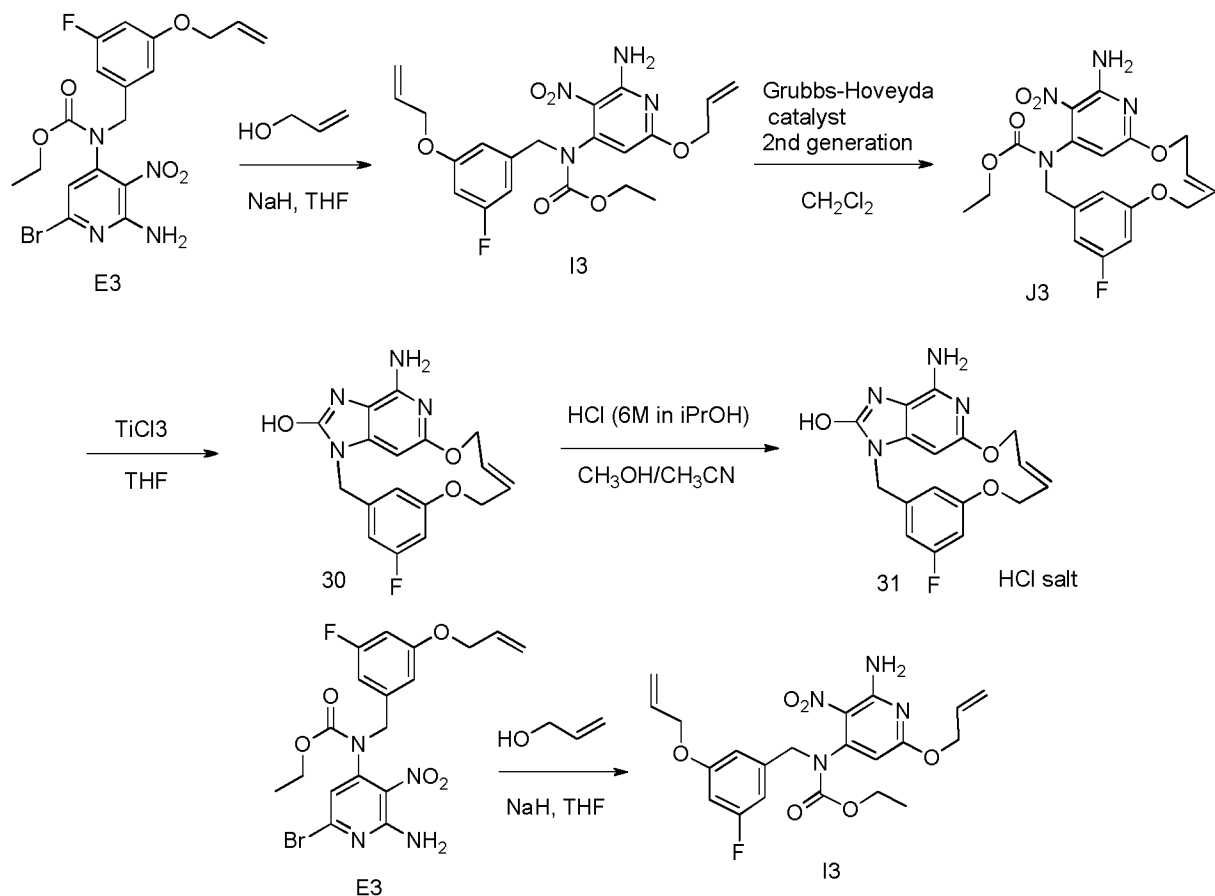
Synthesis of intermediate H3

- 10 A mixture of G3 (600 mg, 1.39 mmol), Wilkinson's catalyst (257 mg; 0.278 mmol) in THF/CH₃OH (50/50) (120 mL) was hydrogenated under 12 bars pressure at rt for 20 h. The solution was concentrated under reduced pressure. Purification was carried out by flash chromatography over silica gel (15-40 μm, 30 g, CH₂Cl₂/CH₃OH: 99.5/0.5). The pure fractions were collected and evaporated to dryness, and then crystallized from CH₃CN to give 150 mg of
- 15 intermediate H3 (25% yield).

Synthesis of final compound 32

- A mixture of H3 (150 mg; 0.345 mmol) and iron (190 mg; 3.45 mmol) in acetic acid (13 mL) and water (1.5 mL) was stirred at 50°C for 5 h. CH₂Cl₂ was added and the reaction mixture was filtered through a pad of celite and concentrated under vacuum. The crude compound was taken
- 20 up with DMF, filtered through a pad of celite and concentrated. The solid was pre-purified by chromatography over silicagel column (SiO₂ 63-200 μm, 80g) in CH₂Cl₂/CH₃OH/NH₄OH(98/2/0.1 to 90/10/0.5). A second purification by achiral SFC (Stationary phase: Welk O1 (S,S) 5μm 250*21.1 mm), Mobile phase: 60% CO₂, 40% CH₃OH (0.3% iPrNH₂)) afforded 27 mg of final compound 32 (22% yield).

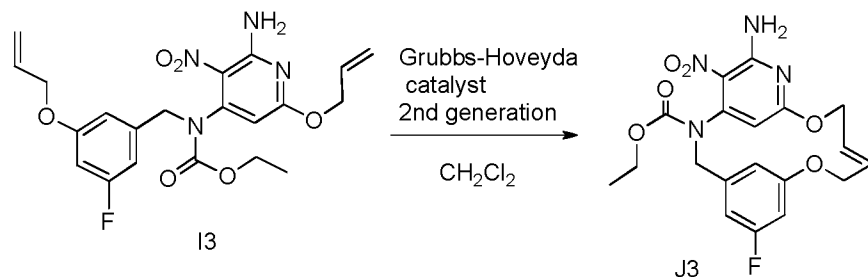
Overall Scheme in the preparation of final products: method 19



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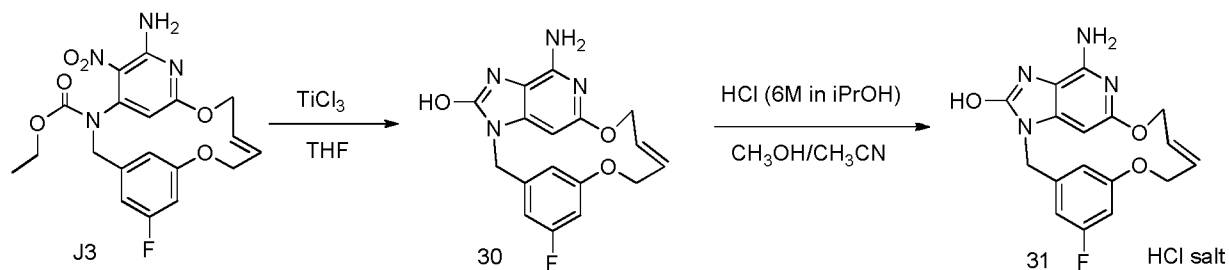
Synthesis of intermediate I3

Intermediate I3 was synthesized using the procedure described for intermediate T2 (4.2 g, 83%).



Synthesis of intermediate J3

- 10 Intermediate J3 was synthesized using the procedure described for intermediate F1 (isomer E, 125 mg, 17%).

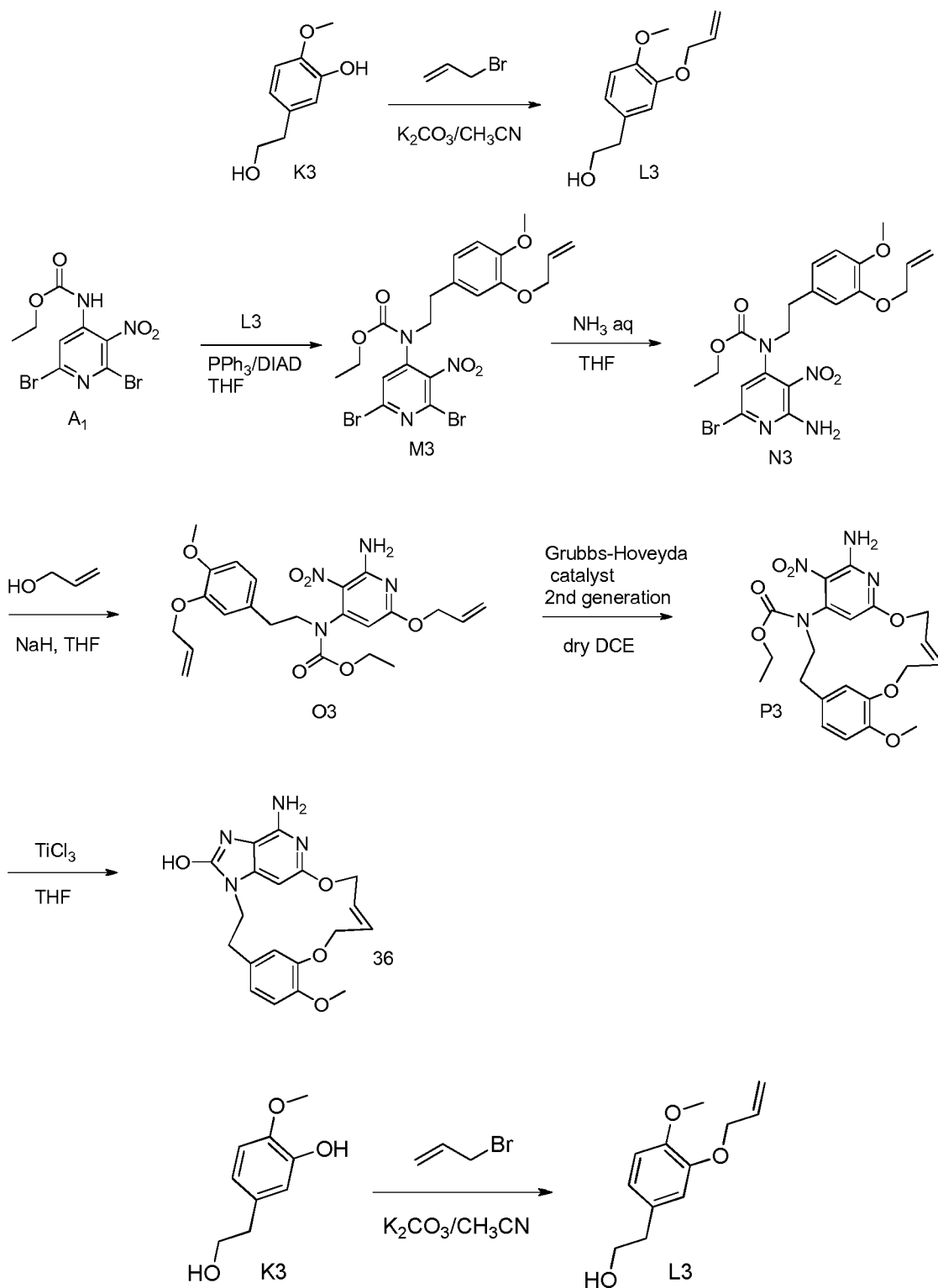


Synthesis of final compound 30

Final compound 30 was synthesized using the procedure described for final compound 21 (72 mg, 44% yield).

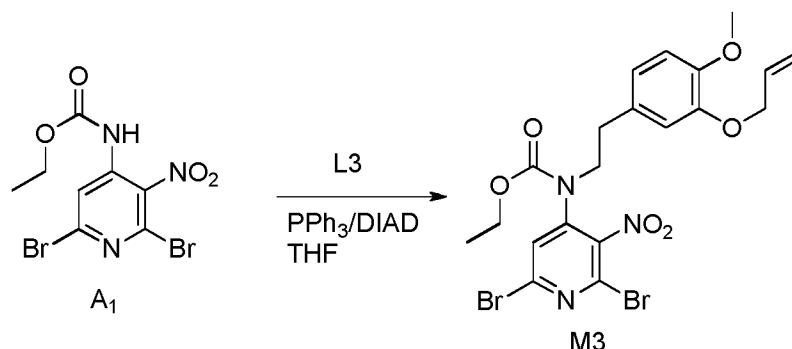
5 Synthesis of final compound 31

Final compound 31 was synthesized using the procedure described for final compound 22 (0.98 HCl 0.15 H₂O, 72 mg, 59% yield).

Overall Scheme in the preparation of final products: method 20**5 Synthesis of intermediate L3**

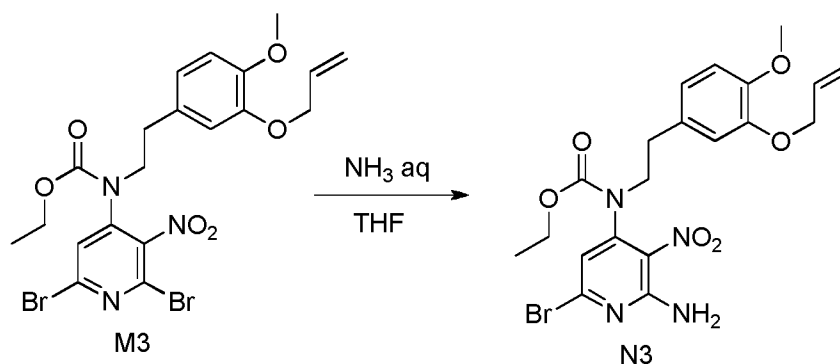
Allyl bromide (1.7 mL; 19.6 mmol) was added to a solution of K3 (3 g; 17.8 mmol) and K_2CO_3 (2.7 g; 19.6 mmol) in CH_3CN (90 ml). The mixture was stirred at 90°C for 20 h, and then filtered. The filtrate was concentrated under vacuum. The crude product was taken up with CH_2Cl_2 and an aqueous solution of NaOH 5%. The layers were separated and the organic layer

was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure to give 3.9 g of intermediate L3 (quantitative yield). The crude compound was used directly in the next reaction step.



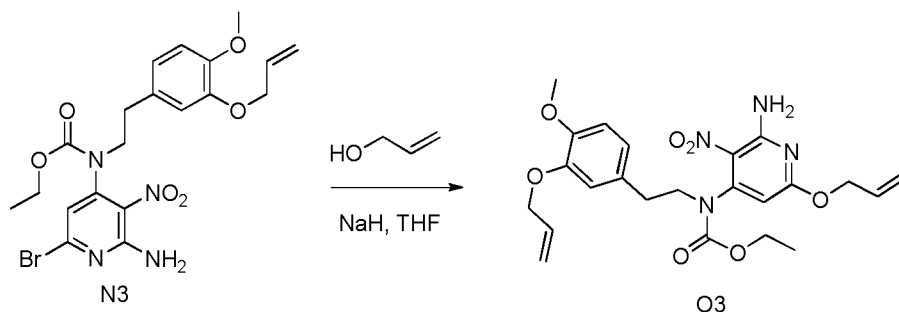
5 Synthesis of intermediate M3

At 0°C, diisopropylazodicarboxylate (4.8 mL; 24.36 mmol) was added drop wise to a mixture of A1 (6 g; 16.2 mmol), L3 (3.2 g; 15.36 mmol) and PPh_3 (6.4 g; 24.36 mmol) in THF (120 mL). The mixture was stirred at rt for 12 h. EtOAc and water were added. The layers were decanted. The organic layer was washed with water, dried over MgSO_4 , filtered and the solvent was evaporated. 20 mL of Heptane/AcOEt 70/30 were added to precipitate a large part of the formed PPh_3O , which was removed by filtration. The crude product was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Interchim, mobile phase Heptane/EtOAc 80/20) to give 8 g of intermediate M3 (88% yield).



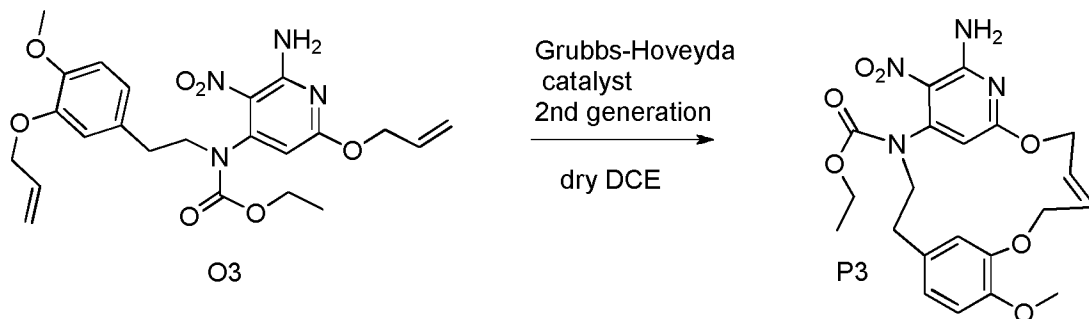
15 Synthesis of intermediate N3

M3 (8.8 g; 15.7 mmol) was stirred in THF (120 mL) and NH_4OH (120 mL) at rt for 24 h. The mixture was concentrated under reduced pressure. The residue was taken up with CH_2Cl_2 , the precipitate (mineral) was filtered off and the filtrate was dried over MgSO_4 , filtered through a pad of celite and concentrated under vacuum. The crude product was purified by preparative LC (Irregular SiOH 15-40 μm , 120 g Interchim, mobile phase Heptane/EtOAc 80/20) to afford 3 g of intermediate N3 (38% yield).



Synthesis of intermediate O3

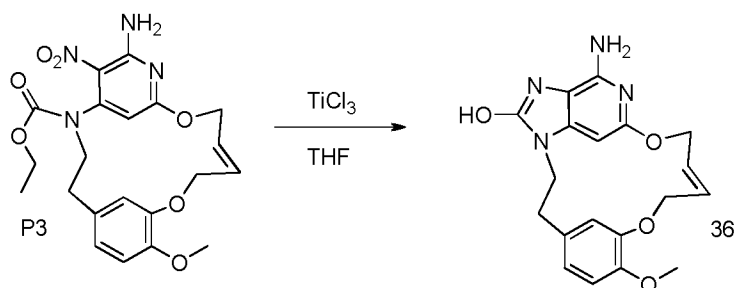
NaH (60% in oil) (0.93 g; 23 mmol) was added portion wise to allyl alcohol (28 mL) at rt. The mixture was stirred at rt for 30 min before being added drop wise to a solution of N3 (2.9 g; 5.85 mmol) in THF (70 mL) at 0°C. The resulting mixture was then stirred at rt for 2 h 30 min and was poured into a saturated aqueous solution of NH₄Cl. EtOAc and a saturated aqueous solution of NaCl were added, the layers were separated and the aqueous layer was extracted with EtOAc (once). The combined organic layers were dried over MgSO₄, filtered and the solvent was removed under reduced pressure to give a yellow oil. The crude product was purified by preparative LC (Irregular SiOH 15-40 μm, 120 g Grace, liquid injection, mobile phase heptane/EtOAc 80/20) to give 2.4 g of intermediate O3 (87% yield).



Synthesis of intermediate P3

The reaction was carried out on three batches.

A solution of O3 (0.8 g; 1.7 mmol) and chlorodicyclohexylborane (1M in hexane) (0.68 mL; 0.68 mmol) in dry dichloroethane (400 mL) was stirred at 80°C and under N₂ atmosphere for 1 h. The mixture was degassed by N₂ bubbling for 15 min, Grubbs-Hoveyda catalyst 2nd generation (110 mg; 0.17 mol) was added, the mixture was degassed again by N₂ bubbling for 15 min and then stirred at 120°C for 16 h. 0.050 eq of catalyst (49 mg, 0.084 mmol) were added and mixture was stirred at 120°C for 7 h. Siliabond DMT (3.3 g; 2.03 mmol) was added and the mixture was stirred rt for 16 h. The mixture was filtered through a pad of celite and the filtrate was concentrated under vacuum to give a black oil. The crude product was purified by preparative LC (Irregular SiOH 15-40 μm, 80 g Interchim, mobile phase Heptane/EtOAc 65/35) to give 190 mg of intermediate P3 (isomer E, 25% yield).



Synthesis of final compound 36

At rt, TiCl_3 (19.3 mL; 22.5 mmol) was added drop wise to a mixture of P3 (500 mg; 1.125 mmol) in THF (90 mL). The mixture was stirred at rt for 2 h. At 0°C , the mixture was basified with K_2CO_3 powder. The resulting muddy mixture was filtered through a pad of celite and celite was washed with a solution of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (90/10). The filtrate was concentrated under reduced pressure. The residue was taken up in MeOH. The white solid was filtered off and dried under vacuum. The product was purified by preparative LC (Irregular SiOH 15-40 μm , 40 g Interchim, mobile phase $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ 98/2/0.1) to give 140 mg of final compound 36 (34% yield).

LCMS methods:

General procedure VDR2 (for methods V300xV30xx.olp)

The LC measurement was performed using a UPLC (Ultra Performance Liquid Chromatography) Acquity (Waters) system comprising a binary pump with degasser, an autosampler, a diode-array detector (DAD) and a column as specified in the respective methods below, the column is hold at a temperature of 40°C. Flow from the column was brought to a MS detector. The MS detector was configured with an electrospray ionization source. The capillary needle voltage was 3 kV and the source temperature was maintained at 130 °C on the Quattro (triple quadrupole mass spectrometer from Waters). Nitrogen was used as the nebulizer gas. Data acquisition was performed with a Waters-Micromass MassLynx-Openlynx data system.

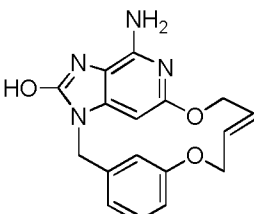
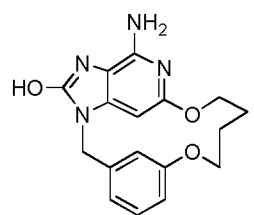
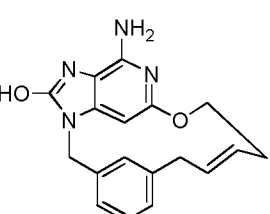
Method V3018V3001

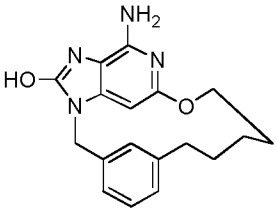
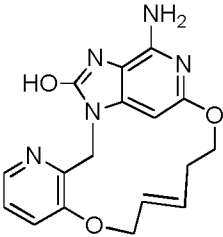
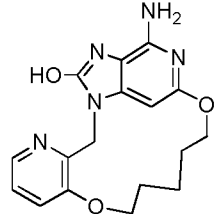
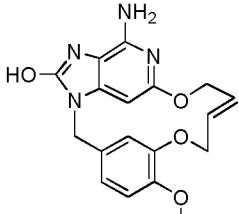
In addition to the general procedure VDR2: Reversed phase UPLC was carried out on a Waters Acquity BEH (bridged ethylsiloxane/silica hybrid) C18 column (1.7 μm , 2.1 x 100 mm) with a flow rate of 0.343 ml/min. Two mobile phases (mobile phase A: 95 % 7 mM ammonium acetate / 5 % acetonitrile; mobile phase B: 100 % acetonitrile) were employed to run a gradient condition from 84.2 % A and 15.8 % B (hold for 0.49 minutes) to 10.5 % A and 89.5 % B in 2.18 minutes, hold for 1.94 min and back to the initial conditions in 0.73 min, hold for 0.73 minutes. An injection volume of 2 μl was used. Cone voltage was 20V for positive and negative

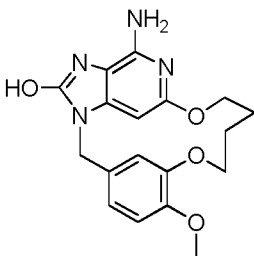
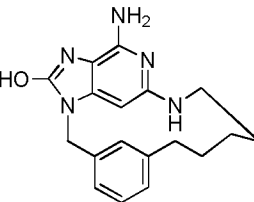
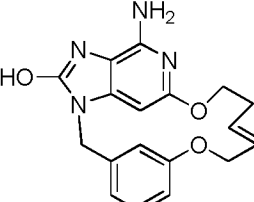
ionization mode. Mass spectra were acquired by scanning from 100 to 1000 in 0.2 seconds using an interscan delay of 0.1 seconds.

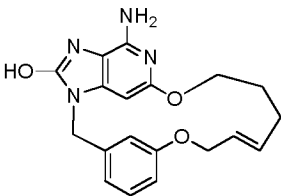
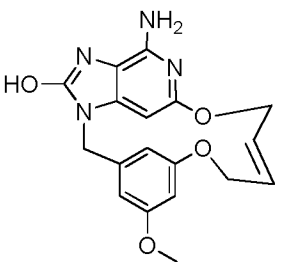
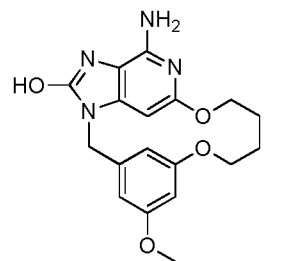
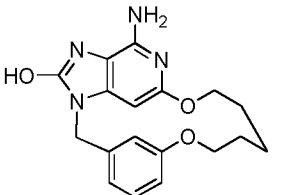
Table 1. Compounds of formula (I).

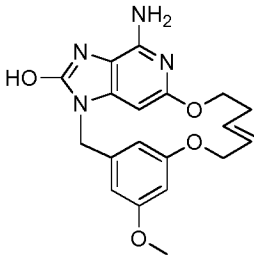
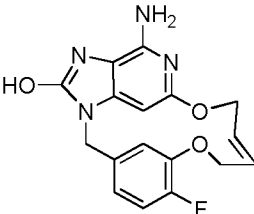
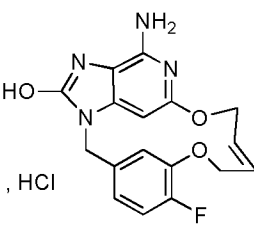
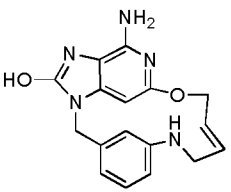
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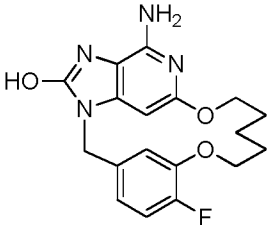
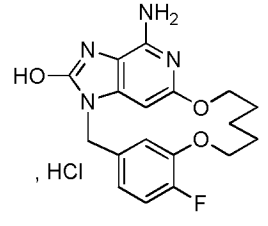
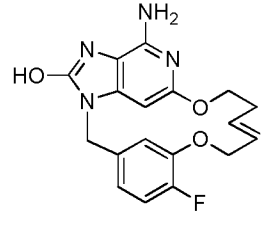
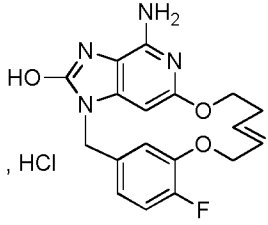
#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
1		324.1	325	1.96, V3018V3 001	Method 1	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.06 (s, 1H), 7.36 (s, 1H), 7.20 (t, <i>J</i> = 7.9 Hz, 1H), 6.76 - 6.93 (m, 2H), 6.05 (s, 1H), 5.94 (dt, <i>J</i> = 6.0, 16.1 Hz, 1H), 5.43 - 5.64 (m, 3H), 4.84 (s, 2H), 4.40 - 4.66 (m, 4H)
2		326.1	327	2.02, V3018V3 001	Method 2	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.04 (br. s., 1H), 7.49 (s, 1H), 7.17 (t, <i>J</i> = 7.9 Hz, 1H), 6.69 - 6.83 (m, 2H), 6.15 (s, 1H), 5.54 (s, 2H), 4.89 (s, 2H), 4.28 (t, <i>J</i> = 6.3 Hz, 2H), 4.14 (t, <i>J</i> = 6.6 Hz, 2H), 1.34 - 1.62 (m, 4H)
3		322.1	323	2.3, V3018V3 001	Method 3	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.11 (br. s., 1H), 7.22 (t, <i>J</i> = 7.6 Hz, 1H), 7.12 (br. s., 1H), 7.09 (d, <i>J</i> = 7.6 Hz, 1H), 7.05 (d, <i>J</i> = 7.6 Hz, 1H), 5.74 (s, 1H), 5.51 - 5.63 (m, 3H), 5.03 (td, <i>J</i> = 7.2, 14.9 Hz, 1H), 4.93 (s, 2H), 4.15 - 4.26 (m, 2H), 3.15 - 3.23 (m, 2H), 2.24 - 2.33 (m, 2H)

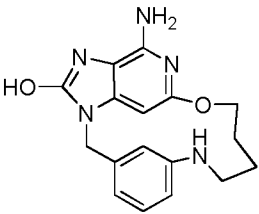
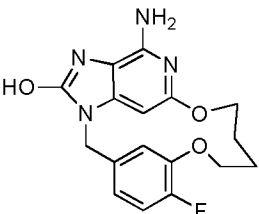
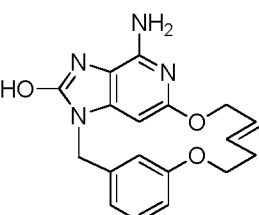
#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
4		324.2	325	2.36, V3018V3 001	Method 4	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.20 (br. s., 1H), 7.34 (s, 1H), 7.21 (t, <i>J</i> = 7.3 Hz, 1H), 7.06 (d, <i>J</i> = 7.3 Hz, 1H), 6.97 (d, <i>J</i> = 7.3 Hz, 1H), 5.59 (s, 2H), 4.93 (s, 2H), 3.91 - 4.03 (m, 2H), 2.55 - 2.62 (m, 2H), 1.59 - 1.71 (m, 2H), 1.12 - 1.27 (m, 4H)
5		339.1	340	1.85, V3018V3 001	Method 5	¹ H NMR (400 MHz, DMSO-d ₆) δ 9.91 (br. s., 1H), 8.05 (d, <i>J</i> = 4.6 Hz, 1H), 7.38 (d, <i>J</i> = 8.6 Hz, 1H), 7.27 (dd, <i>J</i> = 4.6, 8.6 Hz, 1H), 6.20 (s, 1H), 5.82 (dt, <i>J</i> = 7.0, 15.6 Hz, 1H), 5.68 (dt, <i>J</i> = 7.1, 15.6 Hz, 1H), 5.46 (s, 2H), 4.91 (s, 2H), 4.33 (d, <i>J</i> = 7.1 Hz, 2H), 4.12 - 4.27 (m, 2H), 2.36 - 2.46 (m, 2H)
6		341.1	342	1.85, V3018V3 001	Method 6	¹ H NMR (400 MHz, DMSO-d ₆) δ 9.92 (s, 1H), 8.09 (d, <i>J</i> = 4.0 Hz, 1H), 7.43 (d, <i>J</i> = 8.1 Hz, 1H), 7.30 (dd, <i>J</i> = 4.0, 8.1 Hz, 1H), 6.40 (s, 1H), 5.51 (s, 2H), 4.96 (s, 2H), 4.15 (t, <i>J</i> = 6.1 Hz, 2H), 3.97 - 4.10 (m, 2H), 1.81 - 1.91 (m, 2H), 1.68 - 1.78 (m, 2H), 1.52 - 1.65 (m, 2H)
7		354.1	355	1.84, V3018V3 001	Method 1	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.03 (br. s., 1H), 7.36 (d, <i>J</i> = 2.0 Hz, 1H), 6.91 (d, <i>J</i> = 8.1 Hz, 1H), 6.80 (dd, <i>J</i> = 2.0, 8.1 Hz, 1H), 6.06 (dt, <i>J</i> = 6.0,

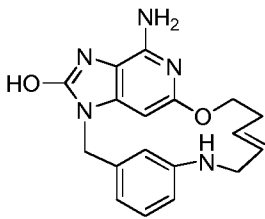
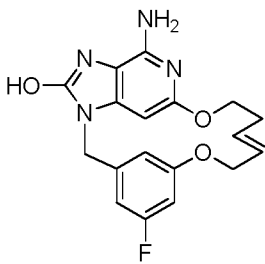
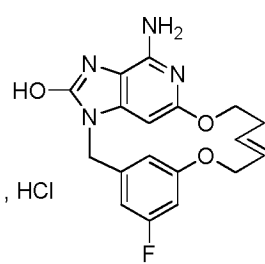
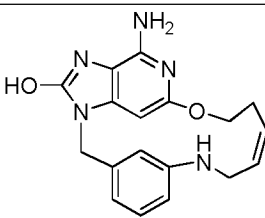
#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
						16.2 Hz, 1H), 5.93 (s, 1H), 5.54 (s, 2H), 5.41 (dt, $J = 5.6$, 16.2 Hz, 1H), 4.78 (s, 2H), 4.50 - 4.67 (m, 2H), 4.28 - 4.48 (m, 2H), 3.71 (s, 3H)
8		356.1	357	1.86, V3018V3 001	Method 2	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.03 (br. s., 1H), 7.54 (br. s., 1H), 6.87 (d, $J = 8.1$ Hz, 1H), 6.76 (d, $J = 8.1$ Hz, 1H), 6.24 (br. s., 1H), 5.53 (br. s., 2H), 4.82 (br. s., 2H), 4.19 - 4.32 (m, 2H), 4.01 - 4.16 (m, 2H), 3.68 (br. s., 3H), 1.34 - 1.60 (m, 4H)
9		323.2	324	2.36, V3018V3 001	Method 1 Method 2	¹ H NMR (400 MHz, DMSO-d ₆) δ 9.73 (s, 1H), 7.57 (s, 1H), 7.16 (t, $J = 7.6$ Hz, 1H), 7.02 (d, $J = 7.6$ Hz, 1H), 6.89 (d, $J = 7.6$ Hz, 1H), 5.33 (t, $J = 6.6$ Hz, 1H), 5.25 (s, 1H), 5.15 (s, 2H), 4.85 (s, 2H), 2.91 (q, $J = 6.6$ Hz, 2H), 2.58 - 2.72 (m, 2H), 1.59 - 1.85 (m, 2H), 0.96 - 1.21 (m, 4H)
10		338.1	339	2.22, V3018V3 001	Method 7	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.05 (br. s., 1H), 7.20 (t, $J = 7.8$ Hz, 1H), 6.92 (d, $J = 7.8$ Hz, 1H), 6.89 (s, 1H), 6.77 (d, $J = 7.8$ Hz, 1H), 6.12 (s, 1H), 5.50 - 5.64 (m, 3H), 5.35 (dt, $J = 4.5$, 16.2 Hz, 1H), 4.87 (s, 2H), 4.58 (d, $J = 4.5$ Hz, 2H), 4.24 (t, $J = 5.1$ Hz, 2H), 2.21 - 2.32 (m, 2H)

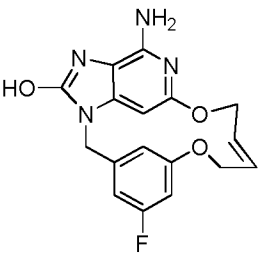
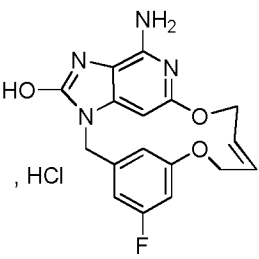
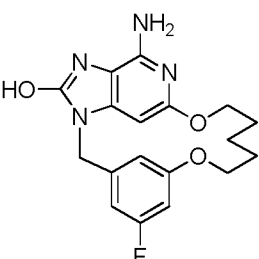
#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
11		352.2	353	2.33, V3018V3 001	Method 1	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.07 (s, 1H), 7.32 (s, 1H), 7.18 (t, <i>J</i> = 7.7 Hz, 1H), 6.82 (d, <i>J</i> = 7.7 Hz, 1H), 6.79 (dd, <i>J</i> = 1.7, 7.7 Hz, 1H), 5.98 (dt, <i>J</i> = 7.7, 15.5 Hz, 1H), 5.80 (s, 1H), 5.56 (s, 2H), 5.51 (td, <i>J</i> = 5.8, 15.5 Hz, 1H), 4.84 (s, 2H), 4.71 (d, <i>J</i> = 5.8 Hz, 2H), 3.96 (t, <i>J</i> = 7.7 Hz, 2H), 1.92 - 2.07 (m, 2H), 1.49 - 1.64 (m, 2H)
12		354.1	355	2.04, V3018V3 001	Method 1	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.05 (br. s., 1H), 6.94 (s, 1H), 6.47 (s, 1H), 6.39 (s, 1H), 6.05 (s, 1H), 5.87 (dt, <i>J</i> = 6.0, 15.7 Hz, 1H), 5.45 - 5.68 (m, 3H), 4.79 (s, 2H), 4.41 - 4.61 (m, 4H), 3.64 (s, 3H)
13		356.1	357	2.1, V3018V3 001	Method 2	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.05 (s, 1H), 7.08 (s, 1H), 6.24 - 6.44 (m, 2H), 6.16 (s, 1H), 5.55 (s, 2H), 4.84 (s, 2H), 4.25 (t, <i>J</i> = 6.3 Hz, 2H), 4.13 (t, <i>J</i> = 6.8 Hz, 2H), 3.64 (s, 3H), 1.47 - 1.62 (m, 2H), 1.29 - 1.46 (m, 2H)
14		340.2	341	2.23, V3018V3 001	Method 8	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.06 (s, 1H), 7.10 - 7.30 (m, 2H), 6.89 (d, <i>J</i> = 7.6 Hz, 1H), 6.81 (dd, <i>J</i> = 2.0, 7.6 Hz, 1H), 6.25 (s, 1H), 5.58 (s, 2H), 4.86 (s, 2H), 3.99 - 4.12 (m, 4H), 1.33 - 1.51 (m, 6H)

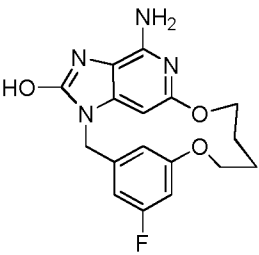
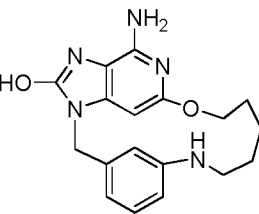
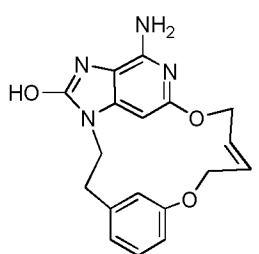
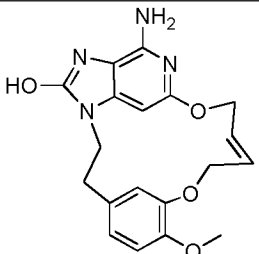
#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
15		368.1	369	2.25, V3018V3 001	Method 9	¹ H NMR (400 MHz, DMSO-d ₆) δ 10.07 (s, 1H), 6.52 (s, 1H), 6.45 (s, 1H), 6.33 (s, 1H), 6.09 (s, 1H), 5.46 - 5.68 (m, 3H), 5.34 (dt, <i>J</i> = 5.2, 15.9 Hz, 1H), 4.83 (s, 2H), 4.54 (d, <i>J</i> = 5.2 Hz, 2H), 4.24 (t, <i>J</i> = 5.3 Hz, 2H), 3.68 (s, 3H), 2.21 - 2.31 (m, 2H)
16		342.1	343	2.04, V3018V3 001	Method 10	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.13 (br. s., 1H), 7.58 (dd, <i>J</i> = 1.7, 7.9 Hz, 1H), 7.14 (dd, <i>J</i> = 8.3, 10.9 Hz, 1H), 6.73 - 6.94 (m, 1H), 5.88 - 6.13 (m, 2H), 5.41 - 5.75 (m, 3H), 4.83 (s, 2H), 4.61 (d, <i>J</i> = 5.4 Hz, 2H), 4.56 (d, <i>J</i> = 6.3 Hz, 2H)
17	 , HCl	342.1	343	2.04, V3018V3 001	Method 10	¹ H NMR (500 MHz, DMSO-d ₆) δ 11.15 (br. s., 1H), 7.70 (dd, <i>J</i> = 1.9, 7.9 Hz, 1H), 6.96 - 7.53 (m, 2H), 6.80 - 6.93 (m, 1H), 6.37 (s, 1H), 6.24 (dt, <i>J</i> = 6.3, 15.8 Hz, 1H), 5.63 (dt, <i>J</i> = 5.9, 15.8 Hz, 1H), 4.77 - 4.99 (m, 4H), 4.58 (d, <i>J</i> = 6.3 Hz, 2H)
18		323.1	324	1.78, V3018V3 001	Method 11	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.01 (s, 1H), 6.93 (t, <i>J</i> = 7.7 Hz, 1H), 6.74 (br. s., 1H), 6.40 - 6.58 (m, 2H), 6.30 (s, 1H), 6.02 (br. s., 1H), 5.83 (dt, <i>J</i> = 5.4, 16.0 Hz, 1H), 5.58 (br. s., 2H), 5.36 - 5.50 (m, 1H), 4.74 (s, 2H), 4.49 (d, <i>J</i> = 5.4

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
						Hz, 2H), 3.56 - 3.87 (m, 2H)
19		358.1	359	2.27, V3018V3 001	Method 14	¹ H NMR (500 MHz, DMSO-d ₆) δ 9.49 (br. s, 1H), 7.44 (d, <i>J</i> = 7.9 Hz, 1H), 7.14 (dd, <i>J</i> = 8.2, 11.4 Hz, 1H), 6.84 - 6.97 (m, 1H), 6.34 (s, 1H), 5.69 (s, 2H), 4.84 (s, 2H), 4.03 - 4.17 (m, 4H), 1.31 - 1.57 (m, 6H)
20	 , HCl	358.1	359	2.27, V3018V3 001	Method 14	¹ H NMR (500 MHz, DMSO-d ₆) δ 11.04 (br. s., 1H), 7.46 (dd, <i>J</i> = 1.9, 8.2 Hz, 1H), 6.86 - 7.32 (m, 3H), 6.78 (s, 1H), 4.94 (s, 2H), 4.41 (t, <i>J</i> = 6.6 Hz, 2H), 4.09 (t, <i>J</i> = 6.9 Hz, 2H), 1.63 - 1.73 (m, 2H), 1.53 - 1.62 (m, 2H), 1.36 - 1.49 (m, 2H)
21		356.1	357	2.21, V3018V3 001	Method 13	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.09 (br. s., 1H), 6.99 - 7.23 (m, 2H), 6.92 (br. s., 1H), 6.15 (s, 1H), 5.53 - 5.73 (m, 3H), 5.23 - 5.48 (m, 1H), 4.85 (s, 2H), 4.67 (d, <i>J</i> = 4.4 Hz, 2H), 4.24 (t, <i>J</i> = 4.6 Hz, 2H), 2.21 - 2.35 (m, 2H)
22	 , HCl	356.1	357	2.21, V3018V3 001	Method 13	¹ H NMR (500 MHz, DMSO-d ₆) δ 11.11 (br. s., 1H), 7.10 - 7.42 (m, 3H), 7.07 (dd, <i>J</i> = 1.9, 8.2 Hz, 1H), 6.85 - 6.99 (m, 1H), 6.64 (s, 1H), 5.80 (dt, <i>J</i> =

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
						7.2, 15.6 Hz, 1H), 5.59 (dt, $J = 5.7, 15.6$ Hz, 1H), 4.98 (s, 2H), 4.68 (d, $J = 5.7$ Hz, 2H), 4.41 - 4.59 (m, 2H), 2.38 - 2.50 (m, 2H)
23		325.2	326	1.83, V3018V3 001	Method 12	^1H NMR (500 MHz, DMSO- d_6) δ 9.97 (br. s., 1H), 7.05 (s, 1H), 6.90 (t, $J = 7.7$ Hz, 1H), 6.42 (d, $J = 7.7$ Hz, 1H), 6.38 (d, $J = 7.7$ Hz, 1H), 6.35 (s, 1H), 5.33 - 5.58 (m, 3H), 4.77 (s, 2H), 4.17 (t, $J = 6.8$ Hz, 2H), 3.20 (q, $J = 6.4$ Hz, 2H), 1.42 - 1.52 (m, 2H), 1.32 - 1.41 (m, 2H)
24		344.1	345	2.08, V3018V3 001	Method 14	^1H NMR (500 MHz, DMSO- d_6) δ 10.04 (br. s., 1H), 7.77 (d, $J = 7.6$ Hz, 1H), 7.10 (dd, $J = 8.7, 11.2$ Hz, 1H), 6.78 (br. s., 1H), 6.28 (s, 1H), 5.56 (s, 2H), 4.86 (s, 2H), 4.28 - 4.47 (m, 2H), 4.04 - 4.23 (m, 2H), 1.52 - 1.66 (m, 2H), 1.31 - 1.50 (m, 2H)
25		338.1	339	2.13, V3018V3 001	Method 15	^1H NMR (500 MHz, DMSO- d_6) δ 10.07 (br. s., 1H), 7.18 (t, $J = 7.9$ Hz, 1H), 7.14 (s, 1H), 6.73 - 6.87 (m, 2H), 5.96 (dt, $J = 5.0, 15.7$ Hz, 1H), 5.90 (s, 1H), 5.57 (s, 2H), 5.37 (dt, $J = 5.8, 15.7$ Hz, 1H), 4.86 (s, 2H), 4.58 (d, $J = 5.8$ Hz, 2H), 4.22 (t, $J = 5.0$ Hz, 2H), 2.27 - 2.42 (m, 2H)

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
26		337.2	338	2.02, V3018V3 001	Method 16	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.04 (br. s., 1H), 6.95 (t, <i>J</i> = 7.2 Hz, 1H), 6.51 (d, <i>J</i> = 7.2 Hz, 1H), 6.46 (dd, <i>J</i> = 1.3, 7.2 Hz, 1H), 6.40 (s, 1H), 6.16 (s, 1H), 5.97 (t, <i>J</i> = 6.3 Hz, 1H), 5.59 (s, 2H), 5.43 (dt, <i>J</i> = 6.3, 15.5 Hz, 1H), 5.22 (dt, <i>J</i> = 5.0, 15.5 Hz, 1H), 4.76 (s, 2H), 4.23 (t, <i>J</i> = 5.2 Hz, 2H), 3.56 (t, <i>J</i> = 5.0 Hz, 2H), 2.18 - 2.30 (m, 2H)
27		356.1	357	2.30, V3018V3 001	Method 17	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.11 (br. s., 1H), 6.77 (d, <i>J</i> = 9.1 Hz, 1H), 6.71 (s, 1H), 6.63 (dt, <i>J</i> = 2.2, 9.1 Hz, 1H), 6.15 (s, 1H), 5.64 (s, 2H), 5.57 (dt, <i>J</i> = 6.6, 15.8 Hz, 1H), 5.34 (dt, <i>J</i> = 5.3, 15.8 Hz, 1H), 4.89 (s, 2H), 4.59 (d, <i>J</i> = 4.7 Hz, 2H), 4.25 (t, <i>J</i> = 5.3 Hz, 2H), 2.20 - 2.34 (m, 2H)
28	 , HCl	356.1	357	2.31, V3018V3 001	Method 17	¹ H NMR (500 MHz, DMSO-d ₆) δ 11.09 (br. s., 1H), 7.15 (br. s., 2H), 6.82 (d, <i>J</i> = 8.8 Hz, 1H), 6.56 - 6.70 (m, 3H), 5.71 (dt, <i>J</i> = 6.3, 15.6 Hz, 1H), 5.52 (dt, <i>J</i> = 5.4, 15.6 Hz, 1H), 4.99 (s, 2H), 4.62 (d, <i>J</i> = 5.4 Hz, 2H), 4.50 (t, <i>J</i> = 4.7 Hz, 2H), 2.42 - 2.47 (m, 2H)
29		337.2	338	2.05, V3018V3 001	Method 16	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.08 (br. s., 1H), 6.96 (t, <i>J</i> = 7.7 Hz, 1H), 6.45 - 6.57 (m, 2H), 6.42 (s, 1H), 6.20 (s, 1H),

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
						5.92 (t, $J = 6.0$ Hz, 1H), 5.65 (s, 2H), 5.31 - 5.48 (m, 1H), 5.08 - 5.26 (m, 1H), 4.75 (s, 2H), 4.31 (t, $J = 5.8$ Hz, 2H), 3.41 - 3.48 (m, 2H), 2.12 - 2.28 (m, 2H)
30		342.1	343	2.08, V3018V3 001	Method 19	^1H NMR (500 MHz, DMSO- d_6) δ 10.10 (br. s., 1H), 7.23 (s, 1H), 6.78 (d, $J = 8.8$ Hz, 1H), 6.62 (d, $J = 8.8$ Hz, 1H), 6.08 (s, 1H), 5.84 (dt, $J = 5.0, 15.8$ Hz, 1H), 5.71 (dt, $J = 5.0, 15.8$ Hz, 1H), 5.60 (s, 2H), 4.86 (s, 2H), 4.63 (d, $J = 5.0$ Hz, 2H), 4.54 (d, $J = 5.0$ Hz, 2H)
31	 , HCl	342.1	343	2.08, V3018V3 001	Method 19	^1H NMR (500 MHz, DMSO- d_6) δ 11.12 (br. s., 1H), 7.34 (s, 1H), 7.15 (br. s., 2H), 6.85 (d, $J = 10.4$ Hz, 1H), 6.64 (d, $J = 10.4$ Hz, 1H), 6.44 (s, 1H), 6.07 (dt, $J = 5.9, 15.8$ Hz, 1H), 5.70 (dt, $J = 5.9, 15.8$ Hz, 1H), 4.95 (s, 2H), 4.84 (d, $J = 5.9$ Hz, 2H), 4.64 (d, $J = 5.9$ Hz, 2H)
32		358.1	359	2.32, V3018V3 001	Method 18	^1H NMR (500 MHz, DMSO- d_6) δ 10.13 (br. s., 1H), 7.05 (s, 1H), 6.71 (d, $J = 9.1$ Hz, 1H), 6.67 (d, $J = 9.1$ Hz, 1H), 6.28 (s, 1H), 5.63 (s, 2H), 4.87 (s, 2H), 3.85 - 4.18 (m, 4H), 1.21 - 1.59 (m, 6H)

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
33		344.1	345	2.14, V3018V3 001	Method 14	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.09 (br. s., 1H), 7.33 (s, 1H), 6.66 (d, <i>J</i> = 9.1 Hz, 1H), 6.59 (d, <i>J</i> = 9.1 Hz, 1H), 6.17 (s, 1H), 5.58 (s, 2H), 4.90 (s, 2H), 4.29 (t, <i>J</i> = 6.5 Hz, 2H), 4.15 (t, <i>J</i> = 6.5 Hz, 2H), 1.48 - 1.59 (m, 2H), 1.36 - 1.46 (m, 2H)
34		339.2	340	2.06, V3018V3 001	Method 12	¹ H NMR (500 MHz, DMSO-d ₆) δ 10.05 (s, 1H), 6.83 - 7.00 (m, 1H), 6.62 (s, 1H), 6.50 (d, <i>J</i> = 8.2 Hz, 1H), 6.43 (dd, <i>J</i> = 1.6, 8.2 Hz, 1H), 6.36 (s, 1H), 5.72 (t, <i>J</i> = 6.6 Hz, 1H), 5.60 (s, 2H), 4.76 (s, 2H), 4.07 (t, <i>J</i> = 5.7 Hz, 2H), 2.95 (q, <i>J</i> = 6.6 Hz, 2H), 1.12 - 1.50 (m, 8H)
35		338.1	339	1.97, V3018V3 001	Method 20	¹ H NMR (500 MHz, DMSO-d ₆) δ 9.92 (br. s., 1H), 6.81 - 7.02 (m, 2H), 6.73 (d, <i>J</i> = 8.2 Hz, 1H), 6.38 (d, <i>J</i> = 8.2 Hz, 1H), 5.91 (dt, <i>J</i> = 4.4, 16 Hz, 1H), 5.38 - 5.57 (m, 3H), 5.33 (s, 1H), 4.65 (d, <i>J</i> = 4.4 Hz, 2H), 4.54 (d, <i>J</i> = 4.4 Hz, 2H), 3.96 (t, <i>J</i> = 5.2 Hz, 2H), 2.87 (t, <i>J</i> = 5.2 Hz, 2H)
36		368.1	369	1.86, V3018V3 001	Method 20	¹ H NMR (500 MHz, DMSO-d ₆) δ 9.92 (br. s., 1H), 7.01 (s, 1H), 6.60 (d, <i>J</i> = 8.2 Hz, 1H), 6.36 (d, <i>J</i> = 8.2 Hz, 1H), 6.02 (dt, <i>J</i> = 5.0, 16 Hz, 1H), 5.46 (s, 2H), 5.28 - 5.42 (m, 2H),

#	STRUCTURE	Exact Mass	Mass Found [M+H]	LCMS Ret Time, Method	Synthesis method	NMR
						4.61 (d, $J = 5.0$ Hz, 2H), 4.52 (d, $J = 5.0$ Hz, 2H), 3.77 - 4.04 (m, 2H), 3.65 (s, 3H), 2.70 - 2.91 (m, 2H)

Biological Activity of compounds of formula (I)

Description of Biological Assays

Assessment of TLR7 and TLR8 activity

- 5 The ability of compounds to activate human TLR7 and TLR8 was assessed in a cellular reporter assay using HEK293 cells transiently transfected with a TLR7 or TLR8 expression vector and NF κ B-luc reporter construct. Briefly, HEK293 cells were grown in culture medium (DMEM supplemented with 10% FCS and 2 mM Glutamine). For transfection of cells in 15 cm dishes, cells were detached with Trypsin-EDTA, transfected with a mix of CMV-TLR7 or TLR8
- 10 plasmid (1,700 ng), NF κ B-luc plasmid (850 ng) and a transfection reagent and incubated 48 hours at 37°C in a humidified 5% CO₂ atmosphere. Transfected cells were then washed in PBS, detached with Trypsin-EDTA, and resuspended in medium to a density of 1.25 x 10⁵ cells/mL. Forty microliters of cells were then dispensed into each well in 384-well plates, where 200 nL of compound in 100% DMSO was already present. Following 6 hours incubation at 37°C, 5% CO₂,
- 15 the luciferase activity was determined by adding 15 μ L of Steady Lite Plus substrate (Perkin Elmer) to each well and readout performed on a ViewLux ultraHTS microplate imager (Perkin Elmer). Dose response curves were generated from measurements performed in quadruplicates. Lowest effective concentrations (LEC) values, defined as the concentration that induces an effect which is at least two fold above the standard deviation of the assay, were determined for each
- 20 compound.

In parallel, a similar dilution series of compound was used (200 nL of compound in 100% DMSO) with 40 μ L per well of cells transfected with NF κ B-luc reporter construct alone (1.25 x 10⁵ cells/mL). Six hours after incubation at 37°C, 5% CO₂, the luciferase activity was determined by adding 15 μ L of Steady Lite Plus substrate (Perkin Elmer) to each well and readout

25 performed on a ViewLux ultraHTS microplate imager (Perkin Elmer). Counterscreen data is reported as LEC.

Measurement of interferon production in human PBMC

Activation of human TLR7 results in robust production of interferon by plasmacytoid dendritic cells present in human blood. The potential of compounds to induce interferon was evaluated by

determination of interferon in the conditioned media from peripheral blood mononuclear cells (PBMC). The presence of interferon in the samples was determined, using an interferon reporter cell line stably expressing an interferon-stimulated responsive elements (ISRE)-luc reporter construct. The ISRE element with sequence TAGTTTCACTTTCCC is highly responsive to the

5 STAT1-STAT2-IRF9 transcription factor, which becomes activated upon binding of IFN-I to the IFN receptor. Briefly, PBMCs were prepared from buffy coats of at least two donors using a standard Ficoll centrifugation protocol. Isolated PBMCs were resuspended in RPMI medium supplemented with 10% human AB serum and 2×10^5 cells/well were dispensed into 384-well

10 plates containing compounds (70 μ L total volume). After overnight incubation of the PBMCs with the compounds, 10 μ L of supernatant was transferred to 384-well plates containing 5×10^3 HEK-ISRE-luc cells/well in 30 μ L (plated the day before). Following 24 hours of incubation, activation of the ISRE elements was measured by assaying luciferase activity using 40 μ L/well Steady Lite Plus substrate (Perkin Elmer) and measured with ViewLux ultraHTS microplate imager (Perkin Elmer). The stimulating activity of each compound on the HEK-ISRE-luc cells

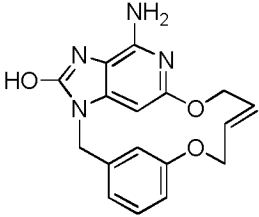
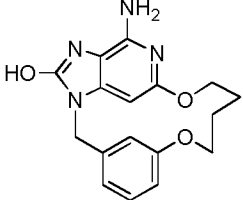
15 was reported as LEC. The LEC in turn indicates the degree of ISRE activation on transfer of a defined amount of PBMC culture medium. Recombinant interferon alfa-2a (Roferon-A) was used as a standard control compound.

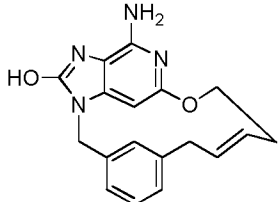
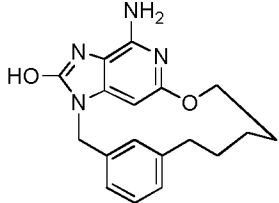
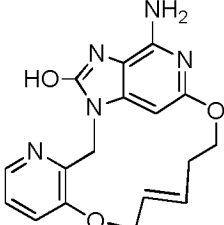
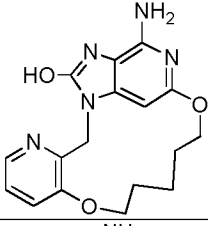
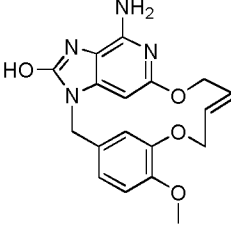
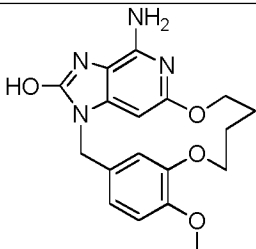
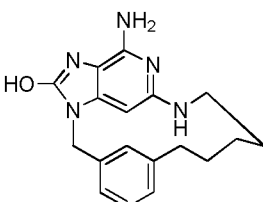
The LEC values for the compounds in table 2 on HEK293 TLR8-NF κ B-luc and HEK293 NF κ B-luc were greater than the highest tested concentration ($> 10 \mu$ M for compound 4 and $> 25 \mu$ M

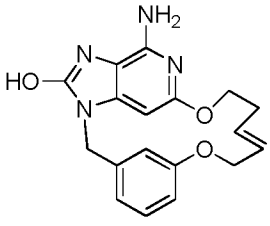
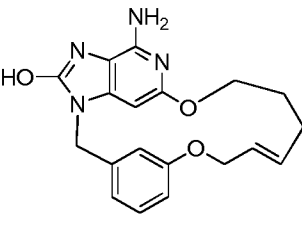
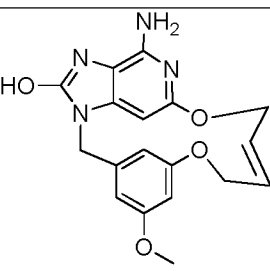
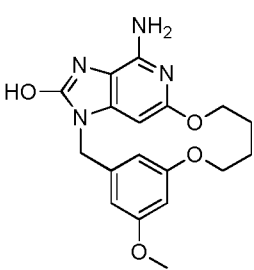
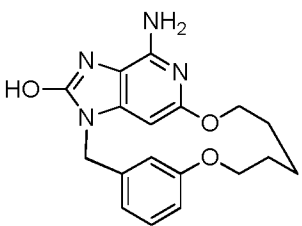
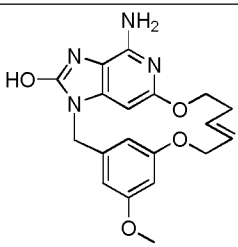
20 for all other compounds).

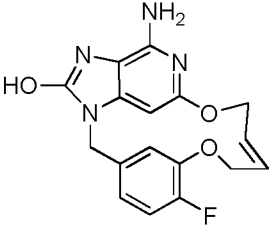
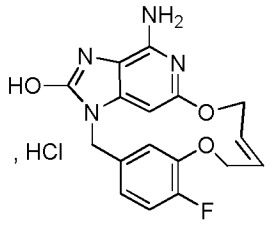
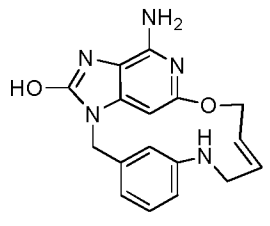
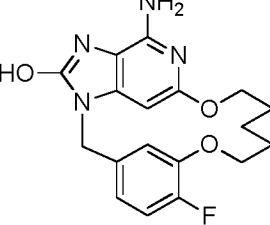
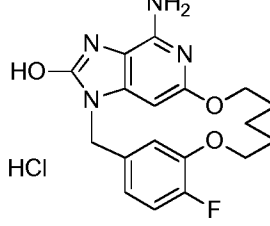
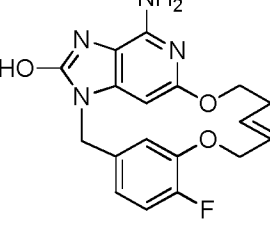
Table 2. Compounds of formula (I)

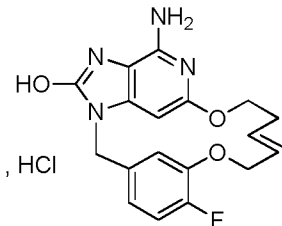
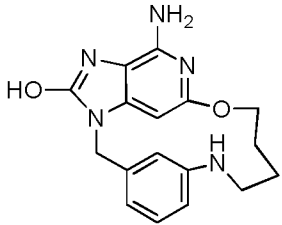
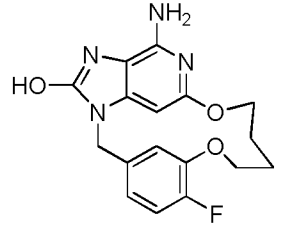
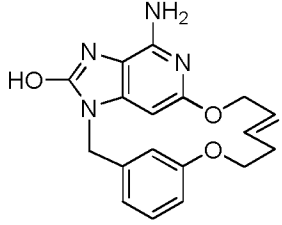
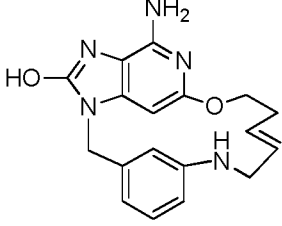
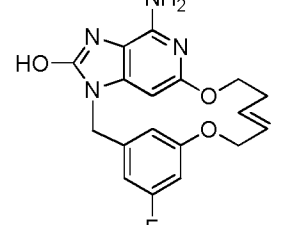
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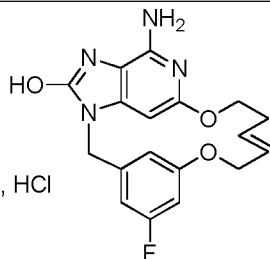
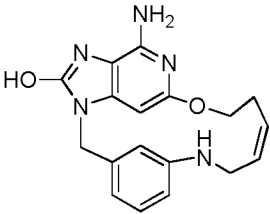
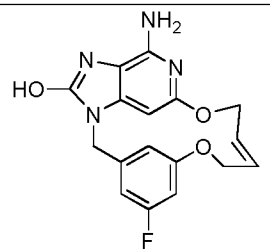
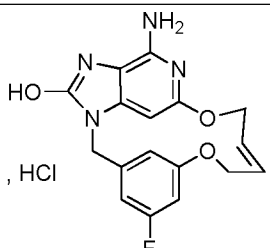
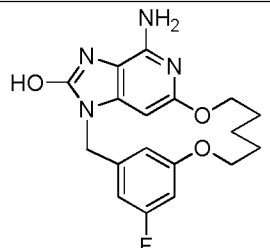
#	STRUCTURE	HEK293 TLR7-NF κ B-luc (LEC; μ M)	n	PBMC HEK-ISRE-luc (LEC; μ M)	n
1		2.88	8	0.30	8
2		4.47	1	0.93	2

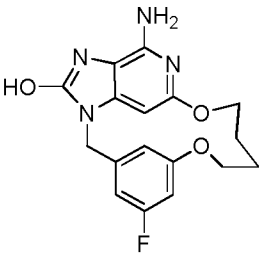
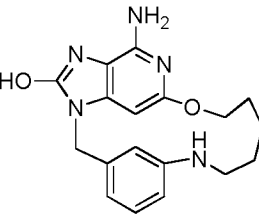
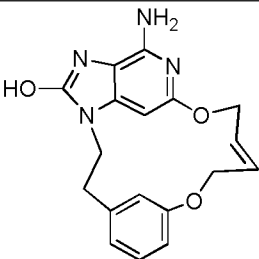
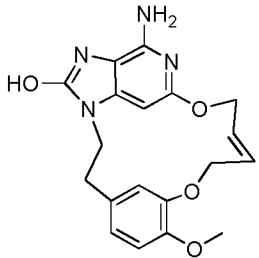
#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
3		0.27	2	0.033	4
4		2.38	1	2.56	2
5		2.14	1	0.082	2
6		1	1	0.16	2
7		3.88	1	0.29	2
8		7.1	1	0.58	2
9		11.09	1	10.87	2

#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
10		0.78	2	0.25	4
11		1.25	1	0.45	2
12		1.08	1	0.3	2
13		1.71	2	0.15	2
14		7.01	2	2.4	2
15		0.18	1	0.04	2

#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
16		2.02	2	0.47	2
17		1.88	2	0.39	4
18		8.02	1	1.11	2
19		14.96	2	2.22	4
20		4.99	1	1.61	2
21		0.9	1	0.25	3

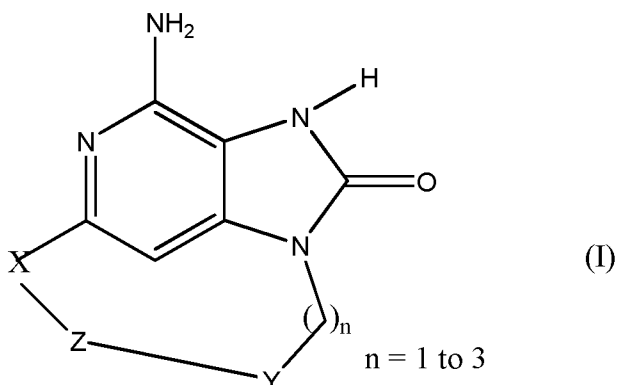
#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
22	 , HCl	1.83	1	0.39	2
23		16.74	2	8.98	4
24				2.14	4
25		1.92	1	0.53	2
26		1.88	2	0.37	4
27		1.35	1	0.14	2

#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
28	 , HCl	0.91	1	0.15	2
29		1.14	2	0.48	2
30		0.64	1	0.15	2
31	 , HCl	1.19	1	0.15	2
32		2.92	2	0.49	2

#	STRUCTURE	HEK293 TLR7-NFκB- <i>luc</i> (LEC; μM)	n	PBMC HEK-ISRE- <i>luc</i> (LEC; μM)	n
33		2.77	1	0.45	2
34		8.1	2	2.11	2
35		17.43	1	1.98	2
36		13.75	1	1.63	4

Claims

1. Compound having formula (I)



and pharmaceutically accepted salts thereof, wherein

X is oxygen, nitrogen or sulfur,

Y represents an aromatic ring or heterocyclic ring comprising at least a nitrogen, optionally substituted by one or more substituents independently selected from C₁₋₆alkyl, C₁₋₄alkoxy, trifluoromethyl or halogen,

Z represents C₁₋₁₀ saturated or unsaturated alkyl optionally substituted by an alkyl or alkylhydroxyl;

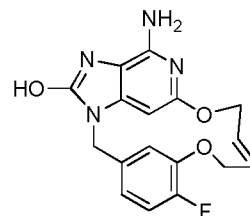
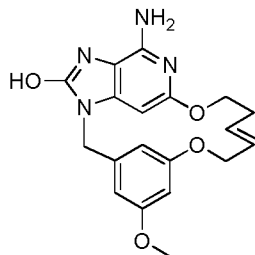
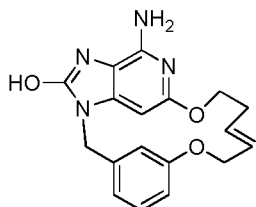
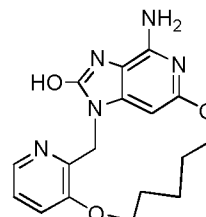
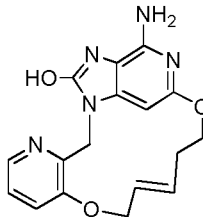
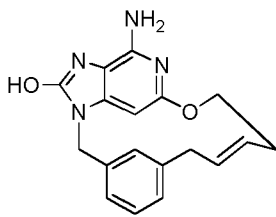
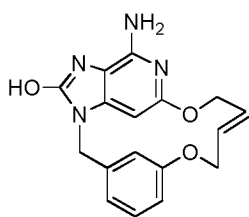
or Z represents C₁₋₆alkyl -NH-C(O)- C₁₋₆alkyl-, C₁₋₆alkyl-NH- or C₁₋₆alkyl -NH-C(O)- C₁₋₆alkyl -O-;

or Z represents C₁₋₁₀alkyl -O- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl ,

or Z represents C₁₋₆alkyl-O-C₁₋₆alkyl- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl

or Z represents C₁₋₆alkyl-O-C₁₋₆alkyl-O- wherein said alkyl is unsaturated or saturated and can optionally be substituted by an alkyl or alkylhydroxyl.

2. Compound according to claim 1 having one of the following formula's selected from the group of:



3. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof according to claim 1 or 2 together with one or more pharmaceutically acceptable excipients, diluents or carriers.

5 4. A compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof according to claim 1 or 2, or a pharmaceutical composition according to claim 3 for use as a medicament.

5. A compound of formula (I) or a pharmaceutically acceptable salt, solvate or polymorph thereof according to claim 1 or 2, or a pharmaceutical composition according to claim 3, for
10 use in the treatment of a disorder in which the modulation of TLR7 is involved.