(12) STANDARD PATENT (11) Application No. AU 2016313228 B2
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Piperidinobenzodiazepine compounds with anti proliferative activity

(51) International Patent Classification(s)
C07D 403/12 (2006.01) ***C07D 403/14*** (2006.01)
A61K 31/5513 (2006.01) ***C07D 409/12*** (2006.01)
A61P 35/00 (2006.01) ***C07D 409/14*** (2006.01)

(21) Application No: **2016313228** (22) Date of Filing: **2016.08.19**

(87) WIPO No: **WO17/032983**

(30) Priority Data

(31) Number (32) Date (33) Country
1514928.9 **2015.08.21** **GB**

(43) Publication Date: **2017.03.02**
(44) Accepted Journal Date: **2020.12.24**

(71) Applicant(s)
Femtogenix Limited

(72) Inventor(s)
Jackson, Paul Joseph Mark;Thurston, David Edwin;Rahman, Khondaker Mirazur

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
MARKANDEYA N et al."Asymmetric syntheses of piperidino-benzodiazepines through 'cation-pool' host/guest supramolecular approach and their DNA-binding studies", 2010, TETRAHEDRON ASYMMETRY, 21(21-22), 2625 - 2630
WO 2016198869 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 March 2017 (02.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/032983 A1

(51) International Patent Classification:
C07D 403/12 (2006.01) *C07D 409/14* (2006.01)
C07D 403/14 (2006.01) *A61K 31/5513* (2006.01)
C07D 409/12 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number: PCT/GB2016/052565

(22) International Filing Date: 19 August 2016 (19.08.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1514928.9 21 August 2015 (21.08.2015) GB

(71) Applicant: **FEMTOGENIX LIMITED** [GB/GB]; Britannia House, 7 Trinity Street, London SE1 1DB (GB).

(72) Inventors: **JACKSON, Paul Joseph Mark**; Britannia House, 7 Trinity Street, London SE1 1DB (GB). **THURSTON, David Edwin**; Britannia House, 7 Trinity Street, London SE1 1DB (GB). **RAHMAN, Khondaker Mirazur**; Britannia House, 7 Trinity Street, London SE1 1DB (GB).

(74) Agents: **SCRIPT IP LIMITED** et al.; Turnpike House, 18 Bridge Street, Frome Somerset BA11 1BB (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

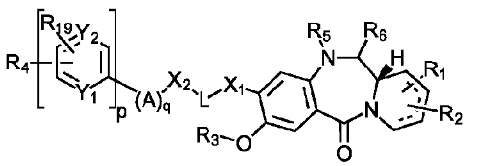
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

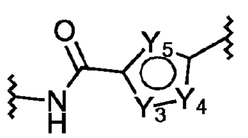
Published:

— *with international search report (Art. 21(3))*

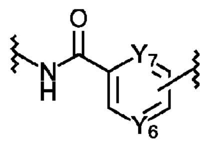
(54) Title: PIPERIDINOBENZODIAZEPINE COMPOUNDS WITH ANTI PROLIFERATIVE ACTIVITY



(I)



and



;

A1 A2

(57) **Abstract**: The invention relates to pyrridinobenzodiazepines (PDDs) comprising three fused 6-7-6-membered rings linked to aromatic groups, and pharmaceutically acceptable salts thereof, which are useful as medicaments, such as anti-proliferative agents. PDDs may be represented by formula (I): and salts and solvates thereof, wherein the dotted lines indicates the optional presence of a double bond between one or more of Ci and C_2, $C_2$and C_3, and $C_3$and C_4; R1 and R2 are substituent groups; R3 is selected from H, C1-12 alkyl and CH2Ph; $R^4$is selected from phenyl and C_{5-9}heteroaryl groups optionally substituted, with the proviso that the optionally substituted C_{5-9}heteroaryl is not indolyl; $R1_9$is selected from H and $(CH2)_t$-$NR_{20}R_{21}$; $Y_i$is N or CH; Y2 is N or CH; and wherein at least one of $Y_1$and Y2 is CH; p is o or$_1$; X_1 is a connecting group; L is a linker group; X2 is a connecting group or is absent; q is selected from $o_{,1,2,3,4,5}$and 6; A is selected from: for each Ai group one of $Y_3$and $Y_4$is selected from N-R_{17},S and O; and the other of $Y_3$and $Y_4$is CH; and $Y_5$is selected from CH, N, S and COH; for each A_2group one of $Y_6$and $Y_7$is independently selected from N and CH; and the other of $Y_6$and $Y_7$is CH; $R1_3$, $R1_4$, R_{17}, $R2_0$and $R2_i$are independently selected from H and C_{1-6}alkyl; and either: (i) $R_5$and R_6together form a double bond; (ii) $R_5$is H and $R_6$is OH; or (iii) $R_5$is H and $R_6$is OC_{1-6}alkyl; with the proviso that when p is o and A is Ai, then: (a) for at least one Ai group one of $Y_3$and $Y_4$is selected from S and O; or (b) for at least one Ai group $Y_5$is S; or (c) $R_4$is not pyrrolyl, imidazolyl, optionally substituted pyrrolyl or optionally substituted imidazolyl.

WO 2017/032983 A1

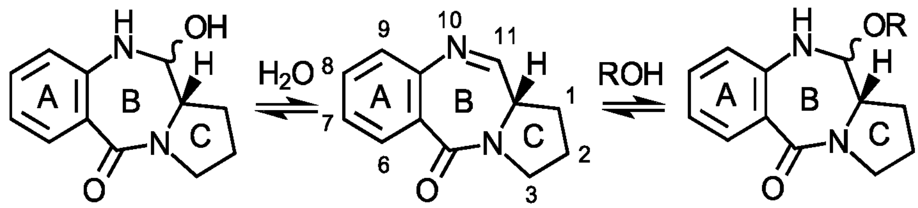
PIPERIDINOBENZODIAZEPINE COMPOUNDS WITH ANTI PROLIFERATIVE ACTIVITY

FIELD OF THE INVENTION

The invention relates to pyrridinobenzodiazepines (PDDs) comprising three fused 6-7-6–membered rings. In particular it relates to compounds comprising a PDD group linked *via* the A-ring to aromatic groups, and to pharmaceutically acceptable salts thereof, which are useful as medicaments, in particular as anti-proliferative agents.

BACKGROUND TO THE INVENTION

Pyrridinobenzodiazepines (PDDs) are related structures to pyrrolobenzodiazepines (PBDs). The pyrrolobenzodiazepines (PBDs) are a group of compounds some of which have been shown to be sequence-selective DNA minor-groove binding agents. The PBDs were originally discovered in *Streptomyces* species (1-5). They are tricyclic in nature, and are comprised of fused 6-7-5–membered rings that comprise an anthranilate (A ring), a diazepine (B ring) and a pyrrolidine (C ring) (3). They are characterized by an electrophilic N10=C11 imine group (as shown below) or the hydrated equivalent, a carbinolamine [NH-CH(OH)], or a carbinolamine alkyl ether ([NH-CH(OR, where R = alkyl)] which can form a covalent bond to a C2-amino group of guanine in DNA to form a DNA adduct (6).



Carbinolamine Imine Carbinolamine alkyl ether

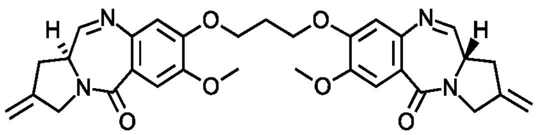
The natural products interact in the minor groove of the DNA helix with excellent fit (i.e., good "isohelicity") due to a right-handed longitudinal twist induced by a chiral C11a-position which has the (*S*)-configuration (6). The DNA adduct has been reported to inhibit a number of biological processes including the binding of transcription factors (7-9) and the function of enzymes such as endonucleases (10, 11) and RNA polymerase (12). PBD monomers (e.g., anthramycin) have been shown by footprinting (6), NMR (13, 14), molecular modeling (15) and X-ray crystallography (16) to span three base pairs and to have a thermodynamic preference for the sequence 5'-Pu-G-Pu-3' (where Pu = purine, and G is the reacting guanine) (17) and a kinetic preference for Py-5-Py (where Py = Pyrimidine).

PBDs are thought to interact with DNA by first locating at a low-energy binding sequence (i.e., a 5'-Pu-G-Pu-3' triplet) through Van der Waals, hydrogen bonding and electrostatic interactions (7). Then, once in place, a nucleophilic attack by the exocyclic C2-amino group of the central guanine occurs to form the covalent adduct (7). Once bound, the PBD remains anchored in the DNA minor groove, avoiding DNA repair by causing negligible distortion of the DNA helix (16). The ability of PBDs to form an adduct in the minor groove and crosslink DNA enables them to interfere with DNA processing and, hence, their potential for use as antiproliferative agents.

A number of monomeric PBD structures have been isolated from *Streptomyces* species, including anthramycin (18) the first PBD, tomamycin (19), and more recently usabamycin (20) from a marine sediment *Streptomyces* species in a marine sediment. This has led to the development of a large range of synthetic analogues which have been reviewed (1, 21). More recently, a number of monomeric PBD structures that are linked through their C8 position to pyrroles and imidazoles have been reported WO 2007/039752, WO 2013/164593 (22-27).

WO 2010/091150 discloses a dimer of a 6-7-6 ring system linked *via* their A-rings. WO 2015/028850 discloses 6-7-5 ring system PBD dimers that are linked *via* phosphine oxide containing linkers attached to their aromatic A-rings. In addition, WO 2015/028850 discloses a dimer compound containing a 6-7-6 ring system linked *via* the key phosphine oxide containing linkers.

Various PBDs have been shown to act as cytotoxic agents *in vitro*, for example, WO 00/12508, WO 2004/087711, and as anti-tumour *in vivo* in animal tumour models, for example, WO 2011/117882, WO 2013/164593. Furthermore, the C8/C8'-linked PBD dimer SJG-136 (28, 29) has completed Phase I clinical trials for leukaemia and ovarian cancer (30) and has shown sufficient therapeutic benefit to progress to Phase II studies.



SJG-136

However, results from a Phase I clinical evaluation of SJG-136 revealed that the drug produced several adverse effects including lower-limb edema and fatigue (31).

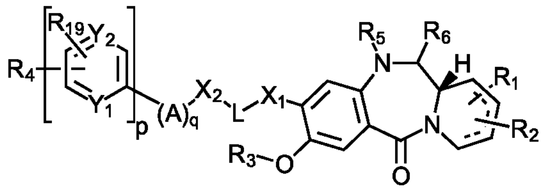
Thus, there exists a need for further compounds related to PBDs that are therapeutically active for treating a variety of proliferative diseases.

The present application reports pyrridinobenzodiazepines (PDDs), which are related to PBDs but contain an expanded 6-membered C-ring as compared to the 5-membered C-ring of PBDs. The inventors have discovered that PDD conjugates provide properties, such as cytoxicity and DNA binding, that results in effective compounds.

The present invention seeks to overcome problem(s) associated with the prior art.

SUMMARY OF THE INVENTION

The present invention provides a compound of formula (I):



(I)

and salts and solvates thereof,

wherein;

the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4;

R_1 is selected from R_7, $=CH_2$, $=CH-(CH_2)_m-CH_3$, $=O$, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-SO_2R_7$, $O-SO_2R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$;

R_2 is selected from R_9, $=CH_2$, $=CH-(CH_2)_r-CH_3$, $=O$, $(CH_2)_r-OR_9$, $(CH_2)_r-CO_2R_9$, $(CH_2)_r-NR_9R_{10}$, $O-(CH_2)_s-NR_9R_{10}$, $NH-C(O)-R_9$, $O-(CH_2)_s-NH-C(O)-R_9$, $O-(CH_2)_s-C(O)-NH-R_9$, $(CH_2)_r-SO_2R_9$, $O-SO_2R_9$, $(CH_2)_r-COR_9$ and $(CH_2)_r-C(O)NR_9R_{10}$;

R_3 is selected from H, C_{1-12} alkyl and CH_2Ph;

R_4 is selected from phenyl and C_{5-9} heteroaryl groups optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j-CO_2R_{11}$, $O-(CH_2)_k-NR_{11}R_{12}$, $(CH_2)_j-NR_{11}R_{12}$, $C(=O)-NH-(CH_2)_k-NR_{11}R_{12}$; $C(=O)-NH-R_{24}$ and $C(=O)-NH-(CH_2)_k-C(=NH)NR_{11}R_{12}$; with the proviso that the optionally substituted C_{5-9} heteroaryl is not indolyl;

R_{19} is selected from H and $(CH_2)_t-NR_{20}R_{21}$;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

p is 0 or 1;

j, m, r and t are independently selected from an integer from 0 to 6;

k, n and s are independently selected from an integer from 1 to 6;

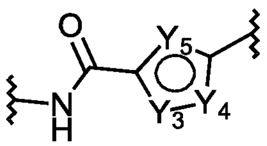
X_1 is selected from O, S, NR_{13}, $CR_{13}R_{14}$, $CR_{13}R_{14}O$, C(=O), C(=O)NR_{13}, NR_{13}C(=O), O-C(O) and C(O)-O;

L is selected from an amino acid, a peptide chain having from 2 to 6 amino acids, an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds, a paraformaldehyde chain $-(OCH_2)_{1-12}-$, a polyethylene glycol chain $-(OCH_2CH_2)_{1-6}-$, which chains may be interrupted by one or more of O, S and/or NH groups and/or C_{3-9} heteroarylene and/or phenylene;

X_2 is selected from O, S, NR_{15}, $CR_{15}R_{16}$, $CR_{15}R_{16}O$, C(=O), C(=O)NR_{15}, NR_{15}C(=O), O-C(O) and C(O)-O or is absent;

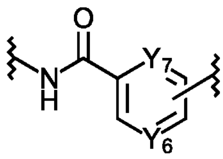
q is selected from 0, 1, 2, 3, 4, 5 and 6;

A is selected from:



A1

and



;

A2

for each A1 group one of Y_3 and Y_4 is independently selected from N-R_{17}, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH; and

for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH;

R_7 and R_9 are independently selected from H, C_{1-12} alkyl, C_{5-9} heteroaryl, C_{6-15} heteroarylalkyl, phenyl and C_{7-12} aralkyl groups; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three optional substituent groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl;

R_{24} is a phenyl optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$ and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$;

R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21} are independently selected from H and C_{1-6} alkyl;

and either:

(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

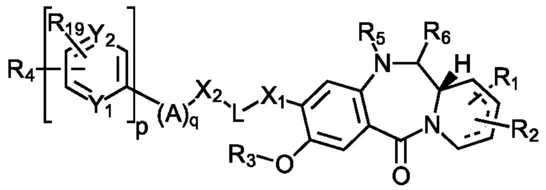
with the proviso that when p is 0 and A is A1, then:

(a) for at least one A1 group one of Y_3 and Y_4 is selected from S and O; or

(b) for at least one A1 group Y_5 is S; or

(c) R_4 is not pyrrolyl, imidazolyl, optionally substituted pyrrolyl or optionally substituted imidazolyl.

The present invention provides a compound of formula (I):



(I)

and salts and solvates thereof,

wherein;

the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4;

R_1 is selected from R_7, $=CH_2$, $=CH-(CH_2)_m-CH_3$, $=O$, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-SO_2R_7$, $O-SO_2R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$;

R_2 is selected from R_9, $=CH_2$, $=CH-(CH_2)_r-CH_3$, $=O$, $(CH_2)_r-OR_9$, $(CH_2)_r-CO_2R_9$, $(CH_2)_r-NR_9R_{10}$, $O-(CH_2)_s-NR_9R_{10}$, $NH-C(O)-R_9$, $O-(CH_2)_s-NH-C(O)-R_9$, $O-(CH_2)_s-C(O)-NH-R_9$, $(CH_2)_r-SO_2R_9$, $O-SO_2R_9$, $(CH_2)_r-COR_9$ and $(CH_2)_r-C(O)NR_9R_{10}$;

R_3 is selected from H, C_{1-12} alkyl and CH_2Ph;

R_4 is selected from phenyl and C_{5-9} heteroaryl groups optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j-CO_2R_{11}$, $O-(CH_2)_k-NR_{11}R_{12}$, $(CH_2)_j-NR_{11}R_{12}$, $C(=O)-NH-(CH_2)_k-NR_{11}R_{12}$; $C(=O)-NH-C_6H_4-(CH_2)_j-R_{18}$ and $C(=O)-NH-(CH_2)_k-C(=NH)NR_{11}R_{12}$; with the proviso that the optionally substituted C_{5-9} heteroaryl is not indolyl;

R_{19} is selected from H and $(CH_2)_t-NR_{20}R_{21}$;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

p is 0 or 1;

j, m, r and t are independently selected from an integer from 0 to 6;

k, n and s are independently selected from an integer from 1 to 6;

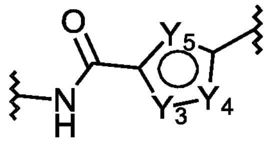
X_1 is selected from O, S, NR_{13}, $CR_{13}R_{14}$, $CR_{13}R_{14}O$, $C(=O)$, $C(=O)NR_{13}$, $NR_{13}C(=O)$, $O-C(O)$ and $C(O)-O$;

L is selected from an amino acid, a peptide chain having from 2 to 6 amino acids, an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds, a paraformaldehyde chain $-(OCH_2)_{1-12}$-, a polyethylene glycol chain $-(OCH_2CH_2)_{1-6}$-, which chains may be interrupted by one or more of O, S and/or NH groups and/or C_{3-9} heteroarylene and/or phenylene;

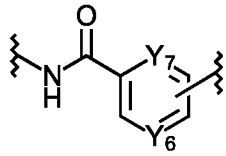
X_2 is selected from O, S, NR_{15}, $CR_{15}R_{16}$, $CR_{15}R_{16}O$, C(=O), C(=O)NR_{15}, NR_{15}C(=O), O-C(O) and C(O)-O or is absent;

q is selected from 0, 1, 2, 3, 4, 5 and 6;

A is selected from:



and



;

A1 A2

for each A1 group one of Y_3 and Y_4 is independently selected from N-R_{17}, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH; and

for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH;

R_7 and R_9 are independently selected from H, C_{1-12} alkyl, C_{5-9} heteroaryl, C_{6-15} heteroarylalkyl, phenyl and C_{7-12} aralkyl groups; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three optional substituent groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl;

R_{18} is selected from CO_2R_{11} and $NR_{11}R_{12}$;

R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21} are independently selected from H and C_{1-6} alkyl;

and either:

(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when p is 0 and A is A1, then:

(a) for at least one A1 group one of Y_3 and Y_4 is selected from S and O; or

(b) for at least one A1 group Y_5 is S; or

(c) R_4 is not pyrrolyl, imidazolyl, optionally substituted pyrrolyl or optionally substituted imidazolyl.

In a further aspect, there is provided a compound of formula (I) and salts and solvates thereof for use in a method of therapy.

In a further aspect, there is provided a compound of formula (I) and salts and solvates thereof for use as a medicament.

In a further aspect, there is provided a compound of formula (I) and salts and solvates thereof for use in the treatment of a proliferative disease.

In a further aspect, there is provided a pharmaceutical composition comprising a compound of formula (I) and salts and solvates thereof and a pharmaceutically acceptable excipient, carrier or diluent.

In a further aspect, the present invention provides the use of a compound of formula (I) and salts and solvates thereof in the manufacture of a medicament for treating a proliferative disease.

In a further aspect, the present invention provides a method of treatment of a patient suffering from a proliferative disease, comprising administering to said patient a therapeutically effective amount of a compound of formula (I) and salts and solvates thereof or a pharmaceutical composition of the present invention.

In a further aspect, the compound of formula (I) and salts and solvates thereof may be administered alone or in combination with other treatments, either simultaneously or sequentially depending upon the condition to be treated.

The pharmaceutical composition of the present invention may further comprise one or more (e.g. two, three or four) further active agents.

In a further aspect, the compound of formula (I) and salts and solvates thereof, may be linked, either directly or indirectly, to a targeting agent (e.g., antibody, antibody fragment, hormone, etc.) to provide a targeted conjugate. The target conjugates of the present disclosure may contain one or multiple compounds of formula (I) (or salts and solvates thereof). A variety of target conjugates are known in the art and may be used with a compound of formula (I) and salts and solvates thereof. For example, in a particular aspect the target conjugate is an antibody-drug conjugate, wherein one or more compounds of formula (I) are linked, directly or indirectly, to the antibody. Therefore, the compound of formula (I) and salts and solvates thereof, may be used as a payload on a targeted conjugate.

Definitions

The following abbreviations are used throughout the specification: Ac acetyl; Alloc allyloxycarbonyl; BAIB bis(acetoxy)iodobenzene/(diacetoxyiodo)benzene; Boc tert-butoxycarbonyl; BPDs benzopyrridodiazecines; CBz benzyloxycarbonyl; DBU 1,8-diazabicyclo[5.4.0]undec-7-ene; DHP dihydropyran; DMAP 4-dimethylaminopyridine; DMF dimethylformamide; DMSO dimethylsulfoxide; EDCl 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; Et ethyl; Et_2O diethyl ether; EtOAc ethyl acetate; EtOH ethanol; HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate); HMDST hexamethyldisilathiane; iBu iso-butyl; KOtBu potassium t-butoxide; L-Selectride Lithium tri-*sec*-butyl(hydride)borate; Me methyl; MeOH methanol; PBDs pyrrolo[2,1-*c*][1,4]benzo-diazepines; PDDs pyrridinobenzodiazepines; PIFA phenyliodine (III) bis[trifluoroacetate]; Ph phenyl; *p*-TSA /PTSA p-Toluenesulfonic acid; Pyr pyridine; TBAF tetrabutylammonium fluoride; TBS-Cl/TBDMSCl tert- butyldimethylsilyl chloride; TEA triethylamine; TEMPO (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl; TFA trifluoroacetic acid; THF tetrahydrofuran; THP tetrahydropyranyl; Troc 2,2,2-Trichloroethyl carbonate and Ts (tosylate) p-toluene sulfonic acid.

“Substituted”, when used in connection with a chemical substituent or moiety (e.g., an alkyl group), means that one or more hydrogen atoms of the substituent or moiety have been replaced with one or more non-hydrogen atoms or groups, provided that valence requirements are met and that a chemically stable compound results from the substitution.

“Optionally substituted” refers to a parent group which may be unsubstituted or which may be substituted with one or more substituents. Suitably, unless otherwise specified, when optional substituents are present the optional substituted parent group comprises from one to three optional substituents. Where a group may be “optionally substituted with up to three groups”, this means that the group may be substituted with 0, 1, 2 or 3 of the optional substituents. Where a group may be “optionally substituted with one or two optional substituents”, this means that the group may be substituted with 0, 1 or 2 of the optional substituents. Suitably groups may be optionally substituted with 0 or 1 optional substituents.

“Independently selected” is used in the context of statement that, for example, “R_1 and R_2 are independently selected from H, C_{1-12} alkyl, phenyl, ...” and means that each instance of the functional group, e.g. R_1, is selected from the listed options independently of any other instance of R_1 or R_2 in the compound. Hence, for example, a C_{1-12} alkyl may be selected for the first instance of R_1 in the compound; a phenyl group may be selected for the next instance of R_1 in the compound; and H may be selected for the first instance of R_2 in the compound.

C_{1-12} alkyl: refers to straight chain and branched saturated hydrocarbon groups, generally having from 1 to 12 carbon atoms; more suitably C_{1-7} alkyl; more suitably C_{1-6} alkyl. Examples of alkyl groups include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl, pent-1-yl, pent-2-yl, pent-3-yl, 3-methylbut-1-yl, 3-methylbut-2-yl, 2-methylbut-2-yl, 2,2,2-trimethyleth-1-yl, n-hexyl, n-heptyl, and the like.

“Alkylene” refers to a divalent radical derived from an alkane which may be a straight chain or branched, as exemplified by $–CH_2CH_2CH_2CH_2$-.

“Aryl”: refers to fully unsaturated monocyclic, bicyclic and polycyclic aromatic hydrocarbons having at least one aromatic ring and having a specified number of carbon atoms that comprise their ring members (e.g., C_{6-14} aryl refers to an aryl group having 6 to 14 carbon atoms as ring members). The aryl group may be attached to a parent group or to a substrate at any ring atom and may include one or more non-hydrogen substituents unless such attachment or substitution would violate valence requirements. Examples of aryl groups include phenyl.

“C_{7-12} aralkyl” refers to an arylalkyl group having 7 to 12 carbon atoms and comprising an alkyl group substituted with an aryl group. Suitably the alkyl group is a C_{1-6} alkyl group and the aryl group is phenyl. Examples of C_{7-12} aralkyl include benzyl and phenethyl. In some cases the C_{7-12} aralkyl group may be optionally substituted and an example of an optionally substituted C_{7-12} aralkyl group is 4-methoxylbenzyl.

“C_{5-9} heteroaryl”: refers to unsaturated monocyclic or bicyclic aromatic groups comprising from 5 to 9 ring atoms, whether carbon or heteroatoms, of which from 1 to 5 are ring heteroatoms. Suitably, any monocyclic heteroaryl ring has from 5 to 6 ring atoms and from 1 to 3 ring heteroatoms. Suitably each ring heteroatom is independently selected from nitrogen, oxygen, and sulfur. The bicyclic rings include fused ring systems and, in particular, include bicyclic groups in which a monocyclic heterocycle comprising 5 ring atoms is fused to a benzene ring. The heteroaryl group may be attached to a parent group or to a substrate at any ring atom and may include one or more non-hydrogen substituents unless such attachment or substitution would violate valence requirements or result in a chemically unstable compound.

Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

N_1: pyrrole, pyridine;
O_1: furan;
S_1: thiophene;
N_1O_1: oxazole, isoxazole, isoxazine;
N_2O_1: oxadiazole (e.g. 1-oxa-2,3-diazolyl, 1-oxa-2,4-diazolyl, 1-oxa-2,5-diazolyl, 1-oxa-3,4-diazolyl);
N_3O_1: oxatriazole;
N_1S_1: thiazole, isothiazole;
N_2: imidazole, pyrazole, pyridazine, pyrimidine, pyrazine;
N_3: triazole, triazine; and,
N_4: tetrazole.

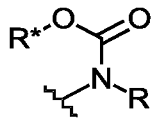
Examples of heteroaryl which comprise fused rings, include, but are not limited to, those derived from:
O_1: benzofuran, isobenzofuran;
N_1: indole, isoindole, indolizine, isoindoline;
S_1: benzothiofuran;
N_1O_1: benzoxazole, benzisoxazole;
N_1S_1: benzothiazole;
N_2: benzimidazole, indazole;
O_2: benzodioxole;
N_2O_1: benzofurazan;
N_2S_1: benzothiadiazole;
N_3: benzotriazole; and
N_4: purine (e.g., adenine, guanine), pteridine;

"Heteroarylene" refers to a divalent radical derived from a heteroaryl group, as exemplified by pyridinylene –(C_5H_3N)-.

"C_{6-15} heteroarylalkyl" refers to an alkyl group substituted with a heteroaryl group. Suitably the alkyl is a C_{1-6} alkyl group and the heteroaryl group is C_{5-9} heteroaryl as defined above. Examples of C_{6-15} heteroarylalkyl groups include pyrrol-2-ylmethyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, pyrrol-3-ylethyl, pyrrol-4-ylethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, imidazol-4-ylethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, pyridin-2-ylmethyl, pyridin-2-ylethyl, thiazol-2-ylmethyl, thiazol-4-ylmethyl, thiazol-2-ylethyl, pyrimidin-2-ylpropyl, and the like.

Nitrogen protecting groups

Nitrogen protecting groups are well known in the art. Preferred nitrogen protecting groups are carbamate protecting groups that have the general formula:



A large number of possible carbamate nitrogen protecting groups are listed on pages 706 to 771 of Wuts, P.G.M. and Greene, T.W., Protective Groups in Organic Synthesis, 4th Edition, Wiley-lnterscience, 2007, and in P. Kocienski, *Protective Groups*, 3rd Edition (2005) which are incorporated herein by reference.

Particularly preferred protecting groups include Alloc (allyloxycarbonyl), Troc (2,2,2-Trichloroethyl carbonate), Teoc [2-(Trimethylsilyl)ethoxycarbony], BOC (tert-butyloxycarbonyl), Doc (2,4-dimethylpent-3-yloxycarbonyl), Hoc (cyclohexyloxy-carbonyl), TcBOC (2,2,2-trichloro-tert-butyloxycarbonyl), Fmoc (9-fluorenylmethyloxycarbonyl), 1-Adoc (1-Adamantyloxycarbonyl) and 2-Adoc (2-adamantyloxycarbonyl).

Hydroxyl protecting groups

Hydroxyl protecting groups are well known in the art, a large number of suitable groups are described on pages 16 to 366 of Wuts, P.G.M. and Greene, T.W., Protective Groups in Organic Synthesis, 4th Edition, Wiley-lnterscience, 2007, and in P. Kocienski, *Protective Groups*, 3rd Edition (2005) which are incorporated herein by reference.

Classes of particular interest include silyl ethers, methyl ethers, alkyl ethers, benzyl ethers, esters, benzoates, carbonates, and sulfonates.

Particularly preferred protecting groups include THP (tetrahydropyranyl ether).

"Compound of formula (I) and salts and solvates thereof" refers to the compounds of formula (I); salts of compounds of formula (I); solvates of compounds of formula (I); and solvates of salts of compounds of formula (I).

"Drug", "drug substance", "active pharmaceutical ingredient", and the like, refer to a compound (e.g., compounds of formula (I) and compounds specifically named above) that may be used for treating a subject in need of treatment.

"Excipient" refers to any substance that may influence the bioavailability of a drug, but is otherwise pharmacologically inactive.

"Pharmaceutically acceptable" substances refers to those substances which are within the scope of sound medical judgment suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit-to-risk ratio, and effective for their intended use.

"Pharmaceutical composition" refers to the combination of one or more drug substances and one or more excipients.

The term "subject" as used herein refers to a human or non-human mammal. Examples of non-human mammals include livestock animals such as sheep, horses, cows, pigs, goats, rabbits and deer; and companion animals such as cats, dogs, rodents, and horses.

"Therapeutically effective amount" of a drug refers to the quantity of the drug or composition that is effective in treating a subject and thus producing the desired therapeutic, ameliorative, inhibitory or preventative effect. The therapeutically effective amount may depend on the weight and age of the subject and the route of administration, among other things.

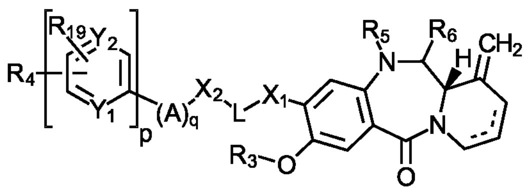
"Treating" refers to reversing, alleviating, inhibiting the progress of, or preventing a disorder, disease or condition to which such term applies, or to reversing, alleviating, inhibiting the progress of, or preventing one or more symptoms of such disorder, disease or condition.

"Treatment" refers to the act of "treating", as defined immediately above.

As used herein the term “comprising” means “including at least in part of” and is meant to be inclusive or open ended. When interpreting each statement in this specification that includes the term “comprising”, features, elements and/or steps other than that or those prefaced by the term may also be present. Related terms such as “comprise” and “comprises” are to be interpreted in the same manner.

$\underline{R_1}$

R_1 is selected from R_7, $=CH_2$, $=CH-(CH_2)_m-CH_3$, $=O$, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-SO_2R_7$, $O-SO_2R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$. For the options where R_1 is selected from $=CH_2$, $=CH-(CH_2)_m-CH_3$ and $=O$, the carbon of the C-ring to which it is attached cannot have an optional double bond in order for the valence requirements of the molecule to be met. For example, if R_1 is $=CH_2$, and is positioned at the C1 position of the C-ring adjacent to the fused carbon of the C-ring, and R_2 is H then the resulting compound of formula (I) may be represented as:



Suitably R_1 is selected from R_7, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-SO_2R_7$, $O-SO_2R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$.

Suitably R_1 is selected from R_7, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$.

Suitably R_1 is selected from R_7, OR_7, CO_2R_7, NR_7R_8, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $C(O)R_7$ and $C(O)NR_7R_8$.

Suitably R_1 is selected from R_7, OR_7, CO_2R_7, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $C(O)R_7$ and $C(O)NR_7R_8$.

Suitably R_1 is selected from R_7, $O-(CH_2)_n-NH-C(O)-R_7$ and $O-(CH_2)_n-C(O)-NH-R_7$.

In some embodiments R_1 is H.

$\underline{R_2}$

R_2 is selected from R_9, $(CH_2)_r$-OR_9, $(CH_2)_r$-CO_2R_9, $(CH_2)_r$-NR_9R_{10}, O-$(CH_2)_s$-NR_9R_{10}, NH-C(O)-R_9,O-$(CH_2)_s$-NH-C(O)-R_9, O-$(CH_2)_s$-C(O)-NH-R_9, $(CH_2)_r$-SO_2R_9, O-SO_2R_9, $(CH_2)_r$-COR_9 and $(CH_2)_r$-C(O)NR_9R_{10}.

Suitably R_2 is selected from R_9, $(CH_2)_r$-OR_9, $(CH_2)_r$-CO_2R_9, $(CH_2)_r$-NR_9R_{10}, O-$(CH_2)_s$-NR_9R_{10}, NH-C(O)-R_9,O-$(CH_2)_s$-NH-C(O)-R_9, O-$(CH_2)_s$-C(O)-NH-R_9, $(CH_2)_r$-COR_9 and $(CH_2)_r$-C(O)NR_9R_{10}.

Suitably R_2 is selected from R_9, OR_9, CO_2R_9, NR_9R_{10}, NH-C(O)-R_9,O-$(CH_2)_s$-NH-C(O)-R_9, O-$(CH_2)_s$-C(O)-NH-R_9, COR_9 and C(O)NR_9R_{10}.

Suitably R_2 is selected from R_9, OR_9, CO_2R_9, O-$(CH_2)_s$-NH-C(O)-R_9, O-$(CH_2)_s$-C(O)-NH-R_9, COR_9 and C(O)NR_9R_{10}.

Suitably R_2 is selected from R_9, O-$(CH_2)_s$-NH-C(O)-R_9 and O-$(CH_2)_s$-C(O)-NH-R_9.

In some embodiments R_2 is H.

$\underline{R_3}$

Suitably R_3 is selected from H, C_{1-6} alkyl and CH_2Ph.

Suitably R_3 is selected from H, methyl, ethyl and CH_2Ph.

More suitably R_3 is selected from methyl and ethyl.

More suitably R_3 is methyl.

$\underline{R_4}$

R_4 is selected from phenyl and C_{5-9} heteroaryl groups optionally substituted with up to three optional substituent groups. Hence, any of the phenyl group or the C_{5-9} heteroaryl groups selected for R_4 may be optionally substituted with up to three optional substituent groups.

Suitably R_4 is selected from phenyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl and benzothiazolyl, optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH- R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$.

Suitably R_4 is selected from phenyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl and benzothiazolyl, optionally substituted with one or two optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$.

Suitably R_4 is selected from phenyl, N-methylpyrrolyl, thiophenyl, N-methylimidazolyl, oxazolyl, thiazolyl, benzothiophenyl, N-methylbenzoimidazolyl and benzothiazolyl, optionally substituted with one or two optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11} O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$.

Suitably R_4 is optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-NH_2, $(CH_2)_j$-NH_2, C(=O)-NH-$(CH_2)_k$-NH_2; C(=O)-NH-R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)NH_2.

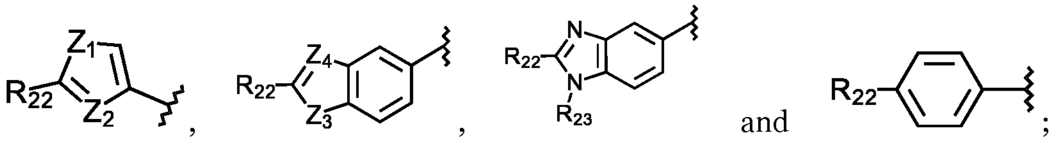
Suitably R_4 is an optionally substituted C(=O)-NH-R_{24}, wherein R_{24} is -C_6H_4-$(CH_2)_j$-R_{18}, and the phenylene group –C_6H_4- is para substituted.

Suitably R_4 is optionally substituted with up to three optional substituent groups selected from OH, methyl, ethyl, OCH_3, OCH_2CH_3, CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, O-$(CH_2)_k$-NH_2 and $(CH_2)_j$-NH_2.

Suitably R_4 is optionally substituted with one or two optional substituent groups.

More suitably R_4 is optionally substituted with one optional substituent group.

More suitably R_4 is selected from:



wherein Z_1 is selected from NH, N-CH_3, S and O;

Z_2 is selected from CH and N;

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{22} is selected from $(CH_2)_jCO_2R_{11}$, $(CH_2)_jNR_{11}R_{12}$ and C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18};

R_{18} is selected from CO_2R_{11} and $NR_{11}R_{12}$;

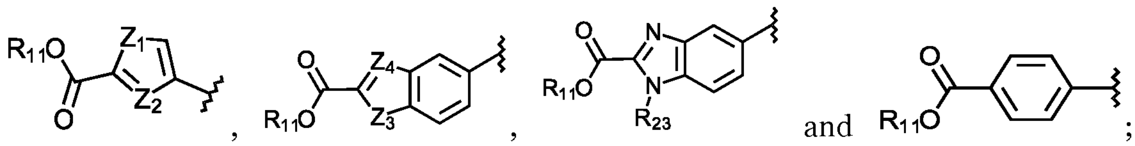
j is selected from an integer from 0 to 6;

R_{11} and R_{12} are independently selected from H and C_{1-6} alkyl; and

R_{23} is selected from H and C_{1-6} alkyl.

The wavy line indicates the point of attachment of the above R_4 group to the rest of the compound of formula (I).

More suitably R_4 is selected from:



wherein Z_1 is selected from NH, N-CH_3, S and O;

Z_2 is selected from CH and N; and

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{11} is selected from H and C_{1-6} alkyl; and

R_{23} is selected from H and C_{1-6} alkyl.

<u>R_5 and R_6</u>

Suitably for (iii) R_5 is H and R_6 is an OC_{1-6} alkyl selected from O-CH_3 and O-CH_2CH_3.

Most suitably, (i) R_5 and R_6 together form a double bond.

<u>R_7</u>

Suitably R_7 is selected from H, C_{1-12} alkyl, C_{5-9} heteroaryl, C_{6-15} heteroarylalkyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_7 is selected from H, C_{1-12} alkyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_7 is selected from H, C_{1-6} alkyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_7 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_7 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, phenyl, benzyl and phenethyl optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

In some embodiments, R_7 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl.

<u>R_9</u>

Suitably R_9 is selected from H, C_{1-12} alkyl, C_{5-9} heteroaryl, C_{6-15} heteroarylalkyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_9 is selected from H, C_{1-12} alkyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_9 is selected from H, C_{1-6} alkyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_9 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, pyrrol-3-ylmethyl, pyrrol-4-ylmethyl, imidazol-2-ylmethyl, imidazol-4-ylmethyl, thiophen-3-ylmethyl, furan-3-ylmethyl, phenyl, benzyl and phenethyl optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

Suitably R_9 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, indolyl, N-methylindolyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl, benzothiazolyl, phenyl, benzyl and phenethyl optionally substituted with up to three groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl.

In some embodiments, R_9 is selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl.

R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21}

Suitably each of R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21} are independently selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl and t-butyl.

Suitably each of R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21} are independently selected from H, methyl, and ethyl.

Suitably R_8 is H.

Suitably R_{10} is H.

Suitably each R_{11} is independently selected from H and methyl.

Suitably each R_{12} is independently selected from H and methyl; more suitably each R_{12} is H.

Suitably R_{13} is H.

Suitably R_{14} is H.

Suitably R_{15} is H.

Suitably R_{16} is H.

Suitably R_{17} is methyl.

Suitably R_{20} is H.

Suitably R_{21} is H.

R_{18}

Suitably R_{18} is selected from CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, $NH(CH_3)$ and NH_2.

R_{19}

Suitably R_{19} is selected from H, $(CH_2)_t$-$N(CH_2CH_3)_2$, $(CH_2)_t$-$N(CH_3)_2$, $(CH_2)_t$-$NH(CH_2CH_3)$, $(CH_2)_t$-$NH(CH_3)$ and $(CH_2)_t$-NH_2.

More suitably R_{19} is selected from H and $(CH_2)_t$-NH_2.

R_{24}

Suitably, R_{24} is a phenyl optionally substituted with up to three optional substituent groups selected from OH, methyl, ethyl, propyl, OCH_3, OCH_2CH_3, CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, O-$(CH_2)_k$-NH_2, O-$(CH_2)_k$-$NH(CH_3)$, $(CH_2)_j$-NH_2, $(CH_2)_j$-$NH(CH_3)$, C(=O)-NH-$(CH_2)_k$-NH_2, C(=O)-NH-$(CH_2)_k$-$NH(CH_3)$, C(=O)-NH-$(CH_2)_k$-C(=NH)$NH(CH_3)$, and C(=O)-NH-$(CH_2)_k$-C(=NH)NH_2.

Suitably, R_{24} is a phenyl optionally substituted with up to three optional substituent groups selected from OH, methyl, ethyl, OCH_3, OCH_2CH_3, CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, O-$(CH_2)_k$-NH_2 and $(CH_2)_j$-NH_2.

Suitably, R_{24} is a para substituted phenyl group.

More suitably, in some aspects R_{24} is -C_6H_4-$(CH_2)_j$-R_{18}, wherein R_{18} is selected from CO_2R_{11} and $NR_{11}R_{12}$.

j

Each instance of j is independently selected from an integer from 0 to 6, hence, each j is independently selected from 0, 1, 2, 3, 4, 5 and 6.

Suitably each j is independently selected from 0, 1, 2 and 3.

More suitably each j is independently selected from 0 and 1.

More suitably each j is 0.

k

Each instance of k is independently selected from an integer from 1 to 6, hence, each k is independently selected from 1, 2, 3, 4, 5 and 6.

Suitably each k is independently selected from 1, 2 and 3.

More suitably each k is 1.

<u>m</u>

m is selected from an integer from 0 to 6, hence, m is selected from 0, 1, 2, 3, 4, 5 and 6.

Suitably m is selected from 0, 1, 2 and 3.

More suitably m is selected from 0 and 1.

More suitably m is 0.

<u>n</u>

n is selected from an integer from 1 to 6, hence, n is selected from 1, 2, 3, 4, 5 and 6.

Suitably n is selected from 1, 2 and 3.

More suitably n is 1.

<u>r</u>

r is selected from an integer from 0 to 6, hence, r is selected from 0, 1, 2, 3, 4, 5 and 6.

Suitably r is selected from 0, 1, 2 and 3.

More suitably r is selected from 0 and 1.

More suitably r is 0.

<u>s</u>

s is selected from an integer from 1 to 6, hence, s is selected from 1, 2, 3, 4, 5 and 6.

Suitably s is selected from 1, 2 and 3.

More suitably s is 1.

t

t is selected from an integer from 0 to 6, hence, t is selected from 0, 1, 2, 3, 4, 5 and 6.

Suitably t is selected from 0, 1, 2 and 3.

More suitably t is selected from 0 and 1.

More suitably t is 0.

Y_1

Y_1 is N or CH; suitably Y_1 is CH.

Y_2

Y_2 is N or CH; suitably Y_2 is CH.

X_1

Suitably X_1 is selected from O, S, NH, CH_2, CH_2O, C(=O), C(=O)NR_{13}, NR_{13}C(=O), O-C(O) and C(O)-O;

Suitably, X_1 is selected from O, C(=O), C(=O)NR_{13} and NR_{13}C(=O).

More suitably X_1 is selected from O, C(=O)NH and NHC(=O).

More suitably X_1 is O.

X_2

Suitably X_2 is selected from O, S, NH, CH_2, CH_2O, C(=O), C(=O)NR_{15}, NR_{15}C(=O), O-C(O) and C(O)-O or is absent.

Suitably X_2 is selected from O, C(=O), C(=O)NR_{15} and NR_{16}C(=O) or is absent.

More suitably X_2 is selected from O, C(=O)NH and NHC(=O).

Suitably X_2 is the same as X_1.

More suitably X_2 is O.

L

L is a linker group. Suitably, any of the peptide chain, alkylene chain, paraformaldehyde chain or polyethylene glycol chain is interrupted by one or more hetero-atoms (e.g., N, O and S) and/or one or more C_{5-9} heteroarylene groups (e.g., pyrrolylene, pyrazolylene, pyrazolylene, 1,2,3-triazolylene, pyridinylene) and/or one or more phenylene group. More suitably, the chains may be interrupted by from one to three hetero-atoms and/or from one to three C_{5-9} heteroarylene groups and/or from one to three phenylene groups.

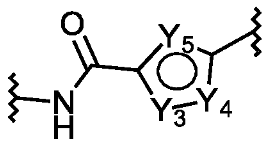
Suitably L is selected from a peptide chain having from 2 to 5 amino acids, from 2 to 4 amino acids, from 2 to 3 amino acids; an alkylene chain containing from 1 to 11 carbon atoms, from 1 to 10 carbon atoms, from 1 to 9 carbon atoms, from 1 to 8 carbon atoms, from 1 to 7 carbon atoms, from 1 to 6 carbon atoms, from 1 to 5 carbon atoms, from 1 to 4 carbon atoms, from 1 to 3 carbon atoms, which may contain one or more carbon-carbon double or triple bonds; a paraformaldehyde chain $-(OCH_2)_{1-12}-$, $-(OCH_2)_{1-11}-$, $-(OCH_2)_{1-10}-$, $-(OCH_2)_{1-9}-$, $-(OCH_2)_{1-8}-$, $-(OCH_2)_{1-7}-$, $-(OCH_2)_{1-6}-$, $-(OCH_2)_{1-5}-$, $-(OCH_2)_{1-4}-$, $-(OCH_2)_{1-3}-$ a polyethylene glycol chain $-(OCH_2CH_2)_{1-5}-$, chain $-(OCH_2CH_2)_{1-4}-$, chain $-(OCH_2CH_2)_{1-3}-$; which chain may be interrupted by one or more hetero-atoms and/or C_{5-9} heteroarylene groups and/or from one to three phenylene groups.

More suitably, L may be selected from an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds.

More suitably, L may be selected from CH=CH, CH_2, CH_2CH_2, $CH_2CH_2CH_2$, $CH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2CH_2CH_2$.

A

In one embodiment A is A1:

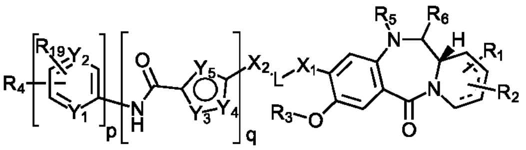


A1

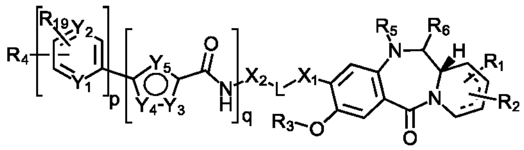
wherein for each A1 group one of Y_3 and Y_4 is independently selected from N-R_{17}, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH.

In this embodiment, when q is selected from 2, 3, 4, 5 and 6 then A will contain multiple A1 groups connected to each other.

Hence, the 5-membered ring containing Y_3, Y_4 and Y_5 is a heteroaryl ring. This A1 group may be attached to the rest of the molecule in either direction. Hence, when A is A1, as in the above embodiment, the compound of formula (I) is selected from:



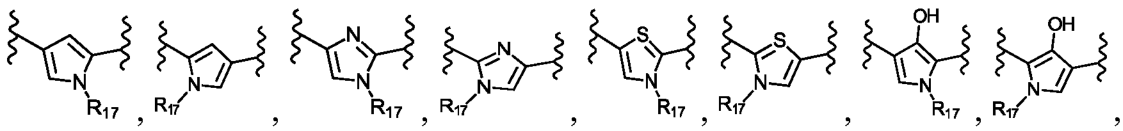
and



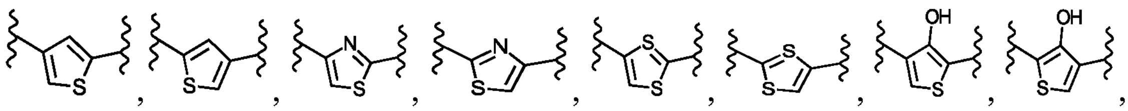
(II) (III).

More suitably, when A is A1 the compound of formula (I) is compound (II).

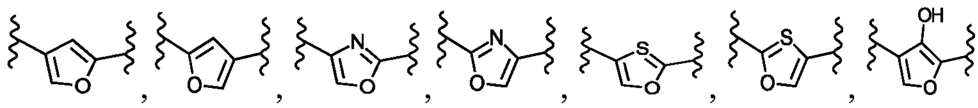
Hence, the heteroaryl ring containing Y_3, Y_4 and Y_5, is selected from one of the following groups:



H1 H2 H3 H4 H5 H6 H7 H8

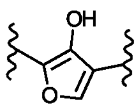


H9 H10 H11 H12 H13 H14 H15 H16



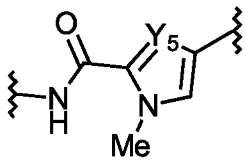
and

H17 H18 H19 H20 H21 H22 H23



H24.

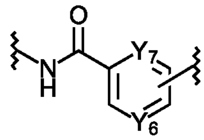
More suitably A is



A3

wherein Y_5 is selected from CH and N.

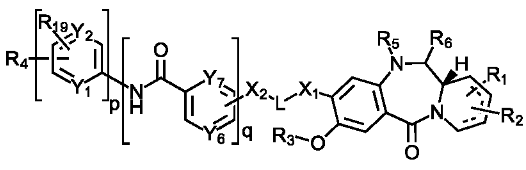
In another embodiment A is A2:



A2

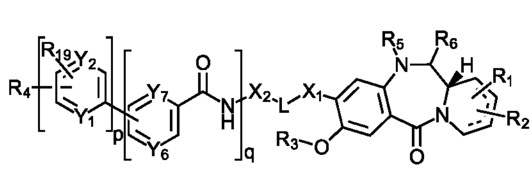
wherein for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH.

Hence, the 6-membered ring containing Y_6 and Y_7 is a phenyl or pyridinyl ring. The A2 group may be attached to the rest of the molecule in either direction. Hence, when A is A2, as in the above embodiment, the compound of formula (I) is selected from:



(IV)

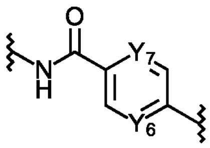
and



(V).

More suitably, when A is A2 the compound of formula (I) is compound (IV).

Suitably, A is A4:



A4.

More suitably Y_6 is CH; and Y_7 is CH.

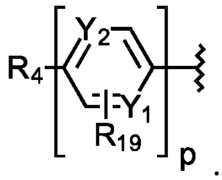
<u>q</u>

Suitably q is selected from 0, 1, 2 and 3.

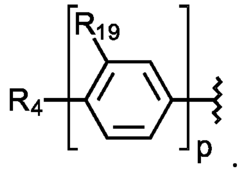
More suitably q is 0 or 1.

<u>6-Membered aromatic-ring</u>

Suitably, the 6-membered aromatic ring of formula (I) is para-substituted:

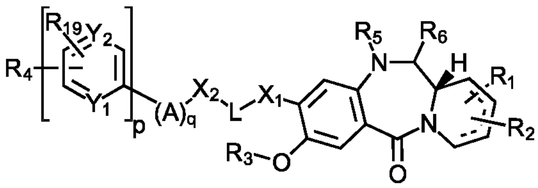


More suitably, the 6-membered aromatic ring of formula (I) is:



Optional double bonds in the C-ring

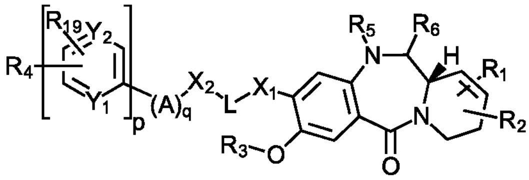
The present invention provides a compound of formula (I):



(I)

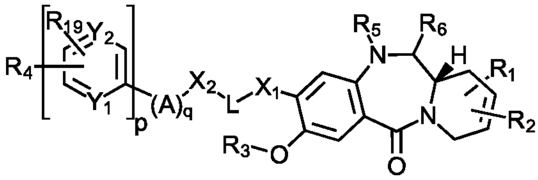
wherein the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4.

In one aspect, the compound of formula (I) has a double bond between C1 and C2 to give a compound of formula (VI):



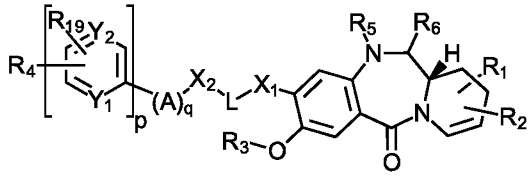
(VI).

In another aspect, the compound of formula (I) has a double bond between C2 and C3 to give a compound of formula (VII):



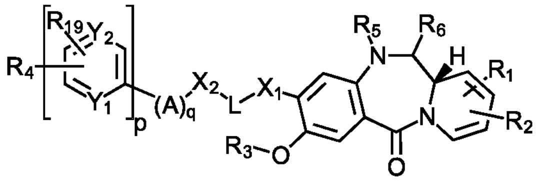
(VII).

In another aspect, the compound of formula (I) has a double bond between C3 and C4 to give a compound of formula (VIII):



(VIII).

In another aspect, the compound of formula (I) has a double bond between C1 and C2 and a double bond between C3 and C4 to give a compound of formula (IX):



(IX).

Other limitations

The options for compounds of formula (I) contain the proviso that when p is 0 and A is A1, then: (a) for at least one A1 group one of Y_3 and Y_4 is selected from S and O; or (b) for at least one A1 group Y_5 is S; or (c) R_4 is not an optionally substituted pyrrolyl or imidazolyl.

There will be more than one A1 group when q is selected from 2, 3, 4, 5 and 6.

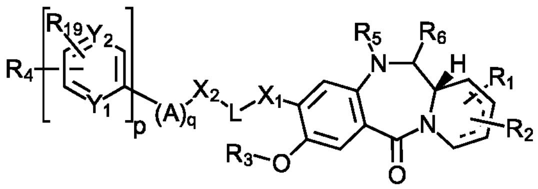
Hence, when p is 0 and A is A1, the proviso requires the presence of at least one aryl group or, alternatively, the presence of a heteroaryl group (either as part of A1 or R_4) which does not contain a 5-membered pyrrole or imidazole ring, or optionally substituted derivatives such as N-methylpyrrole or N-methylimidazole rings. As a result, this proviso prevents the compounds of formula (I) having a purely poly-pyrrole or poly-imidazole or poly-pyrrole-imidazole long chain group attached to the PDD. Compounds having such long chain groups tend to be relatively poorly cytotoxic.

In some aspects, suitably the options for compounds of formula (I) contains the proviso that when p is 0 and A is A1, then: (a) the 5-membered ring of A1 is selected from H9, H10, H11, H12, H13, H14, H15, H16, H17, H19, H20, H21, H22, H23 and H24; or (b) the 5-membered ring of A1 is selected from H5 and H6; or (c) R_4 is selected from phenyl, furanyl, thiophenyl, oxazolyl, thiazolyl, pyridyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl and benzothiazolyl, optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$.

In some aspects, suitably the options for compounds of formula (I) contains the proviso that when p is 0 and A is A1, then: (a) the 5-membered ring of A1 is selected from H9, H10, H11, H12, H13, H14, H15, H16, H17, H19, H20, H21, H22, H23 and H24; or (b) the 5-membered ring of A1 is selected from H5 and H6; or (c) R_4 is selected from phenyl and C_9 heteroaryl groups optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18};C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$, with the proviso that the C_{5-9} heteroaryl is not indolyl.

<u>Suitable structures</u>

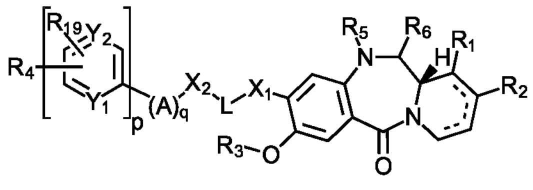
The compound of formula (I):



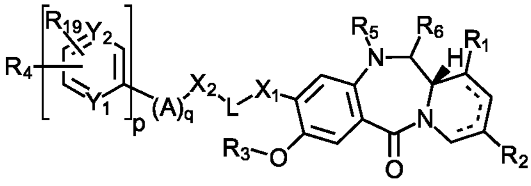
(I)

is drawn without specifying the position of R_1 and R_2 on the C-ring. Hence, R_1 and R_2 may be present on any position of the C-ring provided that the valence requirement are met. As the fused carbon and the nitrogen of the C-ring have all their substituents shown, this means that R_1 and R_2 may be present on any of the non-fused carbons of the C-ring (i.e. the C1, C2, C3 or C4 positions as designated above). Suitably R_1 and R_2 are present on two different non-fused carbons of the C-ring.

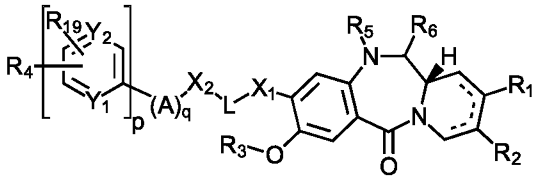
In one aspect, the compound of formula (I) is selected from:



(X);

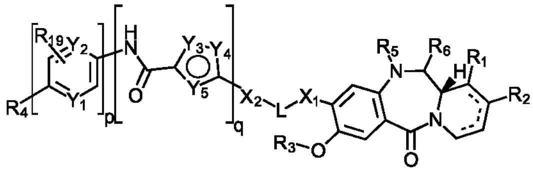


(XI); and

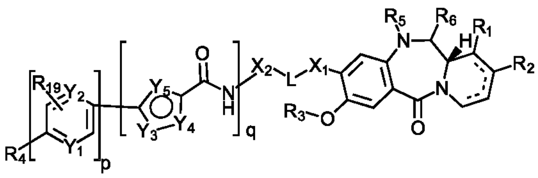


(XII).

In another aspect, the compound of formula (I) is selected from:

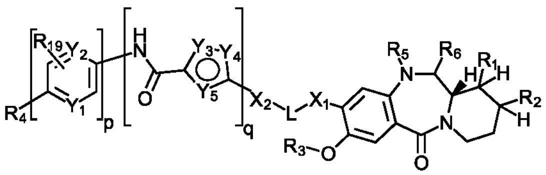


(XIII); and



(XIV).

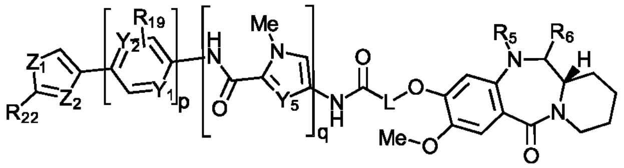
More suitably, the compound of formula (I) has the following structure:



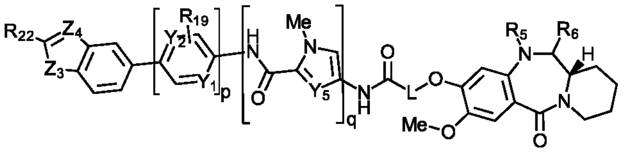
(XV).

For compounds of formula (XV) where R_1 and/or R_2 are substituents other than H, the carbons in the C-ring to which any such substituents are attached will be stereocenters. In formula (XV) R_1 and R_2 are drawn without specifying the stereochemistry of the carbons on the C-ring to which they are attached.

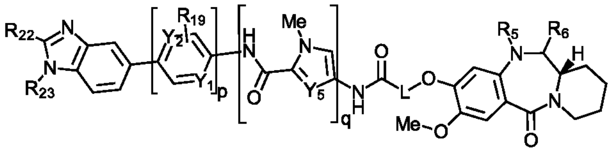
More suitably the compound of formula (I) is selected from:



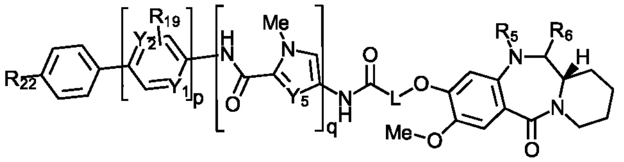
(XVI);



(XVII);



(XVIII); and



(XIX)

wherein q is selected from 0, 1, 2, 3, 4, 5 or 6;

p is 0 or 1;

L is an alkylene chain containing from 1 to 12 carbon atoms;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

Y_5 is selected from CH and N;

Z_1 is selected from O, S, NH and N-CH_3;

Z_2 is selected from CH and N;

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{22} is selected from $(CH_2)_jCO_2H$, $(CH_2)_jCO_2C_{1-6}$ alkyl, $(CH_2)_jNR_{11}R_{12}$ and C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18};

R_{18} is selected from CO_2R_{11} and $NR_{11}R_{12}$;

R_{19} is selected from H and $(CH_2)_t$-$NR_{20}R_{21}$;

j and t are independently selected from an integer from 0 to 6; and

R_{11}, R_{12} and R_{23} are independently selected from H and C_{1-6} alkyl.

and either:

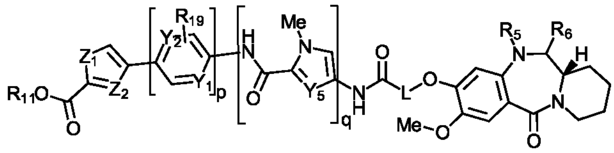
(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

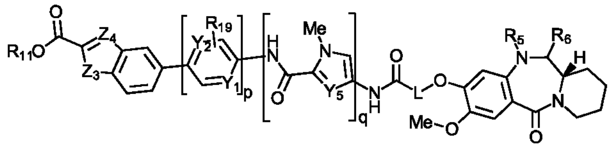
(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when the compound is (XVI) and p is 0, that Z_1 is selected from O and S.

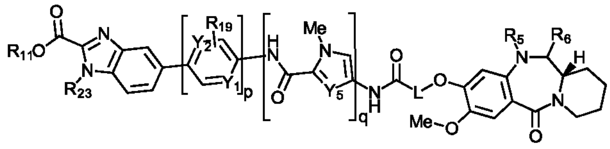
More suitably the compound of formula (I) is selected from:



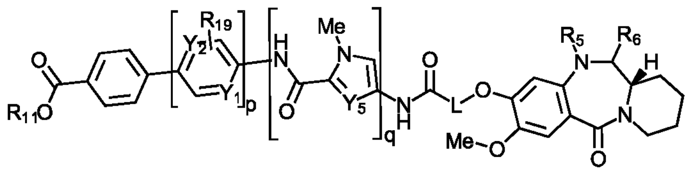
(XX);



(XXI);



(XXII); and



(XXIII);

wherein q is selected from 0, 1, 2, 3, 4, 5 or 6;

p is 0 or 1;

L is an alkylene chain containing from 1 to 12 carbon atoms;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

Y_5 is selected from CH and N;

Z_1 is selected from O, S, NH and N-CH_3;

Z_2 is selected from CH and N;

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{19} is selected from H and $(CH_2)_t$-$NR_{20}R_{21}$;

t is selected from an integer from 0 to 6;

R_{11}, R_{20}, R_{21} and R_{23} are independently selected from H and C_{1-6} alkyl;

and either:

(i) R_5 and R_6 together form a double bond;

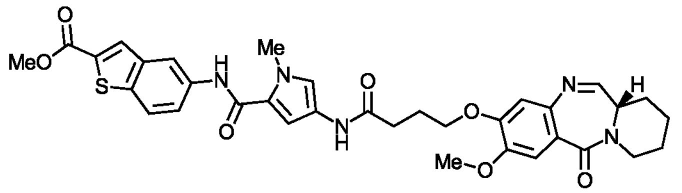
(ii) R_5 is H and R_6 is OH; or

(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when the compound is (XX) and p is 0, that Z_1 is selected from O and S.

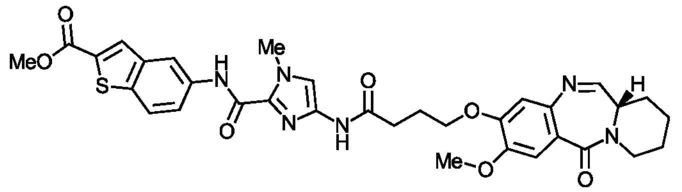
More suitably, the compound of formula (I) is selected from:

(a) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo-[*b*]thiophene-2-carboxylate (13)



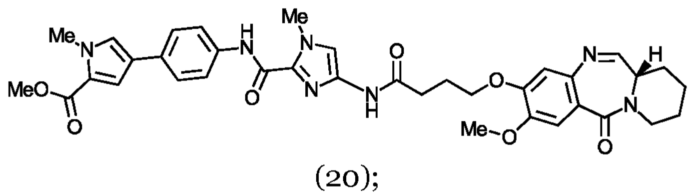
(13);

(b) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)-benzo[*b*]thiophene-2-carboxylate (17)



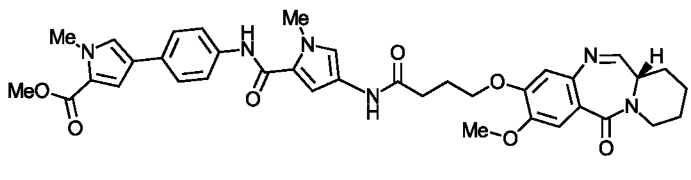
(17);

(c) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (20)



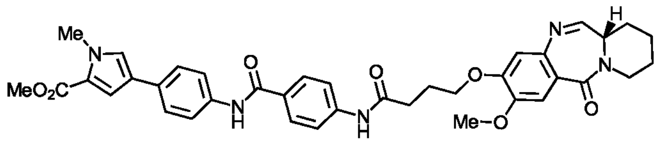
(20);

(d) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (24)



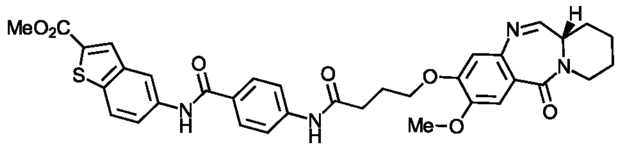
(24);

(e) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (28)



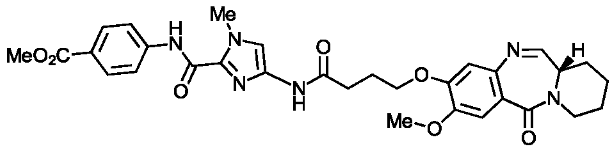
(28);

(f) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)benzo[b]thiophene-2-carboxylate (30)



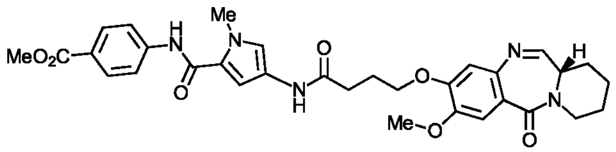
(30);

(g) methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)-benzoate (34)



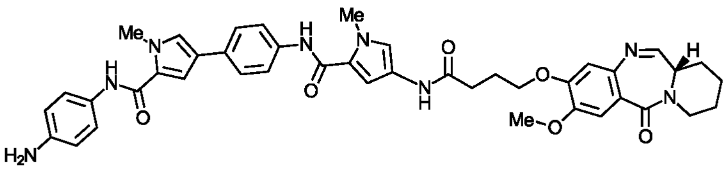
(34);

(h) methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)-benzoate (38)



(38);

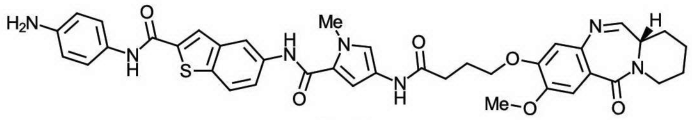
(i) (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7, 8,9,10,12-hexahydro-benzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butan-amido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (41)



(41);

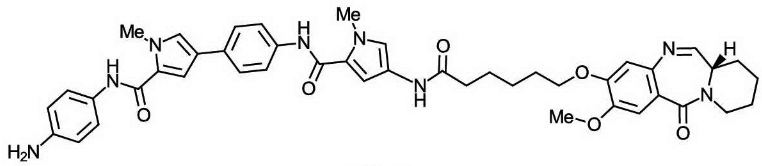
24 Sep 2019 2016313228

(j) (*S*)-*N*-(2-((4-Aminophenyl)carbamoyl)benzo[*b*]thiophen-5-yl)-4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)-butanamido)-1-methyl-1*H*-pyrrole-2-carboxamide (47)



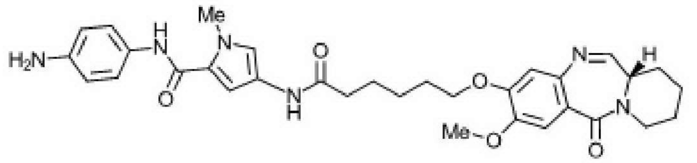
(47);

(k) (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7, 8,9,10,12-hexahydro-benzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexan-amido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (62)



(62);

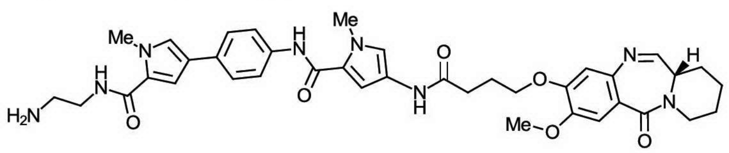
(l) (*S*)-*N*-(4-Aminophenyl)-4-(6-((2-methoxy-12-oxo-6a,7,8,9, 10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexan-amido)-1-methyl-1*H*-pyrrole-2-carboxamide (66)



(66);

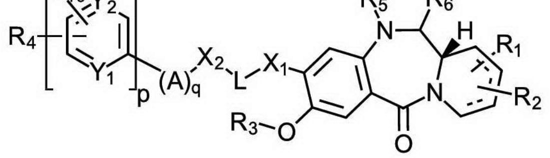
and

(m) (*S*)-*N*-(2-Aminoethyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9, 10,12-hexahydro-benzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (68)



(68).

In a further aspect, there is provided a compound of formula (I):



(I)

and salts and solvates thereof, wherein:

the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4;

R_1 is selected from R_7, $=CH_2$, $=CH-(CH_2)_m-CH_3$, $=O$, $(CH_2)_m-OR_7$, $(CH_2)_m-CO_2R_7$, $(CH_2)_m-NR_7R_8$, $O-(CH_2)_n-NR_7R_8$, $NH-C(O)-R_7$, $O-(CH_2)_n-NH-C(O)-R_7$, $O-(CH_2)_n-C(O)-NH-R_7$, $(CH_2)_m-SO_2R_7$, $O-SO_2R_7$, $(CH_2)_m-C(O)R_7$ and $(CH_2)_m-C(O)NR_7R_8$;

R_2 is selected from R_9, $=CH_2$, $=CH-(CH_2)_r-CH_3$, $=O$, $(CH_2)_r-OR_9$, $(CH_2)_r-CO_2R_9$, $(CH_2)_r-NR_9R_{10}$, $O-(CH_2)_s-NR_9R_{10}$, $NH-C(O)-R_9$, $O-(CH_2)_s-NH-C(O)-R_9$, $O-(CH_2)_s-C(O)-NH-R_9$, $(CH_2)_r-SO_2R_9$, $O-SO_2R_9$, $(CH_2)_r-COR_9$ and $(CH_2)_r-C(O)NR_9R_{10}$;

R_3 is selected from H, C_{1-12} alkyl and CH_2Ph;

R_4 is selected from phenyl and C_{5-9} heteroaryl groups optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j-CO_2R_{11}$, $O-(CH_2)_k-NR_{11}R_{12}$, $(CH_2)_j-NR_{11}R_{12}$, $C(=O)-NH-(CH_2)_k-NR_{11}R_{12}$; $C(=O)-NH-C_6H_4-(CH_2)_j-R_{18}$ and $C(=O)-NH-(CH_2)_k-C(=NH)NR_{11}R_{12}$;

R_{19} is selected from H and $(CH_2)_t-NR_{20}R_{21}$;

Y_1 is N or CH; Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

p is 0 or 1; j, m, r and t are independently selected from an integer from 0 to 6;

k, n and s are independently selected from an integer from 1 to 6;

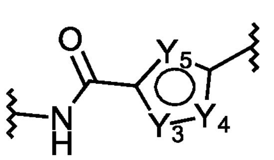
X_1 is selected from O, S, NR_{13}, $CR_{13}R_{14}$, $CR_{13}R_{14}O$, $C(=O)$, $C(=O)NR_{13}$, $NR_{13}C(=O)$, $O-C(O)$ and $C(O)-O$;

L is selected from an amino acid, a peptide chain having from 2 to 6 amino acids, an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds, a paraformaldehyde chain $-(OCH_2)_{1-12}-$, a polyethylene glycol chain $-(OCH_2CH_2)_{1-6}-$, which chains may be interrupted by one or more of O, S and/or NH groups and/or C_{3-9} heteroarylene and/or phenylene;

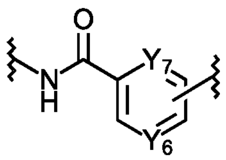
X_2 is selected from O, S, NR_{15}, $CR_{15}R_{16}$, $CR_{15}R_{16}O$, $C(=O)$, $C(=O)NR_{15}$, $NR_{15}C(=O)$, $O-C(O)$ and $C(O)-O$ or is absent;

q is selected from 0, 1, 2, 3, 4, 5 and 6;

A is selected from:



and



;

A1 A2

for each A1 group one of Y_3 and Y_4 is independently selected from $N-R_{17}$, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH; and

for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH;

R_7 and R_9 are independently selected from H, C_{1-12} alkyl, C_{5-9} heteroaryl, C_{6-15} heteroarylalkyl, phenyl and C_{7-12} aralkyl groups; wherein the heteroaryl, heteroarylalkyl, phenyl and aralkyl groups are optionally substituted with up to three optional substituent groups selected from C_{1-6} alkyl, OH, OC_{1-6} alkyl;
R_{18} is selected from CO_2R_{11} and $NR_{11}R_{12}$;
R_8, R_{10}, R_{11}, R_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17}, R_{20} and R_{21} are independently selected from H and C_{1-6} alkyl; and
(i)R_5 and R_6 together form a double bond; or (ii)R_5 is H and R_6 is OH; or (iii) R_5 is H and R_6 is OC_{1-6} alkyl.

<u>Applications</u>

The invention finds application in the treatment of proliferative diseases.

In certain aspects a method of treating a proliferative disease is provided, the method comprising administering to a subject a therapeutically effective amount of a compound of the formula (I) and salts and solvates thereof or a composition comprising a compound of formula (I) and salts and solvates thereof.

In certain aspects a method of treating a proliferative disease is provided, the method comprising administering to a subject a therapeutically effective amount of a targeted conjugate comprising a compound of the formula (I) and salts and solvates thereof.

In certain aspects a method of treating a proliferative disease is provided, the method comprising administering to a subject a therapeutically effective amount of an antibody-drug conjugate comprising a compound of the formula (I) and salts and solvates thereof.

The term “proliferative disease” refers to an unwanted or uncontrolled cellular proliferation of excessive or abnormal cells which is undesired, such as, neoplastic or hyperplastic growth, whether *in vitro* or *in vivo*. Examples of proliferative conditions include, but are not limited to, benign, pre-malignant, and malignant cellular proliferation, including but not limited to, neoplasms and tumours (e.g. histocytoma, glioma, astrocyoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, bowel cancer, colon cancer, hepatoma, breast cancer, glioblastoma, cervical cancer, ovarian cancer, oesophageal [or esophageal] cancer, oral cancer, prostate cancer, testicular cancer, liver cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, uterine cancer, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis. Suitably the proliferative disease is selected from bladder cancer, bone cancer, bowel cancer, brain cancer, breast cancer, cervical cancer, colon cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, oesophageal cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, retinoblastoma, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer and uterine cancer. Suitably the proliferative disease is selected from breast cancer and cervical cancer.

Any type of cell may be treated, including but not limited to, bone, eye, head and neck, lung, gastrointestinal (including, e.g. mouth, oesophagus, bowel, colon), breast (mammary), cervix, ovarian, uterus, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

A skilled person is readily able to determine whether or not a candidate compound treats a proliferative condition for any particular cell type.

Suitably subjects are human, livestock animals and companion animals.

In a further aspect, the compound of formula (I) and salts and solvates thereof, may be linked, either directly or indirectly, to a targeting agent (e.g., antibody, antibody fragment, hormone, etc.) to provide a targeted conjugate. The target conjugates of the present disclosure may contain one or multiple compounds of formula (I) (or salts and solvates thereof). A variety of target conjugates are known in the art and may be used with a compound of formula (I) and salts and solvates thereof. For example, in a particular aspect the target conjugate is an antibody-drug conjugate, wherein one or more compounds of formula (I) are linked, directly or indirectly, to the antibody. Therefore, the compound of formula (I) and salts and solvates thereof, may be used as a payload on a targeted conjugate.

Suitably, a compound of formula (I) and salts and solvates thereof, for use as a drug in targeted conjugate is prepared by attaching a compound of formula (I) and salts and solvates thereof to a targeting agent, either directly or via an optional linker group.

Suitably, the compound of formula (I) and salts and solvates thereof, is attached to a targeting agent via a linker group. Suitably, the targeted conjugate is for use in the treatment of a disease, more specifically of a proliferative disease. Suitably, the drug may be attached by any suitable functional group that it contains to the targeting agent either directly or via a linker group. Typically, the drug contains, or can be modified to contain, one or more functional groups such as amine, hydroxyl or carboxylic acid groups for attaching the drug to the targeting agent either directly or via a linker group. In some aspects, one or more atoms or groups of the compound of formula (I) may be eliminated during the attachment of the drug to the antibody. In some aspects, the targeting agent binds to a cell surface receptor or a tumor-associated antigen. In some aspects, the targeting agent is an antibody. In some aspects, the targeting agent is a hormone. In some aspects, the targeting agent is a protein. In some aspects, the targeting agent is a polypeptide. In some aspects, the targeting agent is a small molecule (for example, folic acid).

The compounds of formula (I) find application as payloads for antibodies or antibody fragments. The compounds of formula (I) readily allow conjugation to antibodies or antibody fragments.

<u>Antibody Drug Conjugates</u>

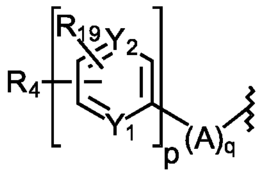
Antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders (Carter, P. (2006) Nature Reviews Immunology 6:343-357). The use of antibody-drug conjugates (ADC), i.e. immunoconjugates, for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer, targets delivery of the drug moiety to tumors, and intracellular accumulation therein, whereas systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells (Xie et al (2006) Expert. Opin. Biol. Ther. 6(3):281 -291 ; Kovtun ef a/ (2006) Cancer Res. 66(6):3214-3121 ; Law et al (2006) CancerRes. 66(4):2328-2337; Wu et al (2005) Nature Biotech. 23(9): 1 137-1 145; Lambert J. (2005) Current Opin. in Pharmacol. 5:543-549; Hamann P. (2005) Expert Opin. Ther. Patents 15(9): 1087-1 103; Payne, G. (2003) Cancer Cell 3:207-212; Trail ef a/ (2003) Cancer Immunol. Immunother. 52:328-337; Syrigos and Epenetos (1999) Anticancer Research 19:605-614).

Maximal efficacy with minimal toxicity is sought thereby. Efforts to design and refine ADC have focused on the selectivity of monoclonal antibodies (mAbs) as well as drug mechanism of action, drug-linking, drug/antibody ratio (loading), and drug-releasing properties (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Doman et al., (2009) Blood 114(13):2721 -2729; US 7521541 ; US 7723485; WO2009/052249; McDonagh (2006) Protein Eng. Design & Sel. 19(7): 299-307; Doronina et al., (2006) Bioconj. Chem. 17:114-124; Erickson et al., (2006) CancerRes. 66(8): 1-8; et al., (2005) Clin. CancerRes. 1 1 :843-852; Jeffrey et al., (2005) J. Med. Chem. 48:1344-1358; Hamblett et al., (2004) Clin. Cancer Res. 10:7063- 7070).

In some aspects, the present invention relates to a compound of formula (I) and salts and solvates thereof, for use as a drug in an antibody-drug conjugate. Suitably, a compound of formula (I) and salts and solvates thereof, for use as a drug in an antibody-drug conjugate is prepared by attaching a compound of formula (I) and salts and solvates thereof to an antibody, either directly or via an optional linker group. Suitably, the compound of formula (I) and salts and solvates thereof, is attached to an antibody via a linker group. Suitably, the antibody-drug conjugate is for use in for treatment of a disease, more specifically of a proliferative disease. Suitably, the antibody-drug conjugate is for use in for treatment of a disease, more specifically of a proliferative disease. Suitably, the drug may be attached by any suitable functional group that it contains to the antibody either directly or via a linker group. Typically, the drug contains, or can be modified to contain, one or more functional groups such as amine, hydroxyl or carboxylic acid groups for attaching the drug to the antibody either directly or via a linker group. In some aspects, the antibody of the antibody drug conjugate is an antibody fragment, such as, but not limited to a single chain antibody. In some aspects, one or more atoms or groups of the compound of formula (I) may be eliminated during the attachment of the drug to the antibody. In some aspects, the antibody binds to a cell surface receptor or a tumor-associated antigen.

In some aspects, the present invention relates to the use of a compound of formula (I) and salts and solvates thereof, as a drug in an antibody-drug conjugate. Suitably, the use of a compound of formula (I) and salts and solvates thereof, as a drug in an antibody-drug conjugate is accomplished by attaching a compound of formula (I) and salts and solvates thereof to an antibody, either directly or via an optional linker group. Suitably, the compound of formula (I) and salts and solvates thereof, is attached to an antibody via a linker group. Suitably, the antibody-drug conjugate is for use in for treatment of a disease, more specifically of a proliferative disease. Suitably, the drug may be attached by any suitable functional group that it contains to the antibody either directly or via a linker group. Typically, the drug contains, or can be modified to contain, one or more functional groups such as amine, hydroxyl or carboxylic acid groups for attaching the drug to the antibody either directly or via a linker group. In some aspects, the antibody of the antibody drug conjugate is an antibody fragment, such as, but not limited to a single chain antibody. In some aspects, one or more atoms or groups of the compound of formula (I) may be eliminated during the attachment of the drug to the antibody. In some aspects, the antibody binds to a cell surface receptor or a tumor-associated antigen.

The substituent groups of the compounds of formula (I) may interact with DNA sequences and may be selected so as to target specific sequences. In particular, the following groups in compounds of formula (I):



may be selected to target specific sequences. Hence, when the substituent groups are tailored in this way, the compounds of formula (I) find application in targeted chemotherapy.

<u>Antibody and antibody fragments</u>

The term “antibody” specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), intact antibodies and antibody fragments, so long as they exhibit the desired biological activity, for example, the ability to bind a desired antigen on a target cell or tissue. Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C, Travers, P., Walport, M., Shlomchik (2001) Immuno Biology, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on the antibody. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin can be of any type (e.g. IgG, IgE, IgM, IgD, and IgA), class (e.g. lgG1 , lgG2, lgG3, lgG4, lgA1 and lgA2) or subclass, or allotype (e.g. human G1 m1 , G1 m2, G1 m3, non-G1 m1 [that, is any allotype other than G1 m1], G1 m17, G2m23, G3m21 , G3m28, G3m1 1 , G3m5, G3m13, G3m14, G3m10, G3m15, G3m16, G3m6, G3m24, G3m26, G3m27, A2m1 , A2m2, Km1 , Km2 and Km3) of immunoglobulin molecule. The immunoglobulins can be derived from any species, including human, murine, or rabbit origin.

As used herein, “binds an epitope” is used to mean the antibody binds an epitope with a higher affinity than a non-specific partner such as Bovine Serum Albumin (BSA, Genbank accession no. CAA76847, version no. CAA76847.1 Gl:3336842, record update date: Jan 7, 201 1 02:30 PM). In some embodiments the antibody binds an epitope with an association constant (Ka) at least 2, 3, 4, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10^4, 10^5 or 10^6-fold higher than the antibody's association constant for BSA, when measured at physiological conditions.

The term “antibody fragment” refers to a portion of a full length antibody, for example, the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, and scFv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), single-chain antibody molecules; and multispecific antibodies formed from antibody fragments and epitope-binding fragments of any of the above which immunospecifically bind to target antigens, such as, for example, cancer cell antigens, viral antigens or microbial antigens,. The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant or epitope on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al (1975) Nature 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597 or from transgenic mice carrying a fully human immunoglobulin system (Lonberg (2008) Curr. Opinion 20(4):450-459).

The antibodies, including monoclonal antibodies, herein specifically include “chimeric” antibodies in which a portion of the antibody structure, for example the heavy and/or light chain, is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4816567; and Morrison et al (1984) Proc. Natl. Acad. Sci. USA, 81 :6851 -6855). Chimeric antibodies include “primatized” antibodies comprising variable domain antigen-binding sequences derived from a non- human primate (e.g. Old World Monkey or Ape) and human constant region sequences. An “intact antibody” herein is one comprising VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1 , CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more “effector functions” which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1 q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

The antibodies disclosed herein may be modified. For example, to make them less immunogenic to a human subject. This may be achieved using any of a number of techniques familiar to the person skilled in the art, such as humanisation.

<u>Administration & Dose</u>

Compounds of formula I may be administered alone or in combination with one or another or with one or more pharmacologically active compounds which are different from the compounds of formula I.

Compounds of the invention may suitably be combined with various components to produce compositions of the invention. Suitably the compositions are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition (which may be for human or animal use). Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. Useful pharmaceutical compositions and methods for their preparation may be found in standard pharmaceutical texts. See, for example, *Handbook for Pharmaceutical Additives*, 3rd Edition (eds. M. Ash and I. Ash), 2007 (Synapse Information Resources, Inc., Endicott, New York, USA) and *Remington: The Science and Practice of Pharmacy*, 21st Edition (ed. D. B. Troy) 2006 (Lippincott, Williams and Wilkins, Philadelphia, USA) which are incorporated herein by reference.

The compounds of the invention may be administered by any suitable route. Suitably the compounds of the invention will normally be administered orally or by any parenteral route, in the form of pharmaceutical preparations comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form.

The compounds of the invention, their pharmaceutically acceptable salts, and pharmaceutically acceptable solvates of either entity can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the invention or salts or solvates thereof can be administered orally, buccally or sublingually in the form of tablets, capsules (including soft gel capsules), ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, sustained-, controlled-release or pulsatile delivery applications. The compounds of the invention may also be administered via fast dispersing or fast dissolving dosages forms.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose

(HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

Modified release and pulsatile release dosage forms may contain excipients such as those detailed for immediate release dosage forms together with additional excipients that act as release rate modifiers, these being coated on and/or included in the body of the device. Release rate modifiers include, but are not exclusively limited to, hydroxypropylmethyl cellulose, methyl cellulose, sodium carboxymethylcellulose, ethyl cellulose, cellulose acetate, polyethylene oxide, Xanthan gum, Carbomer, ammonio methacrylate copolymer, hydrogenated castor oil, carnauba wax, paraffin wax, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, methacrylic acid copolymer and mixtures thereof. Modified release and pulsatile release dosage forms may contain one or a combination of release rate modifying excipients. Release rate modifying excipients maybe present both within the dosage form i.e. within the matrix, and/or on the dosage form i.e. upon the surface or coating.

Fast dispersing or dissolving dosage formulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium, crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol, xylitol.

The compounds of the invention can also be administered parenterally, for example, intravenously, intra-arterially, or they may be administered by infusion techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Suitably formulation of the invention is optimised for the route of administration e.g. oral, intravenously, etc.

Administration may be in one dose, continuously or intermittently (e.g. in divided doses at appropriate intervals) during the course of treatment. Methods of determining the most effective means and dosage are well known to a skilled person and will vary with the formulation used for therapy, the purpose of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and the dose regimen being selected by the treating physician, veterinarian, or clinician.

Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses. For example, a typical dosage for an adult human may be 100 ng to 25 mg (suitably about 1 micro g to about 10 mg) per kg body weight of the subject per day.

Suitably guidance may be taken from studies in test animals when estimating an initial dose for human subjects. For example when a particular dose is identified for mice, suitably an initial test dose for humans may be approx. 0.5x to 2x the mg/Kg value given to mice.

Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms.

Isomers, Salts and Solvates

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l- forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; alpha- and beta-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$.

A reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not apply to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol, imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof.

Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

In some embodiments, the compound of formula (I) and salts and solvates thereof, comprises pharmaceutically acceptable salts of the compounds of formula (I).

Compounds of Formula (I), which include compounds specifically named above, may form pharmaceutically acceptable complexes, salts, solvates and hydrates. These salts include nontoxic acid addition salts (including di-acids) and base salts.

If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then an acid addition salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids hydrochloric acid, nitric acid, nitrous acid, phosphoric acid, sulfuric acid, sulphurous acid, hydrobromic acid, hydroiodic acid, hydrofluoric acid, phosphoric acid and phosphorous acids. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose. Such salts include acetate, adipate, aspartate, benzoate, besylate, bicarbonate, carbonate, bisulfate, sulfate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulfonate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate, hydrogen phosphate, dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate and xinofoate salts.

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be $-COO^-$), then a base salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, metal cations, such as an alkali or alkaline earth metal cation, ammonium and substituted ammonium cations, as well as amines. Examples of suitable metal cations include sodium (Na^+) potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), zinc (Zn^{2+}), and aluminum (Al^{3+}). Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. $NH4^+$) and substituted ammonium ions (e.g. NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$. Examples of suitable amines include arginine, N,N'-dibenzylethylene-diamine, chloroprocaine, choline, diethylamine, diethanolamine, dicyclohexylamine, ethylenediamine, glycine, lysine, N-methylglucamine, olamine, 2-amino-2-hydroxymethyl-propane-1,3-diol, and procaine. For a discussion of useful acid addition and base salts, see S. M. Berge et al., *J. Pharm. Sci.* (1977) 66:1-19; see also Stahl and Wermuth, *Handbook of Pharmaceutical Salts: Properties, Selection, and Use* (2011)

Pharmaceutically acceptable salts may be prepared using various methods. For example, one may react a compound of Formula 1 with an appropriate acid or base to give the desired salt. One may also react a precursor of the compound of Formula 1 with an acid or base to remove an acid- or base-labile protecting group or to open a lactone or lactam group of the precursor. Additionally, one may convert a salt of the compound of Formula 1 to another salt through treatment with an appropriate acid or base or through contact with an ion exchange resin. Following reaction, one may then isolate the salt by filtration if it precipitates from solution, or by evaporation to recover the salt. The degree of ionization of the salt may vary from completely ionized to almost non-ionized.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term “solvate” describes a molecular complex comprising the compound and one or more pharmaceutically acceptable solvent molecules (e.g., EtOH). The term “hydrate” is a solvate in which the solvent is water. Pharmaceutically acceptable solvates include those in which the solvent may be isotopically substituted (e.g., D_2O, acetone-d6, DMSO-d6).

A currently accepted classification system for solvates and hydrates of organic compounds is one that distinguishes between isolated site, channel, and metal-ion coordinated solvates and hydrates. See, e.g., K. R. Morris (H. G. Brittain ed.) Polymorphism in Pharmaceutical Solids (1995). Isolated site solvates and hydrates are ones in which the solvent (e.g., water) molecules are isolated from direct contact with each other by intervening molecules of the organic compound. In channel solvates, the solvent molecules lie in lattice channels where they are next to other solvent molecules. In metal-ion coordinated solvates, the solvent molecules are bonded to the metal ion.

When the solvent or water is tightly bound, the complex will have a well-defined stoichiometry independent of humidity. When, however, the solvent or water is weakly bound, as in channel solvates and in hygroscopic compounds, the water or solvent content will depend on humidity and drying conditions.v In such cases, non-stoichiometry will typically be observed.

Compounds of formula I include imine, carbinolamine and carbinolamine ether forms of the PDD. The carbinolamine or the carbinolamine ether is formed when a nucleophilic solvent (H_2O, ROH) adds across the imine bond of the PDD moiety. The balance of these equilibria between these forms depend on the conditions in which the compounds are found, as well as the nature of the moiety itself.

These compounds may be isolated in solid form, for example, by lyophilisation.

Further particular and preferred aspects are set out in the accompanying independent and dependent claims. Features of the dependent claims may be combined with features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

SYNTHETIC STRATEGIES

The compounds of Formula (I) may be prepared using the techniques described below. Some of the schemes and examples may omit details of common reactions, including oxidations, reductions, and so on, separation techniques (extraction, evaporation, precipitation, chromatography, filtration, trituration, crystallization, and the like), and analytical procedures, which are known to persons of ordinary skill in the art of organic chemistry. The details of such reactions and techniques can be found in a number of treatises, including Richard Larock, *Comprehensive Organic Transformations, A Guide to Functional Group Preparations*, 2nd Ed (2010), and the multi-volume series edited by Michael B. Smith and others, *Compendium of Organic Synthetic Methods* (1974 et seq.). Starting materials and reagents may be obtained from commercial sources or may be prepared using literature methods. Some of the reaction schemes may omit minor products resulting from chemical transformations (e.g., an alcohol from the hydrolysis of an ester, CO_2 from the decarboxylation of a diacid, etc.). In addition, in some instances, reaction intermediates may be used in subsequent steps without isolation or purification (i.e., *in situ*).

In some of the reaction schemes and examples below, certain compounds can be prepared using protecting groups, which prevent undesirable chemical reaction at otherwise reactive sites. Protecting groups may also be used to enhance solubility or otherwise modify physical properties of a compound. For a discussion of protecting group strategies, a description of materials and methods for installing and removing protecting groups, and a compilation of useful protecting groups for common functional groups, including amines, carboxylic acids, alcohols, ketones, aldehydes, and so on, see T. W. Greene and P. G. Wuts, *Protecting Groups in Organic Chemistry*, 4th Edition, (2006) and P. Kocienski, *Protective Groups*, 3rd Edition (2005).

Generally, the chemical transformations described throughout the specification may be carried out using substantially stoichiometric amounts of reactants, though certain reactions may benefit from using an excess of one or more of the reactants. Additionally, many of the reactions disclosed throughout the specification may be carried out at about room temperature (RT) and ambient pressure, but depending on reaction kinetics, yields, and so on, some reactions may be run at elevated pressures or employ higher temperatures (e.g., reflux conditions) or lower temperatures (e.g., -78°C. to 0°C.). Any reference in the disclosure to a stoichiometric range, a temperature range, a pH range, etc., whether or not expressly using the word "range," also includes the indicated endpoints.

Many of the chemical transformations may also employ one or more compatible solvents, which may influence the reaction rate and yield. Depending on the nature of the reactants, the one or more solvents may be polar protic solvents (including water), polar aprotic solvents, non-polar solvents, or some combination. Representative solvents include saturated aliphatic hydrocarbons (e.g., n-pentane, n-hexane, n-heptane, n-octane); aromatic hydrocarbons (e.g., benzene, toluene, xylenes); halogenated hydrocarbons (e.g., methylene chloride, chloroform, carbon tetrachloride); aliphatic alcohols (e.g., methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, 2-

methyl-propan-1-ol, butan-2-ol, 2-methyl-propan-2-ol, pentan-1-ol, 3-methyl-butan-1-ol, hexan-1-ol, 2-methoxy-ethanol, 2-ethoxy-ethanol, 2-butoxy-ethanol, 2-(2-methoxy-ethoxy)-ethanol, 2-(2-ethoxy-ethoxy)-ethanol, 2-(2-butoxy-ethoxy)-ethanol); ethers (e.g., diethyl ether, di-isopropyl ether, dibutyl ether, 1,2-dimethoxy-ethane, 1,2-diethoxy-ethane, 1-methoxy-2-(2-methoxy-ethoxy)-ethane, 1-ethoxy-2-(2-ethoxy-ethoxy)-ethane, tetrahydrofuran, 1,4-dioxane); ketones (e.g., acetone, methyl ethyl ketone); esters (methyl acetate, ethyl acetate); nitrogen-containing solvents (e.g., formamide, N,N-dimethylformamide, acetonitrile, N-methyl-pyrrolidone, pyridine, quinoline, nitrobenzene); sulfur-containing solvents (e.g., carbon disulfide, dimethyl sulfoxide, tetrahydro-thiophene-1,1,-dioxide); and phosphorus-containing solvents (e.g., hexamethylphosphoric triamide).

Further particular and preferred aspects are set out in the accompanying independent and dependent claims. Features of the dependent claims may be combined with features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will now be described further, with reference to the accompanying drawings, in which:

Figure 1 shows a HPLC chromatogram that provides evidence of DNA adduct formation with NFκB transcription factor binding sequence with C8-linked PDD monomer **13**;
Figure 2 shows a HPLC chromatogram that provides evidence of DNA adduct formation with NFκB transcription factor binding sequence with C8-linked PDD monomer **17**;
Figure 3 shows a HPLC chromatogram that provides evidence of DNA adduct formation with NFκB transcription factor binding sequence with C8-linked PDD monomer **20**;
Figure 4 shows a HPLC chromatogram that provides evidence of DNA adduct formation with NFκB transcription factor binding sequence with C8-linked PDD monomer **24**.

EXAMPLES

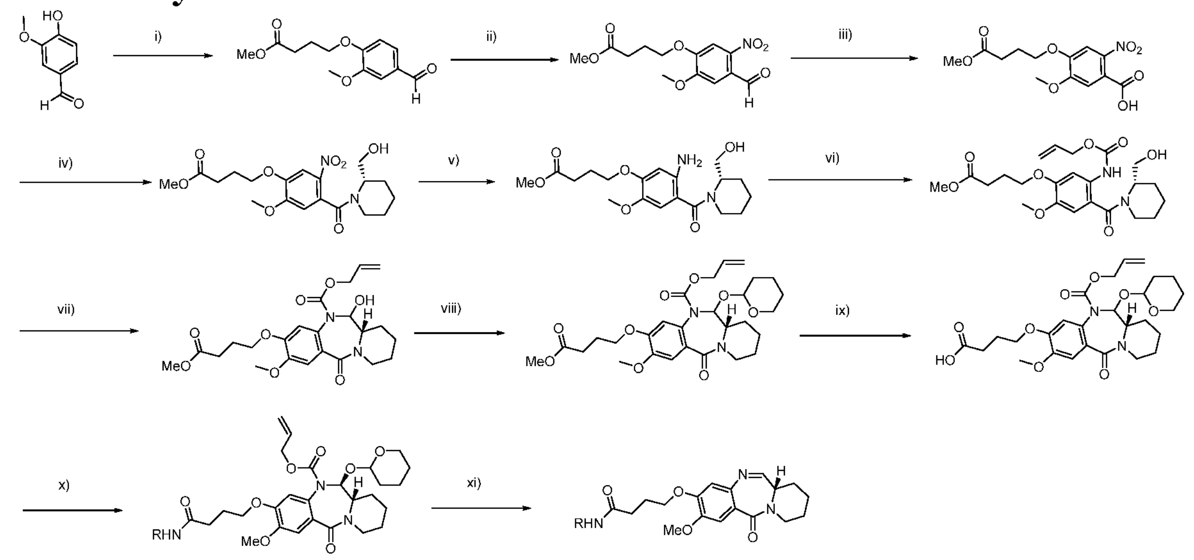
General remarks

Synthetic building blocks and reagents were purchased from Maybridge Chemicals (UK), Fluorochem (USA), ChemShuttle Inc (USA) and Sigma-Aldrich (UK). Solvents were purchased from Sigma-Aldrich (UK) and Fisher Scientific (UK). Anhydrous reactions were carried out in pre-oven-dried glassware under an inert atmosphere of nitrogen. Anhydrous solvents were used as purchased without further drying. Thin Layer Chromatography (TLC) was performed on silica gel aluminium plates (Merck 60, F_{254}), and column chromatography was carried out either manually using silica gel (Merck 9385, 230-400 mesh ASTM, 40-63 μM) (whilst monitoring by thin layer chromatography: UV (254 nm) and an aqueous alkaline solution of potassium permanganate as stain), or using a Grace Reveleris® X2 automated Flash Chromatography System. All NMR spectra were obtained at room temperature using a Bruker DPX400 spectrometer, for which chemical shifts are expressed in ppm relative to the solvent and coupling constants are expressed in Hz. All Liquid Chromatography Mass Spectroscopy (LCMS) analysis was performed on a Waters Alliance 2695 with water (A) and acetonitrile (B) comprising the mobile phases. Formic acid (0.1%) was added to the acetonitrile to ensure acidic conditions throughout the analysis. Function type: Diode array (535 scans). Column type: Monolithic C18 50 X 4.60 mm. Mass spectrometry data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 HPLC with a Waters 2996 PDA. Waters Micromass ZQ parameters used were: Capillary (kV), 3.38; Cone (V), 35; Extractor (V), 3.0; Source temperature (°C), 100; Desolvation Temperature (°C), 200; Cone flow rate (L/h), 50; De-solvation flow rate (L/h), 250. Microwave reactions were carried out on an Anton Paar Monowave 300 microwave synthesis reactor. Yields refer to isolated material (homogeneous by TLC or NMR) unless otherwise stated and names are assigned according to IUPAC nomenclature. LCMS gradient conditions are described as follows.

Method A (10 min): from 95% A/5% B to 50% B over 3 min. Then from 50% B to 80% B over 2 min. Then from 80% B to 95% B over 1.5 min and held constant for 1.5 min. This was then reduced to 5% B over 0.2 min and maintained to 5% B for 1.8 min. The flow rate was 0.5 mL/min, 200 μL was split via a zero dead volume T piece which passed into the mass spectrometer. The wavelength range of the UV detector was 220-400 nm.

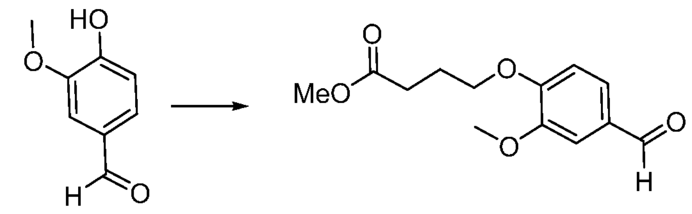
Method B (5 min): from 95% A/5% B to 90% B over 3 min. Then from 90% B to 95% B over 0.5 min and held constant for 1 min. This was then reduced to 5% B over 0.5 min. The flow rate was 1.0 mL/min, 100 μL was split via a zero dead volume T piece which passed into the mass spectrometer. The wavelength range of the UV detector was 220-500 nm.

General synthetic scheme



i) K_2CO_3, DMF, methyl-4-bromobutyrate, r.t.; ii) KNO_3, TFA, 0 - 5 °C; iii) $KMnO_4$, acetone, H_2O, reflux; iv) Oxalyl chloride, (*S*)-piperidin-2-ylmethanol, DMF cat., Et_3N, CH_2Cl_2, 0 °C - r.t.; v) H_2, Pd/C, EtOH / EtOAc; vi) Allylchloroformate, pyridine, CH_2Cl_2, - 10 °C - r.t.; vii) TEMPO, BAIB, CH_2Cl, r.t.; viii) *p*TSA, DHP, EtOAc, r.t.; ix) NaOH, dioxane, H_2O, r.t.; x) RNH_2, EDCI, DMAP, DMF, r.t.; xi) PPh_3, $Pd(PPh_3)_4$, pyrrolidine, CH_2Cl_2, r.t.

Example 1: Methyl 4-(4-formyl-2-methoxyphenoxy)butanoate (1)

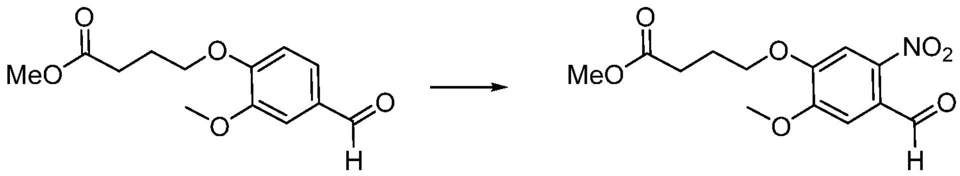


1

A mixture of vanillin (20.0 g, 131 mmol), methyl 4-bromobutanoate (17.5 mL, 139 mmol) and potassium carbonate (27.2 g, 197 mmol) in *N,N*-dimethylformamide (100 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with water (500 mL) and the title compound (30.2 g, 91%) was obtained by filtration as a white solid. The product was carried through to the next step without any further purification.

^1H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.46-7.37 (m, 2H), 6.98 (d, J=8.2 Hz, 1H), 4.16 (t, J=6.3 Hz, 2H), 3.91 (s, 3H), 3.69 (s, 3H), 2.56 (t, J=7.2 Hz, 2H), 2.20 (quin, J=6.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9, 173.4, 153.8, 149.9, 130.1, 126.8, 111.6, 109.2, 67.8, 56.0, 51.7, 30.3, 24.2; MS m/z (EIMS) = 271.9 $(M+Na)^+$; LCMS (**Method A**): t_R = 6.48 min.

Example 2: Methyl 4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoate (2)

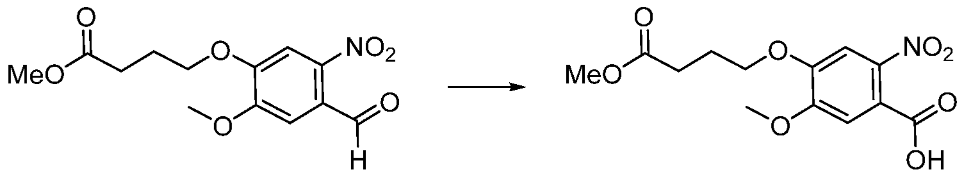


1 **2**

To a stirring solution of potassium nitrate (10.0 g, 98.9 mmol) in TFA (50 mL) at 0 °C was added dropwise a solution of methyl 4-(4-formyl-2-methoxyphenoxy)butanoate (**1**) (20.0 g, 79.2 mmol) in TFA (50 mL). The reaction mixture was stirred at room temperature for 1 h. It was then concentrated *in vacuo* and diluted with ethyl acetate (400 mL). The organic layer was washed with brine (3 x 100 mL) and a saturated aqueous solution of sodium hydrogen carbonate (2 x 80 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (23.5 g, 100%) as a yellow solid. The product was carried through to the next step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.60 (s, 1H), 7.39 (s, 1H), 4.21 (t, J=6.3 Hz, 2H), 3.98 (s, 3H), 3.70 (s, 3H), 2.61-2.53 (m, 2H), 2.22 (quin, J=6.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.8, 173.2, 153.5, 151.7, 143.8, 125.5, 109.9, 108.1, 68.6, 56.6, 51.8, 30.2, 24.1; MS m/z (EIMS) = 296.1 $(M-H)^-$; LCMS (**Method A**): t_R = 6.97 min.

Example 3: 5-Methoxy-4-(4-methoxy-4-oxobutoxy)-2-nitrobenzoic acid (3)

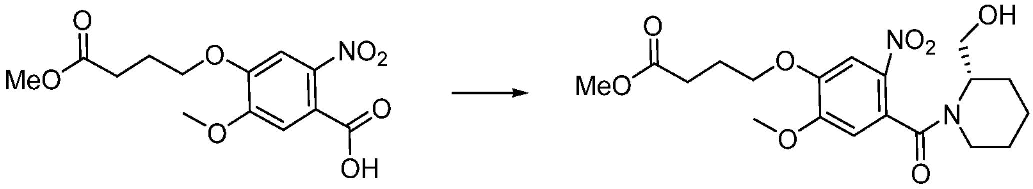


2 **3**

To a solution of methyl 4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoate (**2**) (23.0 g, 77.4 mmol) in acetone (600 mL) was quickly added a hot (70 °C) solution of potassium permanganate (46.0 g, 291 mmol) in water (400 mL). The reaction mixture was stirred at 70 °C for 3 h. The reaction mixture was cooled to room temperature and passed through celite. The cake of celite was washed with hot water (200 mL). A solution of sodium bisulfite in HCl (200 mL) was added to the filtrate which was extracted with dichloromethane (2 x 400 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 50%), to give the title compound (17.0 g, 70%) as a pale yellow solid.

^{1}H NMR (400 MHz, MeOD) δ 7.47 (s, 1H), 7.25 (s, 1H), 4.13 (t, J=6.2 Hz, 2H), 3.94 (s, 3H), 3.68 (s, 3H), 2.54 (t, J=7.2 Hz, 2H), 2.17-2.06 (m, 2H); ^{13}C NMR (100 MHz, MeOD) δ 175.3, 168.6, 153.8, 151.3, 143.1, 122.8, 112.4, 109.2, 69.6, 57.0, 52.2, 31.2, 25.5; MS m/z (EIMS) = 311.9 $(M-H)^-$; LCMS (**Method A**): t_R = 6.22 min.

Example 4: Methyl (*S*)-4-(4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)butanoate (4)

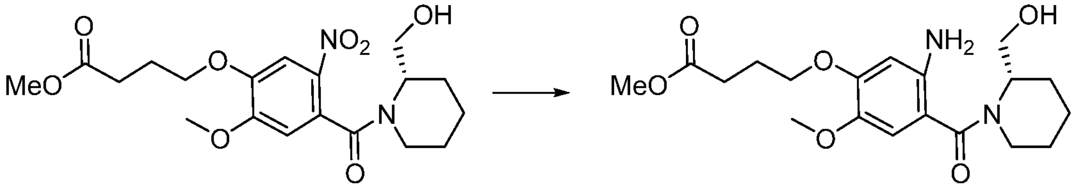


3 **4**

A mixture of 5-methoxy-4-(4-methoxy-4-oxobutoxy)-2-nitrobenzoic acid (**3**) (8.0 g, 25.5 mmol), oxalyl chloride (6.6 mL, 77.0 mmol) and anhydrous *N*,*N*-dimethl-formamide (2 drops) in anhydrous dichloromethane (100 mL) was stirred at room temperature for 1 h. Anhydrous toluene (20 mL) was added to the reaction mixture which was then concentrated *in vacuo*. A solution of the resulting residue in anhydrous dichloromethane (10 mL) was added dropwise to a solution of (*S*)-piperidin-2-ylmethanol (3.8 g, 33.4 mmol) and triethylamine (10.7 mL, 77.0 mmol) in anhydrous dichloromethane (90 mL) at – 10 °C. The reaction mixture was stirred at room temperature for 2 h and then washed with hydrochloric acid (1 M, 50 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with methanol/ dichloromethane (from 0% to 5%), to give the title compound (9.2 g, 73%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.64 (m, 1H), 6.77-6.70 (m, 1H), 4.16-4.07 (m, 3H), 3.93-3.89 (m, 3H), 3.83 (s, 1H), 3.67 (s, 3H), 3.15 (d, *J*=1.4 Hz, 1H), 3.11 (s, 1H), 2.78 (s, 1H), 2.56-2.50 (m, 3H), 2.21-2.12 (m, 4H), 1.74-1.55 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 168.1, 154.6, 148.2, 137.4, 127.6, 111.4, 108.3, 68.3, 60.6, 56.7, 53.5, 51.7, 43.3, 38.0, 34.9, 30.3, 24.1, 19.7; MS m/z (EIMS) = 411.0 $(M+H)^+$; LCMS (**Method A**): t_R = 6.28 min.

Example 5: Methyl (*S*)-4-(5-amino-4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxyphenoxy)butanoate (5)

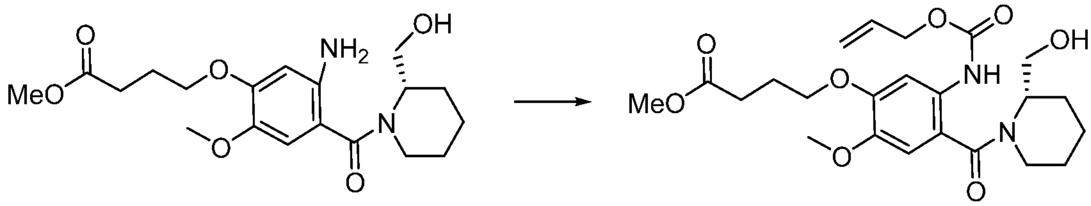


4 **5**

To a solution of methyl (*S*)-4-(4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)butanoate (**4**) (9.2 g, 22.4 mmol) in ethanol (40 mL) and ethyl acetate (10 mL) was added palladium on activated charcoal (10% wt. basis) (920 mg). The reaction mixture was hydrogenated at 35 psi for 3 h in a Parr apparatus. The reaction mixture was filtered through celite and the resulting cake was washed with ethyl acetate. The filtrate was concentrated *in vacuo* to give the title compound (9.0 g, 90%) as a pink solid. The product was carried through to the next step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 1H), 6.27-6.18 (m, 1H), 4.03-3.94 (m, 3H), 3.94-3.82 (m, 3H), 3.81-3.76 (m, 1H), 3.74 (s, 3H), 3.73-3.68 (m, 1H), 3.67-3.65 (m, 3H), 3.56 (d, *J*=4.8 Hz, 1H), 3.03 (s, 1H), 2.51 (t, *J*=7.2 Hz, 2H), 2.11 (quin, *J*=6.7 Hz, 2H), 1.68-1.59 (m, 4H), 1.55-1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 171.2, 150.3, 141.8, 141.1, 113.2, 112.3, 102.4, 67.5, 60.8, 60.4, 56.8, 51.6, 30.4, 25.8, 24.3, 21.0, 19.9, 14.2; MS *m/z* (EIMS) = 381.0 (M+H)$^+$; LCMS (**Method A**): t_R = 5.52 min.

<u>Example 6: Methyl (*S*)-4-(5-(((allyloxy)carbonyl)amino)-4-(2-(hydroxylmethyl)piperidine-1-carbonyl)-2-methoxyphenoxy)butanoate (6)</u>



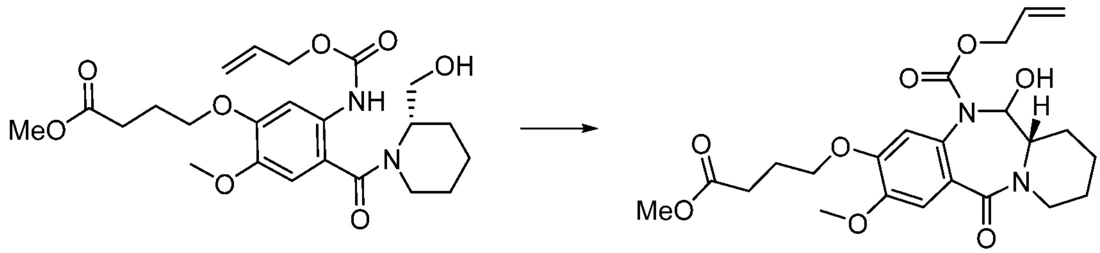
5 **6**

To a solution of methyl (*S*)-4-(5-amino-4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxyphenoxy)butanoate (**5**) (9.0 g, 23.7 mmol) and pyridine (4.4 mL, 54.4 mmol) in anhydrous dichloromethane (100 mL) at – 10 °C was added dropwise a solution of allylchloroformate (2.6 mL, 24.8 mmol) in anhydrous dichloromethane (20 mL). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was sequentially washed with a saturated aqueous solution of copper (II) sulfate (80 mL), water (80 mL) and a saturated aqueous solution of sodium hydrogen carbonate (80 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The resulting residue (2.0 g out of the 11.0 g crude) was purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 1%), to give the title compound (930 mg, 47% based on the amount purified) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (br s, 1H), 7.63 (br s, 1H), 6.76 (br s, 1H), 5.92 (ddt, *J*=17.2, 10.6, 5.4, 5.4 Hz, 1H), 5.37-5.28 (m, 1H), 5.20 (dq, *J*=10.4, 1.3 Hz, 1H), 4.65-

4.56 (m, 2H), 4.06 (t, J=6.2 Hz, 2H), 3.94-3.82 (m, 1H), 3.79 (s, 3H), 3.66 (s, 3H), 3.62-3.54 (m, 1H), 3.40 (br s, 1H), 3.10-2.88 (m, 1H), 2.52 (t, J=7.4 Hz, 2H), 2.22-2.04 (m, 3H), 1.64 (br s, 4H), 1.56-1.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 170.6, 153.9, 149.7, 144.8, 132.6, 130.1, 117.6, 116.9, 110.8, 107.1, 106.0, 67.7, 65.6, 60.7, 56.3, 53.5, 51.6, 43.1, 30.5, 25.7, 24.4, 19.7; MS m/z (EIMS) = 465.1 $(M+H)^+$; LCMS (**Method A**): t_R = 6.47 min.

Example 7: Allyl (6a*S*)-6-hydroxy-2-methoxy-3-(4-methoxy-4-oxobutoxy)-12-oxo-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (7)

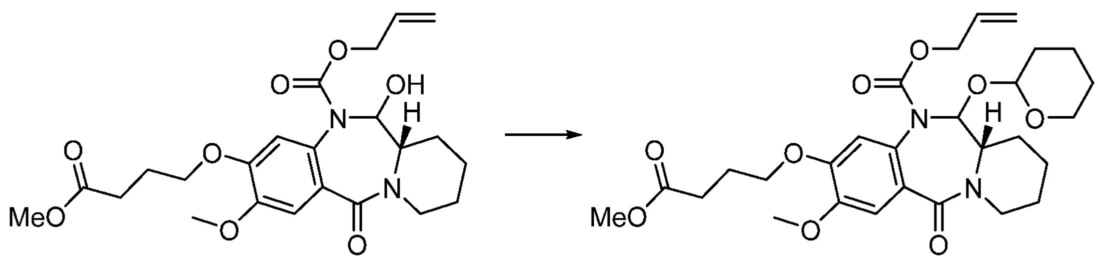


6 7

To a solution of methyl (*S*)-4-(5-(((allyloxy)carbonyl)amino)-4-(2-(hydroxymethyl)-piperidine-1-carbonyl)-2-methoxyphenoxy)butanoate (**6**) (930 mg, 2.0 mmol) in dichloromethane (45 mL) was added TEMPO (32 mg, 0.20 mmol) and (diacetoxyiodo)-benzene (773 mg, 2.4 mmol). The reaction mixture was stirred at room temperature for 16 h, and was then sequentially washed with a saturated aqueous solution of sodium metabisulfite (20 mL), a saturated aqueous solution of sodium hydrogen carbonate (20 mL), water (20 mL) and brine (20 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 5%), to give the title compound (825 mg, 89%) as a cream solid.

MS m/z (EIMS) = 462.9 $(M+H)^+$; LCMS (**Method A**): t_R = 6.30 min.

Example 8: Allyl (6a*S*)-2-methoxy-3-(4-methoxy-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido-[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (8)

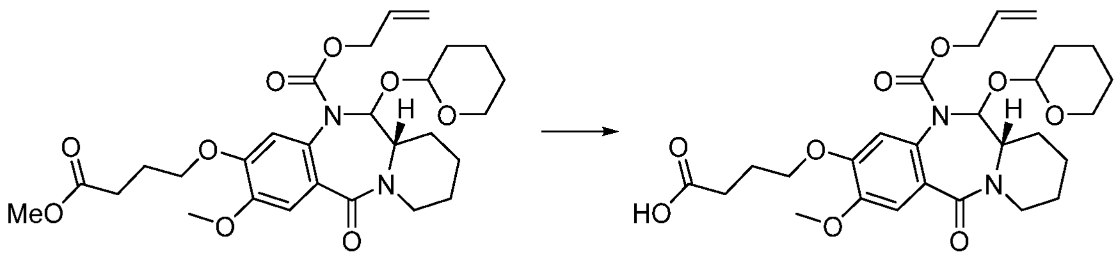


7 **8**

A mixture of allyl (6a*S*)-6-hydroxy-2-methoxy-3-(4-methoxy-4-oxobutoxy)-12-oxo-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (7) (825 mg, 1.8 mmol), 3,4-dihydro-2*H*-pyran (1.7 mL, 18.2 mmol) and *p*TSA (8.5 mg, 1% w/w) in ethyl acetate (12 mL) was stirred at room temperature for 16 h. The reaction mixture was then diluted with ethyl acetate (50 mL) and washed with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and brine (30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with methanol/ dichloromethane (from 0% to 2%), to give the title compound (820 mg, 84%) as a cream solid.

MS m/z (EIMS) = 546.7 $(M+H)^+$; LCMS (**Method A**): t_R = 7.70 min.

Example 9: 4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetra-hydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (9)

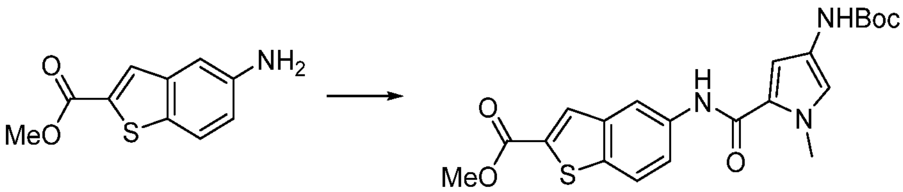


8 **9**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-methoxy-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**8**) (770 mg, 1.4 mmol) in 1,4-dioxane (10 mL) was added a 0.5 M aqueous solution of sodium hydroxide (10 mL, 5.0 mmol). The reaction mixture was stirred at room temperature for 2 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with a 1 M citric acid solution (5 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (700 mg, 93%) as a yellow oil. The product was carried through to the next step without any further purification.

MS m/z (EIMS) = 532.9 $(M+H)^+$; LCMS (**Method A**): t_R = 6.98 min.

Example 10: Methyl 5-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[*b*]thiophene-2-carboxylate (10)

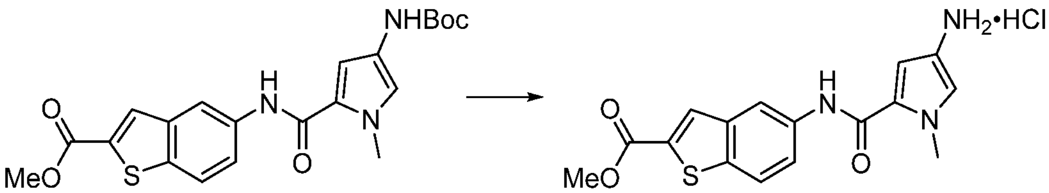


10

A solution of 4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxylic acid (500 mg, 2.1 mmol) in *N,N*-dimethylformamide (10 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (725 mg, 3.8 mmol) and 4-(dimethylamino)pyridine (577 mg, 4.7 mmol). The reaction mixture was stirred at room temperature for 2 h. Methyl 5-aminobenzo[*b*]thiophene-2-carboxylate (392 mg, 1.9 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (20 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were sequentially washed with 1 M citric acid (30 mL), a saturated aqueous solution of sodium hydrogen carbonate (35 mL), water (35 mL) and brine (35 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with ethyl acetate/hexane (from 0% to 50%), to give the title compound (610 mg, 75%) as a beige solid.

MS *m/z* (EIMS) = 430.2 $(M+H)^+$; LCMS (**Method A**): t_R = 7.90 min.

Example 11: Methyl 5-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)-benzo[*b*]thiophene-2-carboxylate hydrochloride (11)

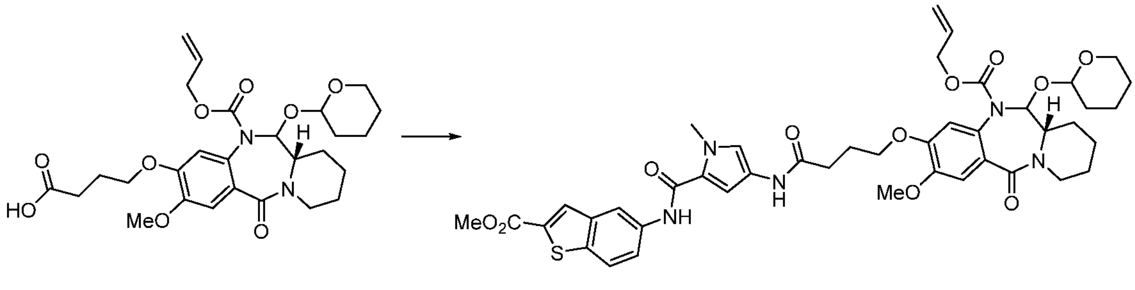


10 **11**

Methyl 5-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo-[*b*]thiophene-2-carboxylate (**10**) (610 mg, 1.4 mmol) was dissolved in hydrochloric acid (4 M in dioxane) (3.6 mL) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to give the title compound (600 mg, 99%) as a brown solid. The product was carried through to the next step without any further purification.

MS *m/z* (EIMS) = 329.9 $(M+H)^+$; LCMS (**Method A**): t_R = 5.52 min.

Example 12: Allyl (6a*S*)-2-methoxy-3-(4-((5-((2-(methoxycarbonyl)benzo-[*b*]thiophen-5-yl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxo-butoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexa-hydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (12)

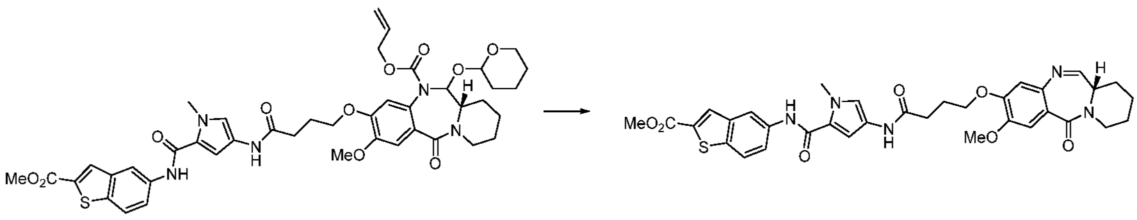


9 **12**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (150 mg, 0.28 mmol) in *N*,*N*-dimethylformamide (4 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (100 mg, 0.52 mmol) and 4-(dimethylamino)pyridine (80 mg, 0.65 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 5-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[*b*]thiophene-2-carboxylate hydrochloride (**11**) (95 mg, 0.26 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (20 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were sequentially washed with 1 M citric acid (30 mL), a saturated aqueous solution of sodium hydrogen carbonate (35 mL), water (35 mL) and brine (35 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (190 mg, 87%) as a yellow oil. The product was carried through to the next step without any further purification.

MS m/z (EIMS) = 844.0 $(M+H)^+$; LCMS (**Method A**): t_R = 8.10 min.

Example 13: Methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexa-hydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[*b*]thiophene-2-carboxylate (13)

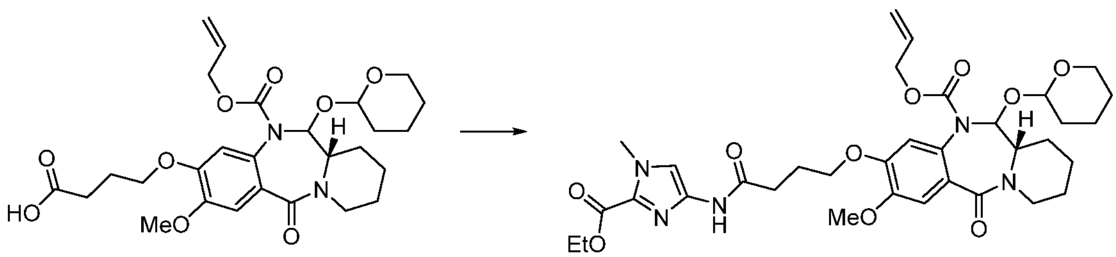


12 **13**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((5-((2-(methoxycarbonyl)benzo[*b*]-thiophen-5-yl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]-diazepine-5(12*H*)-carboxylate (**12**) (190 mg, 0.22 mmol) in dichloromethane (10 mL) was added tetrakis(triphenylphosphine)palladium(0) (13 mg, 5 mol%), triphenyl-phosphine (15 mg, 25 mol%) and pyrrolidine (22 μL, 0.27 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 70%), to give the title compound (60 mg, 40%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (s, 1H), 8.28 (s, 1H), 8.02 (s, 1H), 7.94 (s, 1H), 7.90 (d, *J*=5.7 Hz, 1H), 7.75 (d, *J*=8.8 Hz, 1H), 7.58 (dd, *J*=8.7, 2.1 Hz, 1H), 7.42-7.41 (m, 1H), 7.13 (d, *J*=1.6 Hz, 1H), 6.78 (s, 1H), 6.56 (d, *J*=1.6 Hz, 1H), 4.25-4.18 (m, 1H), 4.08 (t, *J*=6.0 Hz, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.79-3.75 (m, 1H), 3.23-3.16 (m, 1H), 2.52-2.47 (m, 2H), 2.21 (d, *J*=6.4 Hz, 1H), 2.18 (d, *J*=2.1 Hz, 1H), 1.96 (br s, 2H), 1.86-1.81 (m, 2H), 1.77-1.66 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 167.6, 163.4, 163.2, 160.0, 150.7, 148.0, 140.0, 139.2, 137.6, 135.8, 134.2, 130.6, 123.0, 122.9, 121.5, 121.0, 120.1, 116.2, 111.7, 110.3, 104.3, 68.1, 56.1, 53.5, 52.5, 49.7, 40.0, 36.8, 33.0, 24.9, 24.5, 22.9, 18.3; MS *m/z* (EIMS) = 658.0 $(M+H)^+$; LCMS (**Method A**): t_R = 6.92 min.

<u>Example 14: Allyl (6a*S*)-3-(4-((2-(ethoxycarbonyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)-oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (14)</u>

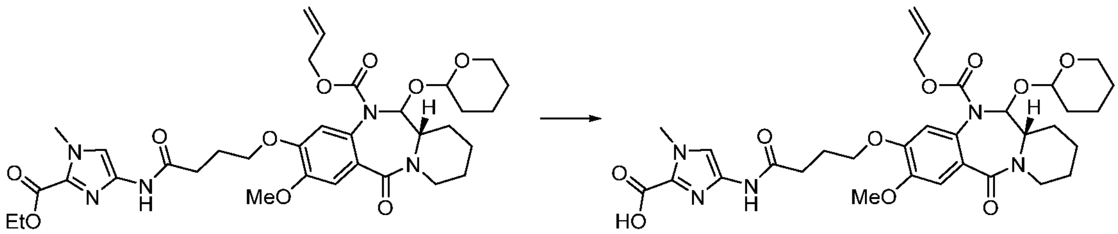


9 **14**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (340 mg, 0.64 mmol) in *N*,*N*-dimethylformamide (10 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (222 mg, 1.2 mmol) and 4-(dimethylamino)pyridine (177 mg, 1.4 mmol). The reaction mixture was stirred at room temperature for 30 min. Ethyl 4-amino-1-methyl-1*H*-imidazole-2-carboxylate hydrochloride (120 mg, 0.58 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (350 mg, 80%) as a yellow oil. The product was carried through to the next step without any further purification.

MS *m/z* (EIMS) = 683.7 $(M+H)^+$; LCMS (**Method A**): t_R = 7.35 min.

Example 15: 4-(4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxylic acid (15)

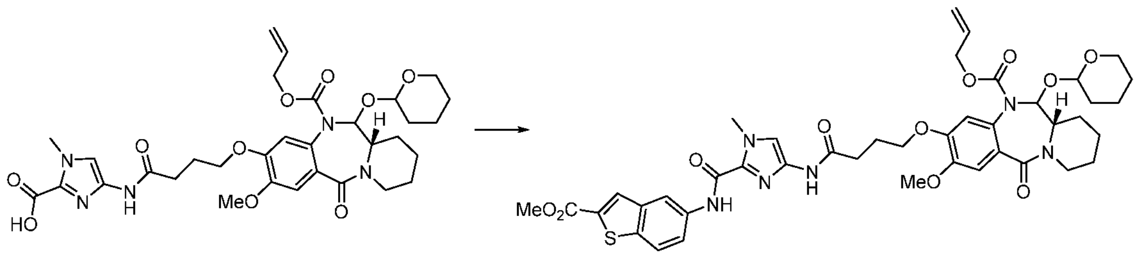


14 **15**

To a solution of allyl (6a*S*)-3-(4-((2-(ethoxycarbonyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobuto xy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**14**) (350 mg, 0.46 mmol) in 1,4-dioxane (10 mL) was added a 0.5 M aqueous solution of sodium hydroxide (10 mL, 5.0 mmol). The reaction mixture was stirred at room temperature for 2 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with a 1 M citric acid solution (10 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated. The resulting residue was triturated in hexane, filtered and dried to give the title compound (220 mg, 74%) as a beige solid. The product was carried through to the next step without any further purification.

MS *m/z* (EIMS) = 656.2 $(M+H)^+$; LCMS (**Method A**): t_R = 6.53 min.

Example 16: Allyl (6a*S*)-2-methoxy-3-(4-((2-((2-(methoxycarbonyl)-benzo[*b*]thiophen-5-yl)carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (16)

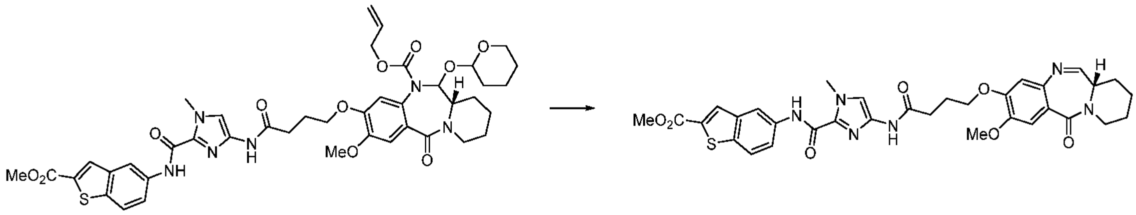


15 **16**

A solution of 4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxylic acid (**15**) (110 mg, 0.17 mmol) in *N*,*N*-dimethylformamide (4 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (59 mg, 0.31 mmol) and 4-(dimethylamino)pyridine (47 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 5-aminobenzo[*b*]thiophene-2-carboxylate (32 mg, 0.15 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was then purified by column chromatography (silica), eluting with ethyl acetate/dichloromethane (0% to 100%), followed by methanol/dichloromethane (from 0% to 10%), to give the title compound (50 mg, 39%) as a yellow oil.

MS *m/z* (EIMS) = 844.9 $(M+H)^+$; LCMS (**Method A**): t_R = 8.22 min.

Example 17: Methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexa-hydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)benzo[*b*]thiophene-2-carboxylate (17)

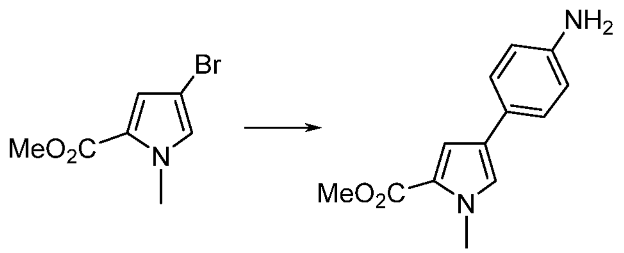


16 **17**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((2-((2-(methoxycarbonyl)benzo[*b*]-thiophen-5-yl)carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]-diazepine-5(12*H*)-carboxylate (**16**) (50 mg, 0.06 mmol) in dichloromethane (3 mL) was added tetrakis(triphenylphosphine)palladium(0) (3.5 mg, 5 mol%), triphenylphosphine (3.9 mg, 25 mol%) and pyrrolidine (5.8 μL, 0.07 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 50%), to give the title compound (10 mg, 26%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 (s, 1H), 8.36 (d, *J*=2.0 Hz, 1H), 8.13 (s, 1H), 8.03 (s, 1H), 7.90 (d, *J*=5.7 Hz, 1H), 7.82 (d, *J*=8.7 Hz, 1H), 7.56 (dd, *J*=8.7, 2.1 Hz, 1H), 7.49-7.43 (m, 2H), 6.81 (s, 1H), 4.26-4.17 (m, 2H), 4.10-4.06 (m, 3H), 3.98-3.93 (m, 6H), 3.93-3.85 (m, 1H), 3.74 (td, *J*=5.8, 4.0 Hz, 1H), 3.27-3.16 (m, 1H), 2.68-2.60 (m, 2H), 2.29 (quin, *J*=6.4 Hz, 2H), 2.10-2.02 (m, 1H), 1.97-1.89 (m, 1H), 1.83-1.77 (m, 2H), 1.76 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 167.5, 163.3, 163.2, 160.3, 156.7, 150.4, 148.0, 140.0, 139.3, 135.8, 135.0, 130.6, 123.2, 120.1, 115.4, 114.9, 110.3, 98.0, 67.8, 65.2, 56.1, 52.6, 49.6, 39.8, 35.9, 32.9, 31.0, 29.3, 24.7, 24.6, 22.9, 18.4; MS *m/z* (EIMS) = 659.1 $(M+H)^+$; LCMS (**Method A**): t_R = 7.00 min.

Example 18: Methyl 4-(4-aminophenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (18)

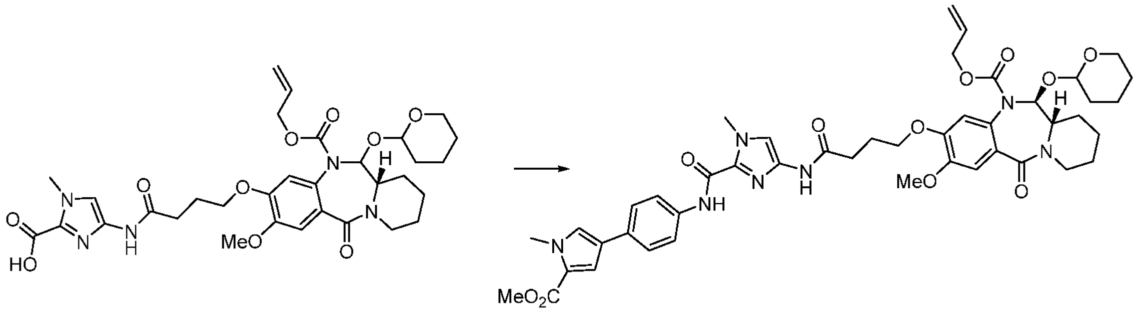


18

A mixture of methyl 4-bromo-1-methyl-1*H*-pyrrole-2-carboxylate (750 mg, 3.44 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (905 mg, 4.13 mmol) and potassium carbonate (1.43 g, 10.3 mmol) in toluene/ethanol/water (9:3:1) (13 mL total) was degassed with nitrogen for 5 mins. Tetrakis(triphenylphosphine)palladium(0) (230 mg, 6 mol%) was then charged and the reaction mixture was irradiated with microwaves at 100 °C for 15 mins. Water (10 mL) was then added to the reaction mixture, which was extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were then dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with ethyl acetate/hexanes (from 0% to 50%), to give the title compound (145 mg, 18%) as a yellow solid.

MS *m/z* (EIMS) = 230.9 $(M+H)^+$; LCMS (**Method A**): t_R = 5.17 min.

Example 19: Allyl (6*S*,6a*S*)-2-methoxy-3-(4-((2-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (19)

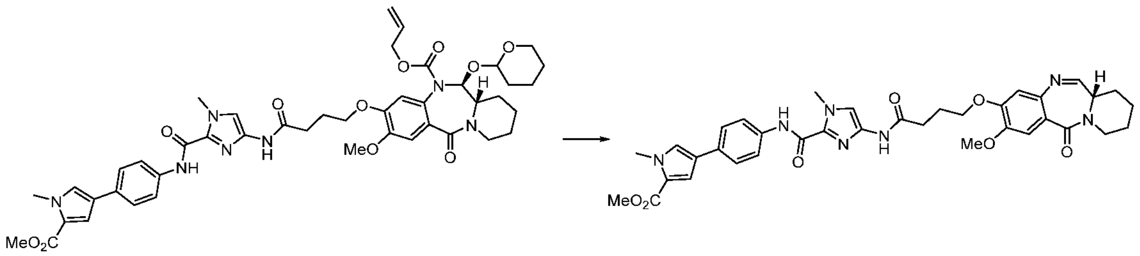


15 **19**

A solution of 4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxylic acid (**15**) (110 mg, 0.17 mmol) in *N*,*N*-dimethylformamide (4 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (59 mg, 0.31 mmol) and 4-(dimethylamino)pyridine (47 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-(4-aminophenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (**18**) (35 mg, 0.15 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (0% to 50%), to give the title compound (54 mg, 37%) as a yellow oil.

MS *m/z* (EIMS) = 868.1 $(M+H)^+$; LCMS (**Method A**): t_R = 8.22 min.

Example 20: Methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (20)

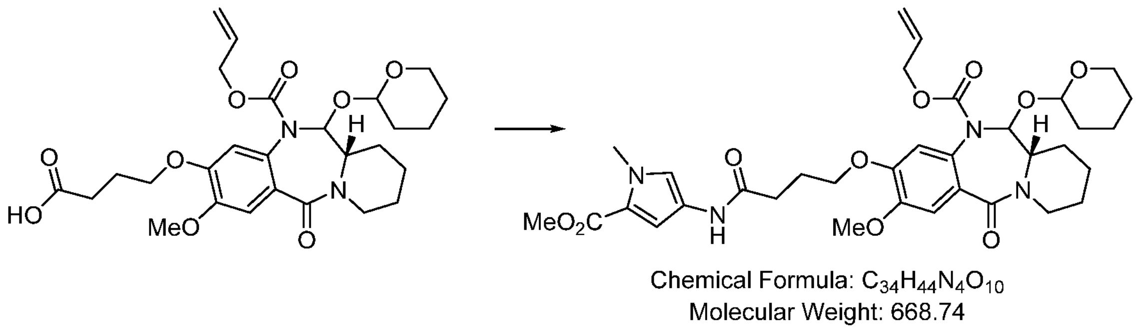


19 **20**

To a solution of allyl (6*S*,6a*S*)-2-methoxy-3-(4-((2-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**19**) (54 mg, 0.06 mmol) in dichloromethane (3 mL) was added tetrakis(triphenylphosphine)palladium(0) (3.6 mg, 5 mol%), triphenylphosphine (4.1 mg, 25 mol%) and pyrrolidine (6.2 μL, 0.07 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 50%), to give the title compound (22 mg, 52%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.95 (s, 1H), 8.27 (s, 1H), 7.89 (d, *J*=5.7 Hz, 1H), 7.59 (d, *J*=8.6 Hz, 2H), 7.47-7.41 (m, 4H), 7.19 (d, *J*=2.0 Hz, 1H), 7.05 (d, *J*=1.9 Hz, 1H), 6.79 (s, 1H), 4.25-4.18 (m, 1H), 4.17-4.12 (m, 1H), 4.12-4.06 (m, 1H), 4.04 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.76-3.71 (m, 1H), 3.26-3.16 (m, 1H), 2.65-2.57 (m, 2H), 2.26 (t, *J*=6.4 Hz, 2H), 2.09-2.01 (m, 2H), 1.96-1.89 (m, 1H), 1.85-1.77 (m, 2H), 1.67 (dd, *J*=10.9, 5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 167.5, 163.3, 161.7, 156.5, 150.4, 148.0, 140.0, 135.8, 135.6, 133.7, 130.6, 126.1, 125.5, 123.1, 122.8, 120.0, 114.6, 111.6, 110.2, 67.8, 56.1, 51.2, 49.6, 39.8, 37.0, 35.8, 32.8, 31.0, 29.7, 24.7, 24.5, 22.9, 18.4; MS *m/z* (EIMS) = 682.1 $(M+H)^+$; LCMS (**Method A**): t_R = 7.03 min.

Example 21: Allyl (6a*S*)-2-methoxy-3-(4-((5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (21)

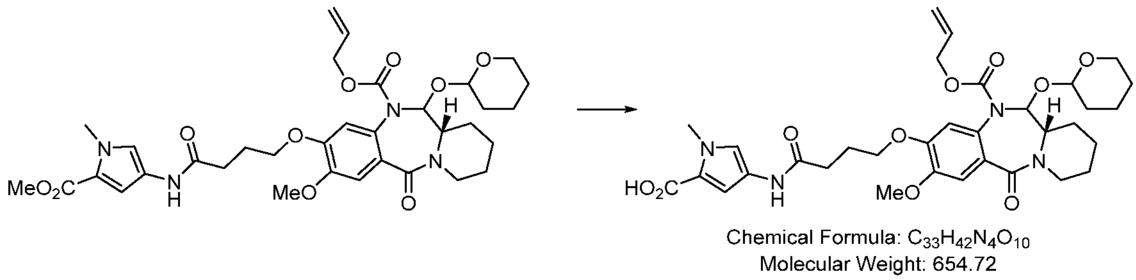


9 **21**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (150 mg, 0.64 mmol) in *N,N*-dimethylformamide (4 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (98 mg, 0.51 mmol) and 4-(dimethylamino)pyridine (79 mg, 0.64 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-amino-1-methyl-1*H*-pyrrole-2-carboxylate hydrochloride (49 mg, 0.26 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (150 mg, 88%) as a yellow oil. The product was carried through to the next step without any further purification.

MS *m/z* (EIMS) = 668.8 $(M+H)^+$; LCMS (**Method A**): t_R = 7.42 min.

<u>Example 22: 4-(4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid (22)</u>



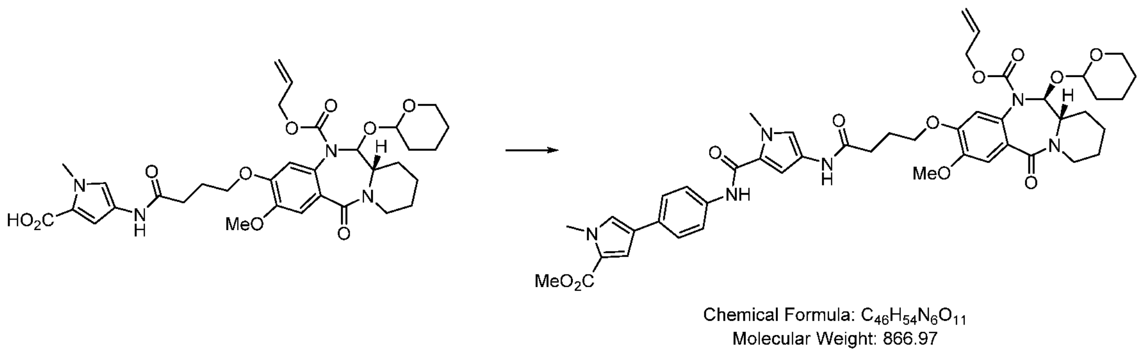
21 **22**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**21**)

(150 mg, 0.22 mmol) in 1,4-dioxane (5 mL) was added a 0.5 M aqueous solution of sodium hydroxide (5 mL, 5.0 mmol). The reaction mixture was stirred at room temperature for 2 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with a 1 M citric acid solution (10 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (140 mg, 90%) as a beige solid. The product was carried through to the next step without any further purification.

MS m/z (EIMS) = 677.0 $(M+Na)^+$; LCMS (**Method A**): t_R = 6.92 min.

Example 23: Allyl (6*S*,6a*S*)-2-methoxy-3-(4-((5-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (23)

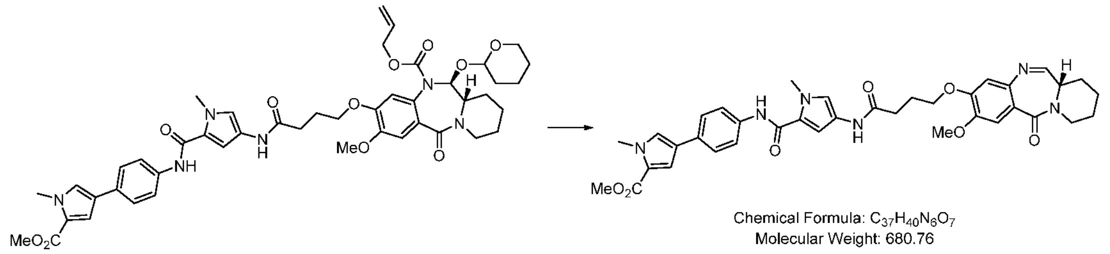


22 **23**

A solution of 4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**22**) (140 mg, 0.21 mmol) in *N,N*-dimethylformamide (4 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (74 mg, 0.39 mmol) and 4-(dimethylamino)pyridine (59 mg, 0.48 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-(4-aminophenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (**18**) (45 mg, 0.19 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (0% to 50%), to give the title compound (160 mg, 95%) as a yellow solid.

MS m/z (EIMS) = 867.0 $(M+H)^+$; LCMS (**Method A**): t_R = 8.10 min.

Example 24: Methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (24)

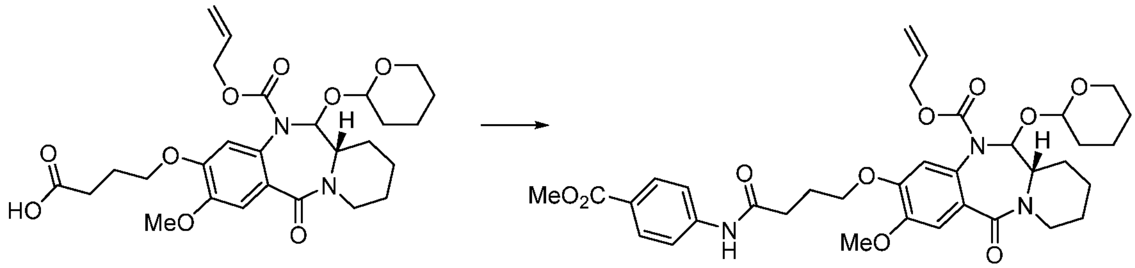


23 **24**

To a solution of allyl (6*S*,6a*S*)-2-methoxy-3-(4-((5-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**23**) (80 mg, 0.09 mmol) in dichloromethane (3 mL) was added tetrakis(triphenylphosphine)palladium(0) (5.3 mg, 5 mol%), triphenylphosphine (6.1 mg, 25 mol%) and pyrrolidine (9.1 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 50%), to give the title compound (23 mg, 37%) as a yellow solid.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.09 (s, 1H), 8.04-8.01 (m, 1H), 7.90 (d, J=5.8 Hz, 1H), 7.58 (s, 1H), 7.56 (s, 1H), 7.44-7.40 (m, 3H), 7.18 (d, J=2.0 Hz, 1H), 7.12 (d, J=1.8 Hz, 1H), 7.04 (d, J=2.0 Hz, 1H), 6.78 (s, 1H), 6.50 (d, J=1.9 Hz, 1H), 4.26-4.18 (m, 1H), 4.07 (t, J=6.0 Hz, 2H), 3.94 (s, 3H), 3.87 (s, 3H), 3.84 (d, J=2.9 Hz, 6H), 3.76 (td, J=5.7, 3.9 Hz, 1H), 3.25-3.15 (m, 1H), 2.49 (t, J=7.0 Hz, 2H), 2.24-2.18 (m, 2H), 2.10-2.03 (m, 1H), 2.01-1.93 (m, 2H), 1.86-1.80 (m, 2H), 1.73-1.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 167.6, 163.5, 161.7, 159.7, 150.7, 147.9, 139.9, 136.4, 130.2, 126.1, 125.4, 123.3, 123.0, 120.6, 119.8, 114.6, 111.7 110.2, 103.9, 68.1, 56.1, 53.8, 51.2, 49.7, 39.9, 37.0, 36.7, 33.0, 31.0, 29.3, 24.9, 24.5, 22.9, 18.4; MS m/z (EIMS) = 681.0 $(M+H)^+$; LCMS (**Method A**): t_R = 6.98 min.

Example 25: Allyl (6a*S*)-2-methoxy-3-(4-((4-(methoxycarbonyl)phenyl)-amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (25)

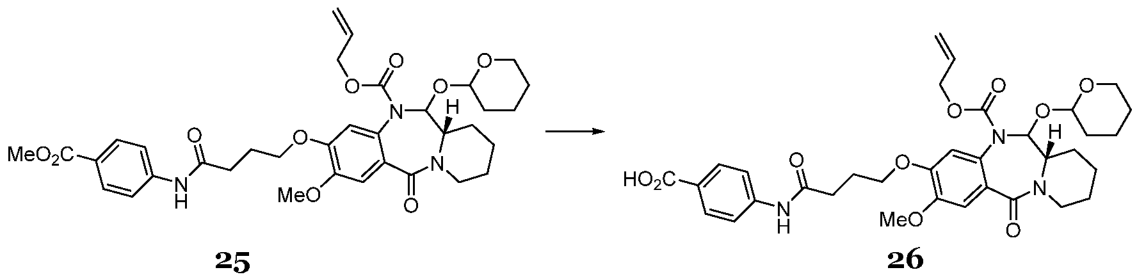


9 **25**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (200 mg, 0.376 mmol) in anhydrous dichloromethane (5 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene] -*N*-methylmethanaminium hexafluorophosphate *N*-oxide (150 mg, 0.394 mmol) and anhydrous triethylamine (220 μL, 1.58 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-aminobenzoate (57.0 mg, 0.376 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (110 mg, 44%) as a yellow solid.

MS (ES+): m/z = 666 $(M+H)^+$; LCMS (**Method A**): t_R = 7.88 min.

Example 26: 4-(4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)benzoic acid (26)

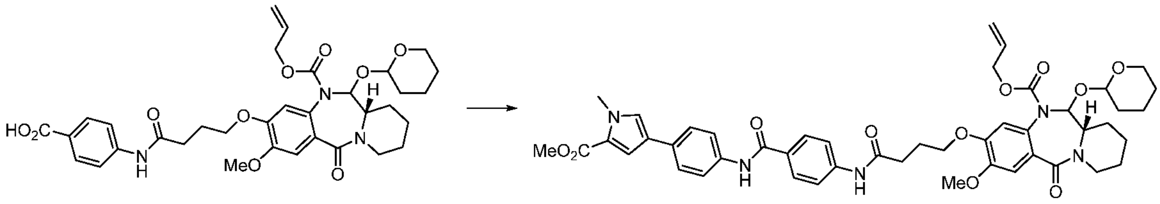


25 **26**

To a solution of allyl (6aS)-2-methoxy-3-(4-((4-(methoxycarbonyl)phenyl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo-[e]pyrido[1,2-a][1,4]diazepine-5(12H)-carboxylate (**25**) (90 mg, 0.14 mmol) in 1,4-dioxane (2.5 mL) was added an aqueous solution of sodium hydroxide (0.5 M, 2.5 mL, 1.3 mmol). The reaction mixture was stirred at room temperature for 16 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with an aqueous solution of citric acid (1 M, 10 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (86 mg, 98%) as a cream solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 652 $(M+H)^+$; LCMS (**Method A**): t_R = 7.13 min.

Example 27: Allyl (6a*S*)-2-methoxy-3-(4-((4-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)phenyl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]-pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (27)

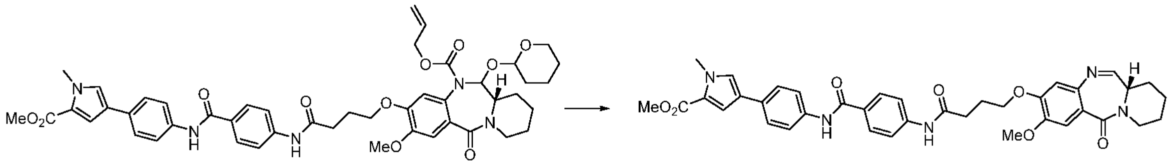


26 **27**

A solution of 4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)benzoic acid (**26**) (40 mg, 0.061 mmol) in anhydrous dichloromethane (1 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (25 mg, 0.064 mmol) and anhydrous triethylamine (36 μL, 0.26 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-(4-aminophenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (**18**) (14 mg, 0.061 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (43 mg, 63%) as a yellow oil.

MS (ES+): m/z = 864 (M+H)+; LCMS (**Method A**): t_R = 8.10 min.

Example 28: Methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (28)

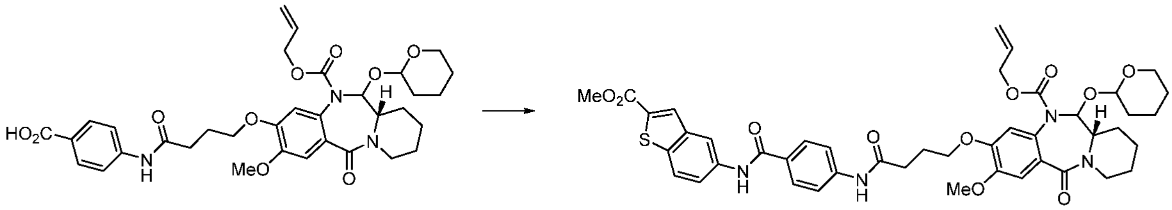


27 **28**

To a solution of allyl (6aS)-2-methoxy-3-(4-((4-((4-(5-(methoxycarbonyl)-1-methyl-1H-pyrrol-3-yl)phenyl)carbamoyl)phenyl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12H)-carboxylate (**27**) (33 mg, 0.038 mmol) in dichloromethane (3 mL) was added tetrakis-(triphenylphosphine)palladium(0) (2.2 mg, 5 mol%), triphenylphosphine (2.5 mg, 25 mol%) and pyrrolidine (4 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%), to give the title compound (5.8 mg, 21%) as a yellow solid.

^{1}H NMR (CDCl$_3$, 400 MHz) δ 8.14 (br s, 1H), 8.06 (br s, 1H), 7.91 (d, J=5.7 Hz, 1H), 7.81 (d, J=8.7 Hz, 2H), 7.65 (d, J=8.6 Hz, 2H), 7.60 (d, J=8.4 Hz, 2H), 7.46-7.50 (m, 2H), 7.41 (s, 1H), 7.21 (d, J=2.1 Hz, 1H), 7.08 (d, J=1.9 Hz, 1H), 6.78-6.82 (m, 1H), 4.24 (d, J=14.0 Hz, 1H), 4.11-4.18 (m, 2H), 3.95-3.98 (m, 3H), 3.83-3.86 (m, 6H), 3.74-3.79 (m, 2H), 3.18-3.30 (m, 2H), 2.60-2.66 (m, 2H), 2.28 (t, J=6.3 Hz, 2H), 1.97 (d, J=6.3 Hz, 2H), 1.82-1.88 (m, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 215.5, 171.1, 167.5, 165.0, 163.4, 161.7, 150.3, 147.8, 141.3, 140.0, 136.2, 130.8, 128.1, 125.6, 123.5, 123.1, 121.5, 120.6, 119.3, 114.7, 111.7, 110.2, 67.9, 56.1, 51.2, 49.7, 39.8, 37.0, 34.3, 30.9, 25.6, 24.5, 23.0, 18.4; MS (ES+): m/z = 678 (M+H)+; LCMS (**Method A**): t_R = 7.05 min.

Example 29: Allyl (6a*S*)-2-methoxy-3-(4-((4-((2-(methoxycarbonyl)benzo-[b]thiophen-5-yl)carbamoyl)phenyl)amino)-4-oxobutoxy)-12-oxo-6-((tetra-hydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a]-[1,4]diazepine-5(12*H*)-carboxylate (29)

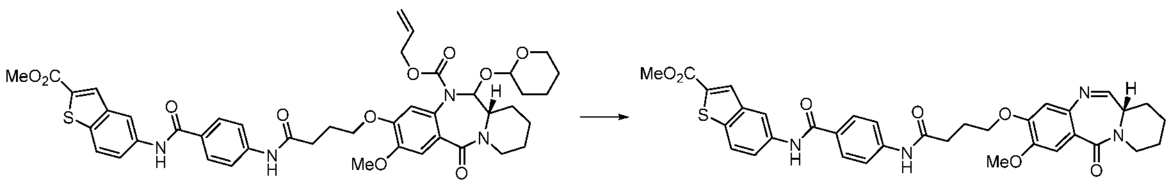


26 **29**

A solution of 4-(4-(((6aS)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)benzoic acid (**26**) (40 mg, 0.061 mmol) in anhydrous dichloromethane (1 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (25 mg, 0.064 mmol) and anhydrous triethylamine (36 μL, 0.26 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 5-aminobenzo[*b*]-thiophene-2-carboxylate (13 mg, 0.063 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (34 mg, 45%) as a brown oil.

MS (ES+): m/z = 841 $(M+H)^+$; LCMS (**Method A**): t_R = 8.15 min.

Example 30: Methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)benzo[b]thiophene-2-carboxylate (30)



29 **30**

To a solution of allyl (6aS)-2-methoxy-3-(4-((4-((2-(methoxycarbonyl)benzo[b]-thiophen-5-yl)carbamoyl)phenyl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12H)-carboxylate (**29**) (23 mg, 0.028 mmol) in dichloromethane (1.5 mL) was added tetrakis(triphenylphosphine)palladium(0) (1.6 mg, 5 mol%), triphenylphosphine (1.8

mg, 25 mol%) and pyrrolidine (3.0 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%) followed by methanol/dichloromethane (from 0% to 100%), to give the title compound (5.4 mg, 30%) as a pink solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.48 (br s, 1H), 8.39 (d, *J*=1.9 Hz, 1H), 8.31 (s, 1H), 7.97 (s, 1H), 7.91 (d, *J*=5.8 Hz, 1H), 7.77-7.84 (m, 3H), 7.65 (dd, *J*=8.8, 2.0 Hz, 1H), 7.57 (d, *J*=8.6 Hz, 2H), 7.38 (s, 1H), 6.79 (s, 1H), 4.24 (dt, *J*=13.7, 4.1 Hz, 1H), 4.09-4.17 (m, 2H), 3.95 (s, 3H), 3.79-3.82 (m, 3H), 3.74-3.79 (m, 1H), 3.49 (d, *J*=3.9 Hz, 1H), 3.29-3.41 (m, 1H), 3.17-3.28 (m, 1H), 2.58-2.64 (m, 2H), 2.26 (quin, *J*=6.2 Hz, 2H), 2.05-2.13 (m, 1H), 1.92-2.01 (m, 1H), 1.83-1.87 (m, 1H), 1.07-1.19 (m, 1H); ^{13}C NMR (CDCl3, 100 MHz) δ 171.2, 167.5, 165.5, 163.4, 163.2, 150.4, 147.8, 141.5, 140.0, 139.3, 138.0, 135.1, 134.4, 130.6, 128.2, 123.0, 121.4, 120.9, 119.2, 116.4, 111.7, 110.1, 67.9, 56.0, 52.6, 49.7, 39.8, 34.2, 30.9, 24.7, 24.5, 22.9, 18.3; MS (ES+): *m/z* = 655 $(M+H)^+$; LCMS (**Method A**): t_R = 7.00 min.

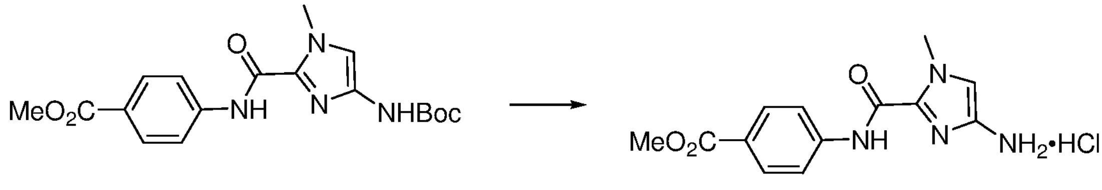
Example 31: Methyl 4-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-imidazole-2-carboxamido)benzoate (31)

31

A solution of 4-((tert-butoxycarbonyl)amino)-1-methyl-1H-imidazole-2-carboxylic acid (100 mg, 0.415 mmol) in anhydrous dichloromethane (3 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (165 mg, 0.435 mmol) and anhydrous triethylamine (242 μL, 1.74 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-aminobenzoate (63 mg, 0.42 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (40 mg, 26%) as a cream solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.16 (s, 1H), 8.01-8.07 (m, 2H), 7.69-7.75 (m, 2H), 7.21 (br s, 1H), 6.84 (s, 1H), 4.07 (s, 3H), 3.92 (s, 3H), 1.53 (s, 9H); MS (ES-): *m/z* = 373 (M-H)$^-$; LCMS (**Method A**): t_R = 7.68 min.

Example 32: Methyl 4-(4-amino-1-methyl-1*H*-imidazole-2-carboxamido)-benzoate hydrochloride (32)

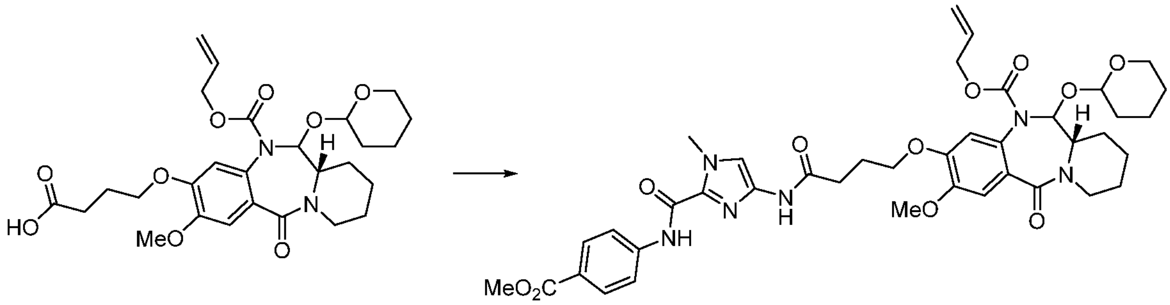


31 **32**

Methyl 4-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-imidazole-2-carboxamido)-benzoate (**31**) (40 mg, 0.11 mmol) was dissolved in hydrochloric acid (4 M in 1,4-dioxane) (2 mL) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to give the title compound (33 mg, 99%) as a brown solid. The product was carried through to the next step without any further purification.

^{1}H NMR (MeOD, 400 MHz) δ 7.89-7.95 (m, 2H), 7.72-7.78 (m, 2H), 7.31 (s, 1H), 4.01 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (MeOD, 100 MHz) δ 168.0, 143.6, 132.5, 131.6, 126.9, 123.3, 120.6, 92.6, 68.1, 52.3, 36.7; MS (ES+): *m/z* = 275 (M+H)$^+$; LCMS (**Method A**): t_R = 5.43 min.

Example 33: Allyl (6a*S*)-2-methoxy-3-(4-((2-((4-(methoxycarbonyl)phenyl) -carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (33)

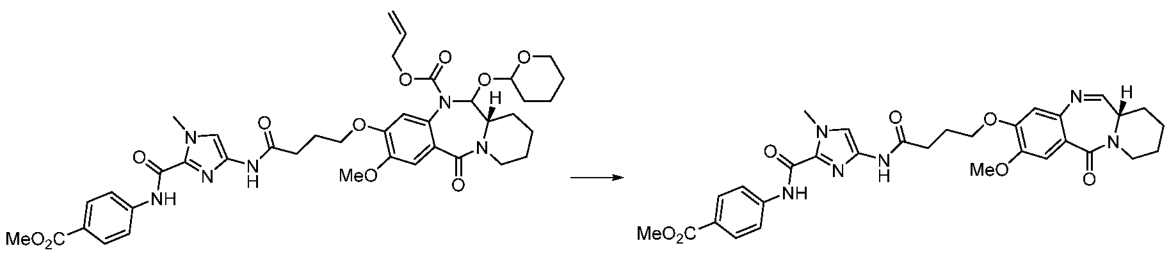


9 **33**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (50 mg, 0.094 mmol) in anhydrous dichloromethane (0.5 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene] -*N*-methylmethanaminium hexafluorophosphate *N*-oxide (38 mg, 0.099 mmol) and anhydrous triethylamine (55 μL, 0.40 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-(4-amino-1-methyl-1*H*-imidazole-2-carboxamido)-benzoate hydrochloride (**32**) (30 mg, 0.094 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (72 mg, 97%) as a brown oil.

MS (ES+): m/z = 789 $(M+H)^+$; LCMS (**Method A**): t_R = 7.87 min.

Example 34: Methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)benzoate (34)

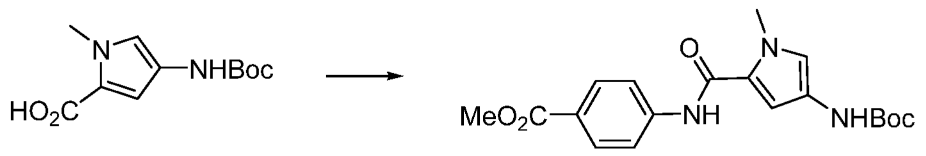


33 **34**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((2-((4-(methoxycarbonyl)phenyl)-carbamoyl)-1-methyl-1*H*-imidazol-4-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**33**) (72 mg, 0.091 mmol) in dichloromethane (2 mL) was added tetrakis(triphenylphosphine)palladium(0) (5.3 mg, 5 mol%), triphenylphosphine (6.0 mg, 25 mol%) and pyrrolidine (9.0 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%), to give the title compound (15 mg, 27%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.13 (s, 1H), 8.24 (s, 1H), 8.03 (d, *J*=8.7 Hz, 2H), 7.90 (d, *J*=5.7 Hz, 1H), 7.65-7.75 (m, 2H), 7.43-7.50 (m, 2H), 6.77-6.83 (m, 1H), 4.12-4.23 (m, 2H), 4.07 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 3.18-3.27 (m, 1H), 2.80 (s, 3H), 2.56-2.68 (m, 3H), 2.23-2.31 (m, 2H), 1.85 (d, *J*=10.1 Hz, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.3, 167.1, 166.2, 162.9, 156.2, 150.1, 147.6, 147.4, 141.4, 139.6, 135.6, 132.8, 130.5, 130.3, 125.2, 121.1, 118.2, 114.8, 111.2, 109.9, 94.1, 67.4, 63.5, 55.7, 53.4, 51.6, 49.2, 39.4, 38.2, 35.5, 32.5, 31.6, 30.9, 28.9, 24.9, 24.3, 24.1, 22.5, 19.9, 18.0; MS (ES+): *m/z* = 603 (M+H)$^+$; LCMS (**Method A**): t_R = 6.57 min.

Example 35: Methyl 4-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)benzoate (35)

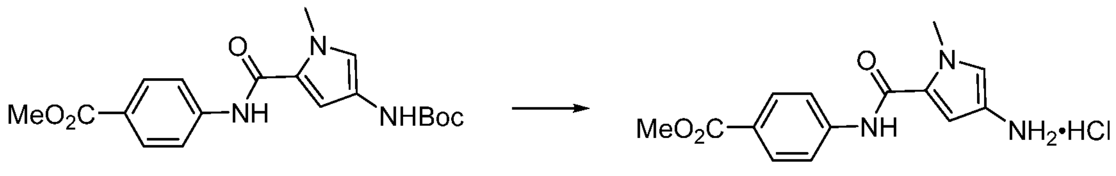


35

A solution of 4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxylic acid (100 mg, 0.416 mmol) in *N,N*-dimethylformamide (3 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (145 mg, 0.756 mmol) and 4-(dimethylamino)pyridine (115 mg, 0.941 mmol). The reaction mixture was stirred at room temperature for 3 h. Methyl 4-aminobenzoate (57 mg, 0.38 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured onto ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with an aqueous solution of citric acid (1 M , 60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (90 mg, 58%) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99-8.07 (m, 2H), 7.69 (s, 1H), 7.61-7.67 (m, 2H), 6.88 (s, 1H), 6.69 (br s, 1H), 6.25 (br s, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 1.52 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.6, 159.4, 153.4, 142.3, 130.9, 125.5, 123.1, 122.5, 119.2, 118.7, 140.1, 80.5, 52.0, 36.8, 28.4; MS (ES+): *m/z* = 374 (M+H)$^+$; LCMS (**Method A**): t_R = 7.52 min.

Example 36: Methyl 4-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)-benzoate hydrochloride (36)

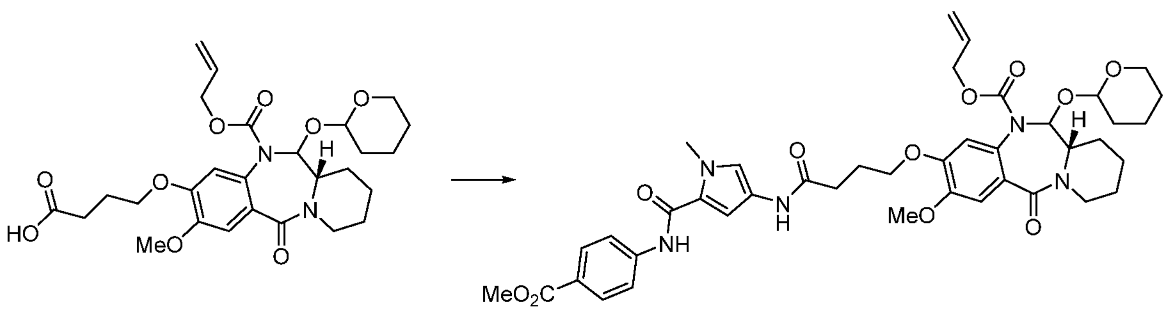


35 **36**

Methyl 4-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)-benzoate (**35**) (90 mg, 0.24 mmol) was dissolved in hydrochloric acid (4 M in 1,4-dioxane) (3 mL) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* to give the title compound (79 mg, 99%) as a cream solid. The product was carried through to the next step without any further purification.

^{1}H NMR (MeOD, 400 MHz) δ 7.99 (d, J=8.7 Hz, 2H), 7.80 (d, J=8.7 Hz, 2H), 7.13 (d, J=1.9 Hz, 1H), 7.09 (d, J=1.9 Hz, 1H), 3.96 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (MeOD, 100 MHz) δ 168.2, 161.2, 144.5, 131.5, 126.9, 126.4, 123.7, 120.8, 114.2, 109.0, 52.5, 37.5; MS (ES+): m/z = 274 (M+H)$^{+}$; LCMS (**Method A**): t_R = 4.98 min.

Example 37: Allyl (6a*S*)-2-methoxy-3-(4-((5-((4-(methoxycarbonyl)phenyl)-carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (37)

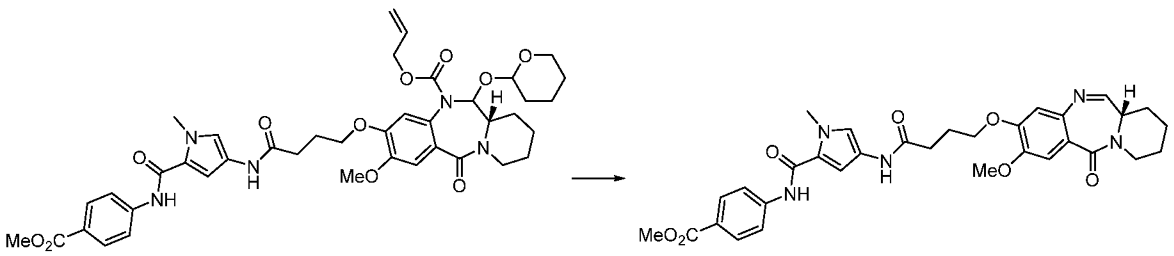


9 **37**

A solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)-oxy)butanoic acid (**9**) (50 mg, 0.094 mmol) in anhydrous dichloromethane (0.5 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (38 mg, 0.099 mmol) and anhydrous triethylamine (55 μL, 0.40 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)benzoate hydrochloride (**36**) (30 mg, 0.094 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (72 mg, 97%) as a brown oil.

MS (ES+): m/z = 788 $(M+H)^+$; LCMS (**Method A**): t_R = 7.77 min.

Example 38: Methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzoate (38)

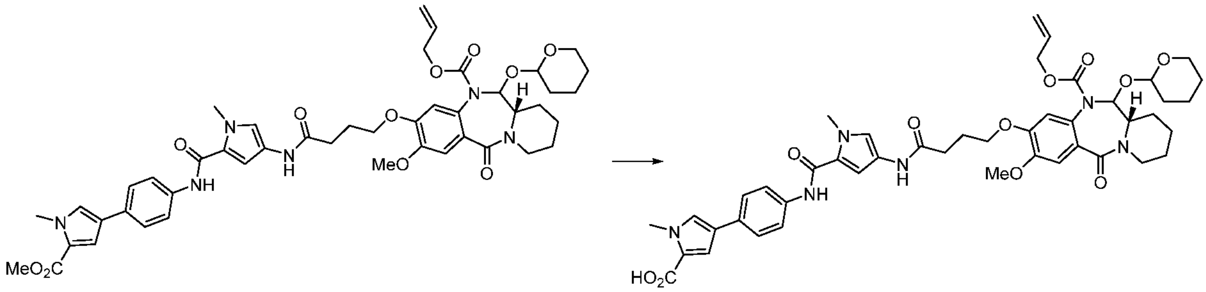


37 38

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((5-((4-(methoxycarbonyl)phenyl)-carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**37**) (72 mg, 0.091 mmol) in dichloromethane (2 mL) was added tetrakis-(triphenylphosphine)palladium(0) (5.3 mg, 5 mol%), triphenylphosphine (6.0 mg, 25 mol%) and pyrrolidine (9.0 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%), to give the title compound (15.0 mg, 27%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.41 (s, 1H), 8.00 (s, 2H), 7.98 (s, 1H), 7.90 (d, J=5.8 Hz, 1H), 7.72-7.74 (m, 1H), 7.70-7.72 (m, 1H), 7.41 (s, 1H), 7.14 (d, J=1.8 Hz, 1H), 6.79 (s, 1H), 6.57 (d, J=1.8 Hz, 1H), 4.22 (d, J=14.1 Hz, 1H), 4.09 (t, J=6.0 Hz, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.74-3.79 (m, 2H), 3.21 (d, J=3.3 Hz, 1H), 2.47-2.52 (m, 2H), 2.17-2.23 (m, 2H), 1.93 (br s, 3H), 1.79-1.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 167.6, 166.8, 163.6, 159.8, 150.7, 147.9, 142.9, 139.9, 130.7, 124.9, 122.8, 121.6, 121.5, 120.8, 119.1, 111.8, 110.4, 104.6, 68.1, 56.1, 52.0, 49.7, 39.9, 36.9, 33.0, 31.0, 25.0, 24.5, 22.9, 18.3; MS (ES+): m/z = 602 (M+H)$^+$; LCMS (**Method A**): t_R = 6.52 min.

Example 39: 4-(4-(4-(4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylic acid (39)

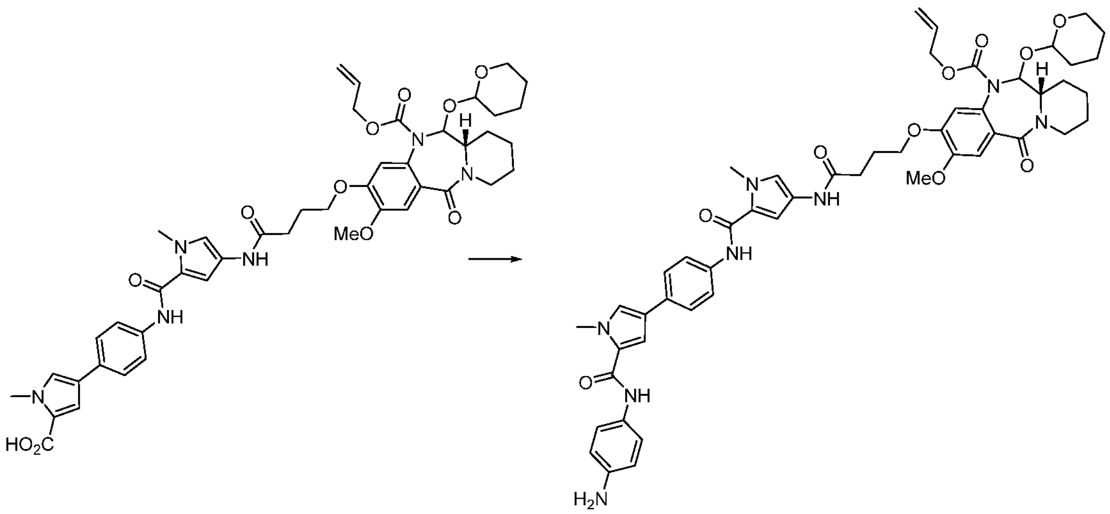


23 **39**

To a solution of allyl (6a*S*)-2-methoxy-3-(4-((5-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)amino)-4-oxobutoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**23**) (195 mg, 0.225 mmol) in 1,4-dioxane (5 mL) was added an aqueous solution of sodium hydroxide (0.5 M, 5 mL, 2.5 mmol). The reaction mixture was stirred at room temperature for 16 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with an aqueous solution of citric acid (1 M, 5 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (190 mg, 99%) as a cream solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 853 (M+H)$^+$; LCMS (**Method B**): t_R = 3.83 min.

Example 40: Allyl (6a*S*)-3-(4-((5-((4-(5-((4-aminophenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (40)

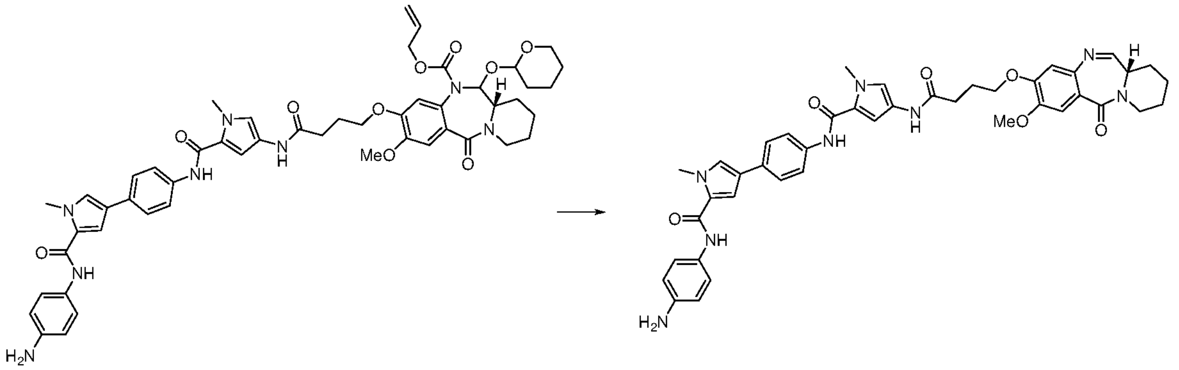


39 **40**

A solution of 4 4-(4-(4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**39**) (320 mg, 0.375 mmol) in anhydrous dichloromethane (1.5 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (150 mg, 0.395 mmol) and anhydrous triethylamine (220 μL, 1.58 mmol). The reaction mixture was stirred at room temperature for 30 min. Benzene-1,4-diamine (41 mg, 0.38 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 10%), to give the title compound (250 mg, 71%) as a cream solid.

MS (ES+): m/z = 944 $(M+H)^+$; LCMS (**Method B**): t_R = 3.45 min.

Example 41: (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (41)

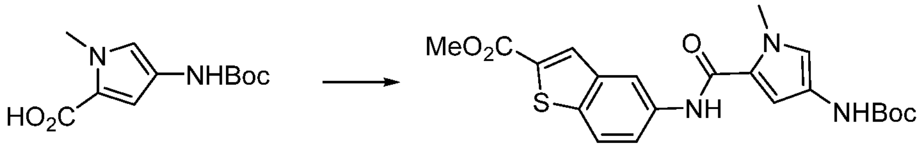


40 **41**

To a solution of allyl (6a*S*)-3-(4-((5-((4-(5-((4-aminophenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**40**) (250 mg, 0.265 mmol) in dichloromethane (3 mL) was added tetrakis(triphenylphosphine)palladium(0) (15 mg, 5 mol%), triphenylphosphine (17 mg, 25 mol%) and pyrrolidine (26 μL, 0.32 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%) followed by methanol/ acetone (from 0% to 100%), to give the title compound (118 mg, 59%) as a yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.88-9.96 (m, 1H), 9.81 (s, 2H), 9.50 (s, 1H), 8.32 (br s, 2H), 8.00 (d, *J*=5.7 Hz, 1H), 7.67-7.73 (m, 2H), 7.48 (d, *J*=8.6 Hz, 2H), 7.39 (d, *J*=1.8 Hz, 1H), 7.31-7.35 (m, 2H), 7.30 (d, *J*=1.6 Hz, 1H), 7.27 (s, 1H), 7.22 (d, *J*=1.5 Hz, 1H), 6.96 (d, *J*=1.6 Hz, 1H), 6.80 (s, 1H), 6.51-6.55 (m, 2H), 4.09-4.17 (m, 1H), 3.99-4.05 (m, 1H), 3.90-3.97 (m, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.68-3.72 (m, 1H), 3.05-3.16 (m, 2H), 2.44 (t, *J*=7.3 Hz, 2H), 2.02-2.07 (m, 2H), 1.81-1.91 (m, 1H), 1.68-1.78 (m, 2H), 1.56 (d, *J*=4.9 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.8, 166.3, 164.7, 159.5, 159.2, 150.2, 147.1, 144.7, 139.8, 137.0, 129.6, 128.2, 126.1, 124.6, 124.3, 122.0, 121.8, 120.4, 120.2, 118.8, 113.7, 111.3, 109.6, 104.7, 67.7, 67.2, 55.6, 51.1, 49.2, 38.5, 36.2, 36.1, 35.4, 31.8, 30.2, 24.7, 23.7, 22.5, 17.7; MS (ES+): *m/z* = 757 $(M+H)^+$; LCMS (**Method A**): t_R = 5.80 min.

<u>Example 42: Methyl 5-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[b] thiophene-2-carboxylate (42)</u>

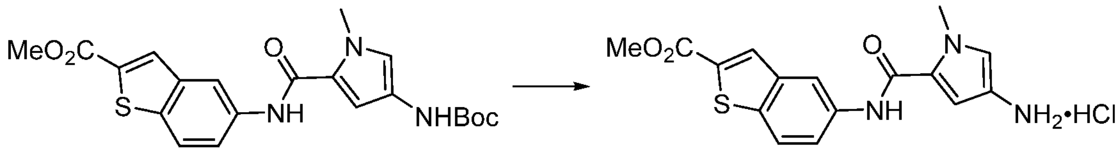


42

A solution of 4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxylic acid (127 mg, 0.530 mmol) in *N,N*-dimethylformamide (1 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (185 mg, 0.960 mmol) and 4-(dimethylamino)pyridine (147 mg, 1.20 mmol). The reaction mixture was stirred at room temperature for 4 h. Methyl 5-aminobenzo[b]thiophene-2-carboxylate (100 mg, 0.480 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured onto ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with an aqueous solution of citric acid (1 M, 60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated to give the title compound (185 mg, 90%) as a cream solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 430 $(M+H)^+$; LCMS (**Method B**): t_R = 4.07 min.

<u>Example 43: Methyl 5-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)-benzo[b]thiophene-2-carboxylate hydrochloride (43)</u>



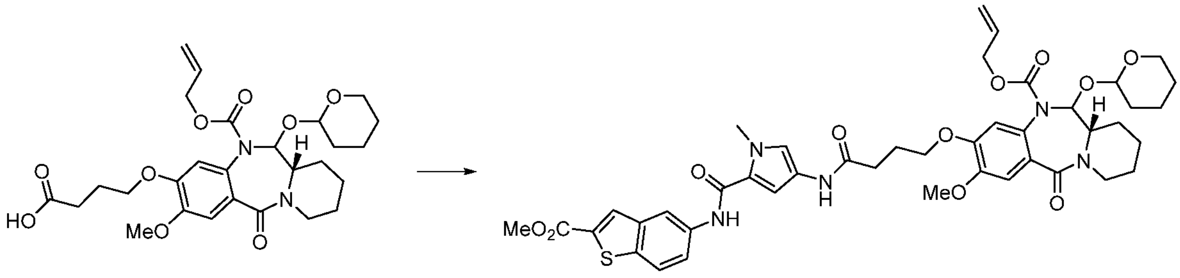
42 **43**

Methyl 5-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo-[b]thiophene-2-carboxylate (**42**) (150 mg, 0.340 mmol) was dissolved in hydrochloric acid (4 M in 1,4-dioxane) (1 mL) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* to give the title compound (118 mg, 95%) as a pale brown solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 364 $(M+H)^+$; LCMS (**Method B**): t_R = 2.78 min.

<u>Example 44: Allyl (6a*S*)-2-methoxy-3-(4-((5-((2-(methoxycarbonyl)benzo-[*b*]thiophen-5-yl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxo-</u>

butoxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (44)

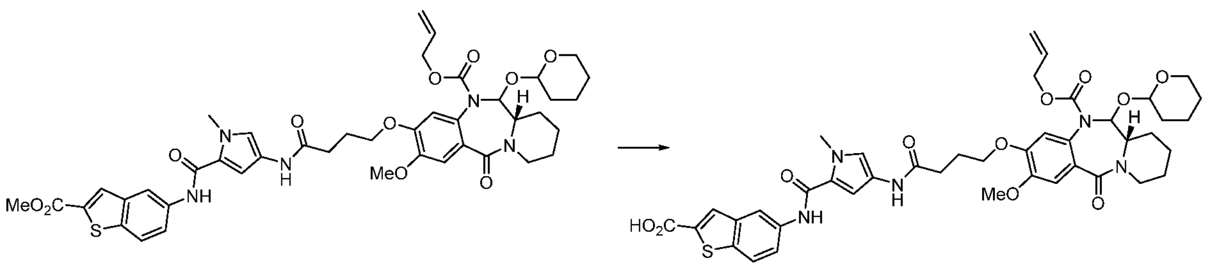


9 **44**

A solution 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanoic acid (**9**) (300 mg, 0.560 mmol) in *N,N*-dimethylformamide (3 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (238 mg, 1.23 mmol) and 4-(dimethylamino)pyridine (189 mg, 1.55 mmol). The reaction mixture was stirred at room temperature for 4 h. Methyl 5-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[b]thiophene-2-carboxylate hydrochloride (**43**) (225 mg, 0.620 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured onto ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were sequentially washed with an aqueous solution of citric acid (1 M, 60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 30%), to give the title compound (348 mg, 66%) as a brown solid.

MS (ES+): m/z = 844 $(M+H)^+$; LCMS (**Method B**): t_R 4.23 min.

Example 45: 5-(4-(4-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[b]thiophene-2-carboxylic acid (45)

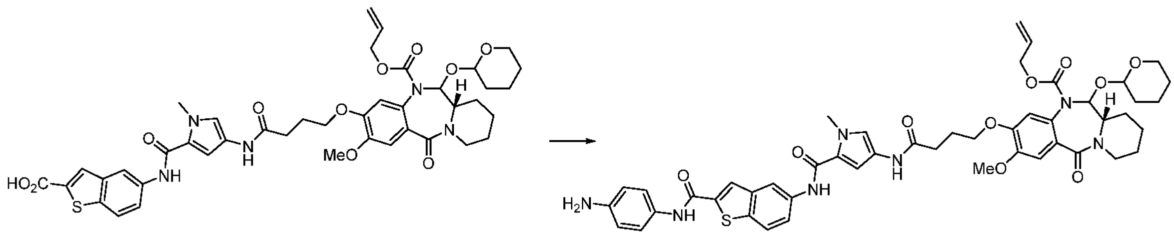


44 **45**

To a solution of 4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanoic acid (**44**) (327 mg, 0.387 mmol) in 1,4-dioxane (5 mL) was added an aqueous solution of sodium hydroxide (0.5 M, 5 mL, 2.5 mmol). The reaction mixture was stirred at room temperature for 3 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with an aqueous solution of citric acid (1 M, 5 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (315 mg, 99%) as a brown solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 831 $(M+H)^+$; LCMS (**Method B**): t_R = 3.82 min.

Example 46: Allyl (6a*S*)-3-(4-((5-((2-((4-aminophenyl)carbamoyl)benzo-[b]thiophen-5-yl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxo-butoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (46)

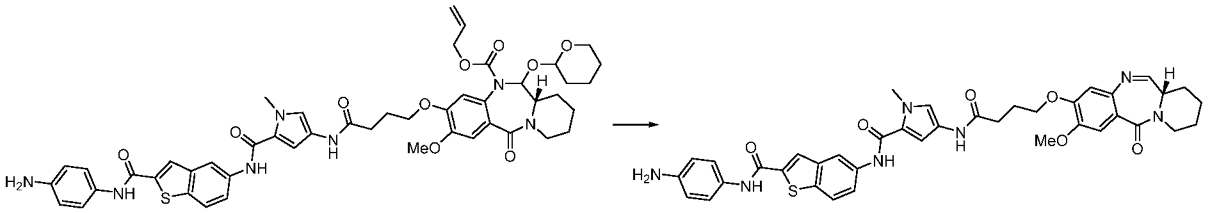


45 **46**

A solution of 5-(4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo[b]thiophene-2-carboxylic acid (**45**) (50 mg, 0.060 mmol) in anhydrous dichloromethane (1 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (28 mg, 0.072 mmol) and anhydrous triethylamine (35 μL, 0.25 mmol). The reaction mixture was stirred at room temperature for 30 min. Benzene-1,4-diamine (7.0 mg, 0.066 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in* *vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 50%), to give the title compound (6.8 mg, 12%) as a yellow solid.

MS (ES+): m/z = 921 $(M+H)^+$; LCMS (**Method B**): t_R = 3.48 min

Example 47: (*S*)-*N*-(2-((4-Aminophenyl)carbamoyl)benzo[*b*]thiophen-5-yl)-4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamide (47)

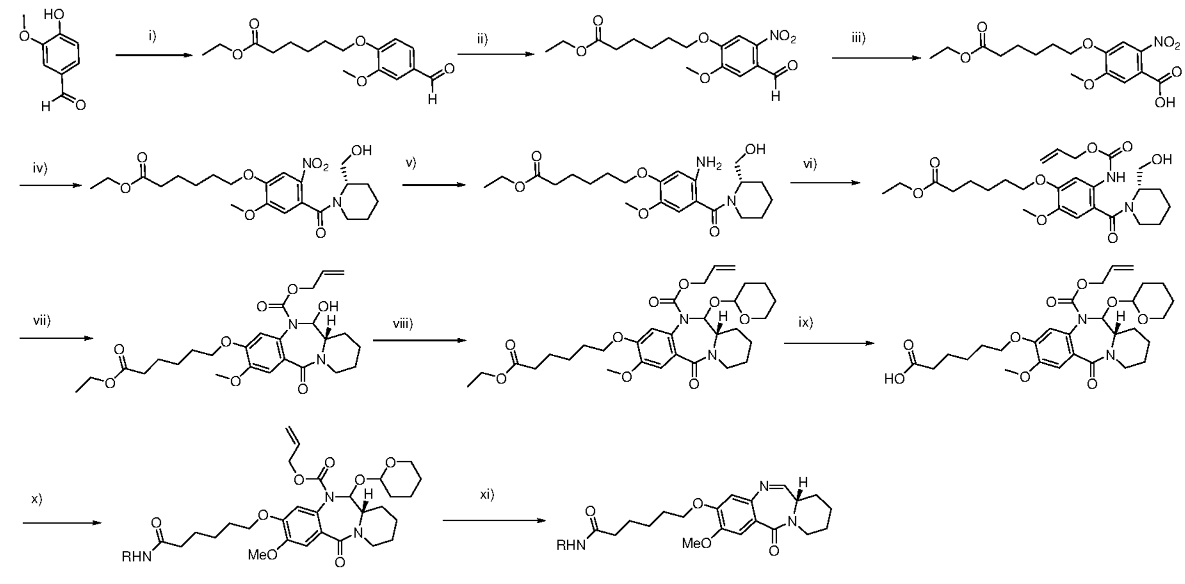


46 **47**

To a solution of allyl (6a*S*)-3-(4-((5-((2-((4-aminophenyl)carbamoyl)benzo[*b*]thiophen-5-yl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]-diazepine-5(12*H*)-carboxylate (**46**) (6.8 mg, 0.0074 mmol) in dichloromethane (1 mL) was added tetrakis(triphenylphosphine)palladium(0) (0.4 mg, 5 mol%), triphenylphosphine (0.5 mg, 25 mol%) and pyrrolidine (1 μL, 0.012 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 100%) followed by methanol/dichloromethane (from 0% to 5%), to give the title compound (1.7 mg, 31%) as a pale yellow solid.

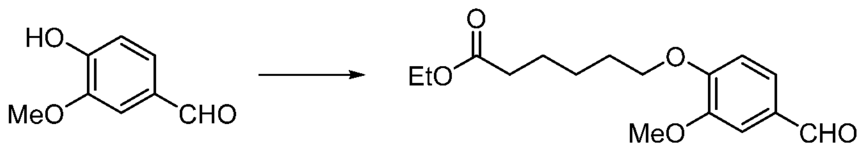
^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.13 (s, 1H), 9.98-10.03 (m, 1H), 9.95 (s, 1H), 8.35 8.42 (m, 1H), 8.19 (s, 1H), 8.01 (d, J=5.7 Hz, 1H), 7.95 (d, J=8.9 Hz, 1H), 7.33-7.40 (m, 2H), 7.23-7.28 (m, 2H), 7.02 (s, 1H), 6.81 (s, 1H), 6.57 (d, J=8.7 Hz, 2H), 5.00 (br. s., 2H), 4.10-4.14 (m, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.65-3.74 (m, 2H), 3.15-3.19 (m, 1H), 3.06-3.14 (m, 1H), 2.45 (t, J=7.5 Hz, 3H), 2.11-2.13 (m, 1H), 2.00-2.08 (m, 4H) 1.74 (dd, J=9.0, 5.3 Hz, 3H); MS (ES+): m/z = 734 $(M+H)^+$; LCMS (**Method A**): t_R = 5.63 min.

General synthetic scheme



i) K_2CO_3, DMF, ethyl 6-bromohexanoate, r.t.; ii) KNO_3, TFA, 0 - 5 °C; iii) $KMnO_4$, acetone, H_2O, reflux; iv) HATU, (S)-piperidin-2-ylmethanol, Et_3N, CH_2Cl_2, 0 °C - r.t.; v) H_2, Ni/Ra, MeOH ; vi) Allylchloroformate, pyridine, CH_2Cl_2, - 10 °C - r.t.; vii) TEMPO, BAIB, CH_2Cl, r.t.; viii) *p*TSA, DHP, EtOAc, r.t.; ix) NaOH, dioxane, H_2O, r.t.; x) RNH_2, EDCI, DMAP, DMF, r.t.; xi) PPh_3, $Pd(PPh_3)_4$, pyrrolidine, CH_2Cl_2, r.t.

Example 48: Ethyl 6-(4-formyl-2-methoxyphenoxy)hexanoate (48)

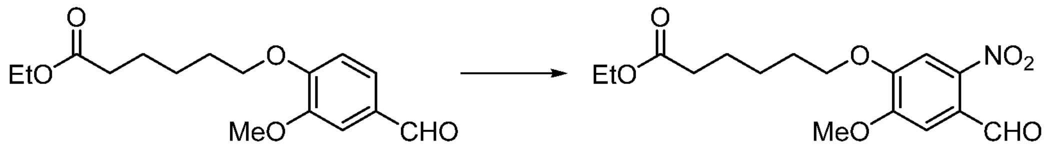


48

A mixture of vanillin (6.5 g, 42.7 mmol), ethyl 6-bromohexanoate (8.0 mL, 45.0 mmol) and potassium carbonate (8.70 g, 63.0 mmol) in *N,N*-dimethylformaldehyde (50 mL) was stirred at room temperature for 18h. The reaction mixture was diluted with water (100 mL), separated and extracted with ethyl acetate (120 mL). The combined organic extracts were sequentially washed with water (100 mL), brine (100 mL), dried over magnesium sulfate, filtered and concentrated to give the title compound as a pale yellow oil (12.5 g, 99%). The product was carried through to the next step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.42-7.44 (dd, *J*=8.2, 1.9 Hz, 1H), 7.40 (d, *J*=1.9 Hz, 1H), 6.96 (d, *J*=8.1 Hz, 1H), 4.08-4.15 (m, 4H), 3.92 (s, 3H), 2.34 (t, *J*=7.5 Hz, 2H), 1.87-1.94 (m, 2H), 1.68-1.75 (m, 2H), 1.49-1.56 (m, 2H), 1.25 (t, *J*=7.2 Hz, 3H); MS (ES+): *m/z* = 317 $(M+Na)^+$; LCMS (**Method B**): t_R = 3.82 min.

Example 49: Ethyl 6-(4-formyl-2-methoxy-5-nitrophenoxy)hexanoate (49)

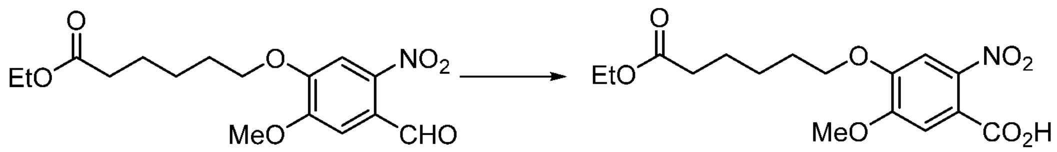


48 **49**

To a stirred solution of potassium nitrate (5.4 g, 53 mmol) in trifluoroacetic acid (25 mL) at room temperature was added dropwise a solution of ethyl 6-(4-formyl-2-methoxyphenoxy)hexanoate (**48**) (12.5, 42 mmol) in trifluoroacetic acid (25 mL). The reaction mixture was stirred for 1 h. It was then concentrated *in vacuo* and the residue was dissolved in ethyl acetate (200 mL). This was washed with brine (3 x 50 mL) followed by a saturated aqueous solution of sodium hydrogen carbonate (2 x 40 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the title compound as a yellow solid (14.4 g, 100%). The product was carried through to the next step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H) 7.58 (s, 1H), 7.40 (s, 1H), 4.10-4.16 (m, 4H), 4.00 (s, 3H), 2.35 (t, *J*=7.4 Hz, 2H), 1.84-1.96 (m, 2H), 1.69-1.76 (m, 2H), 1.50-1.58 (m, 2H), 1.25 (t, *J*=7.2 Hz, 3H); MS (ES+): m/z = 340 $(M+H)^+$; LCMS (**Method B**): t_R = 4.02 min.

Example 50: 4-((6-Ethoxy-6-oxohexyl)oxy)-5-methoxy-2-nitrobenzoic acid (50)

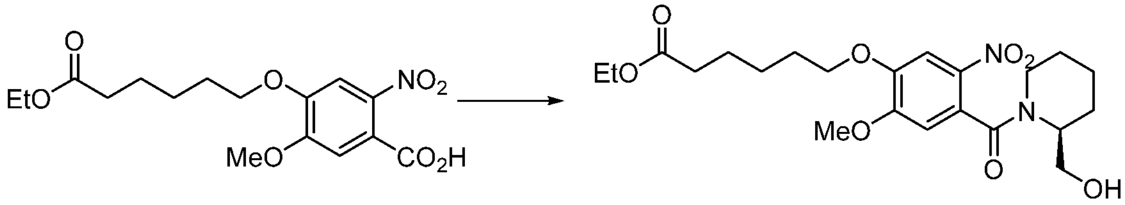


49 **50**

To a solution of ethyl 6-(4-formyl-2-methoxy-5-nitrophenoxy)hexanoate (**49**) (7.8 g, 23.0 mmol) in acetone (200 mL) was added a hot (70 °C) solution of potassium permanganate (13.6 g, 86.0 mmol) in water (100 ml). The mixture was then stirred at 70 °C for 4 h. The reaction mixture was cooled to room temperature and passed through celite. The cake was then washed with hot water (100 mL). A solution of sodium bisulfite in hydrochloric acid (100 mL) was added to the filtrate and extracted with dichloromethane (2 x 200 mL). The combined organic extracts were dried over sodium sulfate, filtrated and concentrated *in vacuo* to give the title compound as a yellow solid (5.0 g, 61%) which was used in the subsequent step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.14 (s, 1H), 3.96-4.03 (m, 4H), 3.84 (s, 3H), 2.24 (t, J=7.4 Hz, 2H), 1.70-1.77 (m, 2H), 1.55-1.62 (m, 2H), 1.39-1.45 (m, 2H), 1.13 (t, J=7.1 Hz, 3H); MS (ES+): m/z = 354 $(M-H)^+$; LCMS (**Method B**): t_R = 3.63 min.

Example 51: Ethyl (*S*)-6-(4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)hexanoate (51)

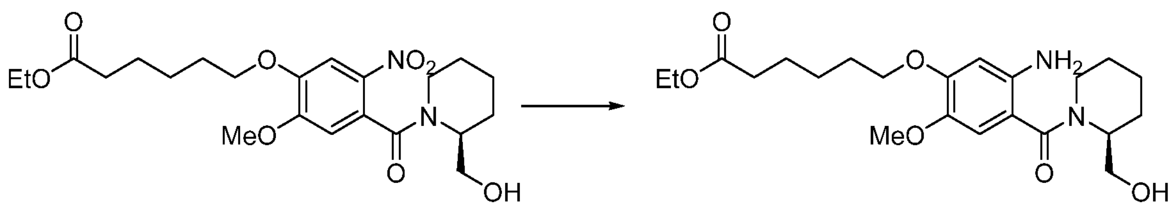


50 **51**

To a stirred solution of 4-((6-ethoxy-6-oxohexyl)oxy)-5-methoxy-2-nitrobenzoic acid (**50**) (2.0 g, 5.6 mmol) and trimethylamine (4.70 mL, 33.8 mmol) in dichloromethane (40 mL) was added *O*-(7-azabenzotriazole-1-yl)-*N,N,N,N'*-tetramethyluronium hexafluorophosphate (2.2 g, 5.9 mmol) in one portion and the resulting mixture was stirred for 2 h at room temperature. A solution of (*S*)-piperidin-2-ylmethanol (647 mg, 5.63 mmol) in dichloromethane (10 mL) was then added dropwise and the resulting mixture was stirred for 16 h at room temperature. The reaction was quenched with a saturated aqueous solution of sodium hydrogen carbonate (40 mL), the phases were separated and the aqueous layer was further extracted with dichloromethane (20 mL). The combined organic extracts were washed with brine (40 mL), dried over magnesium sulfate, filtered and concentrated to give an amber oil. Purification was carried out by column chromatography (silica), eluting with ethyl acetate/hexane (from 0% to 100%), to give the title compound (1.2 g, 48%) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.63 (m, 1H), 6.75-6.77 (m, 1H), 4.02-4.13 (m, 4H), 3.93 (s, 3H), 3.70-3.78 (m, 1H), 3.39-3.68 (m, 1H), 3.11-3.18 (m, 3H), 2.32 (t, J=7.6 Hz, 2H), 1.83-1.91 (m, 2H), 1.39-1.72 (m, 11H), 1.26 (t, J=7.1 Hz, 3H); MS (ES+): m/z = 453 $(M+H)^+$; LCMS (**Method B**): t_R = 3.63 min.

Example 52: Ethyl (*S*)-6-(5-amino-4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxyphenoxy)hexanoate (52)

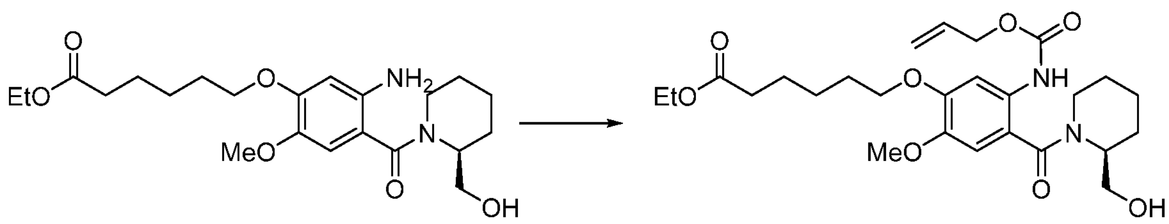


51 **52**

To a solution of ethyl (*S*)-6-(4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)hexanoate (**51**) (1.2 g, 2.7 mmol) in methanol (20 mL) was added Raney®-Nickel (slurry in H_2O) (120 mg). The resulting mixture was hydrogenated at 50 psi for 1.5 h in a Parr apparatus, then filtered through a celite pad and concentrated *in vacuo* to give the title compound (991 mg, 87%) as a grey oil that solidifies upon standing. The resulting material was carried through to the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 1H), 6.32 (s, 1H), 4.13 (m, 4H), 3.98 (t, J=6.5 Hz, 2H), 3.79 (s, 3H), 3.67-3.57 (m, 1H), 3.19-3.22 (m, 4H), 2.87 (s, 2H), 2.32-2.36 (m, 2H), 1.82-1.89 (m, 2H), 1.65-1.73 (m, 6H), 1.47-1.55 (m, 3H), 1.27 (t, J=7.1 Hz, 3H); MS (ES+): m/z = 423 $(M+H)^+$; LCMS (**Method B**): t_R = 3.23 min.

Example 53: Ethyl (*S*)-6-(5-(((allyloxy)carbonyl)amino)-4-(2-(hydroxy-methyl)piperidine-1-carbonyl)-2-methoxyphenoxy)hexanoate (53)

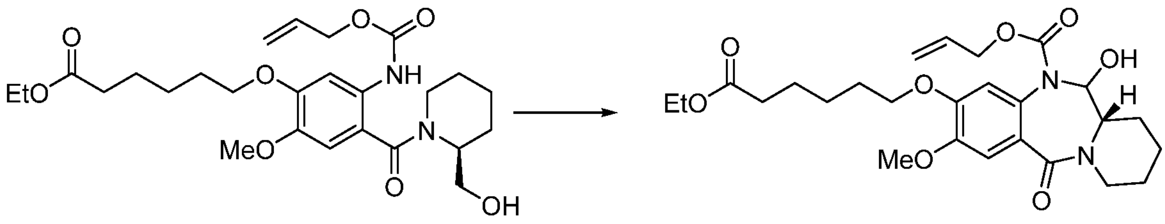


52 **53**

To a solution of ethyl (*S*)-6-(5-amino-4-(2-(hydroxymethyl)piperidine-1-carbonyl)-2-methoxyphenoxy)hexanoate (**52**) (1.23 g, 2.91 mmol) and pyridine (542 μL, 6.69 mmol) in anhydrous dichloromethane (20 mL) at -10 °C, a solution of allyl chloroformate (278 μL, 2.62 mmol) in dichloromethane (12 mL) was added dropwise. The resulting reaction mixture was stirred at room temperature for 0.5 h, quenched with a saturated aqueous solution of copper (II) sulfate (25 mL), diluted with dichloromethane (10 mL), separated, and successively washed with water (20 mL), a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and brine (20 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated *in vacuo* to give the title compound (588 mg, 40%) as an orange oil. The resulting material was carried through to the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (br s, 1H), 7.70 (br s, 1H), 6.78 (s, 1H), 5.90-6.00 (m, 1H), 5.33-5.38 (m, 1H), 5.24 (dd, *J*=10.4, 1.3 Hz, 1H), 4.63 (m, 2H), 4.12 (q, *J*=7.1Hz, 2H) 4.05 (t, J=6.6 Hz, 2H), 3.83 (s, 3H), 3.64-3.72 (m, 1H), 3.02-3.12 (m, 1H), 2.33 (t, *J*=7.6 Hz, 2H), 1.84-1.91 (m, 2H), 1.67-1. 74 (m, 10H), 1.66-1.54 (m, 4H), 1.26 (t, *J*=7.1 Hz, 3H); MS (ES+): m/z = 507 $(M+H)^+$; LCMS (**Method B**): t_R = 3.70 min.

Example 54: Allyl (6a*S*)-3-((6-ethoxy-6-oxohexyl)oxy)-6-hydroxy-2-methoxy-12-oxo-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]-diazepine-5(12*H*)-carboxylate (54)

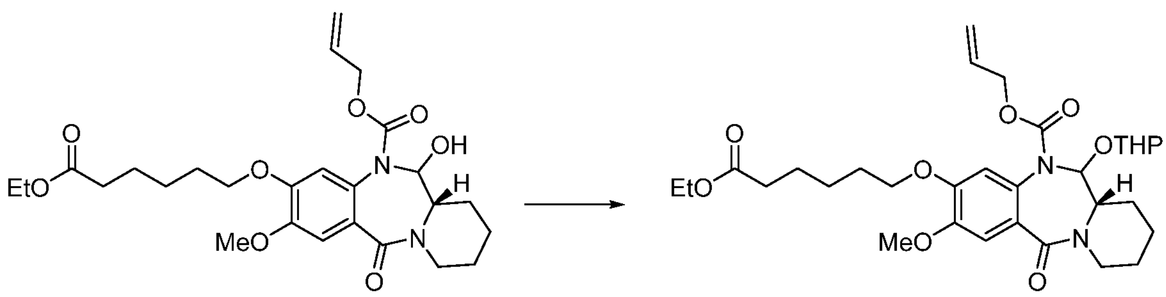


53 **54**

To a solution of ethyl (*S*)-6-(5-(((allyloxy)carbonyl)amino)-4-(2-(hydroxymethyl)-piperidine-1-carbonyl)-2-methoxyphenoxy)hexanoate (**53**) (1.7 g, 3.4 mmol) in dichloromethane (80 mL) was added 2,2,6,6-tetramethyl-1-piperidinyloxy (53 mg, 0.30 mmol) and (diacetoxyiodo)benzene (1.3 g, 4.0 mmol). The reaction mixture was stirred at room temperature for 16 h, and was then placed in an ice bath and quenched with a saturated aqueous solution of sodium metabisulfite (35 mL). The mixture was diluted with dichloromethane (30 mL), separated, and sequentially washed with a saturated aqueous solution of sodium hydrogen carbonate (30 mL), water (30 mL) and brine (30 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification was carried out by column chromatography (silica), eluting with ethyl acetate/hexane (from 0% to 80%) to give the desired compound (1.1 g, 66%) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70-7.72 (m, 1H), 7.09-7.13 (m, 1H), 5.80-5.98 (m, 1H), 5.25-5.38 (m, 1H), 5.14-5.19 (m, 2H), 4.63-4.72 (m, 2H), 4.35-4.50 (m, 1H), 4.13 (q, *J*=7.1 Hz, 2H), 4.03-4.08 (m, 1H), 3.96-4.01 (m, 2H), 3.91 (s, 3H), 3.81-3.83 (m, 1H), 3.45-3.53 (m, 1H), 3.03-3.10 (m, 1H), 2.33 (t, *J*=7.6 Hz, 2H), 1.83-1.90 (m, 2H), 1.62-1.74 (m, 10H), 1.48-1.53 (m, 2H); MS (ES+): *m/z* = 505 (M+H)$^+$; LCMS (**Method B**): t_R = 3.57 min.

Example 55: Allyl (6a*S*)-3-((6-ethoxy-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido-[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (55)

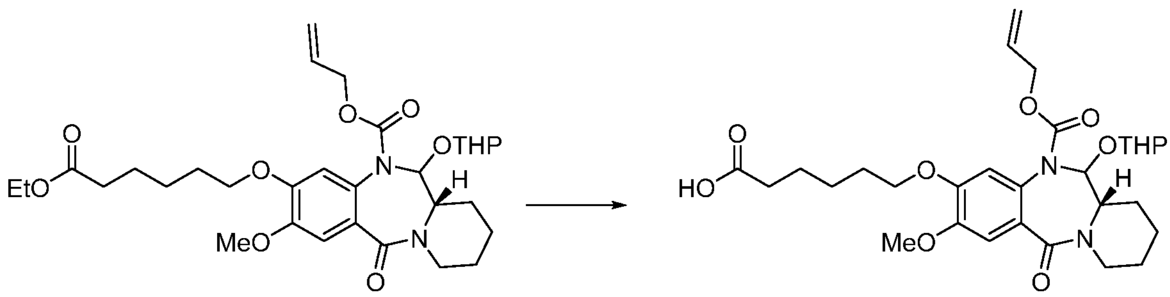


54 **55**

To containing solution of allyl (6a*S*)-3-((6-ethoxy-6-oxohexyl)oxy)-6-hydroxy-2-methoxy-12-oxo-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**54**) (1.1 g, 2.2 mmol) in dichloromethane (50 mL) were added 3,4-dihydro-2*H*-pyran (2.00 mL, 22.4 mmol) and *p*-toluenesulfonic acid monohydrate (113 mg, 1% w/w), and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was then diluted with dichloromethane (50 mL) and washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated to give the title compound as a yellow oil (863 mg, 66%) after purification by column chromatography (silica) eluting with ethyl acetate/hexane (from 0% to 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (m, 1H), 6.50 (s, 1H), 6.10 (m, 1H), 5.76-5.81 (m, 1H), 5.03-5.14 (m, 2H), 4.57-4.69 (m, 2H), 4.37-4.49 (m, 1H), 4.26-4.34 (m, 1H), 4.12 (q, *J*=7.1 Hz, 2H), 3.94-4.01 (m, 3H), 3.90 (s, 3H), 3.62-3.68 (m, 1H), 3.46-3.68 (m, 2H), 3.03-3.12 (m, 1H), 2.33 (t, *J*=7.4 Hz, 2H), 1.66-1.89 (m, 11H), 1.47-1.57 (m, 6H), 1.25 (t, *J*=7.1 Hz, 3H); MS (ES+): *m/z* = 589 $(M+H)^+$; LCMS (**Method B**): t_R = 4.32 min.

Example 56: 6-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetra-hydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanoic acid (56)

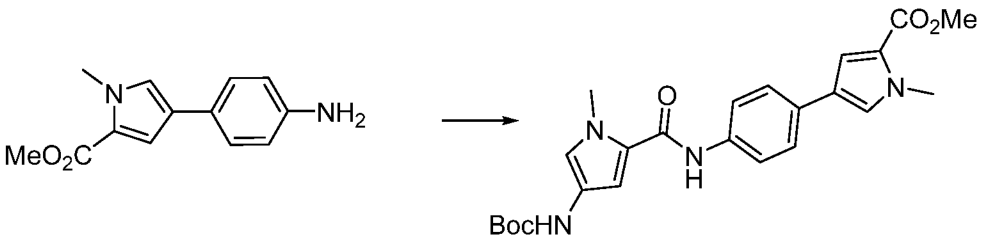


55 **56**

To a solution of allyl (6a*S*)-3-((6-ethoxy-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]-diazepine-5(12*H*)-carboxylate (**55**) (200 mg, 0.34 mmol) in 1,4-dioxane (3 ml) was added an aqueous solution of sodium hydroxide (0.5 M, 1.2 mL). The reaction mixture was stirred at room temperature for 2 h and was then concentrated *in vacuo*, after which water (6 ml) was added and the aqueous layer was then acidified to pH = 1 with acetic acid. The aqueous layer was then extracted with ethyl acetate (2x 40 mL). The combined organic extracts were then washed with brine (40 ml), dried over sodium sulfate, filtered and concentrated to give the title compound as a yellow oil (181 mg, 95%) which was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 6.19 (s, 1H), 5.99-6.19 (m, 1H), 5.71-5.81 (m, 1H), 5.02-5.12 (m, 2H), 4.51-4.67 (m, 1H), 4.36-4.48 (m, 1H), 4.23-4.31 (m, 1H), 3.88-4.00 (m, 7H), 3.46-3.66 (m, 2H), 3.02-3.12 (m, 1H), 2.36 (t, *J*=7.4 Hz, 2H), 1.79-1.81 (m, 2H), 1.65-1.75 (m, 10H), 1.49-1.55 (m, 7H); MS (ES+): *m/z* = 561 $(M+H)^+$; LCMS (**Method B**): t_R = 3.78 min.

Example 57: Methyl 4-(4-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2 carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2 carboxylate (57)

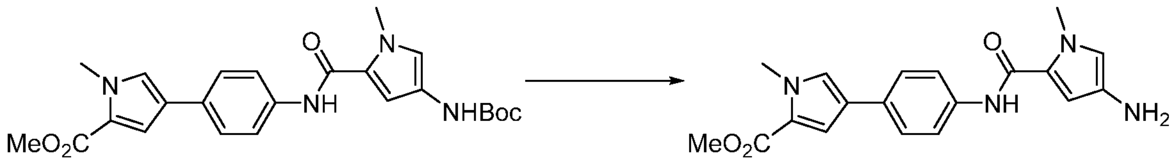


18 **57**

To a solution of 4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**18**) (59 mg, 0.23 mmol) in *N,N*-dimethylformamide (4 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (67 mg, 0.36 mmol) and 4-(dimethylamino)pyridine (65 mg, 0.53 mmol). The reaction mixture was stirred at room temperature for 2 h. Methyl 4-(4-aminophenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (41 mg, 0.18 mmol) was added to the reaction mixture which was then stirred at room temperature for 16 h. The reaction mixture was poured into ice-water (40 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layer was sequentially washed with 1 M citric acid (60 mL), a saturated aqueous solution of sodium hydrogen carbonate (70 mL), water (70 mL) and brine (70 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by column chromatography (silica), eluting with ethyl acetate/dichloromethane (from 0% to 50%), to give the title compound (36 mg, 45%) as a cream solid.

MS (ES+): *m/z* = 453 $(M+H)^+$; LCMS (**Method B**): t_R = 4.07 min.

Example 58: Methyl 4-(4-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)-phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (58)

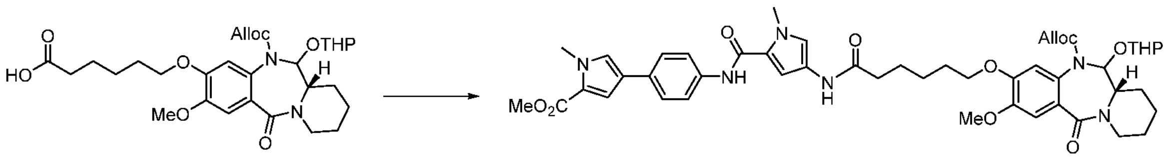


57 **58**

Methyl 4-(4-(4-((tert-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrole-2-carboxamido)-phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (**57**) (150 mg, 0.330 mmol) was dissolved in hydrochloric acid (4 M in 1,4-dioxane) (1 mL) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to give the title compound (114 mg, 99%) as a brown solid. The product was carried through to the next step without further purification.

MS (ES+): m/z = 353 $(M+H)^+$; LCMS (**Method B**): t_R = 2.88 min.

Example 59: Allyl (6aS)-2-methoxy-3-((6-((5-((4-(5-(methoxycarbonyl)-1-methyl-1H-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12H)-carboxylate (59)

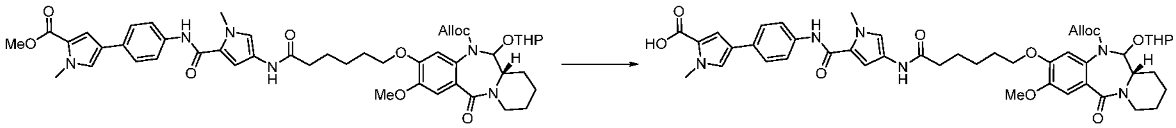


56 **59**

A solution of 6-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanoic acid (**56**) (194 mg, 0.360 mmol) in *N*,*N*-dimethylformamide (5 mL) was charged with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (126 mg, 0.660 mmol) and 4-(dimethylamino)pyridine (121 mg, 0.990 mmol). The reaction mixture was stirred at room temperature for 3 h. Methyl 4-(4-(4-amino-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (**58**) (150 mg, 0.330 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. This was then poured onto ice-water (20 mL) and extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were sequentially washed with an aqueous solution of citric acid (1 M, 50 mL), a saturated aqueous solution of sodium hydrogen carbonate (50 mL), water (50 mL) and brine (50 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (133 mg, 45%) as a yellow oil. The product was carried through to the next step without further purification.

MS (ES+): m/z = 896 $(M+H)^+$; LCMS (**Method B**): t_R = 4.25 min.

Example 60: 4-(4-(4-(6-(((6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[e]-pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylic acid (60)

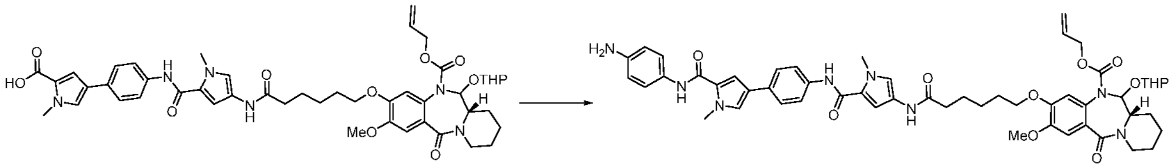


59 **60**

To a solution of allyl (6a*S*)-2-methoxy-3-((6-((5-((4-(5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**59**) (200 mg, 0.340 mmol) in 1,4-dioxane (3 ml) was added an aqueous solution of sodium hydroxide (1 M, 1.2 mL). The reaction mixture was stirred at room temperature for 2 h and was then concentrated *in vacuo*, after which water (6 ml) was added and the aqueous layer was acidified to pH = 1 with acetic acid. The aqueous layer was then extracted with ethyl acetate (2 x 40 mL). The combined organic extracts were then washed with brine (40 ml), dried over sodium sulfate, filtered and concentrated to give the title compound as a yellow oil (181 mg, 95%) which was used in the next step without further purification.

MS (ES+): m/z = 882 $(M+H)^+$; LCMS (**Method B**): t_R = 3.92 min.

Example 61: Allyl (6aS)-3-((6-((5-((4-(5-((4-aminophenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (61)



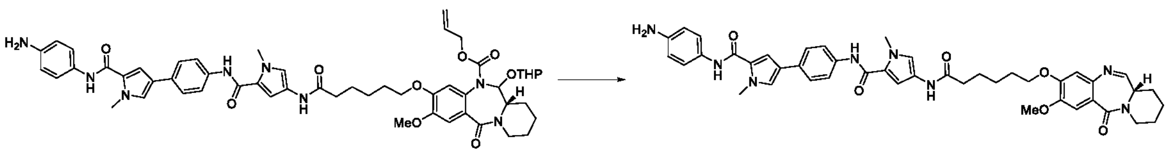
60 **61**

A solution of 4-(4-(4-(6-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-

a][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**60**) (123 mg, 0.14 mmol) in anhydrous dichloromethane (2 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (54 mg, 0.14 mmol) and anhydrous triethylamine (117 μL, 0.84 mmol). The reaction mixture was stirred at room temperature for 30 min. Benzene-1,4-diamine (15.1 mg, 0.14 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 50%), to give the title compound (63 mg, 46%) as a yellow solid.

MS (ES+): m/z = 972 $(M+H)^+$; LCMS (**Method B**): t_R = 3.55 min

Example 62: (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydro-benzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (62)

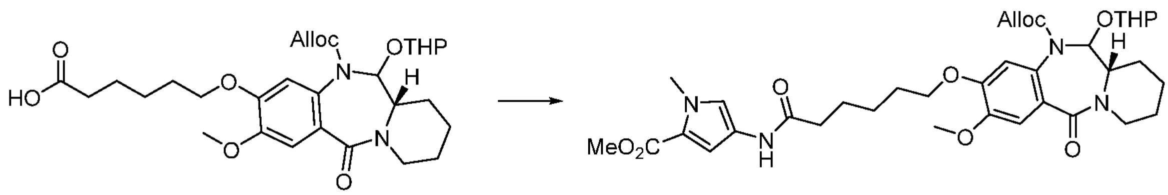


61 **62**

To a solution of Allyl (6aS)-3-((6-((5-((4-(5-((4-aminophenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]-pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**61**) (25 mg, 0.026 mmol) in dichloromethane (1 mL) was added tetrakis(triphenylphosphine)palladium(0) (2.5 mg, 5 mol%), triphenylphosphine (1.7 mg, 25 mol%) and pyrrolidine (21μL, 0.260 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with acetate/hexane (from 0% to 100%) to give the title compound (6.8 mg, 33%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.81-9.85 (m, 1H), 9.58 (s, 1H), 9.51 (s, 2H), 8.00 (d, J=5.7 Hz, 1H), 7.69-7.72 (m, 2H), 7.47-7.49 (m, 2H), 7.38-7.43 (m, 1H), 7.30-7.35 (m, 2H), 7.18-7.24 (m, 1H), 7.11-7.13 (m, 1H), 7.07 (s, 1H), 6.94-6.98 (m, 1H), 6.80 (br s, 1H), 6.63-6.72 (m, 2H), 6.52-6.54 (m, 1H), 3.95-4.14 (m, 3H), 3.89 (s, 3H), 3.83 (s, 3H), 3.70 (s, 3H), 3.65-3.69 (m, 1H), 3.17 (d, J=5.2 Hz, 2H), 2.28 (t, J=6.5 Hz, 2H), 1.72-1.78 (m, 4H), 1.62-1.68 (m, 4H), 1.42-1.48 (m, 3H) ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.5, 166.3, 164.6, 159.5, 159.2, 150.3, 147.1, 144.8, 139.8, 137.0, 129.6, 128.2, 126.6, 124.6, 124.3, 122.7, 122.1, 121.8, 121.7, 120.5, 120.4, 118.7, 113.7, 111.3, 109.6, 109.3, 104.7, 68.1, 55.6, 36.3, 36.1, 35.5, 28.3, 25.2, 25.1, 23.7, 22.5, 17.7; MS (ES+): m/z = 785 $(M+H)^+$; LCMS (**Method A**): t_R = 3.08 min.

Example 63: Allyl (6*S*,6a*S*)-2-methoxy-3-((6-((5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]-diazepine-5(12*H*)-carboxylate (63)

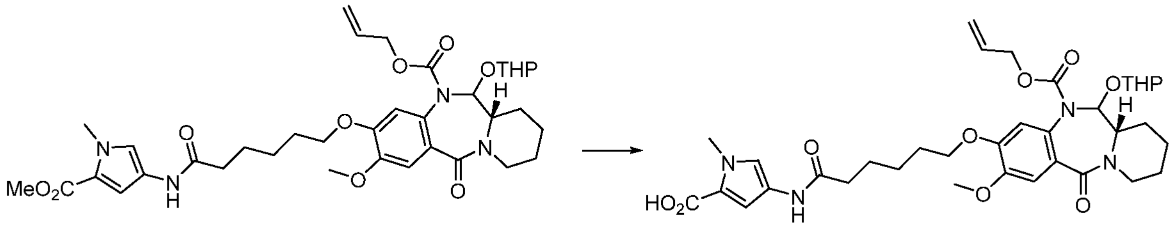


56 **63**

A solution of 6-(((6*S*,6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanoic acid (**56**) (109 mg, 0.190 mmol) in anhydrous dichloromethane (3 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (76 mg, 0.20 mmol) and anhydrous triethylamine (115 μL, 1.14 mmol). The reaction mixture was stirred at room temperature for 30 min. Methyl 4-amino-1-methyl-1*H*-pyrrole-2-carboxylate (37 mg, 0.24 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 0% to 30%), to give the title compound (82 mg, 62%) as a white solid.

MS (ES+): m/z = 697 $(M+H)^+$; LCMS (**Method B**): t_R = 3.98 min.

Example 64: 4-(6-(((6*S*,6a*S*)-5-((Allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid (64)

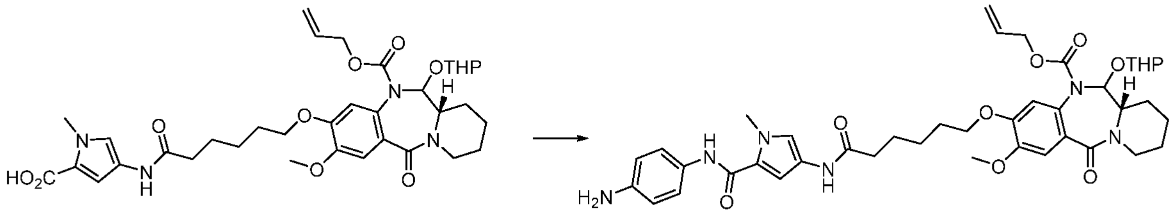


63 **64**

To a solution of allyl (6*S*,6a*S*)-2-methoxy-3-((6-((5-(methoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**63**) (76 mg, 0.11 mmol) in 1,4-dioxane (1 mL) was added an aqueous solution of sodium hydroxide (0.5 M, 1.0 mL, 0.50 mmol). The reaction mixture was stirred at room temperature for 16 h and was then concentrated *in vacuo*, after which water (20 mL) was added and the aqueous layer was acidified to pH = 1 with an aqueous solution of citric acid (1 M, 10 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were then washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated *in vacuo* to give the title compound (74 mg, 98%) as a cream solid. The product was carried through to the next step without any further purification.

MS (ES+): m/z = 683 $(M+H)^+$; LCMS (**Method B**): t_R = 3.68 min.

Example 65: Allyl (6*S*,6a*S*)-3-((6-((5-((4-aminophenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (65)



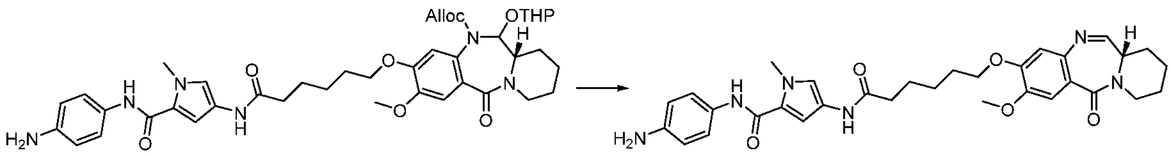
64 **65**

A solution of 4-(6-(((6*S*,6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**64**) (60 mg, 0.090 mmol) in anhydrous dichloromethane (1 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-

triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (67.0 mg, 0.175 mmol) and anhydrous triethylamine (73 µL, 0.52 mmol). The reaction mixture was stirred at room temperature for 30 min. Benzene-1,4-diamine (10 mg, 0.10 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were then washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with acetone/dichloromethane (from 30% to 50% + 5% MeOH), to give the title compound (18 mg, 26%) as a brown solid.

MS (ES+): m/z = 773 $(M+H)^+$; LCMS (**Method B**): t_R = 3.27 min.

Example 66: (*S*)-*N*-(4-Aminophenyl)-4-(6-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexanamido)-1-methyl-1*H*-pyrrole-2-carboxamide (66)

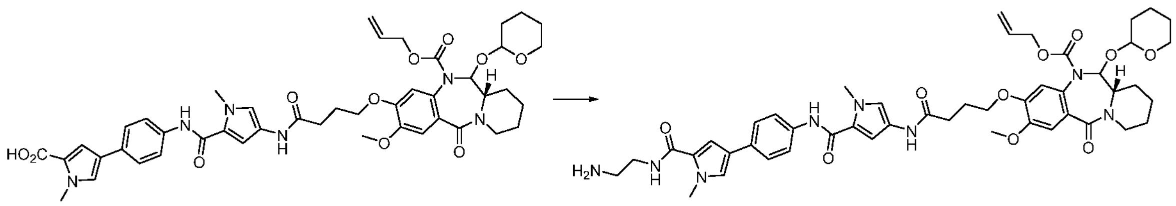


To a solution of allyl (6*S*,6a*S*)-3-((6-((5-((4-aminophenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-6-oxohexyl)oxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepine-5(12*H*)-carboxylate (**65**) (18 mg, 0.020 mmol) in dichloromethane (1 mL) was added tetrakis(triphenylphosphine)palladium(0) (1.3 mg, 5 mol%) and pyrrolidine (2.3 µL, 0.030 mmol). The reaction mixture was stirred at room temperature for 30 min and then subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 100%), to give the title compound (11.6 mg, 86%) as an off-white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.78 (s, 1H), 9.48 (s, 1H), 8.00 (d, J=5.7 Hz, 1H), 7.32 (d, J=8.8 Hz, 2H), 7.25 (s, 1H), 7.17 (d, J=1.8 Hz, 1H), 6.82 (d, J=1.9 Hz, 1H), 6.79 (s, 1H), 6.56 (d, J=8.6 Hz, 2H), 4.13 (dd, J=5.7, 3.4 Hz, 5H), 3.80 (s, 3H), 3.79 (s, 3H), 3.17 (s, 1H), 3.07-3.11 (m, 1H), 2.26 (t, J=7.2 Hz, 3H), 1.75 (dd, J=13.8, 7.0 Hz, 6H), 1.60-

1.65 (m, 5H); MS (ES+): m/z = 587 $(M+H)^+$; LCMS (**Method B**): t_R = 2.72 min, MS (ES+): m/z = 587 $(M+H)^+$; LCMS (**Method A**): t_R = 5.23 min.

Example 67: Allyl (6a*S*)-3-(4-((5-((4-(5-((2-aminoethyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (67)

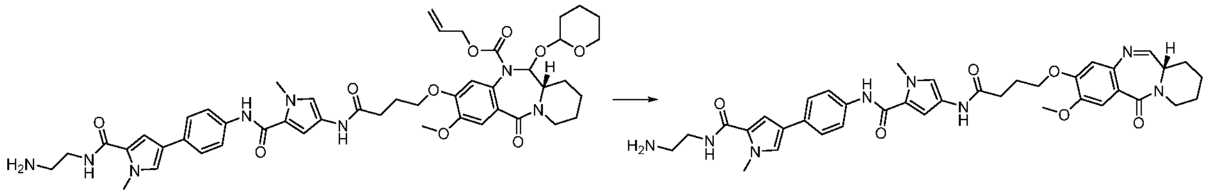


39 **67**

A solution of 4-(4-(4-(4-(((6a*S*)-5-((allyloxy)carbonyl)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,6a,7,8,9,10,12-octahydrobenzo[*e*]pyrido[1,2-*a*][1,4]-diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylic acid (**39**) (270 mg, 0.317 mmol) in anhydrous dichloromethane (6 mL) was charged with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (126 mg, 0.333 mmol) and anhydrous triethylamine (185 μL, 1.33 mmol). The reaction mixture was stirred at room temperature for 30 min. Ethane-1,2-diamine (379 mg, 6.33 mmol) was then added and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate (20 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water containing a few drops of acetic acid (30 mL). The organic layer was then dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (silica), eluting with ammonia in methanol (2 M)/dichloromethane (from 0% to 10%), to give the title compound (180 mg, 63%) as a white solid.

MS (ES+): m/z = 896 $(M+H)^+$; LCMS (**Method B**): t_R = 3.12 min.

Example 68: (*S*)-*N*-(2-Aminoethyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (68)



67 **68**

To a solution of allyl (6a*S*)-3-(4-((5-((4-(5-((2-aminoethyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)phenyl)carbamoyl)-1-methyl-1*H*-pyrrol-3-yl)amino)-4-oxobutoxy)-2-methoxy-12-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-6,6a,7,8,9,10-hexahydrobenzo[e]-pyrido[1,2-a][1,4]diazepine-5(12*H*)-carboxylate (**67**) (22 mg, 0.025 mmol) in dichloromethane (4 mL) was added tetrakis(triphenylphosphine)palladium(0) (1.4 mg, 5 mol%) and pyrrolidine (3.0 μL, 0.037 mmol). The reaction mixture was stirred at room temperature for 2 h and then subjected to high vacuum for 30 min until excess pyrrolidine was thoroughly removed. The resulting residue was then purified by column chromatography (silica), eluting with methanol/dichloromethane (from 0% to 20%), to give the title compound (11 mg, 62%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.01 (s, 1H), 9.84 (s, 1H), 9.21 (br s, 2H), 8.41 (s, 1H), 8.01 (d, J=5.7 Hz, 1H), 7.70 (d, J=8.8 Hz, 2H), 7.44 (d, J=8.7 Hz, 2H), 7.38 (d, J=1.8 Hz, 1H), 7.31 (d, J=1.9 Hz, 1H), 7.27 (s, 1H), 7.22 (d, J=1.8 Hz, 1H), 6.98 (d, J=1.8 Hz, 1H), 6.80 (s, 1H), 4.09-4.19 (m, 2H), 3.99-4.05 (m, 2H), 3.87 (s, 3H), 3.82 (m, 6H), 3.65-3.72 (m, 2H), 3.45-3.50 (m, 2H), 3.16 (d, J=5.3 Hz, 3H), 2.96 (t, J=5.8 Hz, 2H), 2.45 (t, J=7.4 Hz, 2H), 2.00-2.09 (m, 4H); (DMSO-d_6, 100 MHz) δ 203.1, 168.8, 166.3, 164.7, 161.6, 159.6, 150.2, 147.1, 139.8, 137.0, 129.5, 125.9, 124.2, 122.0, 120.6, 120.4, 111.2, 109.8, 109.3, 98.8, 95.4, 85.9, 78.8, 71.0, 67.7, 55.6, 49.2, 48.5, 36.3, 31.8, 30.2, 24.7, 22.5, 17.7; MS (ES+): m/z = 709 $(M+H)^+$; LCMS (**Method B**): t_R = 2.80 min, MS (ES+): m/z = 709 $(M+H)^+$; LCMS (**Method A**): t_R = 5.38 min.

<u>Example 69: Evidence of DNA Adduct formation by HPLC</u>

Interaction of C8-linked PDD monomers with duplex transcription factor consensus sequence was studied with an HPLC assay utilizing a X-bridge MS C18 2.5 μM OST column (2.3 x 50 mm) and a gradient of 40% acetonitrile/water and 100 mM TEAB (Tetraethylammonium bromide)/water as mobile phase with a flow rate of 0.5 mL/min and UV detection at 254 nm. A 4:1 molar ratio of ligand:oligonucleotide was used, with each single-stranded oligonucleotide dissolved in 1 M ammonium acetate to form stock solutions of 1 mM. The oligonucleotides were initially annealed by heating their 1 mM solutions to 70°C for 10 mins followed by gradual cooling over 8 hours and storage overnight at -20°C. Working solutions of oligonucleotides of 25 μM were then prepared by diluting the annealed stock solutions with 100 mM ammonium acetate. The ligands were dissolved in DMSO to form a stock solution of 10 mM which was stored at -20°C for no longer than four months. Working solutions of the drug of 100 µM were prepared by diluting the stock solution with 100 mM ammonium acetate. The working solutions of the ligands were added to the working solution the oligonucleotides at RT, and the mixture incubated for different time intervals at room temperature.

Example 70: Fluorescence Resonance Energy Transfer (FRET) Assay

Oligonucleotide sequences used for the FRET assays were purchased from Eurogentec, Southampton, UK: TAMRA (6-carboxytetramethylrhodamine) and FAM (6-carboxyfluorescein) are acceptor and donor fluorophores, respectively. From 20µM stock solutions, 400nM solutions in FRET buffer (optimized as 50mM potassium, 50mM cacodylate, pH 7.4) were prepared prior to use. The oligonucleotides were annealed through heating the samples to 90°C for 10 mins followed by cooling to room temperature and storing at this temperature for 5h. Dilutions from the initial 5 mM DMSO stock solution were performed using FRET buffer. Annealed DNA (50 µL) and sample solution (50 µL) were added to each well of a 96-well plate (MJ Research, Waltham, MA), and processed in a DNA Engine Opticon (MJ Research). Fluorescence readings were taken at intervals of 0.5°C over the range 30-100°C, with a constant temperature maintained for 30 seconds prior to each reading. Incident radiation of 450-495 nm was used, with detection at 515-545 nm. The raw data were imported into the program Origin (Version 7.0, OringinLab Corp.), and the graphs were smoothed using a 10-point running average, and then normalized. Determination of melting temperatures was based on values at the maxima of the first derivative of the smoothed melting curves using a script. The difference between the melting temperature of each sample and that of the blank (ΔTm) was used for comparative purposes.

Table 1: ΔTm determined after 24 hours incubation with Transcription Factor duplex DNA sequences

	ΔTm at 1 µM ligand concentration			
Compound	**NFκB (1st transition)**	**NFκB (2ND transition)**	**AP-1 (1st transition)**	**AP-1 (2ND transition)**
13	12	23	11	19
17	11	26	13	18
20	9	12	8	13

24	10	14	9	15

Example 71: Cytotoxicity Analysis of C8-linked PDD monomers by MTT Assay

Cell culture

MDA MB231 (triple negative human breast cancer) was obtained from the American Type Culture Collection. The cell-line was maintained in monolayer culture in 75 cm^2 flasks (TPP, Switzerland) under a humidified 5% CO_2 atmosphere at 37°C. The MDA MB231 cell line was maintained in high glucose DMEM (4.5g\l; Invitrogen), foetal bovine serum (10%, Biosera UK), non-essential amino acids (1x; Invitrogen), L-glutamine (2mM; Invitrogen) and Penicillin-Streptomycin (1% v/v, Invitrogen). The HeLa cell line was maintained in Dulbecco's Modified Eagles Media (DMEM; Invitrogen) supplemented with foetal bovine serum (10% v/v; Invitrogen), L-glutamine (2mM; Invitrogen), non-essential amino acids (1x; Invitrogen) and Penicillin-Streptomycin (1% v/v, Invitrogen). For passaging, cells were washed with PBS (GIBCO 14040, Invitrogen, UK), incubated with trypsine (GIBCO 25300, Invitrogen, UK), and re-seeded into fresh medium. For seeding, cells were counted using a Neubauer haemocytometer (Assistant, Germany) by microscopy (Nikon, USA) on a non-adherent suspension of cells that were washed in PBS, trypsinised, centrifuged at 8°C at 8000 rpm for 5 min and re-suspended in fresh medium.

MTT Assay

The cells were grown in normal cell culture conditions at 37 °C under a 5% CO_2 humidified atmosphere using appropriate medium. The cell count was adjusted to 10^5 cells/ml and 5,000-20,000 cells were added per well depending on the cell line. The cells were incubated for 24 hours and 1 μl of the appropriate inhibitor concentrations were added to the wells in triplicates. After 72 h of continuous exposure to each compound, the cytotoxicity was determined using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Lancaster Synthesis Ltd, UK) colorimetric assay.[34 Absorbance was quantified by spectrophotometry at λ = 570 nm (Envision Plate Reader, PerkinElmer, USA). IC_{50} values were calculated by a dose-response analysis using the GraphPad Prism® software.

Table 2: IC_{50} values (nM) determined after 72 hours exposure for the C8-linked PDD monomers.

	IC_{50} (nanomolar)	
Compound	**MDA MB 231 (Triple negative breast cancer cell line)**	**HeLa (Cervical cancer cell line)**
13	64± 9.6	0.6± 0.4
17	21± 1.8	1.2± 0.8
20	0.3± 0.22	0.14± 0.09
24	0.8± 0.66	1± 0.12

REFERENCES

1. Antonow, D., and Thurston, D. E. (2011) *Chem Rev* 111, 2815-2864.
2. Cipolla, L., Araujo, A. C., Airoldi, C., and Bini, D. (2009) *Anticancer Agents Med Chem* 9, 1-31.
3. Gerratana, B. (2012) *Med Res Rev* 32, 254-293.
4. Hartley, J. A. (2011) *Expert Opin Investig Drugs* 20, 733-744.
5. Kamal, A., Reddy, K. L., Devaiah, V., Shankaraiah, N., and Reddy, D. R. (2006) *Mini Rev Med Chem* 6, 53-69.
6. Hurley, L. H., Reck, T., Thurston, D. E., Langley, D. R., Holden, K. G., Hertzberg, R. P., Hoover, J. R., Gallagher, G., Jr., Faucette, L. F., Mong, S. M., (1988) *Chem Res Toxicol* 1, 258-268.
7. Wells, G., Martin, C. R., Howard, P. W., Sands, Z. A., Laughton, C. A., Tiberghien, A., Woo, C. K., Masterson, L. A., Stephenson, M. J., Hartley, J. A., Jenkins, T. C., Shnyder, S. D., Loadman, P. M., Waring, M. J., and Thurston, D. E. (2006) *J Med Chem* 49, 5442-5461.
8. Brucoli, F., Hawkins, R. M., James, C. H., Jackson, P. J., Wells, G., Jenkins, T. C., Ellis, T., Kotecha, M., Hochhauser, D., Hartley, J. A., Howard, P. W., and Thurston, D. E. (2013) *J Med Chem* 56, 6339-6351.
9. Kotecha, M., Kluza, J., Wells, G., O'Hare, C. C., Forni, C., Mantovani, R., Howard, P. W., Morris, P., Thurston, D. E., Hartley, J. A., and Hochhauser, D. (2008) *Mol Cancer Ther* 7, 1319-1328.
10. Puvvada, M. S., Hartley, J. A., Jenkins, T. C., and Thurston, D. E. (1993) *Nucleic Acids Res* 21, 3671-3675.
11. Clingen, P. H., De Silva, I. U., McHugh, P. J., Ghadessy, F. J., Tilby, M. J., Thurston, D. E., and Hartley, J. A. (2005) *Nucleic Acids Res* 33, 3283-3291.
12. Puvvada, M. S., Forrow, S. A., Hartley, J. A., Stephenson, P., Gibson, I., Jenkins, T. C., and Thurston, D. E. (1997) *Biochemistry* 36, 2478-2484.
13. Barkley, M. D., Cheatham, S., Thurston, D. E., and Hurley, L. H. (1986) *Biochemistry* 25, 3021-3031.
14. Seifert, J., Pezeshki, S., Kamal, A., and Weisz, K. (2012) *Organic & Biomolecular Chemistry* 10, 6850-6860.
15. Smellie, M., Bose, D. S., Thompson, A. S., Jenkins, T. C., Hartley, J. A., and Thurston, D. E. (2003) *Biochemistry* 42, 8232-8239.
16. Kopka, M. L., Goodsell, D. S., Baikalov, I., Grzeskowiak, K., Cascio, D., and Dickerson, R. E. (1994) *Biochemistry* 33, 13593-13610.
17. Kizu, R., Draves, P. H., and Hurley, L. H. (1993) *Biochemistry* 32, 8712-8722.
18. Leimgruber, W., Stefanovic, V., Schenker, F., Karr, A., and Berger, J. (1965) *J Am Chem Soc* 87, 5791-5793.
19. Arima, K., Kosaka, M., Tamura, G., Imanaka, H., and Sakai, H. (1972) *J Antibiot (Tokyo)* 25, 437-444.
20. Sato, S., Iwata, F., Yamada, S., Kawahara, H., and Katayama, M. (2011) *Bioorg Med Chem Lett* 21, 7099-7101.
21. Thurston D.E. and Bose D.S., *Chem Rev* (1994); 94:433-465.
22. Damayanthi, Y., et al.; *Journal of Organic Chemistry* (1999), 64, 290-292;
23. Kumar, et al., *Heterocyclic Communications* (2002) 8, 19-26.
24. Kumar, R, Lown, J. W.; *Oncology Research*, (2003) 13, 221-233.
25. Baraldi, P. G. et al., *Journal of Medicinal Chemistry* (1999) 42, 5131-5141.
26. Wells, G., et al., *Proc. Am. Assoc. Canc. Res.* (2003) 44, 452.
27. Thurston, D. E.; Howard, P. W. WO 2004/043963.
28. Bose, D. S., Thompson, A. S., Ching, J. S., Hartley, J. A., Berardini, M.D., Jenkins, T. C., Neidele, S., Hurley, L. H., and Thurston, D. E. (1992) *J. Am. Chem. Soc.* 114, 4939.

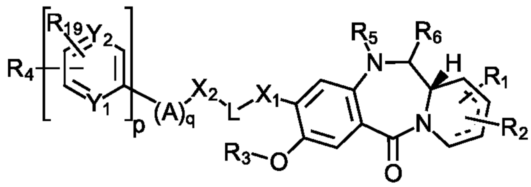
24 Sep 2019 2016313228

29. Wu, J., Clingen, P. H., Spanswick, V. J., Mellinas-Gomez, M., Meyer, T., Puzanov, I., Jodrell, D., Hochhauser, D., and Hartley, J. A. (2013) *Clin Cancer Res* 19, 721-730.
30. Jenkins, T. C., Hurley, L. H., Neidle, S., and Thurston, D. E. (1994) *J Med Chem* 37, 4529-4537.
31. Hochhauser, D., Meyer, T., Spanswick, V. J., Wu, J., Clingen, P. H., Loadman, P., Cobb, M., Gumbrell, L., Begent, R. H.,Hartley, J. A., Jodrell, D., (2009) *Clin Cancer Res* 15, 2140-2147.

All publications mentioned in the above specification are herein incorporated by reference. Although illustrative embodiments of the invention have been disclosed in detail herein, with reference to the accompanying drawings, it is understood that the invention is not limited to the precise embodiment and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope of the invention as defined by the appended claims and their equivalents.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge.

The present invention also provides a compound of formula (I):



(I)

and salts and solvates thereof,

wherein;

the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4;

R_1 is selected from R_7, OR_7, CO_2R_7, NR_7R_8, NH-C(O)-R_7, O-$(CH_2)_n$-NH-C(O)-R_7, O-$(CH_2)_n$-C(O)-NH-R_7, C(O)R_7 and C(O)NR_7R_8;

R_2 is H;

R_3 is selected from H, C_{1-12} alkyl and CH_2Ph;

R_4 is selected from phenyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl and benzothiazolyl, optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$;

R_{19} is selected from H and $(CH_2)_t$-$NR_{20}R_{21}$;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

p is 0 or 1;

j and t are independently selected from an integer from 0 to 6;

k and n are independently selected from an integer from 1 to 6;

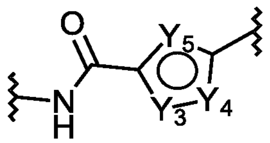
X_1 is selected from O, C(=O), C(=O)NR_{13}, NR_{13}C(=O);

L is selected from an amino acid, a peptide chain having from 2 to 6 amino acids, an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds, a paraformaldehyde chain $-(OCH_2)_{1-12}$-, a polyethylene glycol chain $-(OCH_2CH_2)_{1-6}$-, which chains may be interrupted by one or more of O, S and/or NH groups and/or C_{5-9} heteroarylene and/or phenylene;

X_2 is selected from O, C(=O), C(=O)NR_{15}, NR_{15}C(=O) or is absent;

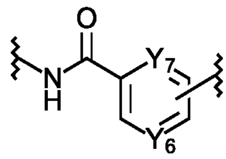
q is 1;

A is:



A1

and



A2

;

for each A1 group one of Y_3 and Y_4 is independently selected from N-R_{17}, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH;

for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH;

R_7 is independently selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl;

R_{24} is a phenyl optionally substituted with up to three optional substituent groups selected from OH, methyl, ethyl, propyl, OCH_3, OCH_2CH_3, CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, O-$(CH_2)_k$-NH_2, O-$(CH_2)_k$-NH(CH_3), $(CH_2)_j$-NH_2, $(CH_2)_j$-NH(CH_3), C(=O)-NH-$(CH_2)_k$-NH_2, C(=O)-NH-$(CH_2)_k$-NH(CH_3), C(=O)-NH-$(CH_2)_k$-C(=NH)NH(CH_3), and C(=O)-NH-$(CH_2)_k$-C(=NH)NH_2;

R_8, R_{11}, R_{12}, R_{13}, R_{15}, R_{17}, R_{20} and R_{21} are independently selected from H and C_{1-6} alkyl;

and either:

(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when p is 0 and A is A1, then:

(a) for at least one A1 group one of Y_3 and Y_4 is selected from S and O; or

2016313228 24 Sep 2019

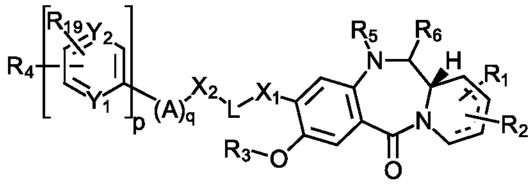
(b) for at least one A1 group Y_5 is S; or

(c) R_4 is not pyrrolyl, imidazolyl, optionally substituted pyrrolyl or optionally substituted imidazolyl.

2016313228 24 Sep 2019

CLAIMS

1. A compound of formula (I):



(I)

and salts and solvates thereof,

wherein;

the dotted lines indicates the optional presence of a double bond between one or more of C1 and C2, C2 and C3, and C3 and C4;

R_1 is selected from R_7, OR_7, CO_2R_7, NR_7R_8, NH-C(O)-R_7, O-$(CH_2)_n$-NH-C(O)-R_7, O-$(CH_2)_n$-C(O)-NH-R_7, C(O)R_7 and C(O)NR_7R_8;

R_2 is H;

R_3 is selected from H, C_{1-12} alkyl and CH_2Ph;

R_4 is selected from phenyl, pyrrolyl, N-methylpyrrolyl, furanyl, thiophenyl, imidazolyl, N-methylimidazolyl, oxazolyl, thiazolyl, pyridyl, benzofuranyl, benzothiophenyl, benzimidazolyl, N-methylbenzoimidazolyl, benzooxazolyl and benzothiazolyl, optionally substituted with up to three optional substituent groups selected from OH, C_{1-6} alkyl, OC_{1-6} alkyl, $(CH_2)_j$-CO_2R_{11}, O-$(CH_2)_k$-$NR_{11}R_{12}$, $(CH_2)_j$-$NR_{11}R_{12}$, C(=O)-NH-$(CH_2)_k$-$NR_{11}R_{12}$; C(=O)-NH-R_{24} and C(=O)-NH-$(CH_2)_k$-C(=NH)$NR_{11}R_{12}$;

R_{19} is selected from H and $(CH_2)_t$-$NR_{20}R_{21}$;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

p is 0 or 1;

j and t are independently selected from an integer from 0 to 6;

k and n are independently selected from an integer from 1 to 6;

X_1 is selected from O, C(=O), C(=O)NR_{13}, NR_{13}C(=O);

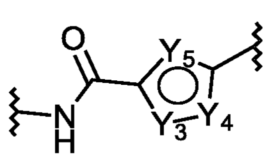
L is selected from an amino acid, a peptide chain having from 2 to 6 amino acids, an alkylene chain containing from 1 to 12 carbon atoms which may contain one or more carbon-carbon double or triple bonds, a paraformaldehyde chain $-(OCH_2)_{1-12}-$, a polyethylene glycol chain $-(OCH_2CH_2)_{1-6}-$, which chains may be interrupted by one or more of O, S and/or NH groups and/or C_{5-9} heteroarylene and/or phenylene;

X_2 is selected from O, C(=O), C(=O)NR_{15}, NR_{15}C(=O) or is absent;

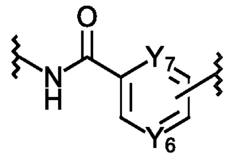
q is 1;

A is:

2016313228 24 Sep 2019



and



;

A1 A2

for each A1 group one of Y_3 and Y_4 is independently selected from N-R_{17}, S and O; and the other of Y_3 and Y_4 is CH; and Y_5 is independently selected from CH, N, S and COH;

for each A2 group one of Y_6 and Y_7 is independently selected from N and CH; and the other of Y_6 and Y_7 is CH;

R_7 is independently selected from H, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl, t-butyl;

R_{24} is a phenyl optionally substituted with up to three optional substituent groups selected from OH, methyl, ethyl, propyl, OCH_3, OCH_2CH_3, CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, O-$(CH_2)_k$-NH_2, O-$(CH_2)_k$-NH(CH_3), $(CH_2)_j$-NH_2, $(CH_2)_j$-NH(CH_3), C(=O)-NH-$(CH_2)_k$-NH_2, C(=O)-NH-$(CH_2)_k$-NH(CH_3), C(=O)-NH-$(CH_2)_k$-C(=NH)NH(CH_3), and C(=O)-NH-$(CH_2)_k$-C(=NH)NH_2;

R_8, R_{11}, R_{12}, R_{13}, R_{15}, R_{17}, R_{20} and R_{21} are independently selected from H and C_{1-6} alkyl; and either:

(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when p is 0 and A is A1, then:

(a) for at least one A1 group one of Y_3 and Y_4 is selected from S and O; or

(b) for at least one A1 group Y_5 is S; or

(c) R_4 is not pyrrolyl, imidazolyl, optionally substituted pyrrolyl or optionally substituted imidazolyl.

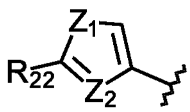
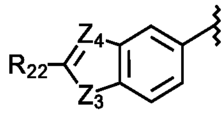
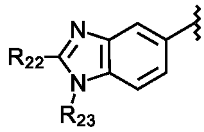
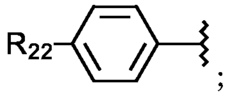
2. A compound of formula (I) and salts and solvates thereof according to claim 1, wherein R_{24} is -C_6H_4-$(CH_2)_j$-R_{18}, wherein R_{18} is selected from CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, NH(CH_3) and NH_2.

3. A compound of formula (I) and salts and solvates thereof according to claim 1 or 2, wherein R_1 is selected from R_7, O-$(CH_2)_n$-NH-C(O)-R_7 and O-$(CH_2)_n$-C(O)-NH-R_7.

4. A compound of formula (I) and salts and solvates thereof according to any of the previous claims, wherein R_3 is selected from methyl and ethyl.

2016313228 24 Sep 2019

5. A compound of formula (I) and salts and solvates thereof according to any of the previous claims, wherein R_4 is selected from:


,

,

and

;

wherein Z_1 is selected from NH, N-CH_3, S and O;

Z_2 is selected from CH and N;

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{22} is selected from $(CH_2)_jCO_2R_{11}$, $(CH_2)_jNR_{11}R_{12}$ and C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18};

R_{18} is selected from CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, $NH(CH_3)$ and NH_2;

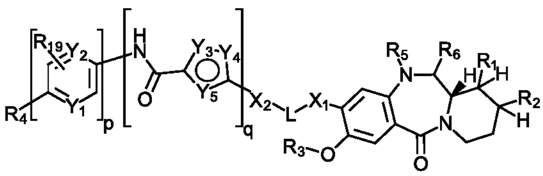
j is selected from an integer from 0 to 6; and

R_{11} and R_{12} are independently selected from H and C_{1-6} alkyl; and

R_{23} is selected from H and C_{1-6} alkyl.

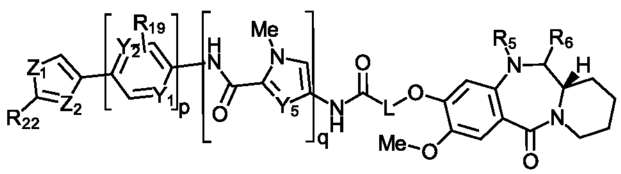
6. A compound of formula (I) and salts and solvates thereof according to any of the previous claims, wherein R_5 and R_6 together form a double bond.

7. A compound of formula (I) and salts and solvates thereof according to any of the previous claims, wherein compound has the following structure:

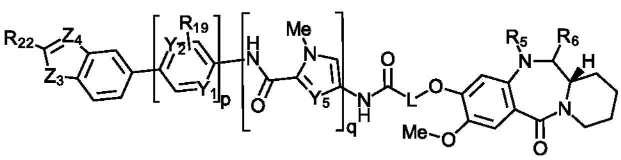


(XV).

8. A compound of formula (I) and salts and solvates thereof according to any of the previous claims, wherein the compound has the following structure:

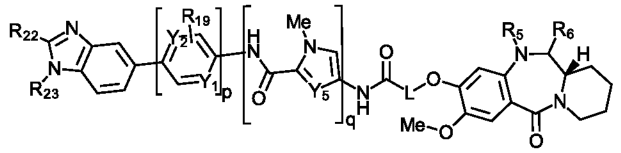


(XVI);

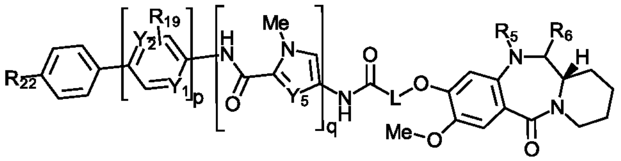


(XVII);

2016313228 24 Sep 2019



(XVIII); and



(XIX)

wherein q is 1;

p is 0 or 1;

L is an alkylene chain containing from 1 to 12 carbon atoms;

Y_1 is N or CH;

Y_2 is N or CH; and wherein at least one of Y_1 and Y_2 is CH;

Y_5 is selected from CH and N;

Z_1 is selected from O, S, NH and N-CH_3;

Z_2 is selected from CH and N; and

Z_3 is selected from S and O;

Z_4 is selected from CH and N;

R_{22} is selected from $(CH_2)_jCO_2H$, $(CH_2)_jCO_2C_{1-6}$ alkyl, $(CH_2)_jNR_{11}R_{12}$ and C(=O)-NH-C_6H_4-$(CH_2)_j$-R_{18};

R_{18} is selected from CO_2H, CO_2CH_3, $CO_2CH_2CH_3$, $NH(CH_3)$ and NH_2;

R_{19} is selected from H and $(CH_2)_t$-$NR_{20}R_{21}$;

j and t are independently selected from an integer from 0 to 6; and

R_{11}, R_{12} and R_{23} are independently selected from H and C_{1-6} alkyl.

and either:

(i) R_5 and R_6 together form a double bond;

(ii) R_5 is H and R_6 is OH; or

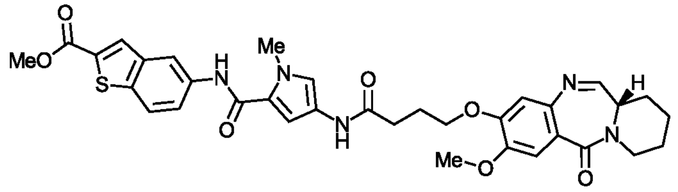
(iii) R_5 is H and R_6 is OC_{1-6} alkyl;

with the proviso that when the compound is (XVI) and p is 0, that Z_1 is selected from O and S.

9. A compound of formula (I) and salts and solvates thereof according to any of the preceding claims, wherein the compound is selected from:

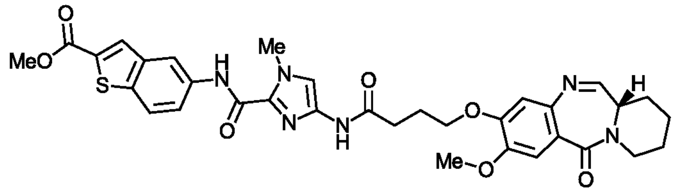
(a) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)benzo-[*b*]thiophene-2-carboxylate (13)

2016313228 24 Sep 2019



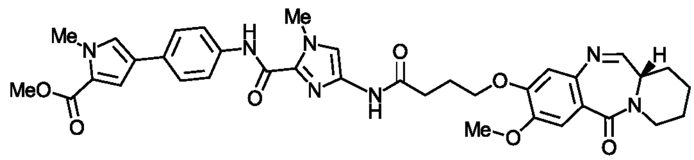
(13);

(b) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)-benzo[*b*]thiophene-2-carboxylate (17)



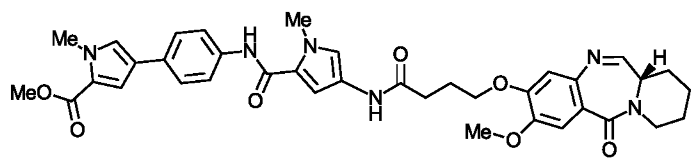
(17);

(c) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (20)



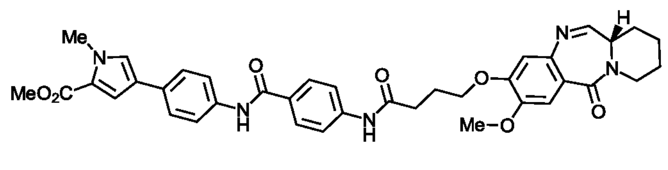
(20);

(d) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (24)



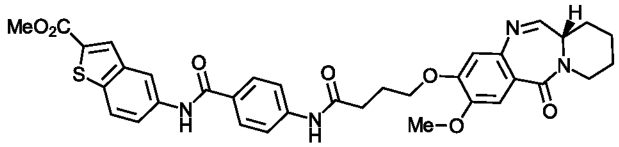
(24);

(e) methyl (*S*)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]-pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxylate (28)



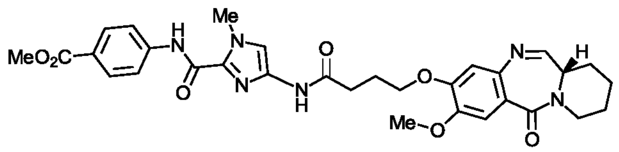
(28);

(f) methyl (*S*)-5-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-benzamido)benzo[b]thiophene-2-carboxylate (30)



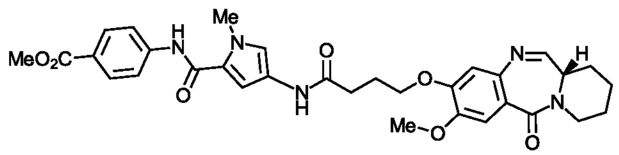
(30);

(g) methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-*a*][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-imidazole-2-carboxamido)-benzoate (34)



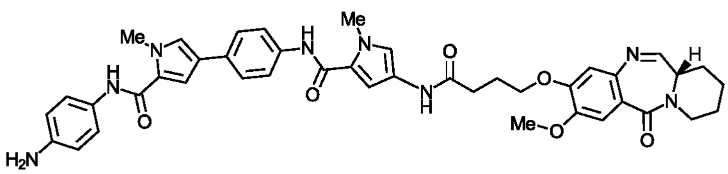
(34);

(h) methyl (*S*)-4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[e]pyrido-[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)-benzoate (38)



(38);

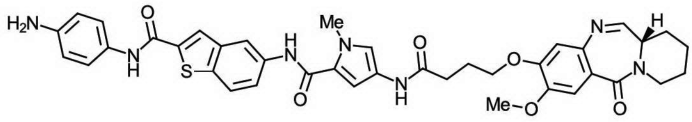
(i) (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7, 8,9,10,12-hexahydro-benzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)butan-amido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (41)



(41);

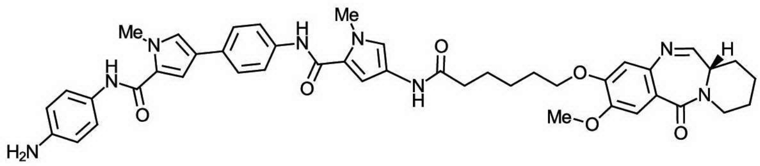
(j) (*S*)-*N*-(2-((4-Aminophenyl)carbamoyl)benzo[*b*]thiophen-5-yl)-4-(4-((2-methoxy-12-oxo-6a,7,8,9,10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)-butanamido)-1-methyl-1*H*-pyrrole-2-carboxamide (47)

2016313228 24 Sep 2019



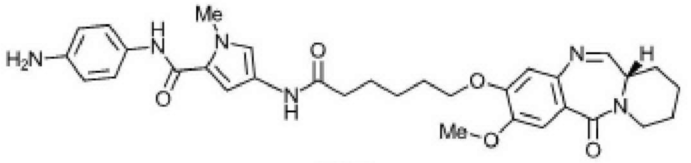
(47);

(k) (*S*)-*N*-(4-aminophenyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7, 8,9,10,12-hexahydro-benzo[e]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexan-amido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (62)



(62);

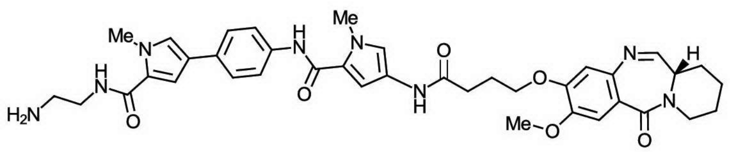
(l) (*S*)-*N*-(4-Aminophenyl)-4-(6-((2-methoxy-12-oxo-6a,7,8,9, 10,12-hexahydrobenzo[*e*]pyrido[1,2-*a*][1,4]diazepin-3-yl)oxy)hexan-amido)-1-methyl-1*H*-pyrrole-2-carboxamide (66)



(66);

and

(m) (*S*)-*N*-(2-Aminoethyl)-4-(4-(4-(4-((2-methoxy-12-oxo-6a,7,8,9, 10,12-hexahydro-benzo[e]pyrido[1,2-a][1,4]diazepin-3-yl)oxy)butanamido)-1-methyl-1*H*-pyrrole-2-carboxamido)phenyl)-1-methyl-1*H*-pyrrole-2-carboxamide (68)



(68).

10. A compound of formula (I) and salts and solvates thereof according to any one of claims 1 to 9 for use as a medicament.

11. A compound of formula (I) and salts and solvates thereof according to any one of claims 1 to 9 for use in the treatment of a proliferative disease.

2016313228 24 Sep 2019

12. A compound for use of formula (I) and salts and solvates thereof according to claim 11 for use in the treatment of a proliferative disease, wherein the proliferative disease is selected from bladder cancer, bone cancer, bowel cancer, brain cancer, breast cancer, cervical cancer, colon cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, oesophageal cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, retinoblastoma, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer and uterine cancer.

13. A pharmaceutical composition comprising a compound of formula (I) and salts and solvates thereof of any one of claims 1 to 9 and a pharmaceutically acceptable excipient, carrier or diluent.

14. Use of a compound of formula (I) and salts and solvates thereof according to any one of claims 1 to 9 in the manufacture of a medicament for treating a proliferative disease.

15. Use of a compound of formula (I) and salts and solvates thereof in the manufacture of a medicament for treating a proliferative disease according to claim 14, wherein the proliferative disease is selected from bladder cancer, bone cancer, bowel cancer, brain cancer, breast cancer, cervical cancer, colon cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, oesophageal cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, retinoblastoma, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer and uterine cancer.

16. A method of treatment of a patient suffering from a proliferative disease, comprising administering to said patient a therapeutically effective amount of a compound of any one of claims 1 to 11 or a pharmaceutical composition of claim 15.

17. A method of treatment of a patient suffering from a proliferative disease according to claim 16, wherein the proliferative disease is selected from bladder cancer, bone cancer, bowel cancer, brain cancer, breast cancer, cervical cancer, colon cancer, head and neck cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, oesophageal cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, retinoblastoma, sarcoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer and uterine cancer.

2016313228 24 Sep 2019

18. A compound of formula (I) and salts and solvates thereof of any one of claims 1 to 9, linked, either directly or indirectly, to a targeting agent to provide a targeted conjugate.

Figure 1

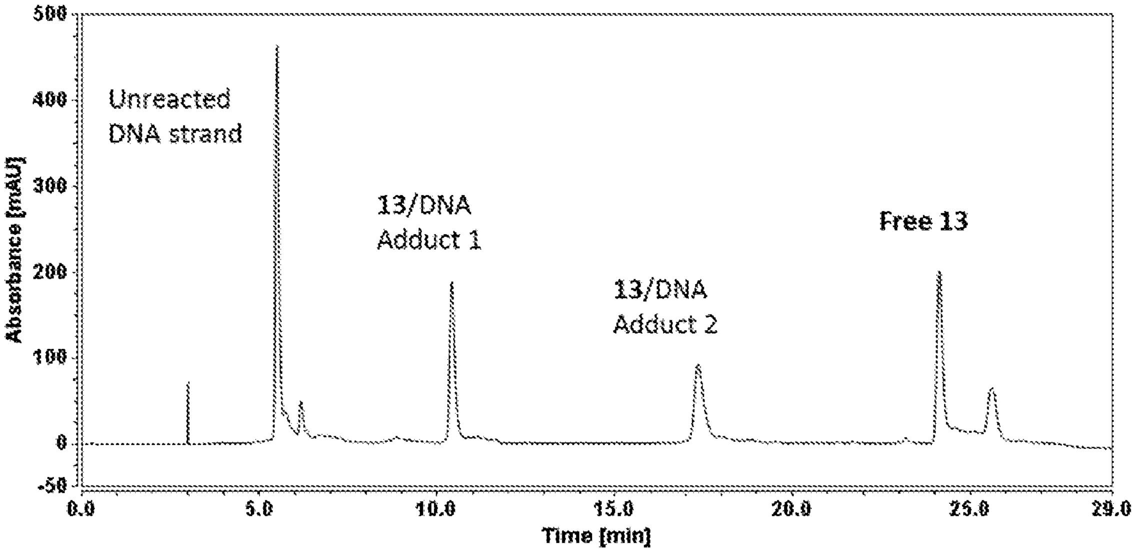


Figure 2

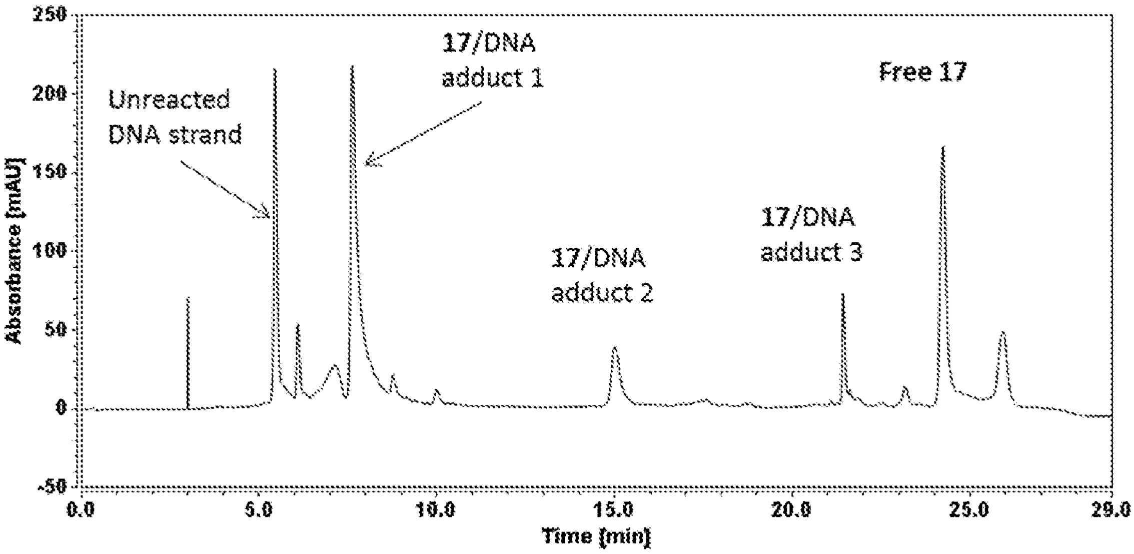


Figure 3

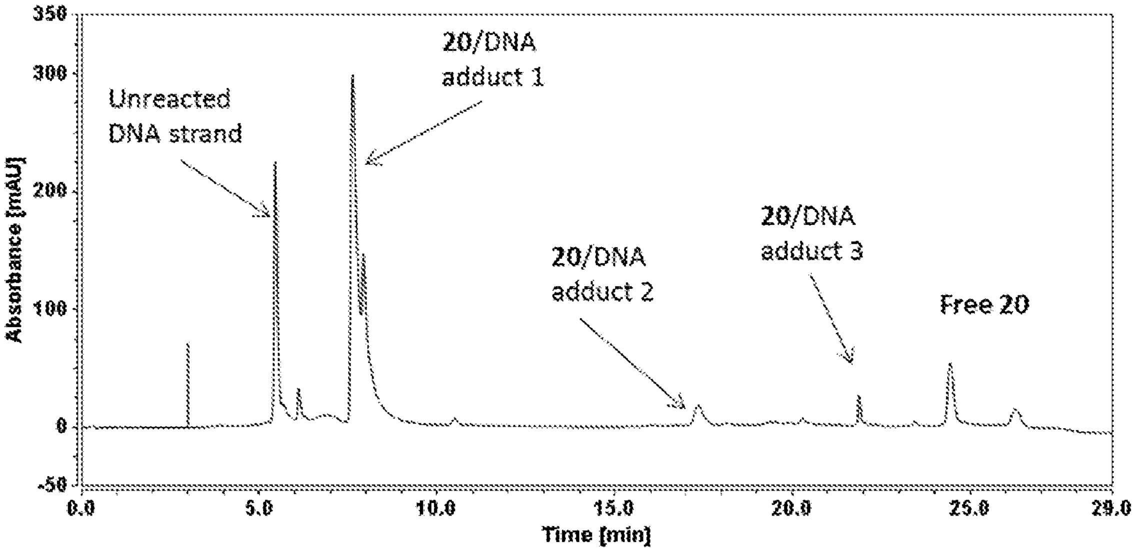


Figure 4