or any pharmaceutically acceptable salt thereof.

The compound of the preceding clause wherein B-L$(D)_X$ is capable of binding to the cell surface receptor.

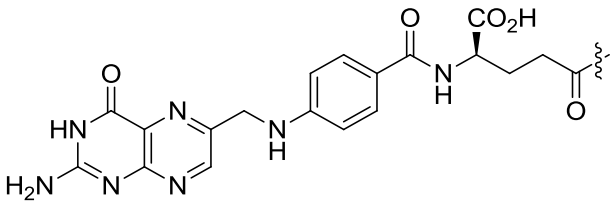
The compound of any one of the preceding clauses wherein the ligand is a vitamin receptor binding ligand.

The compound of any one of the preceding clauses wherein the ligand is a folate receptor binding ligand.

The compound of any one of the preceding clauses wherein the ligand is a folate.

The compound of any one of the preceding clauses wherein the ligand is a folate comprising D-glutamyl, also referred to herein as D-folate, or pteroyl-D-glutamic acid. It is to be understood herein that when B is a radical of D-folate, the included D-glutamyl portion of B is not part of the linker L.

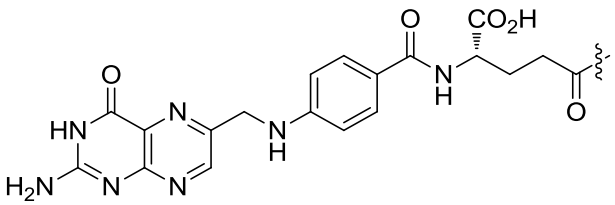
The compound of any one of the preceding clauses wherein B is an unnatural folate radical of the formula



.

The compound of any one of the preceding clauses wherein the ligand is folic acid.

The compound of any one of the preceding clauses wherein B is a radical of the formula

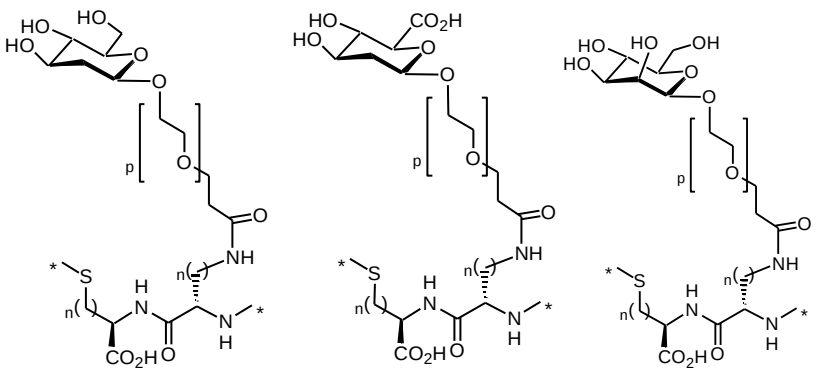


.

The compound of any one of the preceding clauses wherein at least one unnatural amino acid has the D-configuration.

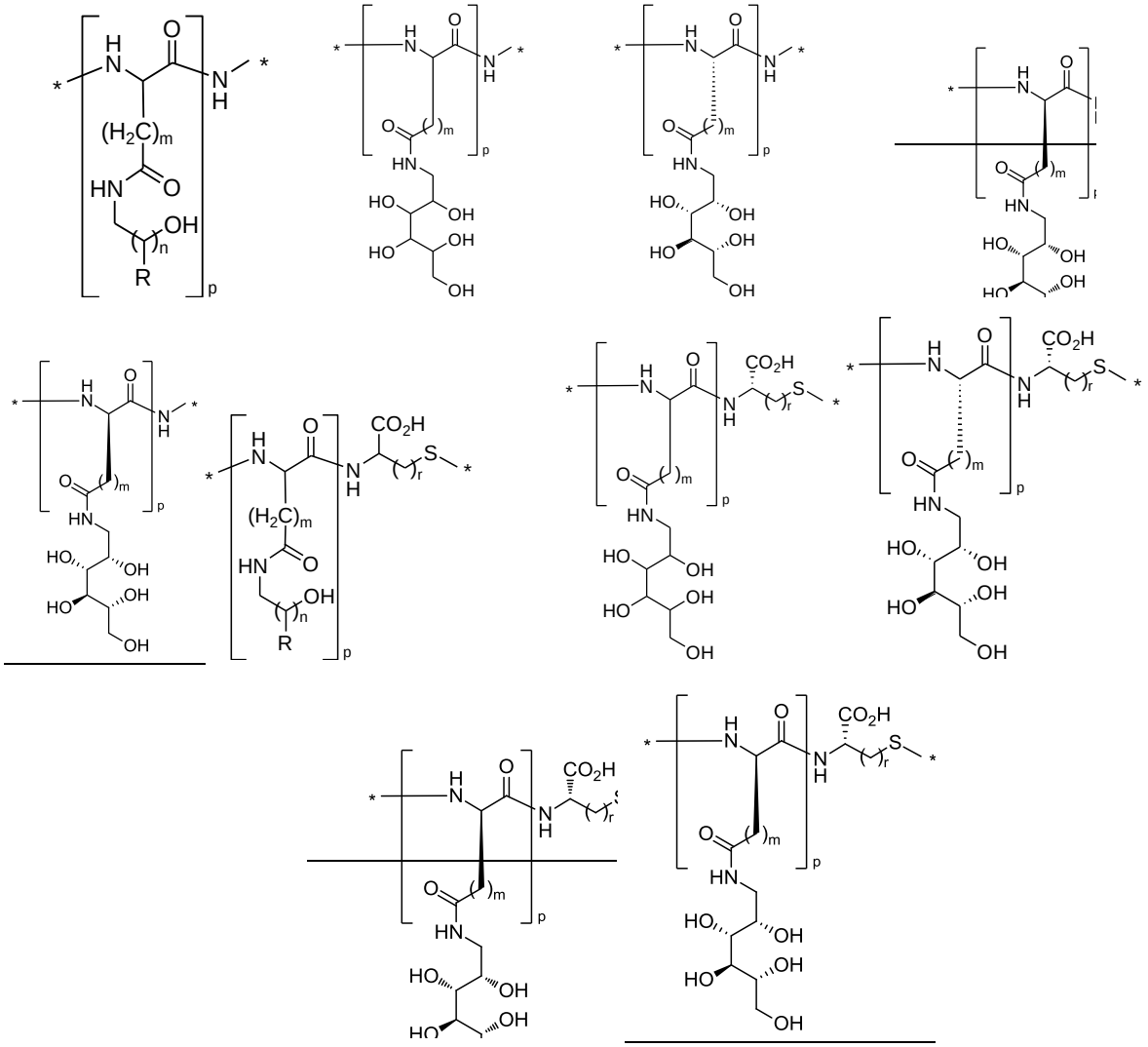
The compound of any one of the preceding clauses wherein at least one unnatural amino acid is selected from D-alanine, D-aspartic acid, D-asparagine, D-cysteine, D-glutamic acid, D-phenylalanine, D-histidine, D-isoleucine, D-lysine, D-leucine, D-methionine, D-proline, D-glutamine, D-arginine, D-serine, D-threonine, D-valine, D-tryptophan, D-tyrosine, and D-ornithine, and any amino acid derivatives thereof.

The compound of any one of the preceding clauses wherein at least one

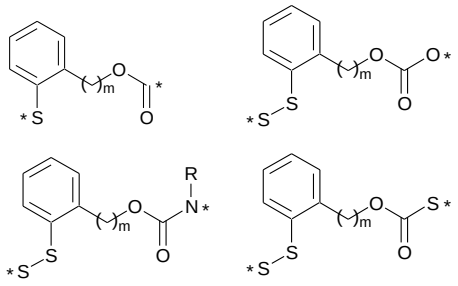


wherein n and r are integers and are each independently selected in each instance from 1 to about 5; and p is an integer selected from 1 to about 4.

In another embodiment, the polyvalent linker L comprises one or more of the following fragments:



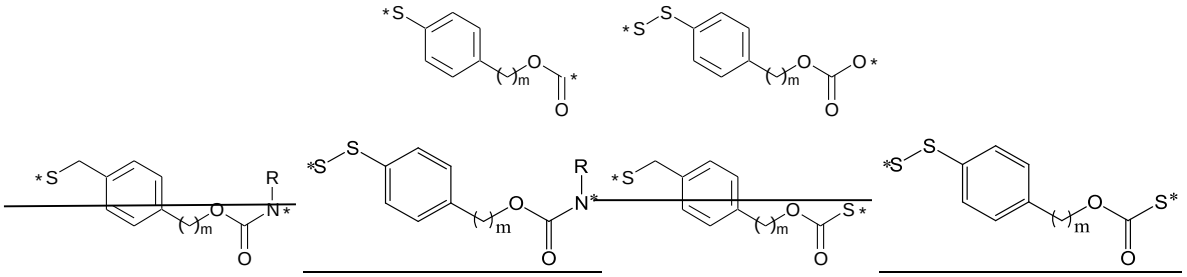
divalent radicals of formulae (III)



(III)

where m is an integer selected from 1 to about 4; R is an optionally substituted alkyl group, an optionally substituted acyl group, or a suitably selected nitrogen protecting group; and (*) indicates points of attachment for the drug, vitamin, imaging agent, diagnostic agent, other bivalent linkers, or other parts of the conjugate.

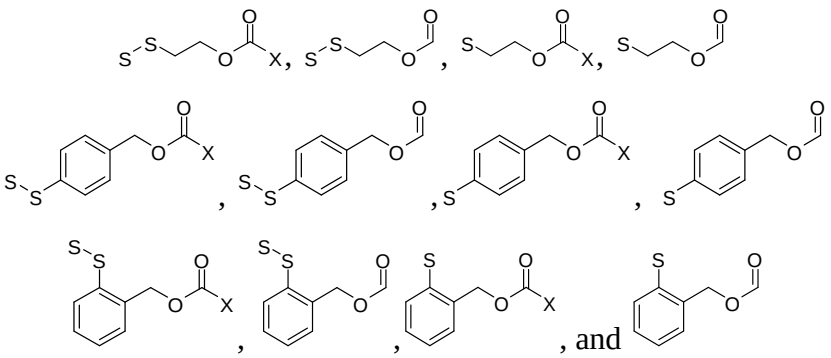
In another embodiment, the polyvalent linkers described herein comprise divalent radicals of formulae (IV)



(IV)

where m is an integer selected from 1 to about 4; R is an optionally substituted alkyl group, an optionally substituted acyl group, or a suitably selected nitrogen protecting group; and (*) indicates points of attachment for the drug, vitamin, imaging agent, diagnostic agent, other divalent linkers, or other parts of the conjugate.

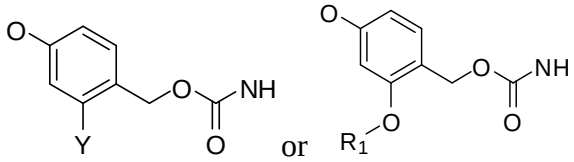
In another embodiment, the compounds described herein comprise one or more radicals ~~linkers of~~ selected from the formulae:



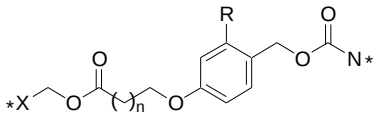
wherein X is NH, O, or S.

In another embodiment, the polyvalent linkers herein described comprise a

radical having the formula:



Another embodiment, the polyvalent linkers described herein comprise a radical of having the formula:



where X is an heteroatom, such as nitrogen, oxygen, or sulfur, n is an integer selected from 0, 1, 2, and 3, R is hydrogen, or a substituent, including a substituent capable of stabilizing a positive charge inductively or by resonance on the aryl ring, such as alkoxy, and the like, and the symbol (*) indicates points of attachment. It is appreciated that other substituents may be present on the aryl ring, the benzyl carbon, the alkanoic acid, or the methylene bridge, including but not limited to hydroxy, alkyl, alkoxy, alkylthio, halo, and the like.

In another embodiment, the polyvalent linkers described herein comprise ~~radicalsf~~ radicals selected from carbonyl, thionocarbonyl, alkylene, cycloalkylene, alkylenecycloalkyl, alkylenecarbonyl, cycloalkylenecarbonyl, carbonylalkylcarbonyl, ~~1~~ ~~alkylenesuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl,~~ 1-alkylenesuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl, alkylenesulfoxyl, sulfonylalkyl, alkylenesulfoxylalkyl, alkylenesulfonylalkyl, carbonyltetrahydro-2H-pyranyl, carbonyltetrahydrofuranyl, 1-(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl, and 1-(carbonyltetrahydrofuranyl)succinimid-3-yl, wherein each of said spacer linkers is optionally substituted with one or more substituents X^1;

wherein each substituent X^1 is independently selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, hydroxyalkyl, amino, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, halo, haloalkyl, sulfhydrylalkyl, alkylthioalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroaryl, substituted heteroaryl, carboxy, carboxyalkyl, alkyl carboxylate, alkyl alkanoate, guanidinoalkyl, R^4-carbonyl, R^5-carbonylalkyl, R^6-acylamino, and R^7-acylaminoalkyl, wherein R^4 and R^5 are each independently selected from the group consisting of an amino acid, an amino acid derivative, and a peptide, and wherein R^6 and R^7 are each independently selected from the group consisting of an amino acid, an amino acid derivative, and a peptide.

The compounds described herein may contain one or more chiral centers, or may

otherwise be capable of existing as multiple stereoisomers. It is to be understood that in one

embodiment, the invention described herein is not limited to any particular sterochemical requirement, except where specifically indicated, and that the compounds, and compositions, methods, uses, and medicaments that include them may be optically pure, or may be any of a variety of stereoisomeric mixtures, including racemic and other mixtures of enantiomers, other mixtures of diastereomers, and the like. It is also to be understood that such mixtures of stereoisomers may include a single stereochemical configuration at one or more chiral centers, while including mixtures of stereochemical configuration at one or more other chiral centers.

Similarly, the compounds described herein may ~~be~~ include geometric centers, such as cis, trans, E, and Z double bonds. It is to be understood that in another embodiment, the invention described herein is not limited to any particular geometric isomer requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be pure, or may be any of a variety of geometric isomer mixtures. It is also to be understood that such mixtures of geometric isomers may include a single configuration at one or more double bonds, while including mixtures of geometry at one or more other double bonds.

As used herein, the term "cell surface receptor binding or targeting ligand" generally refers to compounds that bind to and/or target receptors that are found on cell surfaces, and in particular those that are found on, over-expressed by, and/or preferentially expressed on the surface of pathogenic cells. Illustrative ligands include, but are not limited to, vitamins and vitamin receptor binding compounds.

Illustrative vitamin moieties include carnitine, inositol, lipoic acid, pyridoxal, ascorbic acid, niacin, pantothenic acid, folic acid, riboflavin, thiamine, biotin, vitamin B_{12}, and the lipid soluble vitamins A, D, E and K. These vitamins, and their receptor-binding analogs and derivatives, constitute the targeting entity from which a radical can be formed for covalent attachment to the polyvalent linker L. Illustrative biotin analogs that bind to biotin receptors include, but are not limited to, biocytin, biotin sulfoxide, oxybiotin, and the ~~like)~~ <u>like</u>.

Illustrative folic acid analogs that bind to folate receptors include, but are not limited to folinic acid, pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates, and their deaza and dideaza analogs. The terms "deaza" and "dideaza" analogs refer to the art-recognized analogs having a carbon atom substituted for one or two nitrogen atoms in the naturally occurring folic acid structure, or analog or derivative thereof. For example, the deaza analogs include the 1-deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs of folate, folinic acid, pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, and tetrahydrofolates. The dideaza analogs include, for example, 1,5-dideaza, 5,10-dideaza, 8,10-dideaza, and 5,8-

dideaza analoges of folate, folinic acid, peteropolyglutamic acid, and folate receptor binding

n, p, r, s and t are each independently either 0 or 1.

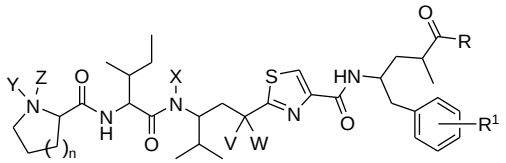
As used herein, the term "amino acid" refers generally to beta, gamma, and longer amino acids, such as amino acids of the formula:

$$-N(R)-(CR'R'')_q-C(O)-$$

where R is hydrogen, alkyl, acyl, or a suitable nitrogen protecting group, R′ and R″ are hydrogen or a substituent, each of which is independently selected in each occurrence, and q is an integer such as 1, 2, 3, 4, or 5. Illustratively, R′ and/or R″ independently correspond to, but are not limited to, hydrogen or the side chains present on naturally occurring amino acids, such as methyl, benzyl, hydroxymethyl, thiomethyl, carboxyl, carboxylmethyl, guanidinopropyl, and the like, and derivatives and protected derivatives thereof. The above described formula includes all stereoisomeric variations. For example, the amino acid may be selected from asparagine, aspartic acid, cysteine, glutamic acid, lysine, glutamine, arginine, serine, ornitine, threonine, and the like.

As used herein, the term "amino acid derivative" generally refers to an amino acid as defined herein where either, or both, the amino group and/or the side chain is substituted. Illustrative amino acid derivatives include prodrugs and protecting groups of the amino group and/or the side chain, such as amine, amide, hydroxy, carboxylic acid, and thio prodrugs and protecting groups. Additional Illustrative amino acid derivatives include substituted variations of the amino acid as described herein, such as, but not limited to, ethers and esters of hydroxy groups, amides, carbamates, and ureas of amino groups, esters, amides, and cyano derivatives of carboxylic acid groups, and the like.

As used herein, the terms "tubulysin" and "tubulysins" refer generally to tetrapeptide compounds of the formula



and pharmaceutical salts thereof, where

n is 1-3;

V is H, OR^2, or halo, and W is H, OR^2, or alkyl, where R^2 is independently selected in each instance from H, alkyl, and $C(O)R^3$, where R^3 is alkyl, cycloalkyl, alkenyl, aryl, or arylalkyl, each of which is optionally substituted; providing that R^2 is not H when both V and W are OR^2; or V and W are taken together with the attached carbon to form a carbonyl;

X=H, C_{1-4} alkyl, alkenyl, each of which is optionally substituted, or CH_2QR^9; where Q is ~~–N–,~~ -O-, or –S-; R^9=H, C_{1-4} alkyl, alkenyl, aryl, or $C(O)R^{10}$; and R^{10}=C_{1-6} alkyl, alkenyl, aryl, or heteroary, each of which is optionally substituted;

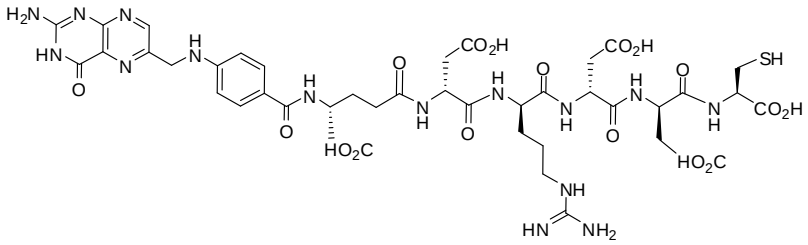
to as lower alkenylene and/or alkynylene. It is appreciated herein that shorter alkylene, alkenylene, and/or alkynylene groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. In embodiments of the invention described herein, it is to be understood, in each case, that the recitation of alkylene, alkenylene, and alkynylene refers to alkylene, alkenylene, and alkynylene as defined herein, and optionally lower alkylene, alkenylene, and alkynylene. Illustrative alkyl groups are, but not limited to, methylene, ethylene, n-propylene, isopropylene, n-butylene, isobutylene, sec-butylene, pentylene, 1,2-pentylene, 1,3-pentylene, hexylene, heptylene, octylene, and the like.

As used herein, the term "cycloalkyl" includes a chain of carbon atoms, which is optionally branched, where at least a portion of the chain ~~in~~ is cyclic. It is to be understood that cycloalkylalkyl is a subset of cycloalkyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkyl include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, 2-methylcyclopropyl, cyclopentyleth-2-yl, adamantyl, and the like. As used herein, the term "cycloalkenyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond, where at least a portion of the chain in cyclic. It is to be understood that the one or more double bonds may be in the cyclic portion of cycloalkenyl and/or the non-cyclic portion of cycloalkenyl. It is to be understood that cycloalkenylalkyl and cycloalkylalkenyl are each subsets of cycloalkenyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkenyl include, but are not limited to, cyclopentenyl, cyclohexylethen-2-yl, cycloheptenylpropenyl, and the like. It is to be further understood that chain forming cycloalkyl and/or cycloalkenyl is advantageously of limited length, including C_3-C_{24}, C_3-C_{12}, C_3-C_8, C_3-C_6, and C_5-C_6. It is appreciated herein that shorter alkyl and/or alkenyl chains forming cycloalkyl and/or cycloalkenyl, respectively, may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior.

As used herein, the term "heteroalkyl" includes a chain of atoms that includes both carbon and at least one heteroatom, and is optionally branched. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. As used herein, the term "cycloheteroalkyl" including heterocyclyl and heterocycle, includes a chain of atoms that includes both carbon and at least one heteroatom, such as heteroalkyl, and is optionally branched, where at least a portion of the chain is cyclic. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. Illustrative cycloheteroalkyl include, but are not limited to, tetrahydrofuryl, pyrrolidinyl, tetrahydropyranyl, piperidinyl, morpholinyl, piperazinyl, homopiperazinyl, quinuclidinyl, and the like

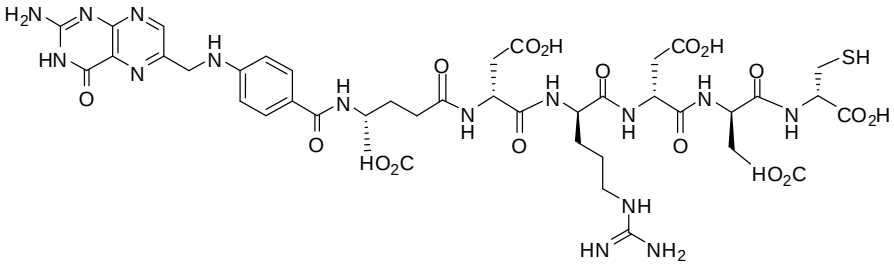
As used herein, the term "aryl" includes monocyclic and polycyclic aromatic

4.6(m), 2.8-3.2(m), 2.2-2.8(m), 1-2.2(m)



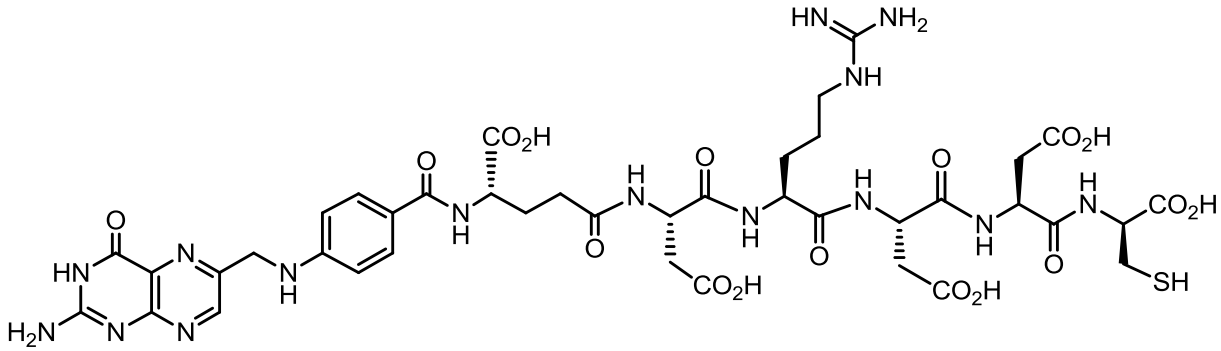
EC819

MS (ESI, $[M+H]^{+1}$) = 1046.4. Partial 1H-NMR (DMSO) δ(ppm): 8.6(s), 7.6(d), 6.6(d), 4-4.6(m), 3.4-3.8(m), 3-3.15(m), 1-2.8(m)



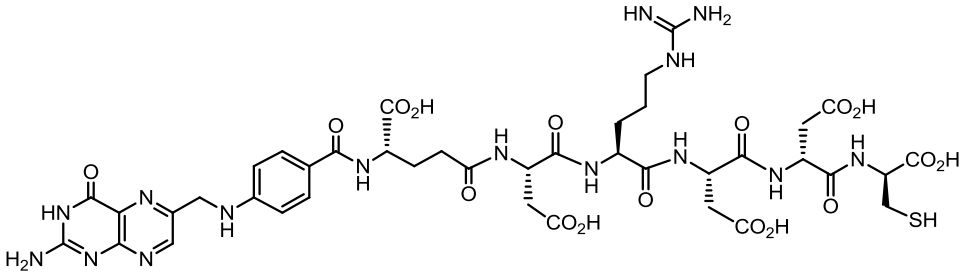
EC259

$[M+H]^+$ = 1047.52. Partial 1H NMR (<u>D_2O</u> ~~D2O~~): 8.6 (s, 1H), 7.5 (d, 2H), 6.65 (d, 2H), 4.4 (dd, 2H), 4.18 (m, 4H), 2.9 (t, 2H), 2.75 (t, 2H), 2.6-2.15 (m, 10H), 2.1-1.8 (m, 3H), 1.7-1.4 (m, 3H), 1.3 (m, 3H).



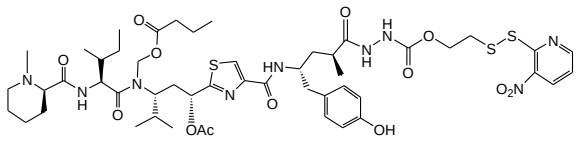
EC1544

MS (ESI $[M + H]^+$): 1046. Partial ^1H NMR data (D_2O, 300 MHz): δ(ppm) 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).



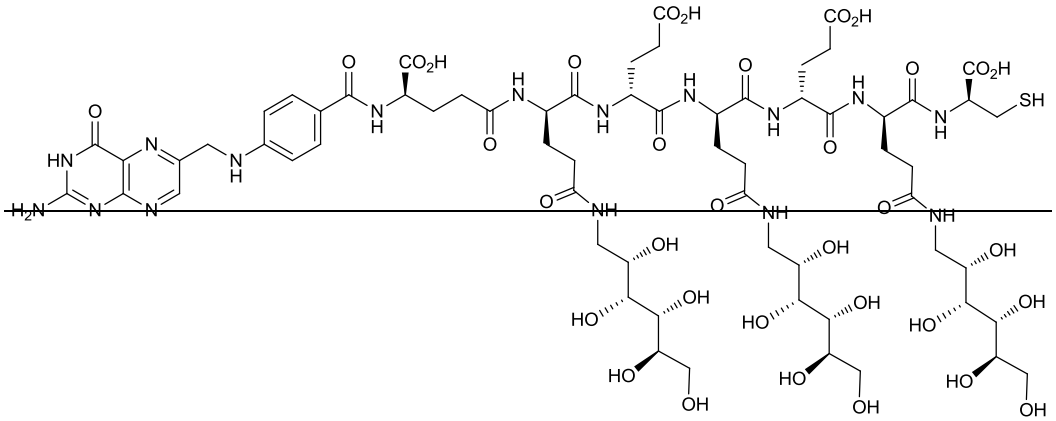
EC1547

MS (ESI, $[M + H]^+$) = 1046.7. Partial 1H-NMR (<u>D_2O</u> ~~D2O~~) δ(ppm): 8.6(s), 7.5(d), 6.6(d), 4.4-



Similarly, Tubulysin B pyridyldisulfide is prepared as described herein.

~~EXAMPLE. D-EC0488.~~



~~This compound was prepared by SPPS according to the general peptide synthesis procedure described herein starting from H-Cys(4-methoxytrityl)-2-chlorotrityl-Resin, and the following SPPS reagents:~~

~~Reagents~~	~~mmol~~	~~equivalent~~	~~MW~~	~~amount~~
~~H-D-Cys(4-methoxytrityl)-2-chlorotrityl-Resin (loading 0.6mmol/g)~~	~~0.10~~			~~0.17 g~~
~~D-EC0475~~	~~0.13~~	~~1.3~~	~~612.67~~	~~0.082 g~~
~~Fmoc-D-Glu(OtBu)-OH~~	~~0.19~~	~~1.9~~	~~425.47~~	~~0.080 g~~
~~D-EC0475~~	~~0.13~~	~~1.3~~	~~612.67~~	~~0.082 g~~
~~Fmoc-D-Glu(OtBu)-OH~~	~~0.19~~	~~1.9~~	~~425.47~~	~~0.080 g~~
~~D-EC0475~~	~~0.13~~	~~1.3~~	~~612.67~~	~~0.082 g~~
~~Fmoc-D-Glu-OtBu~~	~~0.19~~	~~1.9~~	~~425.47~~	~~0.080 g~~
~~N^{10}TFA-Pteroic Acid (dissolve in 10ml DMSO)~~	~~0.16~~	~~1.6~~	~~408.29~~	~~0.066 g~~
~~DIPEA~~		~~2.0 eq of AA~~		
~~PyBOP~~		~~1.0 eq of AA~~		

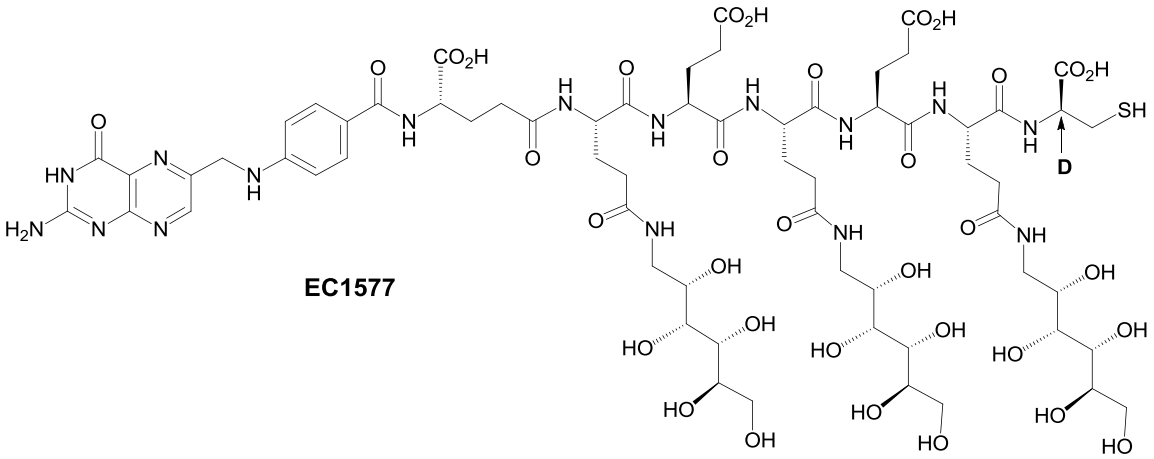
~~Coupling steps. In a peptide synthesis vessel add the resin, add the amino acid solution, DIPEA, and PyBOP. Bubble argon for 1hr. and wash 3X with DMF and IPA. Use 20% piperidine in DMF for Fmoc deprotection, 3X (10min), before each amino acid coupling. Continue to complete all 9 coupling steps. At the end treat the resin with 2% hydrazine in DMF 3X (5min) to cleave TFA protecting group on Pteroic acid, wash the resin with DMF (3X), IPA (3X), MeOH (3X), and bubble the resin with argon for 30 min.~~

~~Cleavage step. Reagent: 92.5% TFA, 2.5% H_2O, 2.5% triisopropylsilane, 2.5% ethanedithiol. Treat the resin with cleavage reagent 3X (10 min, 5 min, 5 min) with argon bubbling, drain, wash the resin once with cleavage reagent, and combine the solution. Rotavap~~

~~until 5ml remains and precipitate in diethyl ether (35 mL). Centrifuge, wash with diethyl ether, and dry. About half of the crude solid (~100 mg) was purified by HPLC.~~

~~HPLC Purification step. Column: Waters Xterra Prep MS C18 10 µm 19x250 mm; Solvent A: 10mM ammonium acetate, pH 5; Solvent B: ACN; Method: 5 min 0% B to 25 min 20% B 26mL/min. Fractions containing the product was collected and freeze-dried to give 43 mg EC0488 (51% yield). ^{1}H NMR and LC/MS (exact mass 1678.62) were consistent with the product.~~

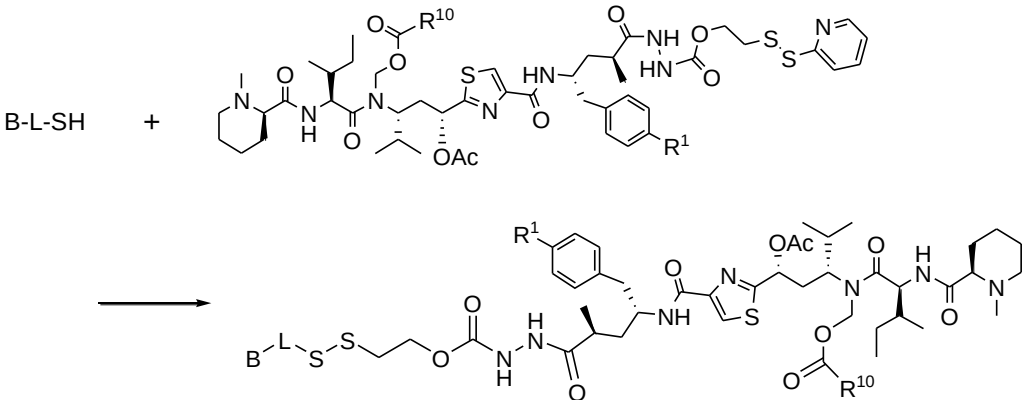
EXAMPLE.



EC1577

MS (ESI, $[M + H]^+$) = 1681. Partial ^{1}H NMR (D_2O): 8.96 (s), 7.65 (d), 6.81 (d), 4.66 (s), 4.40-4.15 (m), 3.90-3.54 (m), 3.50-3.18 (m), 2.97-2.90 (m), 2.51-1.80 (m).

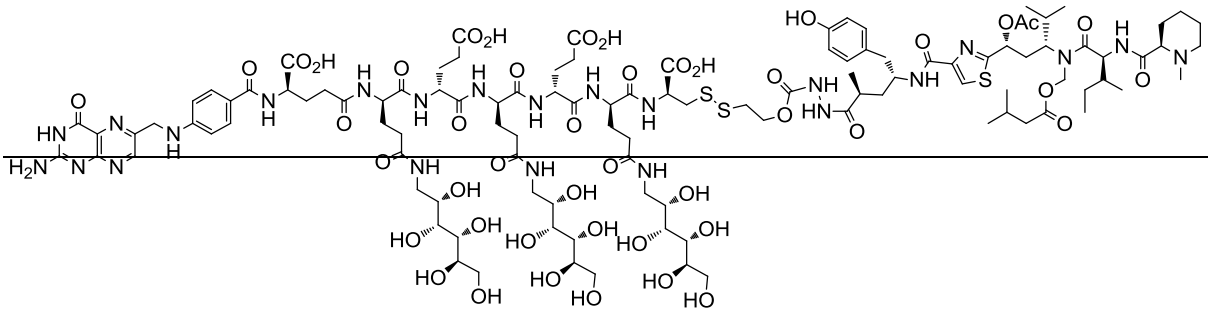
EXAMPLE. General Synthesis of Disulfide Containing Tubulysin Conjugates.



Illustrated with pyridinyl disulfide derivatives of certain naturally occurring tubulysins, where R^1 is H or OH, and R^{10}, is alkyl or alkenyl. A binding ligand-linker intermediate containing a thiol group is taken in deionized water (ca. 20 mg/mL, bubbled with argon for 10 minutes prior to use) and the pH of the suspension was adjusted by saturated $NaHCO_3$ (bubbled with argon for 10 minutes prior to use) to about 6.9 (the suspension may become a solution when the pH increased). Additional deionized water is added (ca. 20-25%) to the solution as needed, and to the aqueous solution is added immediately a solution of

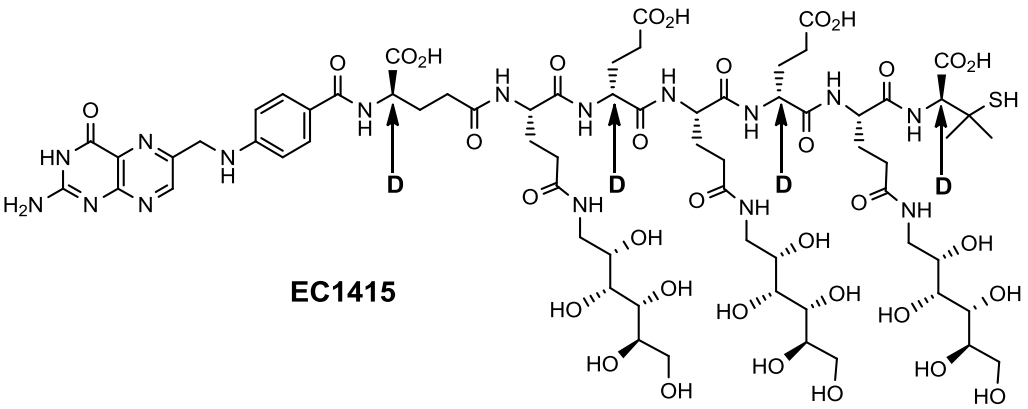
EC0312 in THF (ca. 20 mg/mL). The reaction mixture becomes homogenous quickly. After stirring under argon, e.g. for 45 minutes, the reaction mixture is diluted with 2.0 mM sodium phosphate buffer (pH 7.0, ca 150 volume percent) and the THF is removed by evacuation. The resulting suspension is filtered and the filtrate may be purified by preparative HPLC (as described herein). Fraction are lyophilized to isolate the conjugates. The foregoing method is equally applicable for preparing other tubulysin conjugates by the appropriate selection of the tubulysin starting compound.

~~EXAMPLE. General Method 2 for Preparing Conjugates (one-pot).~~



~~Illustrated with preparation of EC1456. DIPEA (7.8 µL) and isobutyl chloroformate (3.1 µL) were added with the help of a syringe in tandem into a solution of tubulysin A (18 mg) in anhydrous EtOAc (0.50 mL) at -15 ºC. After stirring for 35 minutes at -15 ºC under argon, to the reaction mixture was added a solution of EC0311 (5.8 mg) in anhydrous EtOAc (0.50 mL). The cooling was removed and the reaction mixture was stirred under argon for an additional 45 minutes, concentrated, vacuumed, and the residue was dissolved in THF (2.0 mL). Meanwhile, D-EC0488 (40 mg) was dissolved in deionized water (bubbled with argon for 10 minutes prior to use) and the pH of the aqueous solution was adjusted to 6.9 by saturated $NaHCO_3$. Additional deionized water was added to the D-EC0488 solution to make a total volume of 2.0 mL and to which was added immediately the THF solution containing the activated tubulysin. The reaction mixture, which became homogeneous quickly, was stirred under argon for 50 minutes and quenched with 2.0 mM sodium phosphate buffer (pH 7.0, 15 mL). The resulting cloudy solution was filtered and the filtrate was injected into a preparative HPLC for purification. Column: Waters XTerra Prep MS C_{18} 10 µm, 19×250 mm; Mobile phase A: 2.0 mM sodium phosphate buffer, pH 7.0; Mobile phase B: acetonitrile; Method: 1%B for 5 minutes, then 1%B to 60%B over the next 30 minutes, flow rate = 26mL/min. Fractions from 20.75-24.50 minutes were collected and lyophilized to afford EC1456 as a pale yellow fluffy solid (26 mg). The foregoing method is equally applicable for preparing other tubulysin and other conjugates by the appropriate selection of the tubulysin or other drug starting compound.~~

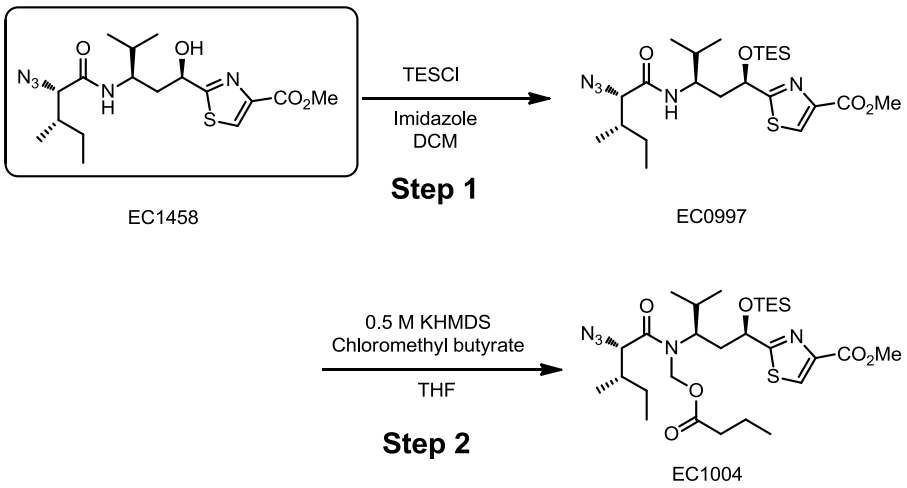
EXAMPLE. EC1663 and EC1664 The following additional compounds are



EC1415

EC1415: $[M + H]^+$ = 1709.69, $[M+2H]^{2+}$ = 855.22. Partial ^{1}H NMR (D_2O ~~$D2O$~~, 300 MHz) δ(ppm): 8.6 (s, 1H), 7.45 (d, 2H), 6.5 (d, 2H), 4.5 (s, 2H), 4.3-4.1 (m, 6H), 3.95 (t, 1H), 3.8-3.4 (m, 19H), 3.4-2.95 (m, 7H), 2.4-1.7 (m, 26H), 1.6 (m, 1H), 1.25 (s, 2H), 1.05 (s, 3H).

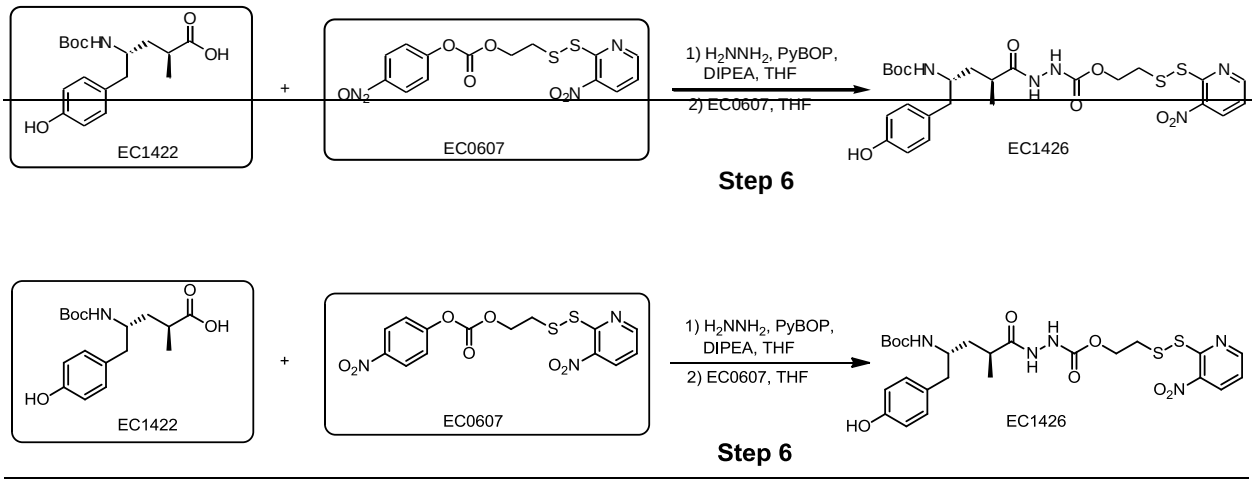
EXAMPLE. EC1004 is prepared according to the following process.



Into a round bottomed flask equipped with magnetic stir bar and temperature probe dipeptide EC1458, imidazole, and methylene chloride is added. Once all the solids have dissolved, the solution is cooled using an ice bath. Chlorotriethylsilane (TESCl) is added drop wise and the ice bath is removed. The reaction is monitored for completion. A second portion of chlorotriethylsilane and/or imidazole is added if necessary. The imidazole HCl salt is removed by filtration and methylene chloride is added. The organics are washed with a saturated solution of sodium chloride (brine), the aqueous layer is back extracted once with methylene chloride, and the combined organic layers are washed with brine. The organic layer is dried over sodium sulfate and concentrated on a rotary evaporator. The residue is dissolved in tetrahydrofuran (THF) and cooled to approximately -45 ^oC. A solution of potassium bis(trimethylsilyl)amide (KHMDS) in toluene is added drop wise. With stirring, chloromethyl butyrate is added and the reaction is monitored. The reaction is quenched with methanol and then ethyl acetate and brine are added. The aqueous layer is discarded and the organics are washed once with brine. The organic layer is concentrated on a rotary evaporator and the oily

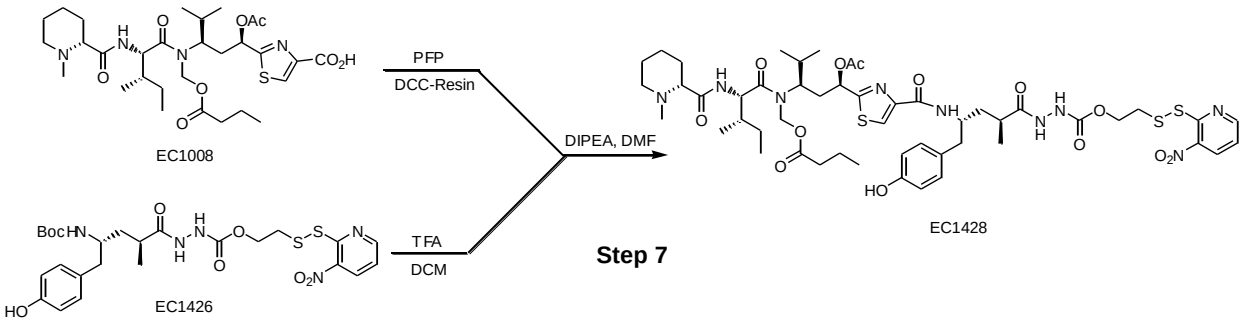
Pyridine, dimethylaminopyridine (DMAP), and acetic anhydride are added. The reaction is stirred and monitored by LC. The reaction mixture is concentrated to a residue and the product is purified by C18 column chromatography with acetonitrile and water as eluents. Product fractions are collected, concentrated, and lyophilized to yield a white to off-white powder.

EXAMPLE. EC1426 is prepared according to the following process.



EC1422 is dissolved in tetrahydrofuran (THF) and (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) and diisopropylethylamine (DIPEA) are added. Once all the solids have dissolved hydrazine is added and the reaction is stirred and monitored for completion. EC0607 is added and the mixture stirred and monitored for completion by LC. Ethyl acetate is added and the organics are washed once with saturated ammonium chloride, twice with saturated sodium bicarbonate, and once with saturated sodium chloride. The organics are dried over sodium sulfate and concentrated on a rotary evaporator. The crude EC1426 is purified by silica column chromatography with dichloromethane and methanol as eluents. Fractions are collected and the combined product fractions are concentrated on a rotary evaporator to yield a yellow solid.

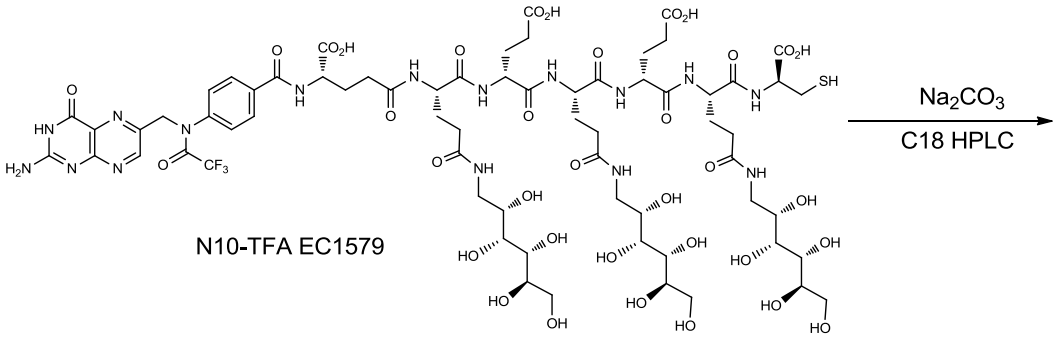
EXAMPLE. EC1428 is prepared according to the following process.

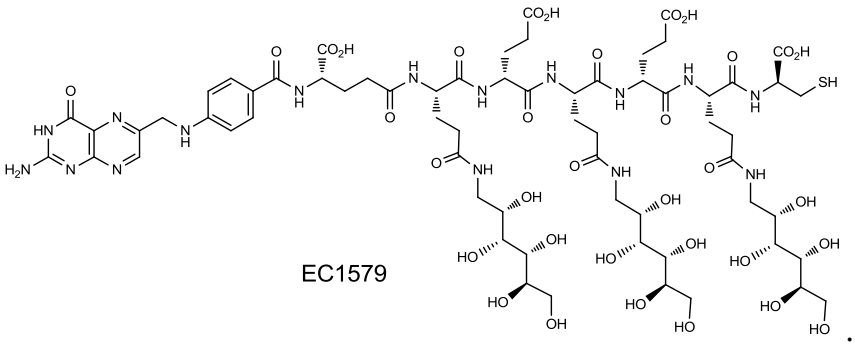


EC1008 is dissolved in dichloromethane and pentafluorophenol dissolved in DCM along with N-cyclohexylcarbodiimide,N'-methyl polystyrene (DCC-resin) are added. The

mixture is stirred and reaction completion is monitord by LC. The mixture is filtered to remove the resin and the organic layer is concentrated on a rotary evaporator to yield activated EC1008. In a separate flask, EC1426 is dissolved in dichloromethane and trifluoroacetic acid is added. The reaction mixture is stirred and monitored for completion by LC. The reaction mixture is concentrated on a rotary evaporator to yield deprotected EC1426. The activated

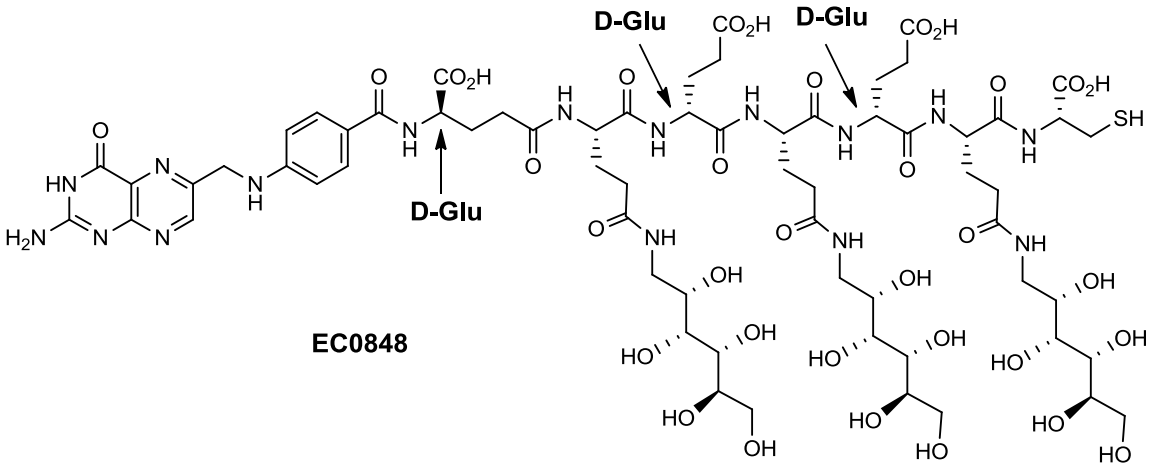
EXAMPLE. EC1579 is prepared according to the following process.



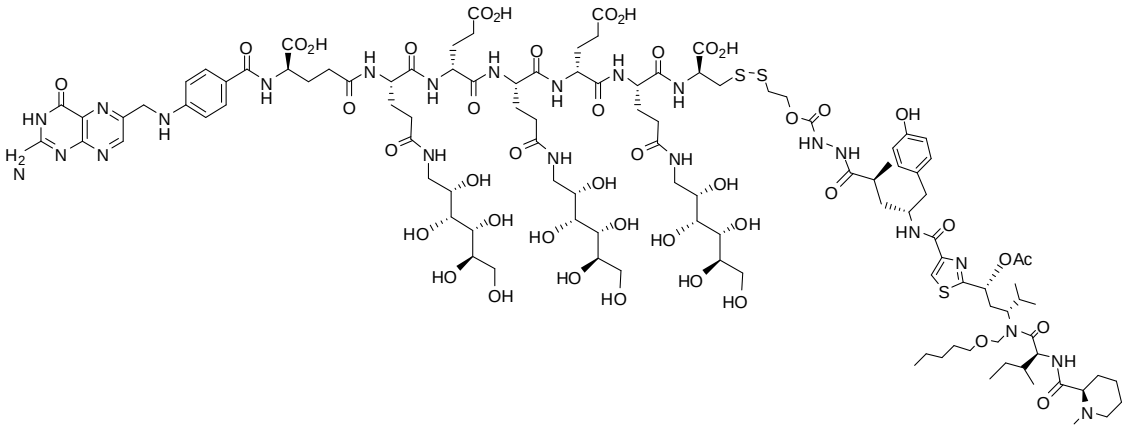


EC1579 MS (ESI, $[M+2H]^{2+}$) = 840.89 (M+1H)1+ = 1681.0. Partial 1H-NMR (<u>D_2O</u> ~~D2O~~) δ(ppm): 8.6(s), 7.5(d), 6.65(d), 4.4-4.8(m), 4-4.2(m), 3.4-3.8(m) 3-3.3(m) 2.75(s), 1.6-2.4(m).

EXAMPLE. EC0948 is made by the processes described herein.

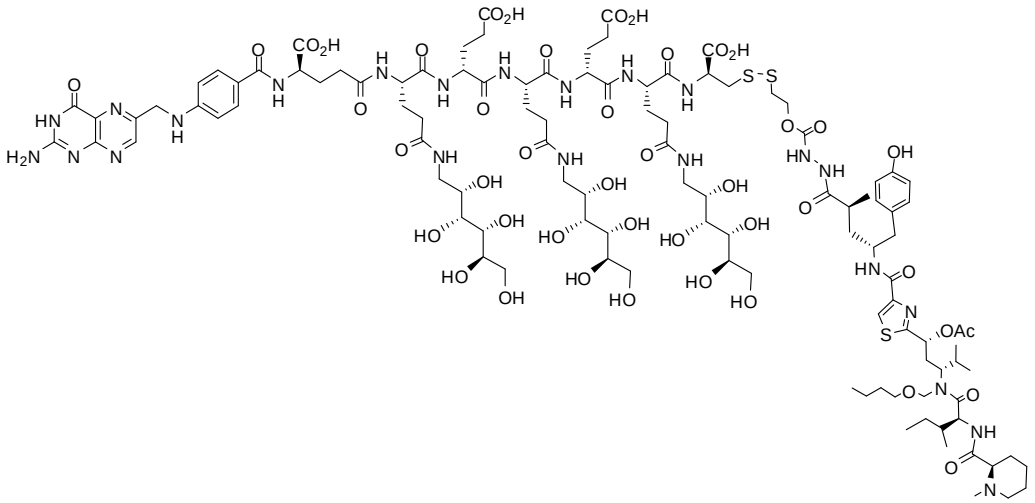


EC0848: MS (ESI, [M+2H]2+)= 840.8. [M+H]+ = 1681.1. Selected 1H-NMR (DMSO) δ(ppm): s, 8.6; d, 7.6; d, 6.6; s, 4.45; m, 4-4.2; m, 3.3-3.8; m, 3.1-3.3; m, 3-3.1; m, 2.7-2.9; m, 1.7-2.3; s, 1.15



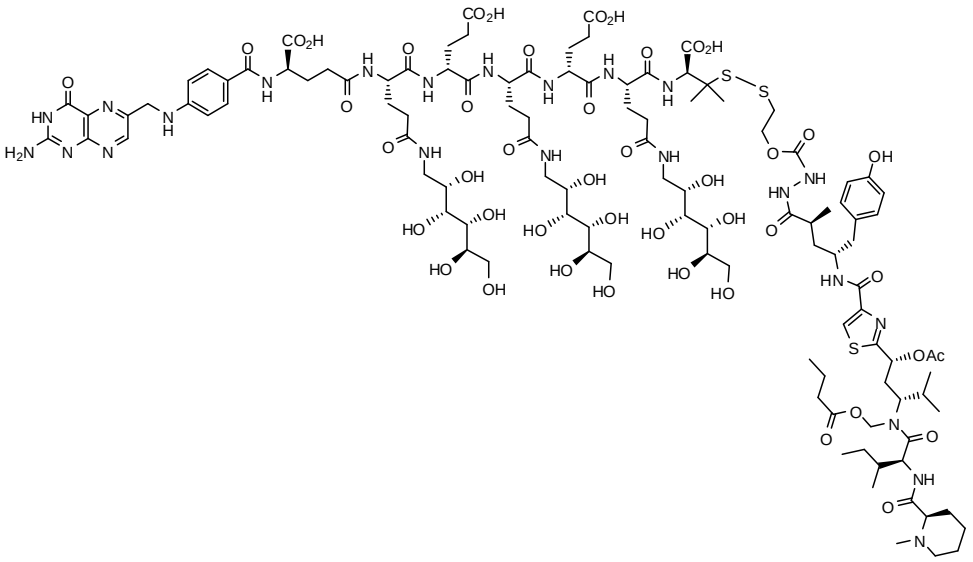
EC1664 ($C_{111}H_{169}N_{23}O_{44}S_3$; Molecular Weight 2625.85)

EC1454 (5.5mg, 1.0eq) was dissolved in degassed (Ar bubbling) 20mM phosphate pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1662 (3.6mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1664 (4.6mg, 54%) was purified by preparative HPLC in 10-100% acetonitrile/50mM NH_4HCO_3 pH7 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) Predicted 1313.05, Found 1313.37. Partial ^1H NMR (DMSO w/ 10% D_2O, 300 MHz) δ (ppm) 8.61 (s), 8.15 (s), 7.58 (d), 6.94 (d), 6.60 (m), 5.78 (d), 5.22 (d), 4.47 (m), 4.09-4.33 (m), 0.99 (d), 0.93 (d), 0.76 (t), 0.71 (t), 0.61 (d).



EC1663 (Chemical Formula: $C_{110}H_{167}N_{23}O_{44}S_3$; MW 2611.83)

EC1454 (16.1mg, 1.2eq) was dissolved in degassed (Ar bubbling) 20mM phosphate pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1661 (8.7mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1663 (15.8mg, 76%) was purified by preparative HPLC in 10-100% acetonitrile/50mM NH_4HCO_3 pH7 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) Predicted 1306.04, Found 1306.82.



EC1416 ($C_{112}H_{169}N_{23}O_{45}S_3$; MW 2653.87)

EC1415 (20 mg) was dissolved in pH7 phosphate (pH 7.75, purged with argon). To this solution was added a suspension of EC0312 (14 mg) in equal volume of MeOH. The reaction mixture was stirred at ambient temperature under argon for 45 min, and then loaded onto a preparatory HPLC (Mobile phase A = 50 mM NH_4HCO_3 buffer, pH = 7.0. B = ACN. Method: 5-80% B in 20 min.) for purification. Fractions containing the desired product were collected, combined, and freeze-dried to afford the product (18 mg) as a pale yellow solid. MS(ESI, $[M+2H]^{2+}$) 1328, 1H NMR (DMSO-d6, $\underline{D_2O}$ ~~D2O~~, 300MHz): 8.6 (s, 1H), 8.15 (s, 1H), 7.85 (bd, 1H), 7.55 (d, 2H), 6.95 (d, 2H), 6.6 (m, 4H), 6.2 (d, 1H), 5.68 (d, 1H), 5.2 (d, 1H), 4.5 (bs, 3H), 4.5-4.3 (m, 4H), 4.3-4.0 (m, 10H), 3.5-3.3 (m, 13H), 3.2 (bd, 5H), 3.1-2.8 (m, 8H), 2.75 (bs, 5H), 2.6-1.6 (m, 50H), 1.4 (m, 9H), 1.2 (m, 9H), 1.0 (dd, 9H), 0.7 (m, 11H), 0.6 (d, 3H).

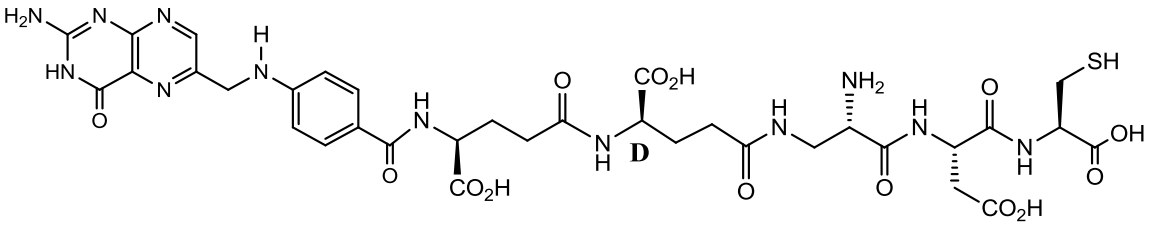
The pH of a solution of EC1392 (20 mg) in 40 mM pH7 phosphate buffer was adjusted to 8 with a saturated $NaHCO_3$ solution. To the solution was added a suspension of EC0312 (20 mg) in equal volume of MeOH. The reaction mixture was stirred at ambient temperature under argon for 30 min, and then loaded onto a preparatory HPLC (Mobile phase A = 50 mM NH_4HCO_3 buffer, pH = 7.0. B = ACN. Method: 5-80% B in 20 min.) for purification. Fractions containing the desired product were collected, combined, and freeze-dried to afford the product (15 mg) as a pale yellow solid. MS(ESI, $[M+2H]^{2+}$) 1011.39. ^{1}H NMR (DMSO-d6, $\underline{D_2O}$ ~~D2O~~, 300 MHz): 8.6 (s, 1H), 8.15 (s, 1H), 7.85 (bd, 1H), 7.55 (d, 2H), 6.95 (d, 2H), 6.6 (m, 4H), 6.2 (d, 1H), 5.68 (d, 1H), 5.2 (d, 1H), 4.6 (t, 1H), 4.5 (m, 3H), 4.5-4.0 (m, 11H), 3.2-2.8 (m, 6H), 2.8-2.5 (m, 8H), 2.4 (m, 5H), 2.2-2.0 (m, 14H), 2.0-1.7 (m, 7H), 1.6-1.3 (m, 13H), 1.25 (d, 8H), 1.1-0.95 (dd, 8H), 0.75 (m, 10H), 0.6 (d, 2H).

EXAMPLE. The compounds described herein can also be prepared by following two methods:

Method A: Folate spacer is dissolved in water by adjusting the pH of the solution with $NaHCO_3$ solution to a pH = 7 with argon purging. The thiophilic agent in organic solvent (MeOH, ACN, THF or DMSO) is then added. The reaction mixture is stirred at room temperature with argon purging. The progress of reaction is monitored by analytical HPLC (Mobile phase A = 50 mM NH_4HCO_3 buffer, pH = 7.0; B = ACN). After the reaction is complete, the organic solvent is evaporated and the resulted solution is then purified by prep-HPLC with C18 column (Mobile phase A = 50 mM NH_4HCO_3 buffer or 2 mM phosphate buffer, pH = 7.0; B = ACN).

Method B: Folate spacer is dissolved in water and the pH is adjusted to 2 with acid (AcOH or dilute HCl). The resulting pH adjusted spacer is lyophilized, and then redissolved in DMSO. The reaction mixture is purged with argon, and 10 molar equivalents of Et_3N (or DIPEA) are added. To this solution is added the thiophilic agent in organic solvent (DMSO, THF, ACN, etc.). The progress of the reaction is monitored by HPLC (Mobile phase A = 50 mM NH_4HCO_3 buffer or 2 mM phosphate buffer, pH = 7.0. B = ACN). After the reaction is complete, the reaction mixture is purified by prep-HPLC with C18 column (Mobile phase A = 50 mM NH_4HCO_3 buffer or 2 mM phosphate buffer, pH = 7.0; B = ACN).

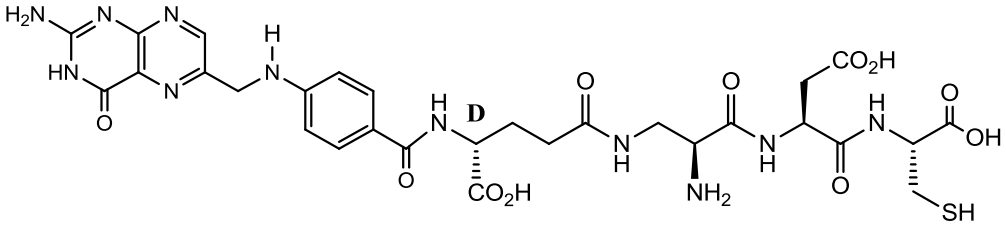
EXAMPLE. Additional illustrative linker intermediates (also referred as folate spacers) are described herein:



EC 014

EC0014: ^{1}H NMR (D_2O, 500 MHz) δ(ppm) 8.73(s, 1H, FA H-7), 7.56 (d, 2H, FA H-12&H16), 6.73(d, 2H, FA H-13&H15), 4.45(m, 2H), 4.1(m, 2H), 3.61(d, 2H), 2.82(m, 3H), 2.74(dd, 1H), 2.37(m, 2H), 2.18(m, 1H), 2.09(m, 3H), 1.74(m, 1H)

EXAMPLE.

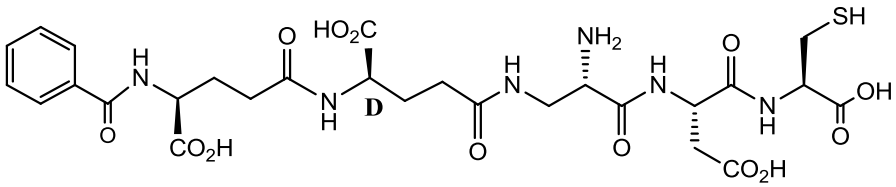


EC 20

Pte-γ–D-Glu-β-Dap-Asp-Cys

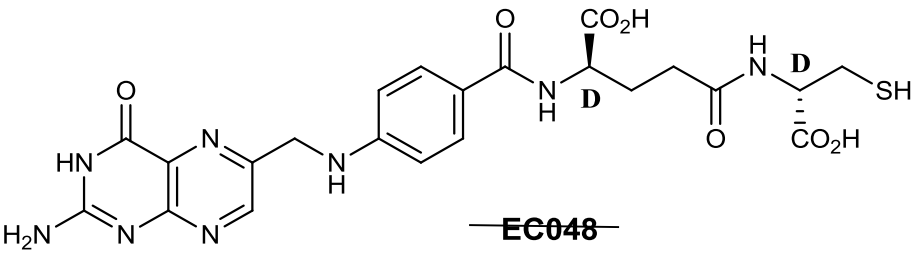
EC0020: MS (ESI, $[M+H]^+$) 746. ^{1}H NMR (D_2O, 500 MHz) δ(ppm) 8.76(s, 1H, FA H-7), 7.68(d, 2H, FA H-12&H16), 6.8(d, 2H, FA H-13&H15), 4.71(dd, 1H, Asp H-2), 4.64(s, 2H FA H-9), 4.41(dd, 1H, D-Glu H-2), 4.3(dd, 1H, Cys H-2), 4.1(dd, Dpr H-2),3.72(dd, 1H, Dpr H-3A), 3.52(dd, 1H, Dpr H-3B), 2.89(dd, 1H, Cys H-3A), 2.85(dd, 1H, Cys H-3B), 2.81(dd, 1H, Asp H-3A), 2.62(dd, 1H, Asp H-3B), 2.44(dd, 2H, D-Glu H-4), 2.27(m, 1H, D-Glu H-3A), 2.08(m, 1H, D-Glu H-3B). ^{13}C NMR (DMSO-d6+ $\underline{D_2O}$ ~~D2O~~, 75 MHz): □ 174.78, 174.42, 172.68 (2C), 170.45, 168.25, 167.08, 162.24, 156.24, 154.38, 151.24, 149.41 (2C), 129.52, 128.14, 121.74, 111.98, 55.76, 53.02 (2C), 52.77, 50.89, 46.16, 36.61, 32.26, 27.32, 26.60

EXAMPLES.



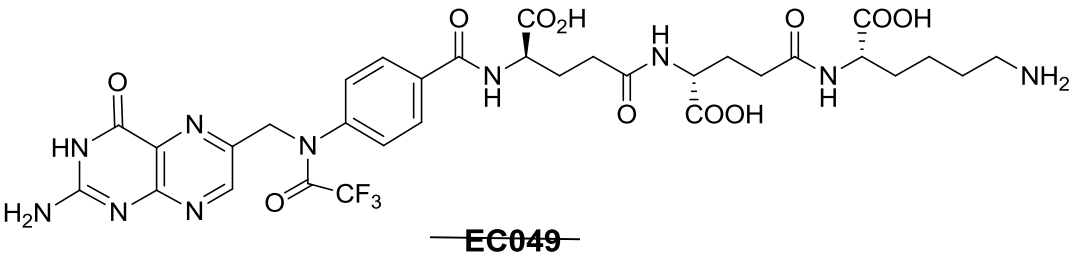
~~**EC 028**~~

EC0028



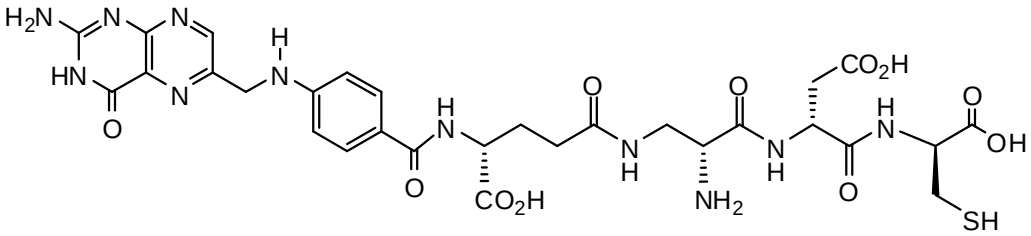
~~EC048~~

EC0048



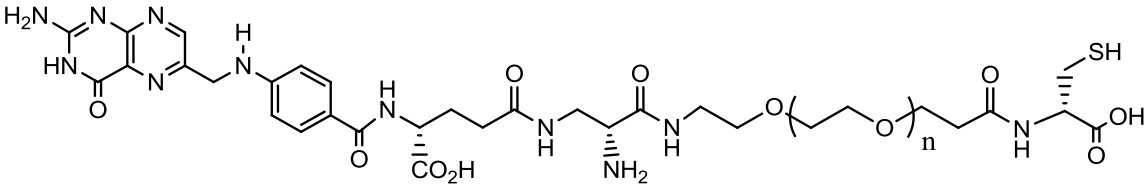
~~EC049~~

EC0049: MS (ESI, $[M+H]^+$) 795.



Pte-γ-D-Glu-β-D-Dap-D-Asp-D-Cys

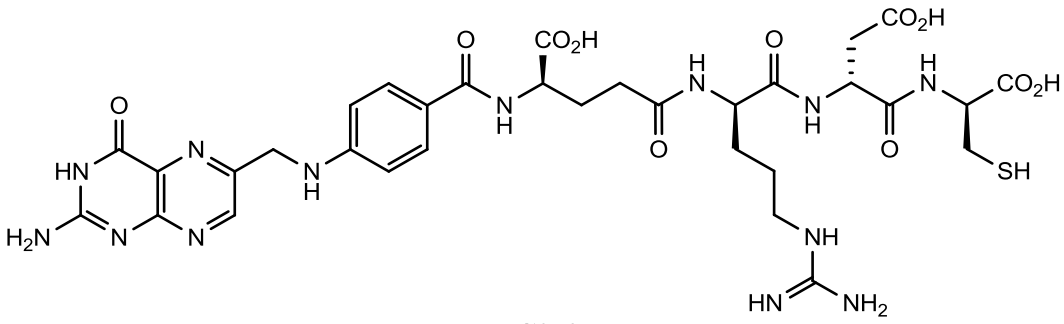
EC0053: MS (ESI, $[M+H]^+$) 746



~~EC055~~

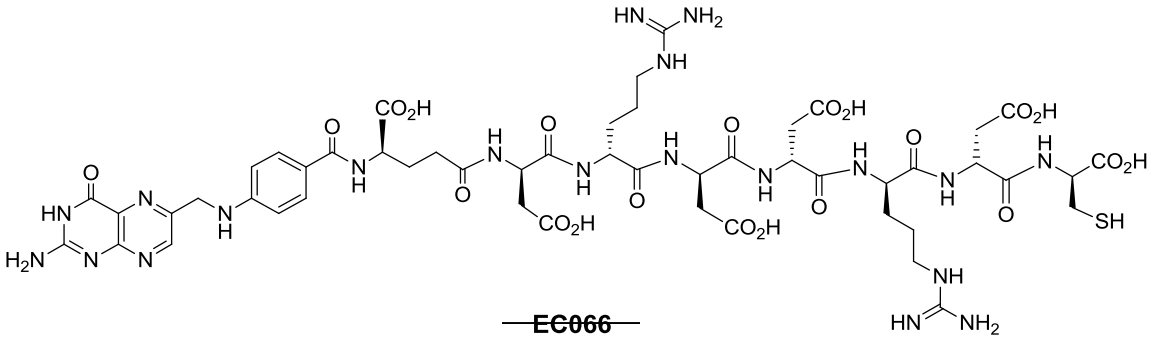
Pte-γ-D-Glu-β-D-Dap-PEG_{3400}-D-Cys

EC0055



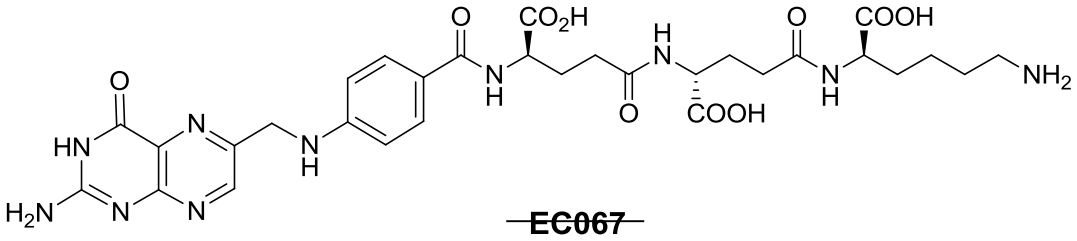
~~EC059~~

EC0059: MS (ESI, $[M+H]^+$) 816



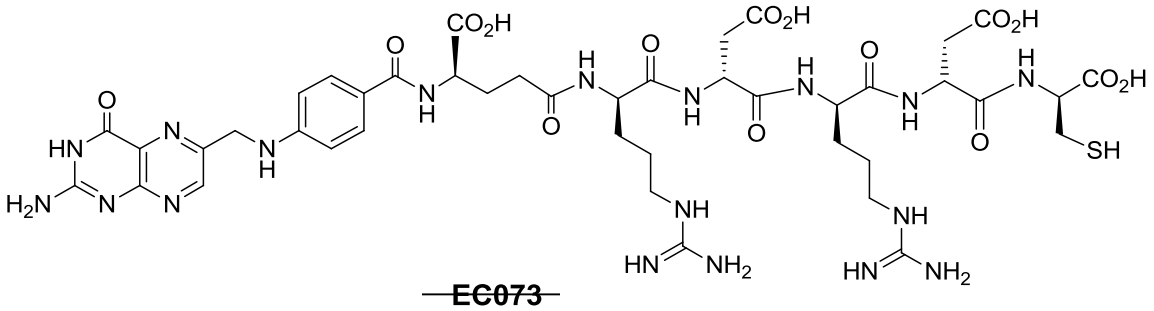
~~EC066~~

EC0066: MS (ESI, $[M+H]^+$) 1317



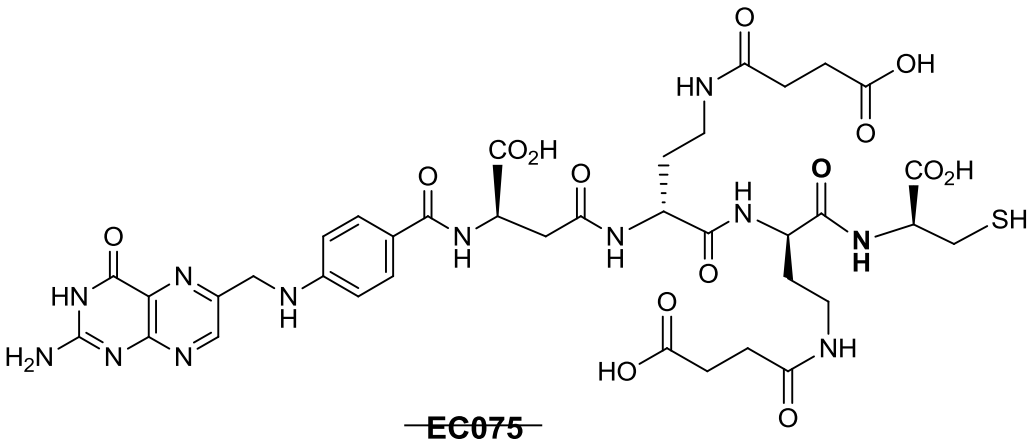
~~EC067~~

EC0067: MS (ESI, $[M+H]^+$) 699



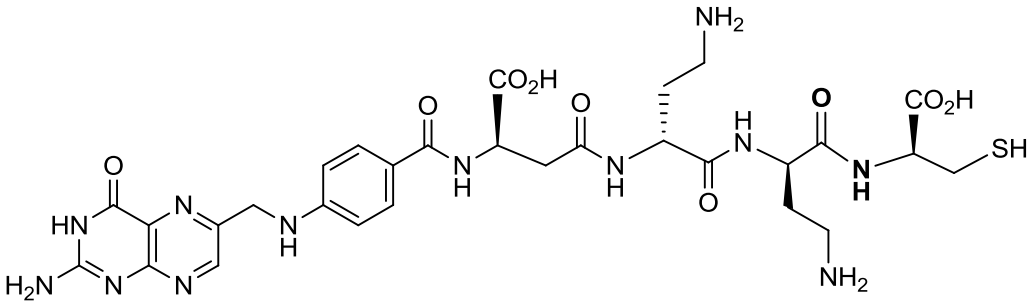
~~EC073~~

EC0073: N^{10}-TFA-EC073 MS (ESI, $[M+H]^+$) 1183.



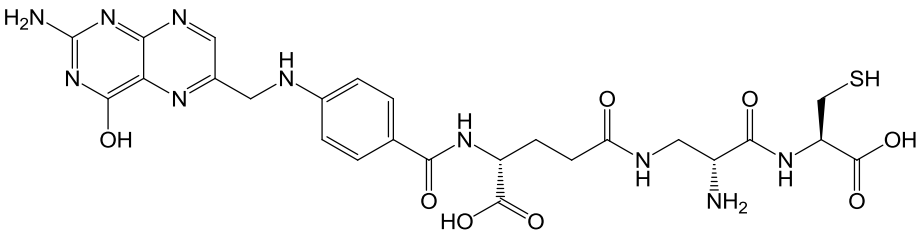
~~EC075~~

EC0075: MS (ESI, $[M+H]^+$) 931



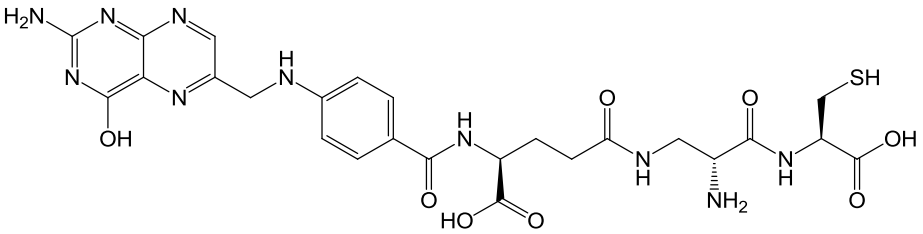
~~EC076~~

EC0076: MS (ESI, $[M+H]^+$) 731



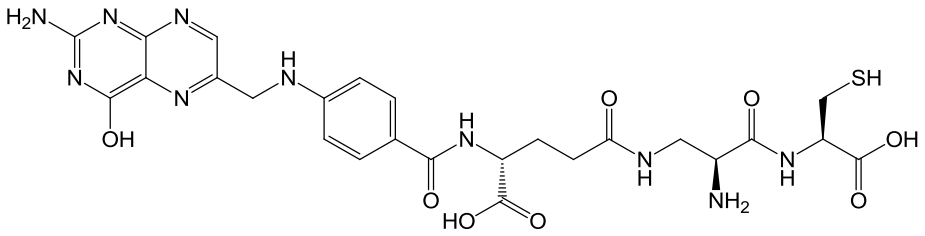
EC149 Pte-Glu-D-Dap-D-Glu-Pte

EC0149: $[M+H]^+$ = 631. ^1H NMR (D_2O): 8.55 (s, 1H), 7.5 (d, 2H), 6.61 (d, 2H), 4.42 (s, 2H), 4.35 (dd, 1H), 4.25 (m, 2H), 4.1 (s, 1H), 3.68 (m, 1H), 3.5 (m, 1H), 3.35-3.2 (m, 3H), 3.1 (dd, 1H), 2.4-2.1 (m, 3H), 2.1-1.9 (m, 4H).



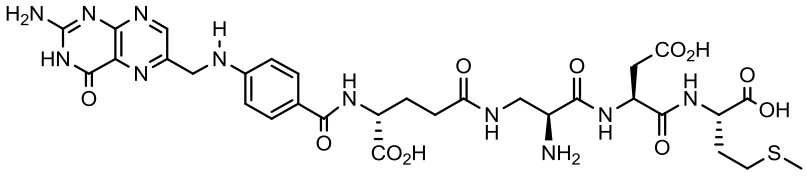
EC150 Pte-Glu-D-Dap-Cys

EC0150: MS (ESI, $[M+H]^+$) 631. Selected ^1H NMR (D_2O) δ(ppm) 8.42(s, 1H, FA H-7), 7.50(d, 2H, FA H-12&16), 6.65(d, 2H, FA H-13&15), 4.42 (s, 2H), 4.3-4.1(m, 2H), 4.0-3.85 (m, 1H), 3.35-3.30(m, 1H), 3.30-3.10(m, 2H), 3.10-2.90(m, 2H), 2.80-2.70(m, 1H), 2.65-2.50(m, 2H), 2.30-2.10(m, 3H), 2.10-1.85(m, 2H), 1.95-1.80(m, 2H).



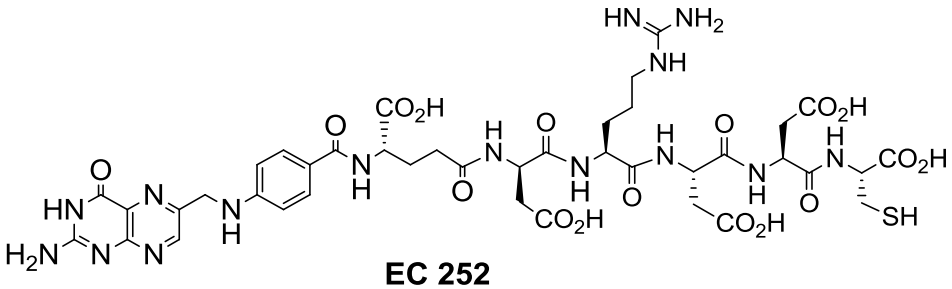
EC151 Pte-D-Glu-Dap-Cys

EC0151: MS (ESI, $[M+H]^+$) 630. Selected ^1H NMR (D_2O) δ(ppm) 8.42(s, 1H, FA H-7), 7.50(d, 2H, FA H-12&16), 6.65(d, 2H, FA H-13&15), 4.42 (s, 2H), 4.3-4.1(m, 2H), 4.0-3.85 (m, 1H), 3.35-3.30(m, 1H), 3.30-3.10(m, 2H), 3.10-2.90(m, 2H), 2.80-2.70(m, 1H), 2.65-2.50(m, 2H), 2.30-2.10(m, 3H), 2.10-1.85(m, 2H), 1.95-1.80(m, 2H).



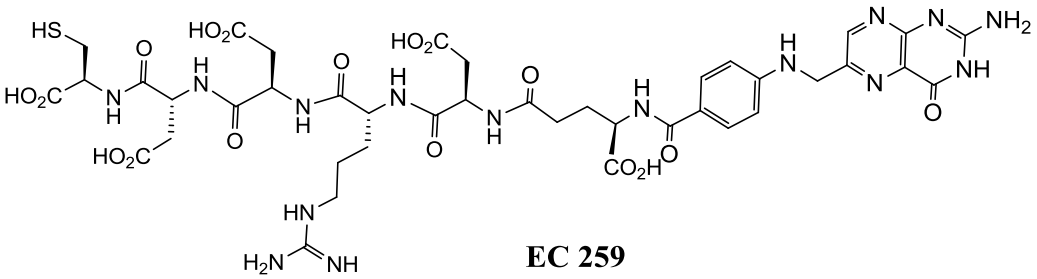
EC 232

EC0232: MS (ESI, $[M+H]^+$) 774. ^1H NMR ($\underline{D_2O}$ ~~D2O~~): 8.56 (s), 7.50 (d), 6.65 (d), 4.48-4.41 (m), 4.21 (dd), 4.08 (dd), 3.48-3.42 (m), 3.28-3.09 (m), 2.61-2.35 (m), 2.28-2.18 (m), 2.16-2.02 (m), 1.97-1.62 (m).



EC 252

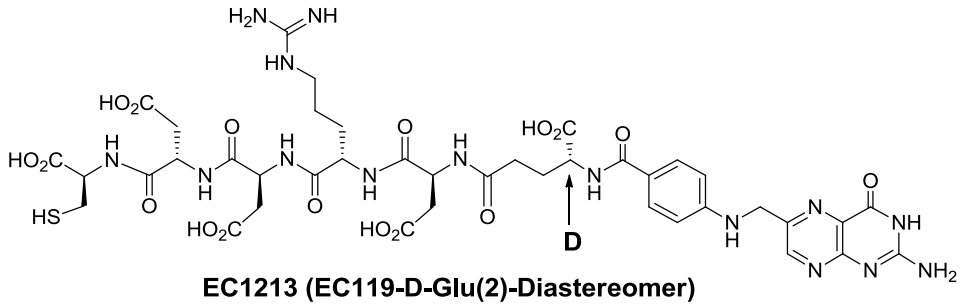
EC0252: $[M+H]^+$ = 1046.83. 1H NMR ($\underline{D_2O}$ ~~D2O~~): 8.58 (s, 1H), 7.5 (d, 2H), 6.6 (d, 2H), 3.05-2.6 (m, 5H), 2.3-1.9 (m, 4H), 1.8-1.2 (m, 7H).



EC 259
(All D-EC119)

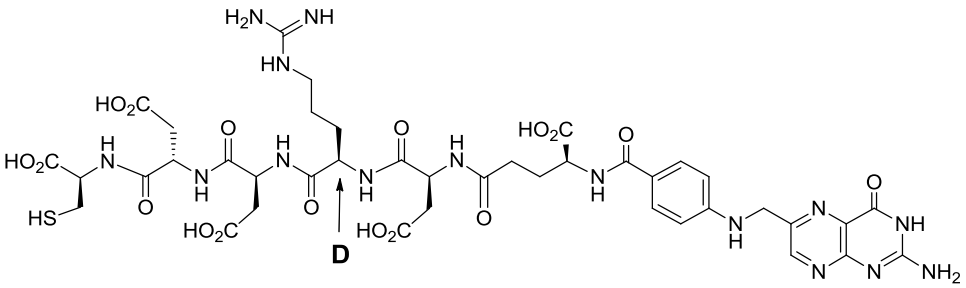
EC0259: $[M+H]^+$ = 1047.52. 1H NMR (D2O ~~D2O~~): 8.6 (s, 1H), 7.5 (d, 2H), 6.65 (d, 2H), 4.4 (dd, 2H), 4.18 (m, 4H), 2.9 (t, 2H), 2.75 (t, 2H), 2.6-2.15 (m, 10H), 2.1-1.8 (m, 3H), 1.7-1.4 (m, 3H), 1.3 (m, 3H).

5



EC1213 (EC119-D-Glu(2)-Diastereomer)

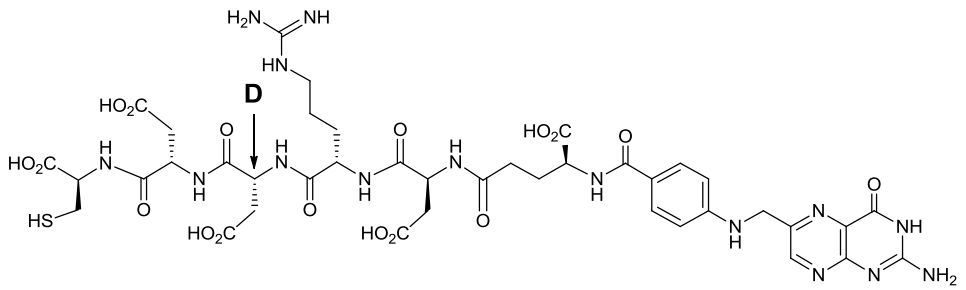
EC1213: LCMS (ESI $[M + H]^+$): 1046. Selected ^1H NMR data (D_2O, 300 MHz): δ 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).



EC1214 (EC119-D-Arg(4)-Diastereomer)

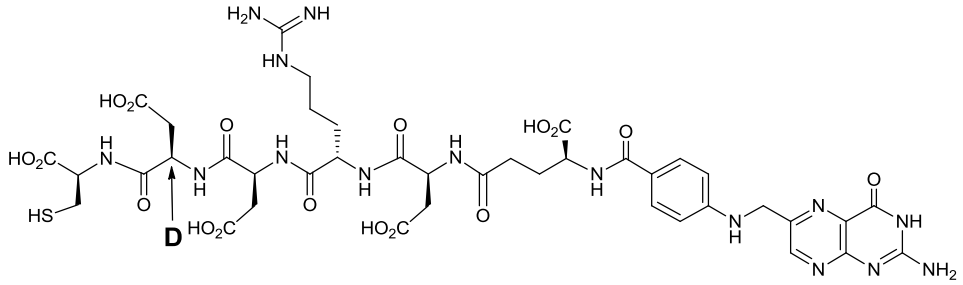
10

EC1214: LCMS (ESI $[M + H]^+$): 1046. Selected ^1H NMR data (D_2O, 300 MHz): δ 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).



EC1215 (EC119-D-Asp(5)-Diastereomer)

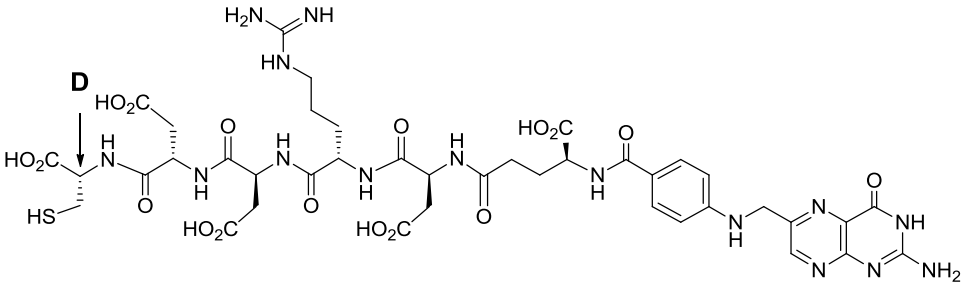
EC1215: LCMS (ESI $[M + H]^+$): 1046. Selected ^1H NMR data (D_2O, 300 MHz): δ 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).



EC1216 (EC119-D-Asp(6)-Diastereomer)

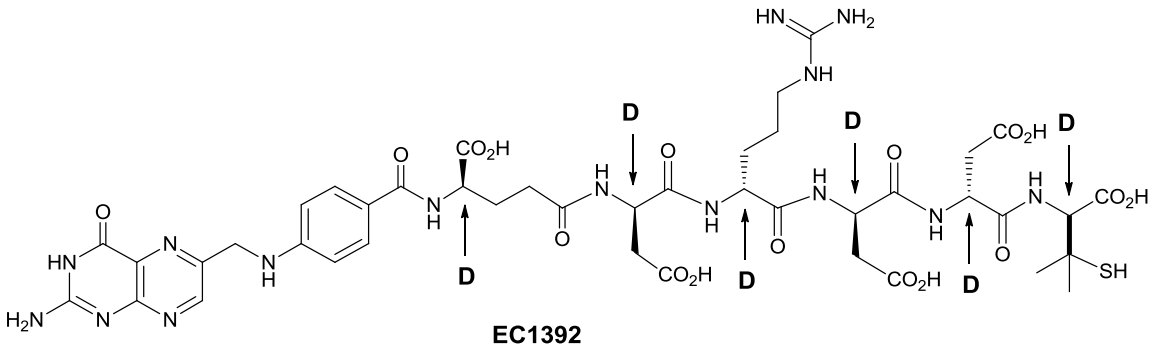
EC1216: LCMS (ESI $[M + H]^+$): 1046

Selected ^1H NMR data (D_2O, 300 MHz): δ 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).



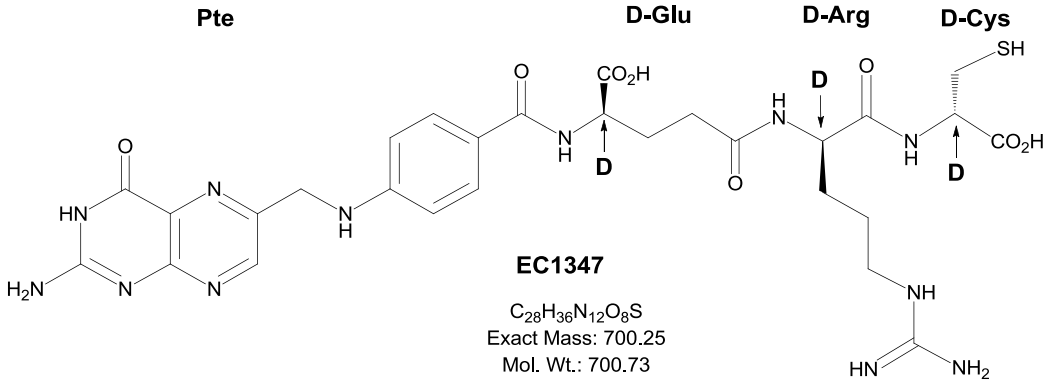
EC1217 (EC119-D-Cys-Diastereomer)

EC1217: LCMS (ESI $[M + H]^+$): 1046. Selected ^1H NMR data (D_2O, 300 MHz): δ 8.68 (s, 1H, FA H-7), 7.57 (d, 2H, J = 8.4 Hz, FA H-12 &16), 6.67 (d, 2H, J = 9 Hz, FA H-13 &15).

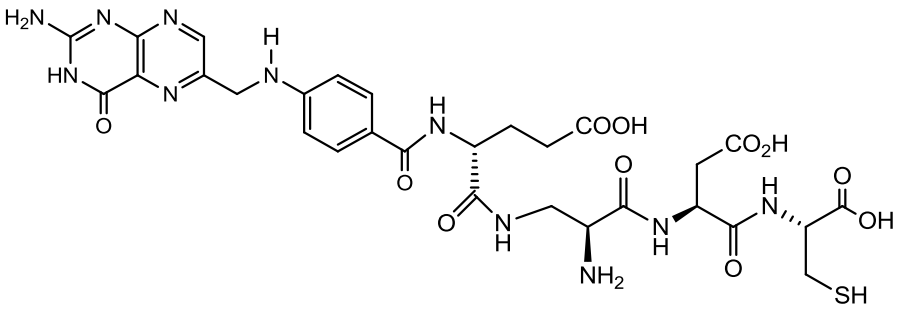


EC1392

EC1392: $[M + H]^+$ = 1074.85. 1H NMR (<u>D_2O</u> ~~D2O~~, 300 MHz) δ(ppm): 8.55 (s, 1H), 7.45 (d, 2H), 6.5 (d, 2H), 4.6 (m, 2H), 4.45 (t, 1H), 4.35 (bs, 2H), 4.2 (m, 1H), 4.1 (s, 1H), 4.05 (m, 1H), 2.9 (t, 2H), 2.75-2.4 (m, 6H), 2.3 (m, 2H), 2.2-1.9 (m, 2H), 1.8-1.4 (m, 2H), 1.2 (m, 2H), 1.3 (s, 3H), 1.2 (s, 3H).

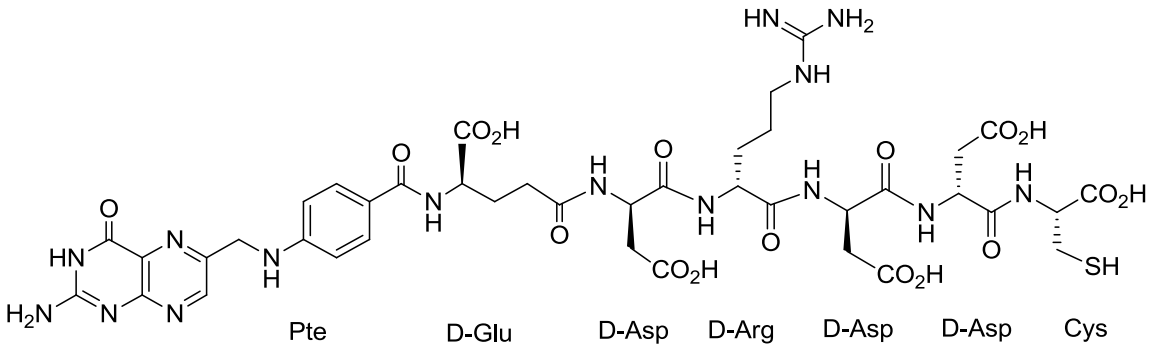


EC1347: MS (ESI, $[M + H]^+$) = 701.57. Selected 1H-NMR (DMSO, 300 MHz) δ(ppm): 8.65(s), 7.6(d), 6.6(d), 4.2-4.6(m), 2.6-3.2(m), 1.8-2.6(m), 1.1-1.7(m)



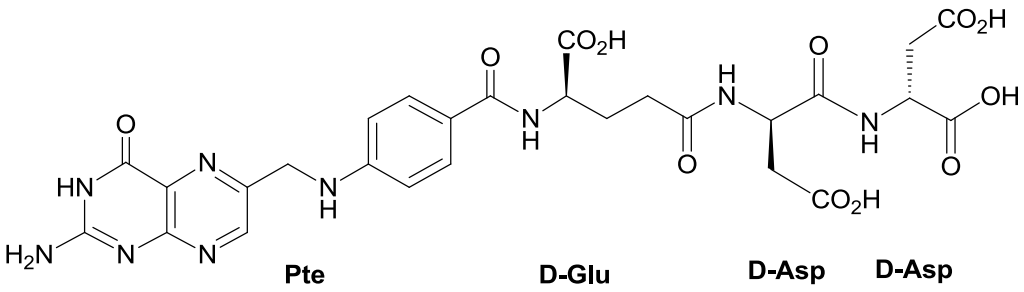
EC0589 (α-isomer of EC20)

EC0589: MS (ESI, $[M+H]^+$) 746. Selected ^{1}H NMR (DMSO-d6+ $\underline{D_2O}$ ~~D2O~~, 300 MHz): $\underline{\delta}$ ~~□~~ 8.46(s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 6.47 (d, J = 8.4 Hz, 2H), 4.39 (t, J = 6.6 Hz, 1H).



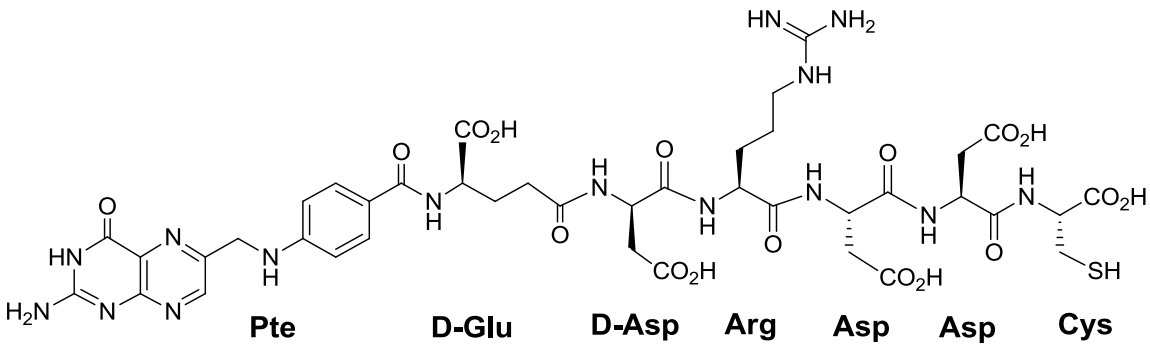
EC0819

EC0819: MS (ESI, $[M+H]^+$)= 1046.4. Selected 1H-NMR (DMSO) δ(ppm): 8.6(s), 7.6(d), 6.6(d), 4-4.6(m), 3.4-3.8(m), 3-3.15(m), 1-2.8(m).



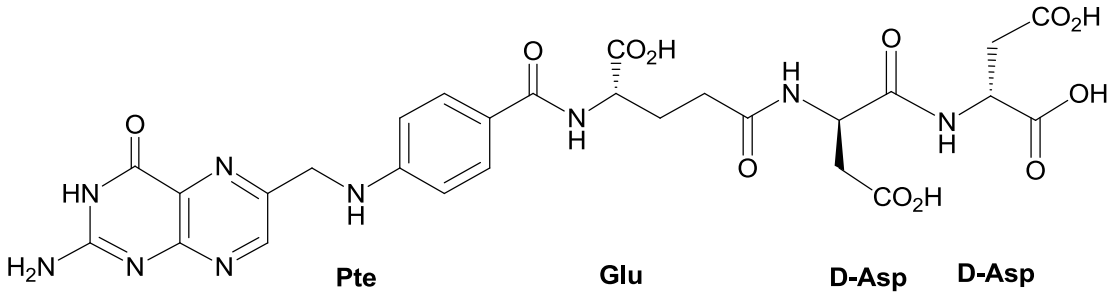
EC0823

EC0823: MS (ESI, $[M+H]^+$) = 672.3. Selected 1H-NMR (DMSO) δ(ppm): 8.8(s), 7.6(d), 6.6(d), 4.4-4.6(m), 4.2-4.4(m), 3.4-3.8(m), 1.8-2.8(m), 1.15(s)



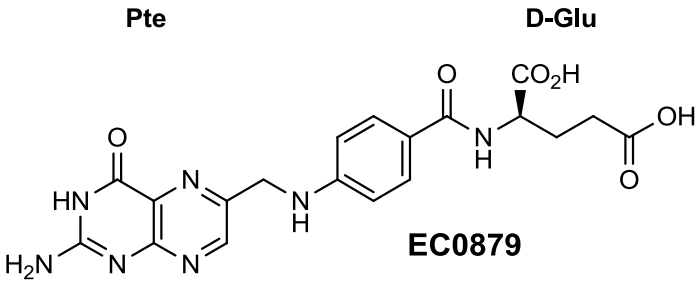
EC0835

EC0835: MS (ESI, [M+H]+)=1046.5. Selected 1H-NMR (DMSO) δ(ppm): 8.6(s), 7.5(d), 6.6(d), 3.8-4.6(m), 2.8-3.2(m), 2.2-2.8(m), 1-2.2(m)



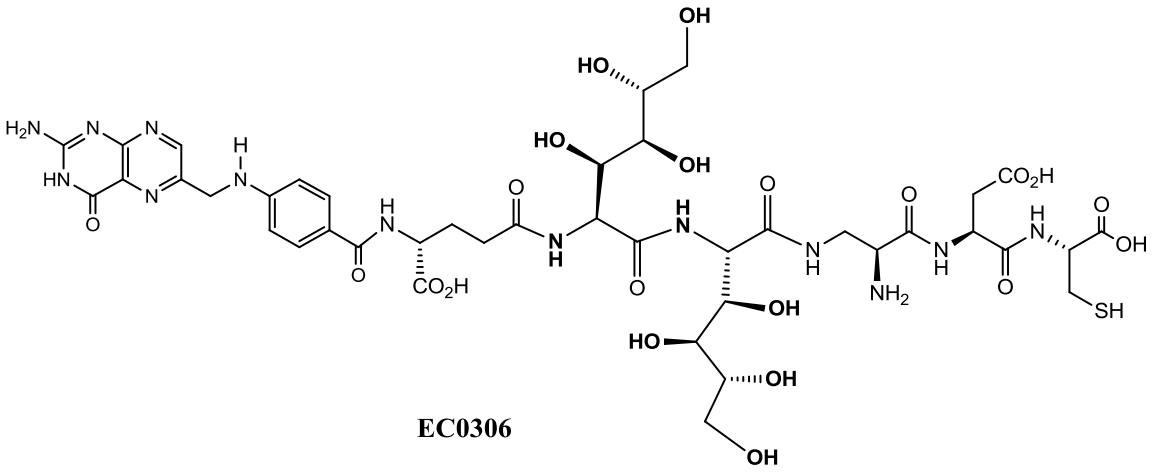
EC0923

EC0923: MS (ESI, $[M+H]^+$) = 672.3. Selected 1H-NMR (<u>D_2O</u> ~~D2O~~) δ(ppm): 8.8(s), 7.75(d), 6.85(d), 4.4-5(m), 2.6-2.9(m), 2.4-2.6(m), 2-2.6(m)

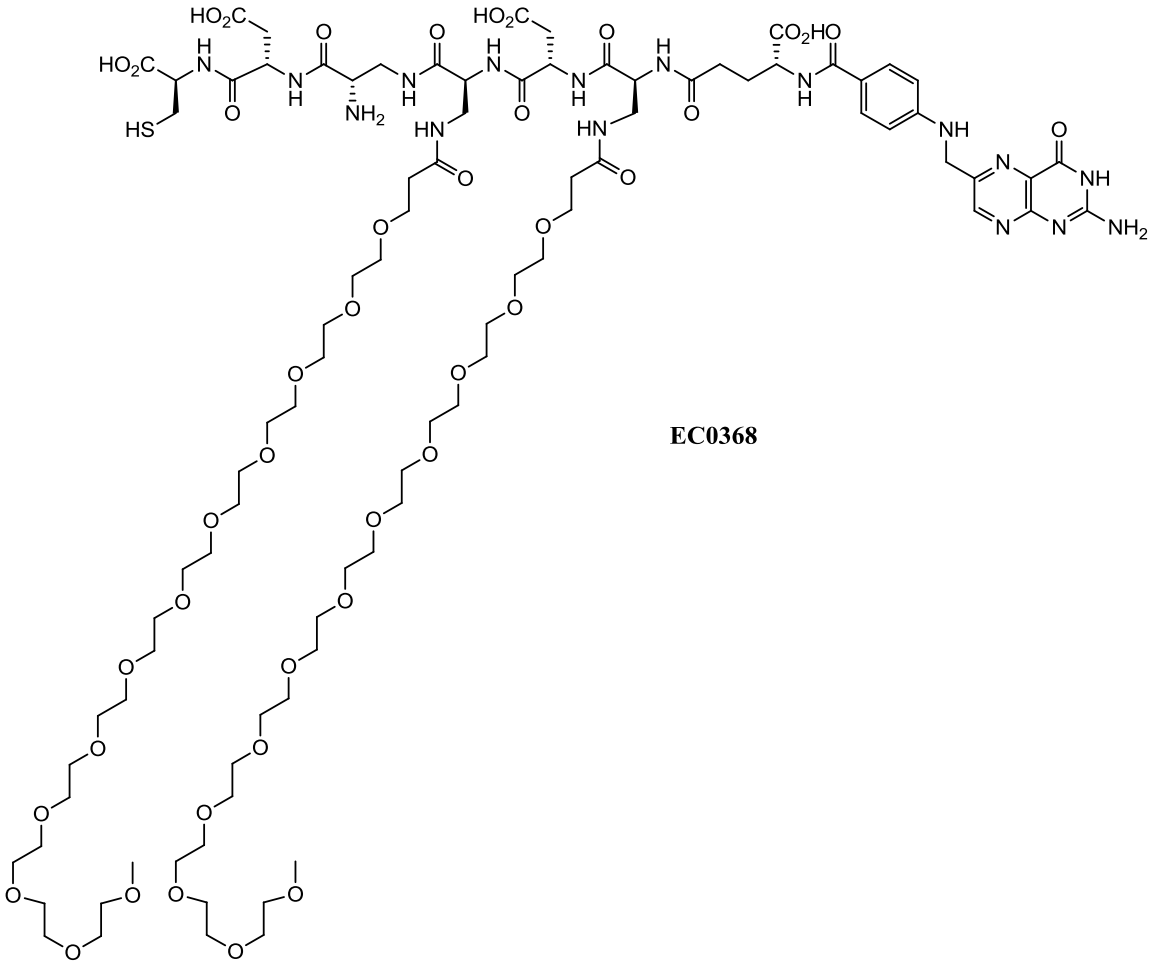


D-Folic acid

EC0879: MS (ESI, $[M+H]^+$)= 442.3. Selected 1H-NMR (DMSO) δ(ppm): 8.7(s), 7.6(d), 6.6(d), 4.55(s), 4.3(m), 2.2-2.6(m), 1.8-2.2(m), 1-1.2(m)



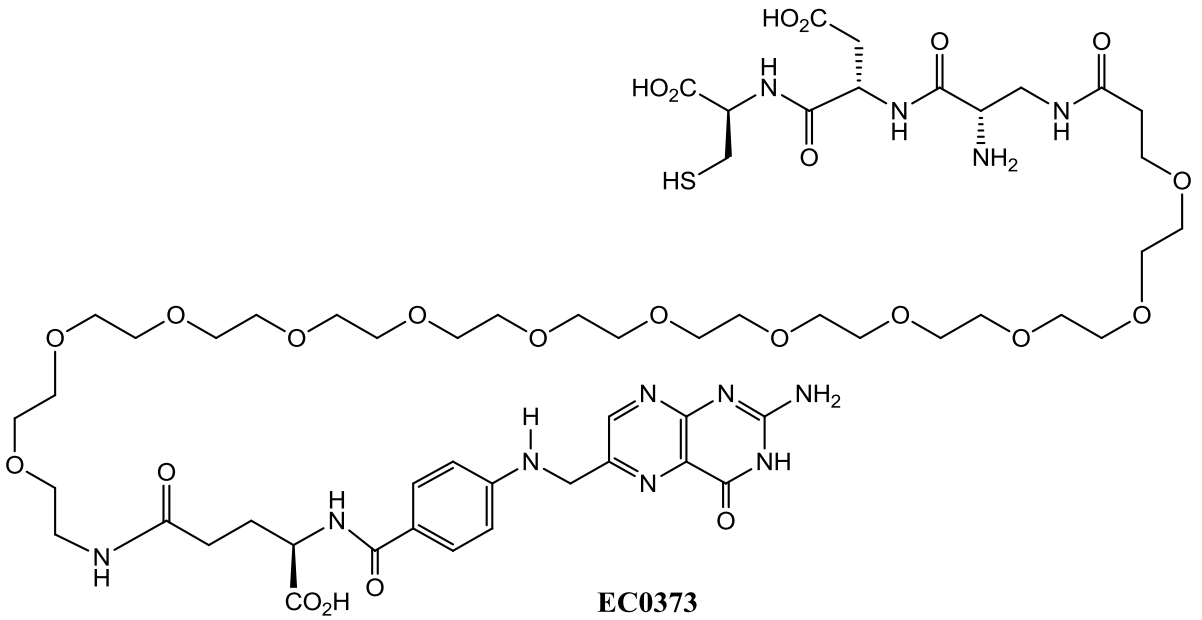
EC0306: $[M+H]^+$ = 1100.51. 1H NMR (<u>D_2O</u> ~~D2O~~): δ 8.75 (s, 1H), 7.6 (d, 2H), 6.75 (d, 2H), 4.7-4.5 (m, 5H), 4.38 (m, 2H), 4.2 (m, 2H), 4.1 (d, 1H), 3.85-3.5 (m, 10H), 2.95-2.6 (m, 4H), 2.45 (m, 2H), 2.3-2.0 (m, 2H).



EC0368

EC0368: [M+H]+ = 2175.5. 1H NMR (<u>D_2O</u> ~~D2O~~): 8.6 (s, 1H), 7.5 (d, 2H), 6.6 (d, 2H), 4.45 (bs, 3H), 4.35-4.2 (m, 4H), 4.05 (t, 1H), 3.6-3.35 (bs, 114H), 3.2 (s, 6H), 2.77 (t, 2H), 2.65 (dd, 1H), 2.55-2.45 (m, 3H), 2.4-2.2 (m, 6H), 2.1-1.8 (m, 2H).

5

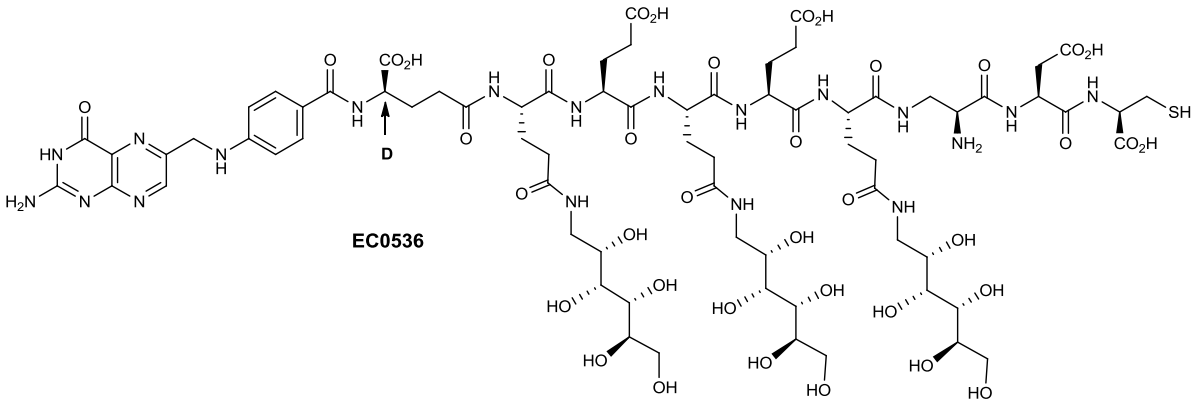


EC0373

EC0373: $[M+H]^+$ = 1346.0. 1H NMR (<u>D_2O</u> ~~D2O~~): 8.55 (s, 1H), 7.5 (d, 2H), 6.6 (d, 2H), 4.4

(s,

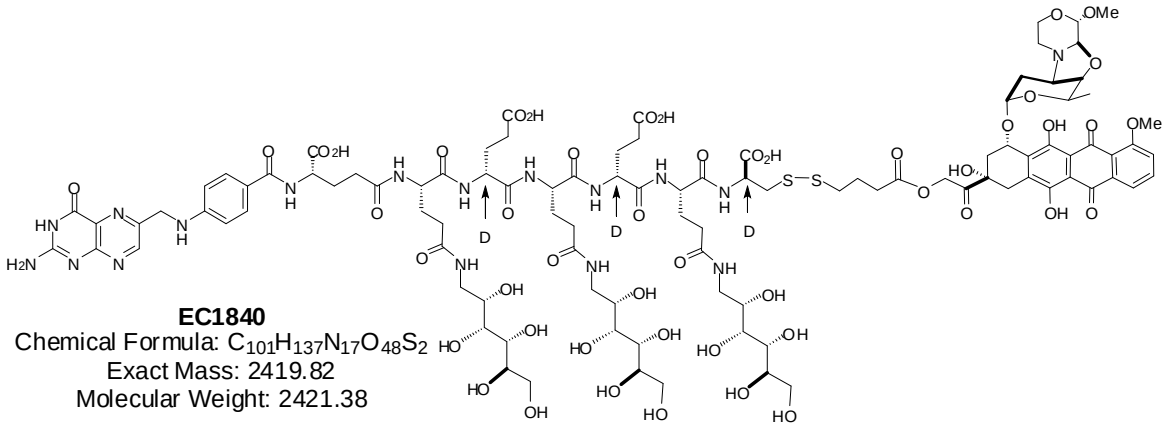
2H), 4.25 (m,2H), 4.05 (t, 1H), 3.7 (dd, 1H), 3.6-3.3 (m, 50H), 3.25 (dd, 3H), 3.05 (dd, 3H), 2.8 (t, 2H), 2.7 (dd, 2H), 2.6 (dd, 1H), 2.4 (t, 2H), 2.2-1.9 (m, 4H).



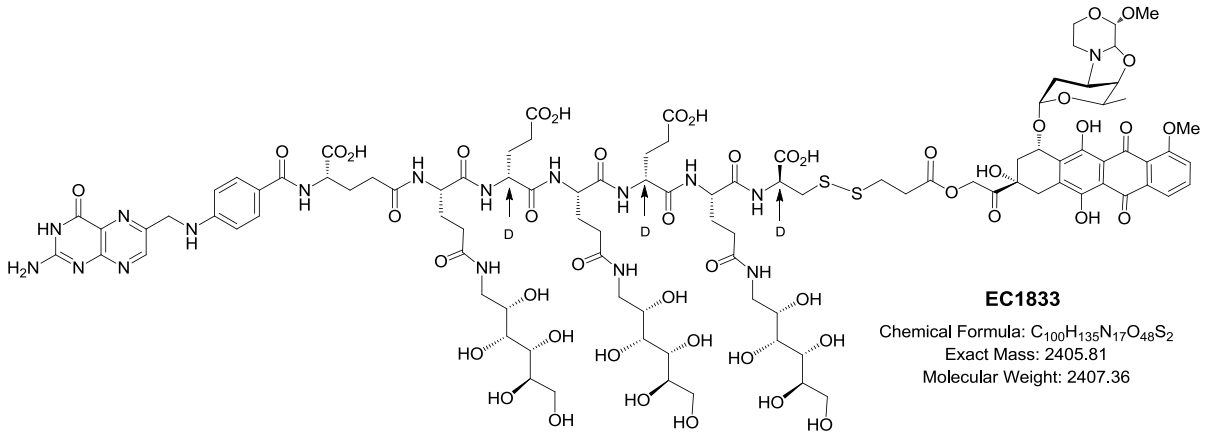
EC0536: $[M+2H]^{2+}$ = 941.2. 1H NMR ($\underline{D_2O}$ ~~D2O~~): 8.55 (s, 1H), 7.5 (d, 2H), 6.6 (d, 2H), 4.4 (s, 2H), 4.25 (m,2H), 4.1 (m, 5H), 3.85 (t, 1H), 3.8-3.4 (m, 21H), 3.4-2.95 (m, 7H), 2.8 (s, 2H), 2.7-2.4 (ddd, 2H), 2.4-1.7 (m, 22H), 1.55 (m, 1H).

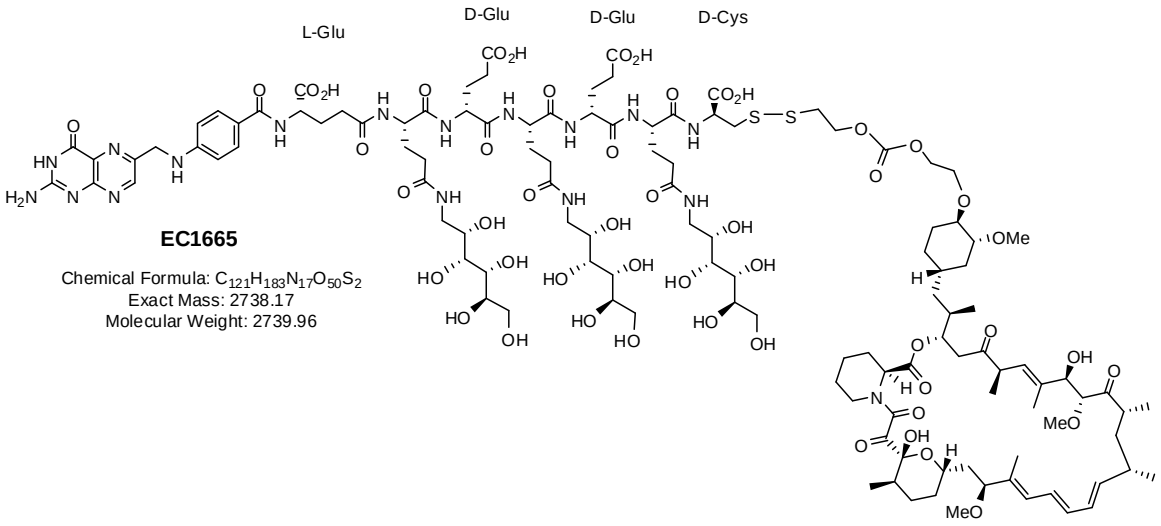
EXAMPLE. Additional illustrative compounds and processes for preparing the compounds are described herein:

Conjugates of EC1579



~~EC1840~~

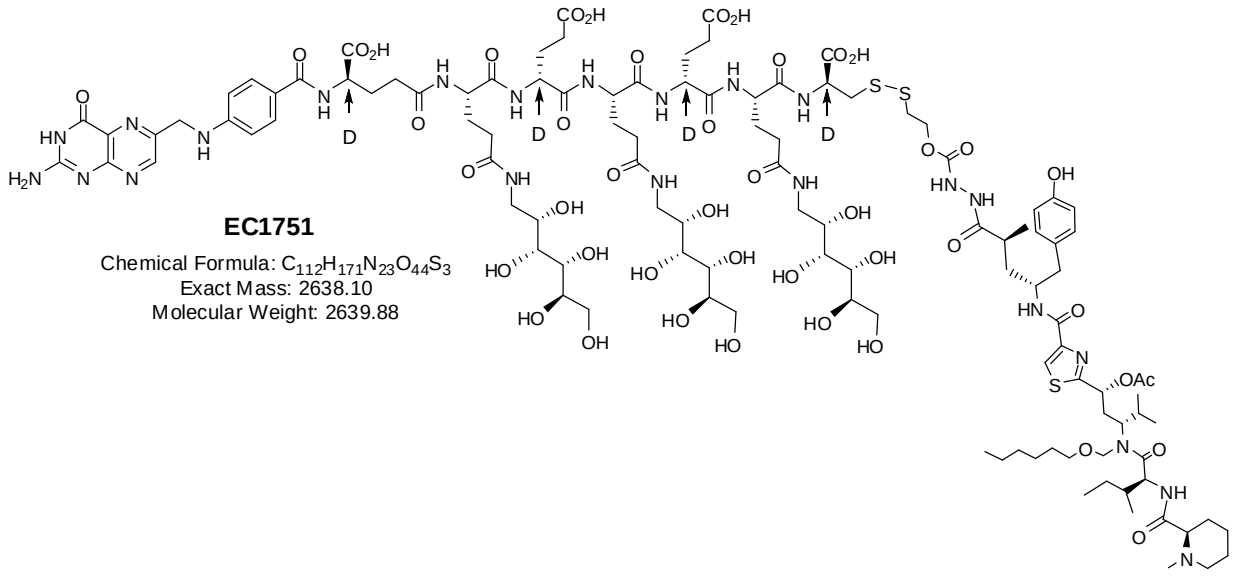




EC1579 (15mg, 1.0eq) was dissolved in degassed (Ar bubbling) 20mM PO_4 pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC0564 (10.5mg, 1.0eq) in dry dimethylsulfoxide (4.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1665 (13.4mg, 55%) was purified by preparative HPLC in 10-100% acetonitrile/10mM NH_4OAc pH5 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) Predicted 1368.09, Found 1368.30

Conjugates of EC1454

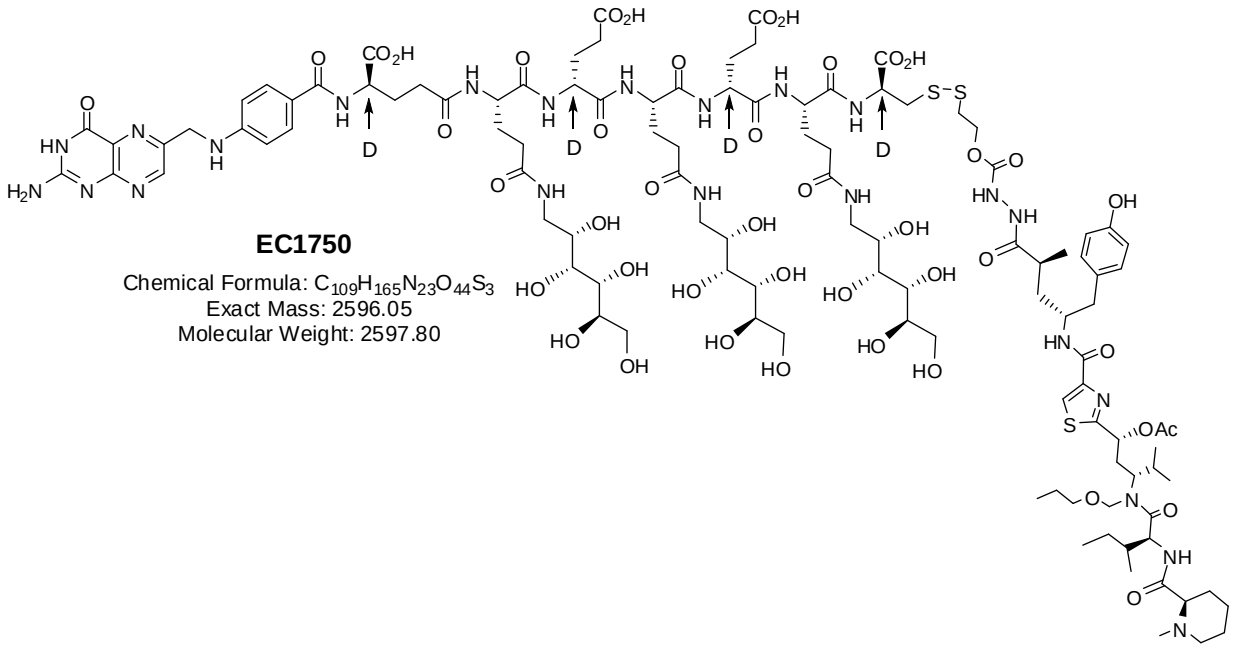
EXAMPLE. Synthesis of EC1751



EC1454 (21.1mg, 1.3eq) was dissolved in degassed (Ar bubbling) 20mM PO_4 pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1716 (10.8mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1751 (8.5mg, 33%) was purified by preparative HPLC in 10-100% acetonitrile/50mM NH_4HCO_3 pH7 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) Predicted 1320.05, Found 1320.72. Selected ^1H NMR (DMSO w/ 10% D_2O) $\underline{\delta}$ ~~d~~(ppm) 8.61 (s), 8.15 (s), 7.58 (d), 6.94 (d),

6.60 (m), 5.79 (d), 5.22 (d), 4.47 (m), 4.09-4.33 (m), 0.98 (d), 0.93 (d), 0.75 (m), 0.61 (d)

EXAMPLE. Synthesis of EC1750

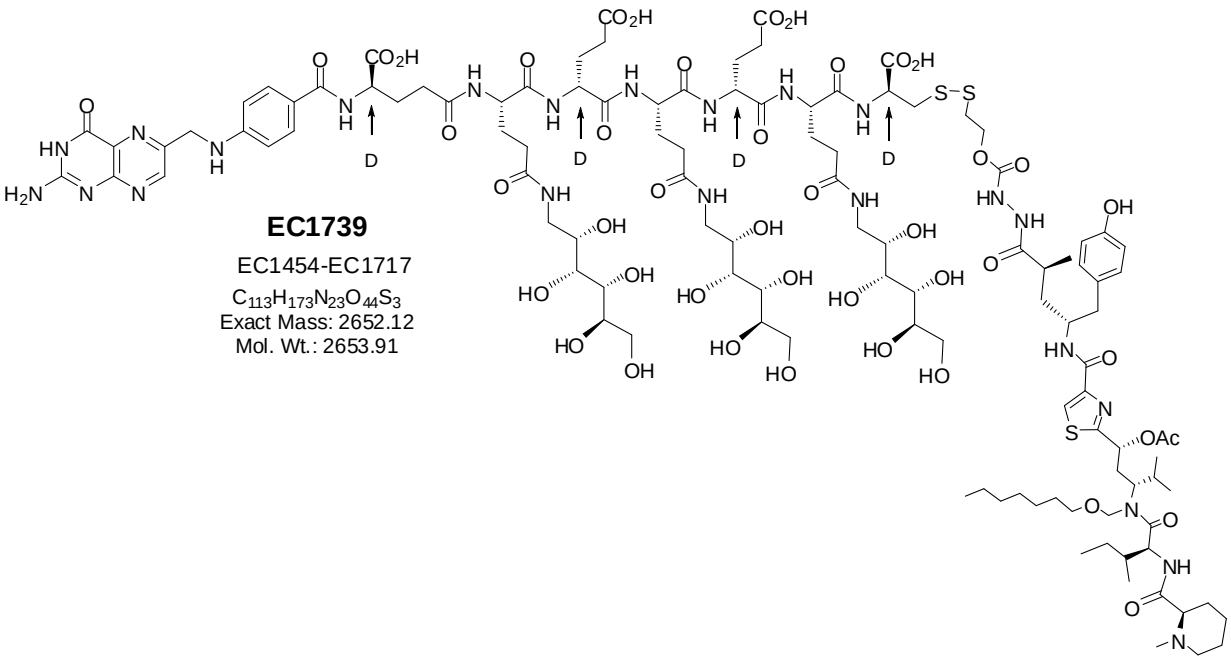


EC1750

Chemical Formula: $C_{109}H_{165}N_{23}O_{44}S_3$
Exact Mass: 2596.05
Molecular Weight: 2597.80

EC1454 (31.1mg, 1.3eq) was dissolved in degassed (Ar bubbling) 20mM PO_4 pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1715 (15.3mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1750 (18.0mg, 97%) was purified by preparative HPLC in 10-100% acetonitrile/50mM NH_4HCO_3 pH7 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) Predicted 1299.03, Found 1299.19. Partial ^{1}H NMR (DMSO w/ 10% D_2O) δ ~~d~~(ppm) 8.61 (s), 8.14 (s), 7.57 (d), 6.93 (d), 6.60 (m), 5.77 (d), 5.23 (d), 4.47 (m), 0.98 (d), 0.92 (d), 0.76 (m), 0.71 (t), 0.61 (d)

EXAMPLE. Synthesis of EC1739



EC1739

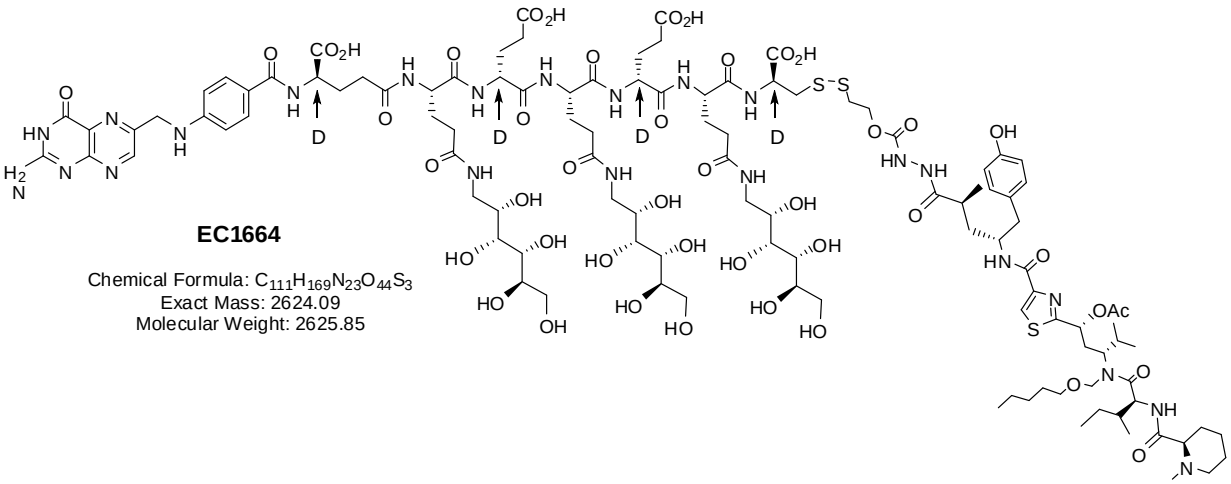
EC1454-EC1717

$C_{113}H_{173}N_{23}O_{44}S_3$

Exact Mass: 2652.12

Mol. Wt.: 2653.91

EC1454 (8.5mg, 1.5eq) was dissolved in degassed (Ar bubbling) 20mM PO_4 pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1717 (3.8mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min, EC1739 (5.3mg, 59%) was purified by preparative HPLC in 10-100% acetonitrile/50mM NH_4HCO_3 pH7 buffer and lyophilized. MS (ESI, $[M+2H]^{2+}$) predicted 1327.06, Found 1327.73



EC1664

Chemical Formula: $C_{111}H_{169}N_{23}O_{44}S_3$

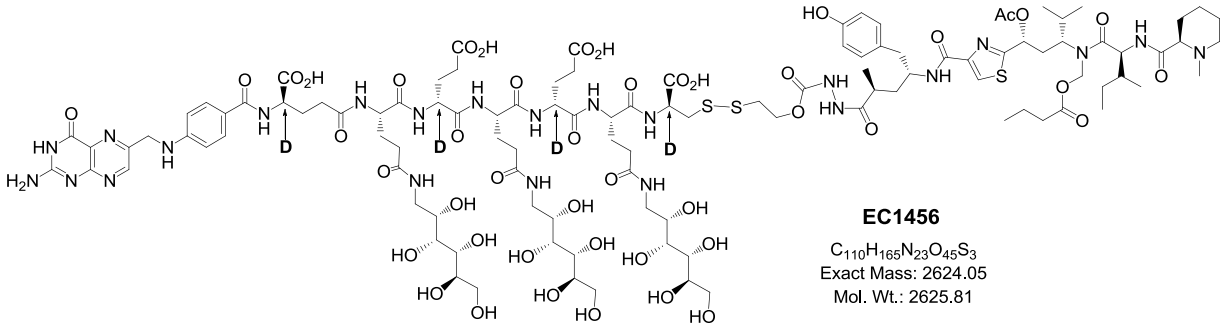
Exact Mass: 2624.09

Molecular Weight: 2625.85

MS (ESI, $[M+2H]^{2+}$) Predicted 1313.05, Found 1313.37. Selected ^1H NMR (DMSO w/ 10% D_2O) $\underline{\delta}$ ~~d~~(ppm) 8.61 (s), 8.15 (s), 7.58 (d), 6.94 (d), 6.60 (m), 5.78 (d), 5.22 (d), 4.47 (m), 4.09-4.33 (m), 0.99 (d), 0.93 (d), 0.76 (t), 0.71 (t), 0.61 (d)

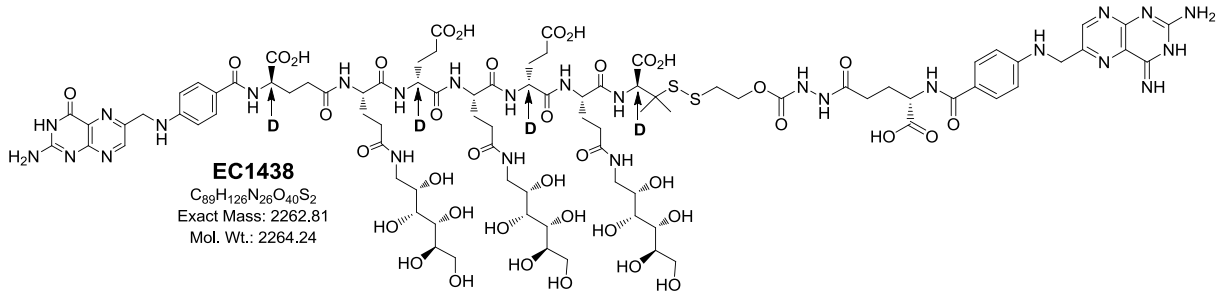
EXAMPLE. Synthesis of EC1664

EC1454 (5.5mg, 1.0eq) was dissolved in degassed (Ar bubbling) 20mM PO_4 pH7 buffer (2.0mL) and added dropwise to a stirring solution of EC1662 (3.6mg, 1.0eq) in dry dimethylsulfoxide (2.0mL, Aldrich) at room temperature with Ar bubbling. After 30min,



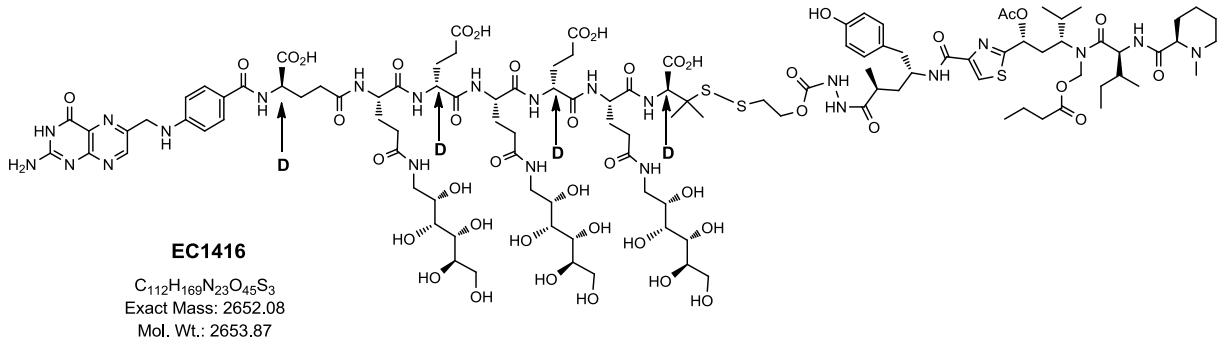
~~EC1456~~

EXAMPLES. Conjugates of EC1415



~~EC1438~~

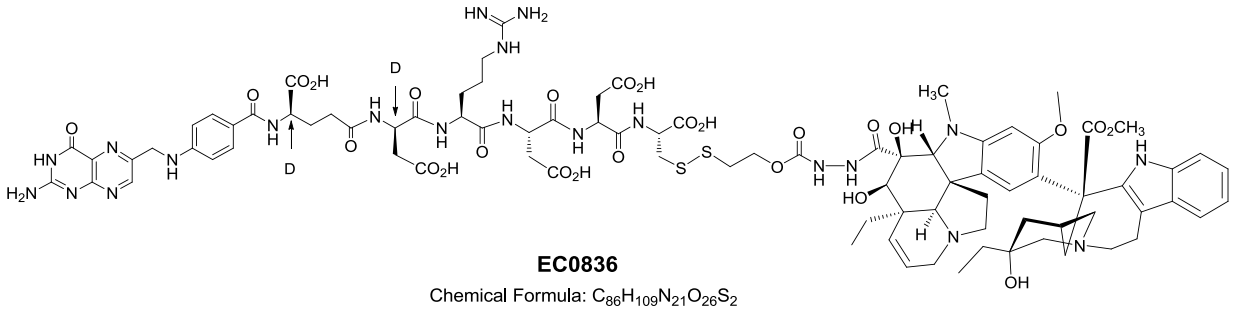
EXAMPLE. Synthesis of EC1416



EC1415 (20 mg) was dissolved in pH7 phosphate (pH 7.75, purged with argon). To this solution was added a suspension of EC0312 (14 mg) in equal volume of MeOH. The reaction mixture was stirred at ambient temperature under argon for 45 min, and then loaded onto a preparatory HPLC (Mobile phase A = 50 mM NH_4HCO_3 buffer, pH = 7.0. B = ACN. Method: 5-80% B in 20 min.) for purification. Fractions containing the desired product were collected, combined, and freeze-dried to afford the product (18 mg) as a pale yellow solid. MS(ESI, $[M+2H]^{2+}$) 1328. 1H NMR (DMSO-d6, $\underline{D_2O}$ ~~D2O~~, 300MHz): 8.6 (s, 1H), 8.15 (s, 1H), 7.85 (bd, 1H), 7.55 (d, 2H), 6.95 (d, 2H), 6.6 (m, 4H), 6.2 (d, 1H), 5.68 (d, 1H), 5.2 (d, 1H), 4.5 (bs, 3H), 4.5-4.3 (m, 4H), 4.3-4.0 (m, 10H), 3.5-3.3 (m, 13H), 3.2 (bd, 5H), 3.1-2.8 (m, 8H), 2.75 (bs, 5H), 2.6-1.6 (m, 50H), 1.4 (m, 9H), 1.2 (m, 9H), 1.0 (dd, 9H), 0.7 (m, 11H), 0.6 (d, 3H).

the product (25 mg) as a pale yellow solid. MS (ESI, M+1) 1993

EXAMPLE.

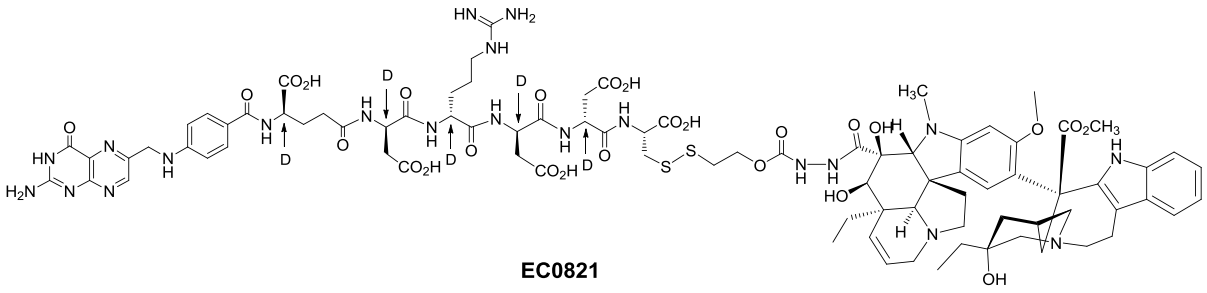


EC0836

Chemical Formula: $C_{86}H_{109}N_{21}O_{26}S_2$
Exact Mass: 1915.73
Molecular Weight: 1917.04

~~EC0836~~

EXAMPLE.

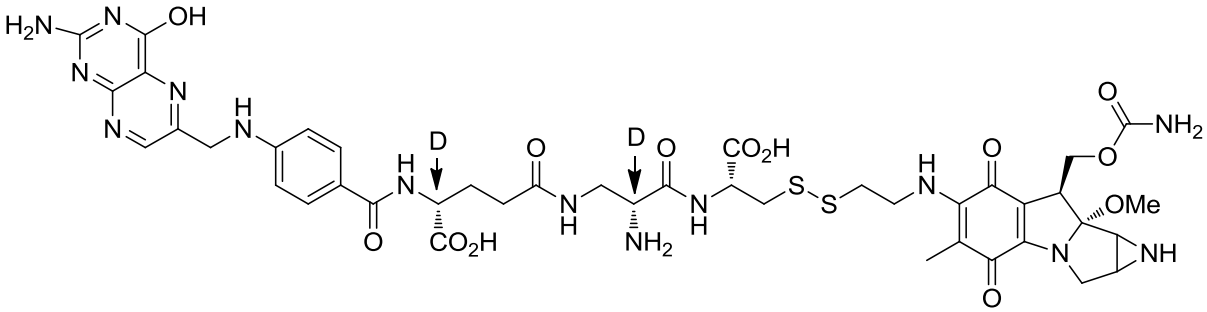


EC0821

Chemical Formula: $C_{86}H_{109}N_{21}O_{26}S_2$
Exact Mass: 1915.73
Molecular Weight: 1917.04

~~EC0821~~

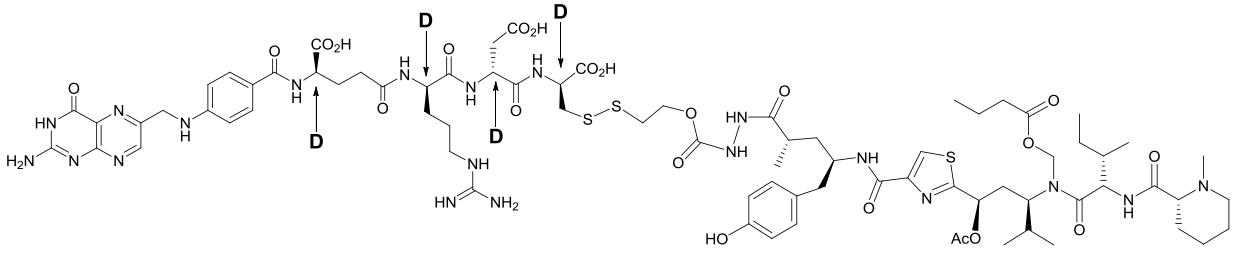
EXAMPLE.



EC 153

EC153: MS(ESI, $[M + H]^+$) 1023; (ESI, $[M - H]^-$) 1021; ^1H NMR (DMSO-d6, 300 MHz): 8.84 (s, 1H), 7.70 (d, 2H), 6.80(d, 2H), 4.60(m, 1H), 4.56 (s, 2H), 4.34 (m, 2H), 4.10(m, 2H), 3.85(m, 2H), 3.60-3.30(m, 5H), 3.20(s, 2H), 318-3.05(m, 1H), 3.0(br, 2H), 2.90-2.70(m, 2H), 2.40-2.00 (m, 4H), 1.95(m, 3 H),

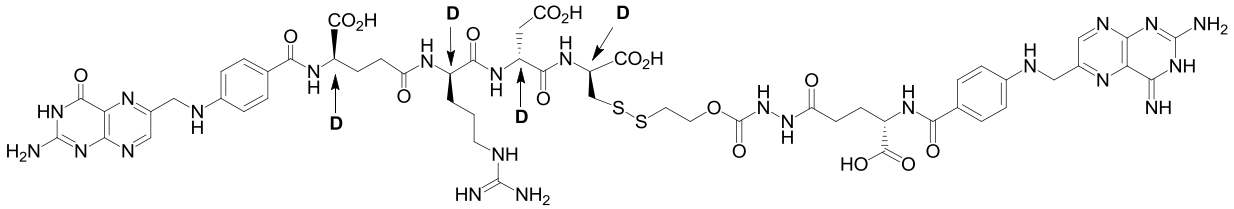
EXAMPLES. The following compounds were prepared according to the processes described herein starting from EC0059:



EC1391

$C_{77}H_{108}N_{20}O_{22}S_3$
Exact Mass: 1760.71
Mol. Wt.: 1762.00

~~EXAMPLE. EC1391~~

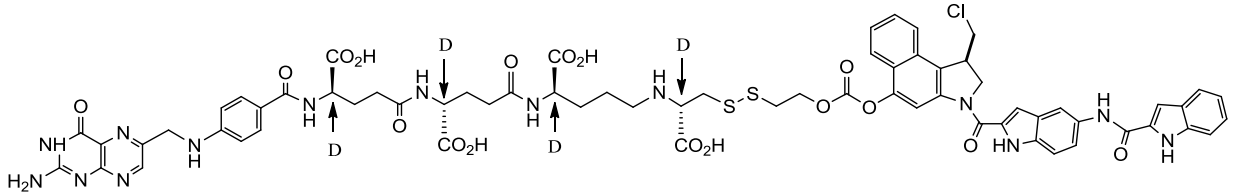


EC1390

Exact Mass: 1371.44
Mol. Wt.: 1372.37

~~EXAMPLE. EC1390~~

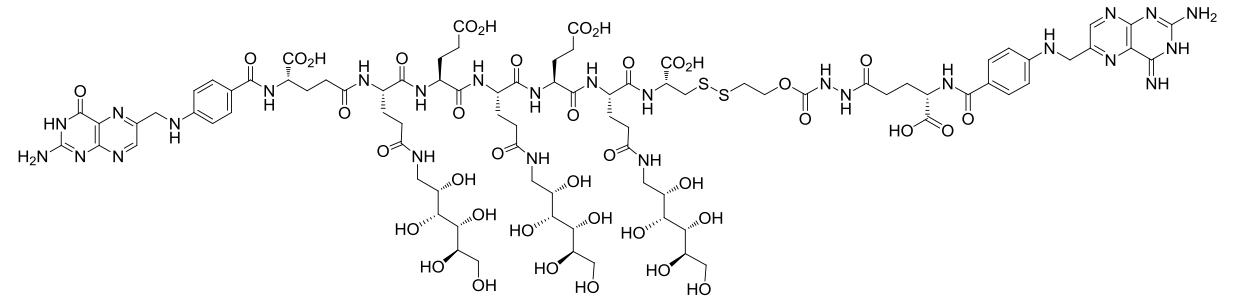
EXAMPLE.



EC74: MS(ESI, $[M + H]^+$) 1438.3; (ESI, $[M - H]^-$) 1436.4

COMPARATIVE EXAMPLES. The following comparative compounds are disclosed:

COMPARATIVE EXAMPLE.



EC0746 ($C_{87}H_{122}N_{26}O_{40}S_2$; MW 2236.18)

COMPARATIVE EXAMPLE.

compete against ^{3}H-folic acid for binding to cell surface folate receptors (FR). EC1669 (). The relative affinity values of EC1669 (normalized against folic acid, which is set to (1) are determined to be 0.53 and 0.13 on KB and CHO-FRβ cells, respectively (see, FIG. 1A and FIG. 1B). In comparison, methotrexate (MTX) showed poor binding to the cell surface FRs. Without being bound by theory, it is believed herein that the high binding affinity of EC1669 allows for efficient cellular uptake via FR-mediated endocytosis.

METHOD. Inhibition of Cellular DNA Synthesis. The compounds described herein are evaluated using an *in vitro* cytotoxicity assay that predicts the ability of the drug to inhibit the growth of folate receptor-positive cells, such as KB cells, RAW264.7 macrophages, and the like. It is to be understood that the choice of cell type can made on the basis of the susceptibility of those selected cells to the drug that forms the conjugate. The test compounds are comprised of folate linked to a respective chemotherapeutic drug, as prepared according to the processes described herein. The test cells are exposed to varying concentrations of folate-drug conjugate, and also in the absence or presence of at least a 100-fold excess of folic acid to assess activity as being specific to folate receptor mediation.

EXAMPLE. Conjugates of cytotoxic drugs described herein are active against KB cells. The activity is mediated by the folate receptor as indicated by competition experiments using co-administered folic acid. KB cells are exposed for up to 7 h at 37^{o}C to the indicated concentrations of folate-drug conjugate in the absence or presence of at least a 100-fold excess of folic acid. The cells are then rinsed once with fresh culture medium and incubated in fresh culture medium for 72 hours at 37^{o}C. Cell viability was assessed using a ^{3}H-thymidine incorporation assay. For compounds described herein, dose-dependent cytotoxicity is generally measurable, and in most cases, the IC_{50} values (concentration of drug conjugate required to reduce ^{3}H-thymidine incorporation into newly synthesized DNA by 50%) are in the low nanomolar range. Though without being bound by theory, when the cytotoxicities of the conjugates are reduced in the presence of excess free folic acid, it is believed herein that such results indicate that the observed cell death is mediated by binding to the folate receptor.

EXAMPLE. EC1669 shows a potent cytostatic effect against murine RAW264.7 macrophages. The anti-proliferative activity of EC1669 is measured in a XTT cell viability assay (FIG. 2) on RAW264.7 cells after a 2-h exposure and a total of 72 h incubation. RAW264.7 macrophages are susceptible to the drug forming the EC1669 conjugate, aminopterin. The cell viability is assessed by adding XTT (2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide) following the manufacturer's instructions. EC1669 showed a dose-dependent inhibition of cell proliferation with a relative $\underline{IC_{50}}$ ~~IC50~~ value of ~1.2 nM. The observed anti-proliferative effect was 100% competable in the presence of 100-fold excess

folic acid (FA), indicating a FR-specific mode of action for EC1669.

METHOD. In vitro activity against various cancer cell lines. $\underline{IC_{50}}$ ~~IC50~~ values are generated for various cell lines. Cells are heavily seeded in 24-well Falcon plates and allowed to form nearly confluent monolayers overnight. Thirty minutes prior to the addition of the test compound, spent medium is aspirated from all wells and replaced with fresh folate-deficient RPMI medium (FFRPMI). A subset of wells are designated to receive media containing 100 μM folic acid. The cells in the designated wells are used to determine the targeting specificity. Without being bound by theory it is believed herein that the cytotoxic activity produced by test compounds in the presence of excess folic acid, i.e. where there is competition for FR binding, corresponds to the portion of the total activity that is unrelated to FR-specific delivery. Following one rinse with 1 mL of fresh FFRPMI containing 10% heat-inactivated fetal calf serum, each well receives 1 mL of medium containing increasing concentrations of test compound (4 wells per sample) in the presence or absence of 100 μM free folic acid as indicated. Treated cells are pulsed for 2 h at 37 °C, rinsed 4 times with 0.5 mL of media, and then chased in 1 mL of fresh medium up to 70 h. Spent medium is aspirated from all wells and replaced with fresh medium containing 5 μCi/mL ^{3}H-thymidine. Following a further 2 h 37 °C incubation, cells are washed 3 times with 0.5 mL of PBS and then treated with 0.5 mL of ice-cold 5% trichloroacetic acid per well. After 15 min, the trichloroacetic acid is aspirated and the cell material solubilized by the addition of 0.5 mL of 0.25 N sodium hydroxide for 15 min. A 450 μL aliquot of each solubilized sample is transferred to a scintillation vial containing 3 mL of Ecolume scintillation cocktail and then counted in a liquid scintillation counter. Final results are expressed as the percentage of ^{3}H-thymidine incorporation relative to untreated controls.

EXAMPLE. Compounds described herein exhibit potent in vitro activity against pathogenic cells, such as KB cells. Compounds described herein exhibit greater specificity for the folate receptor compared to compounds that do not include at least one unnatural amino acid. For Example, EC1456 exhibits ca. 1000-fold specificity for the folate receptor as determined by folic acid competition (specificity = difference in IC_{50} between competed group and non-competed group), and a 4-fold improvement in specificity compared to comparator compound EC0531, which does not include a linker L having an unnatural amino acid.

EXAMPLE. Selectivity for folate receptor expressing cells. Compounds described herein show high activity for folate receptor expressing cells. Compounds described herein do not show significant binding to folate receptor negative cells. EC1456 show high competable binding to low and high FR expressing cells (FR+), and does not show binding to cells that do not express FR (FR-).

Activity of EC1456 in (FR+) and (FR-) Cell Lines

Example	Relative Affinity (a)	In vitro $\underline{IC_{50}}$ ~~IC50~~ (nM) (b)	50% competition (nM) (c)	In vitro specificity (fold) (d)	In vivo activity (e)	Therapeutic index over unconjugated drug (f)
EC1299	0.29	0.9	700	778	CR	Yes
EC1393	0.25	2.2	600	300	NT	NT
EC1456	0.27	1.5	1416	944	CR	Yes
EC1548	0.23	4.4	350	78	CR	Yes
EC1549	0.90	4.5	350	78	CR	Yes
EC1586	0.56	NT	NT	NT	NT	NT
EC0531 (comparator example)	NT	1.5	355	237	CR	Yes

(a) compared to folic acid; (b) as determined by thymidine incorporation; (c) $\underline{IC_{50}}$ ~~IC50~~ for test compound when competed with excess folic acid; the higher the $\underline{IC_{50}}$ ~~IC50~~ the more specific is the folate mediation.; (d) in vitro specificity = difference in IC_{50} between competed group and non-competed group; (e) as determined in subcutaneous KB tumor in nu/nu mice; CR=complete response, where tumor volume, as defined herein, during the observation period was zero for all test animals in the group; (f) parent tubulysin; NT=not tested.

METHOD. Adjuvant-Induced Arthritis (AIA) Model. Female Lewis rats are fed a folate-deficient diet (Harlan Teklad, Indianapolis, IN) for 9-10 days prior to arthritis induction. The adjuvant-induced arthritis (AIA) is induced by intradermal inoculation (at the base of tail) of 0.4-0.5 mg of heat-killed *Mycobacteria butyricum* (BD Diagnostic Systems, Sparks, MD) in 100 µL light mineral oil (Sigma). Ten days after arthritis induction, paw edema (degree of arthritis) in rats is assessed using a modified arthritis scoring system: 0 = no arthritis; 1 = swelling in one type of joint; 2 = swelling in two types of joint; 3 = swelling in three types of joint; 4 = swelling of the entire paw. A total score for each rat is calculated by summarizing the scores for each of the four paws, giving a maximum score of 16 for each rat. On Day 10 post arthritis induction, rats with a total arthritis score of ≥2 were removed from the study and the remaining rats are distributed evenly across the control and treatment groups (n = 5 for all groups except that n = 2-3 for healthy controls). All treatments are started on Day 10 unless indicated otherwise. Rat paws are also evaluated radiographically to assess and determine bone damage.

EXAMPLE. Compounds described herein are potent in treating inflammatory diseases, such as inflammation and bone damage accompanying arthritis. EC1496 is potent and efficacious in the reducing paw inflammation in a rat model of adjuvant-induced arthritis, as shown in FIG. 7. FIG. 7 shows that EC1496 is efficacious in preventing the development of

arthritis based on the evaluation of paw edema. EC1496 (trace (d)) is significantly different

from untreated control (trace (b)). In addition, the data indicate that the effect is folate receptor mediated because EC1496 (trace (d)) is also significantly different from the competition control group where EC1496 is co-administered with excess folic acid (trace (d)).

EXAMPLE. Compounds described herein are potent in treating inflammatory diseases, such as inflammation and bone damage accompanying arthritis. Illustratively, EC1496 is potent and efficacious in reducing and/or preventing bone damage in a rat model of adjuvant-induced arthritis, as determined by radiographic analysis. The radiography shows that the treated animals do not exhibit the bone damage seen in the untreated control animals, based on visual scoring. Instead, the treated animals and the healthy animals show similar bone structure.

EXAMPLE. EC1669 displays folate receptor-specific activity against adjuvant arthritis. Starting 9 days after induction, rats with developing AIA are distributed according to arthritis scores into three groups (n = 5): (1) the untreated AIA control, (2) the EC1669 treated group, and (3) the EC1669 plus EC0923 competition group. All treatments last for 2 consecutive weeks. The animals in the AIA control group are left untreated. The animals in the EC1669 treatment group are given twice-a-week subcutaneous doses of EC1669 at a dosage of 375 nmol/kg. The animals in the EC1669 plus EC0923 group are given twice-a-week subcutaneous doses of EC1669 at a dosage of 375 nmol/kg in conjunction to EC0923 at a dosage of 187.5 μmol/kg. The study endpoints are shown in FIG. 8A, FIG. 8B, FIG. 9A, and FIG. 9B are: (a) arthritis score; (b) change in body weight; and (c) paw swelling, assessed by percent increase in paw weight (collected 4 days after the last dose), and (d) bone radiography. EC1669 is found to be highly effective in alleviating paw swelling (by ~80% compared to control) and bone damage (by ~80% compared to control). The anti-arthritic activity of EC1669 is competable (blocked) with the folate competitor (EC0923).

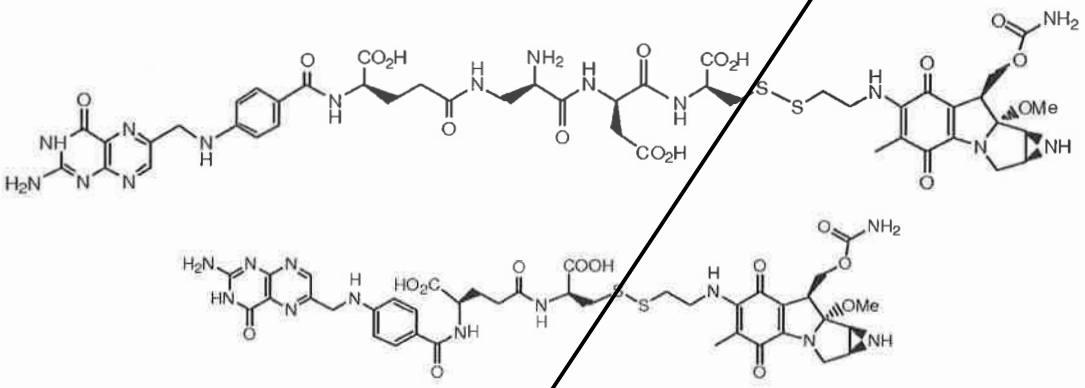
EXAMPLE. In a subsequent dosing study, various EC1669 dosing regiments were evaluated including once-a-week at 1000 nmol/kg ~~nml/kg~~, twice-a-week at 250 nmol/kg, and twice-a-week at 500 nmol/kg ~~nml/kg~~. Surprisingly, twice-a-week dosing at 250 nmol/kg was superior to once-a-week dosing at 1000 nmol/kg ~~nml/kg~~, a two-fold decrease in total dose. EC1669 was found more efficacious when dosed biweekly than once weekly in reducing paw swelling at 81% reduction at 500 nmol/kg, biw, and 64% reduction at 250 nmol/kg, biw, compared to a 44% reduction at 1000 nmol/kg, siw.

EXAMPLE. EC1669 plus CellCept is more effective than either agent alone against adjuvant-induced arthritis. CellCept is a prodrug of mycophenolic acid, an immunosuppressant drug used to prevent organ rejection in transplantation. CellCept is activated in vivo and releases its active product that can inhibit T cell proliferation and interfere

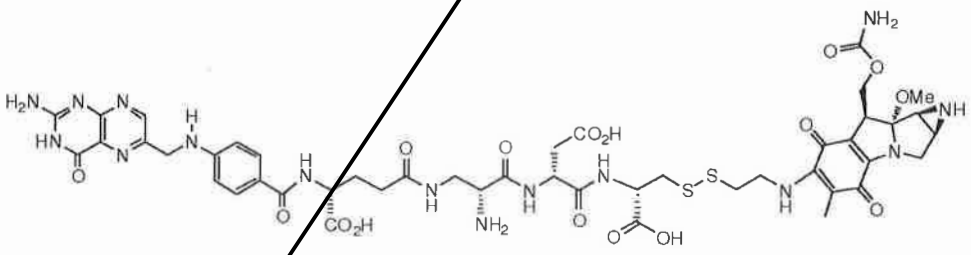
WHAT IS CLAIMED IS:

1. A compound of the formula B-L(D)$_x$, or a pharmaceutically acceptable salt thereof, wherein B is a radical of a cell surface receptor binding and/or targeting ligand, D is in each instance a radical of an independently selected drug, x is an integer selected from 1, 2, 3, 4 and 5; and L is a polyvalent releasable linker comprising one or more unnatural amino acids; and where B is covalently attached to L, and L is covalently attached to each D; and

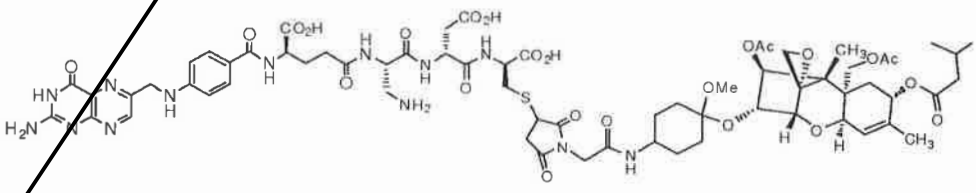
where the compound is not any one of or any subgroup or subset of the following formulae



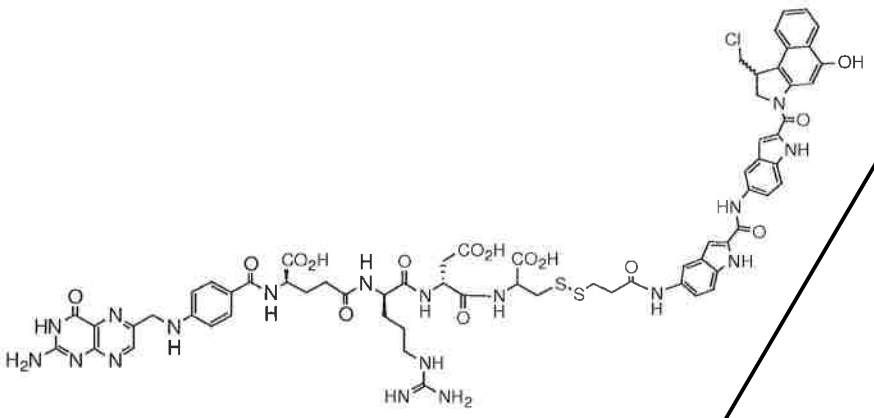
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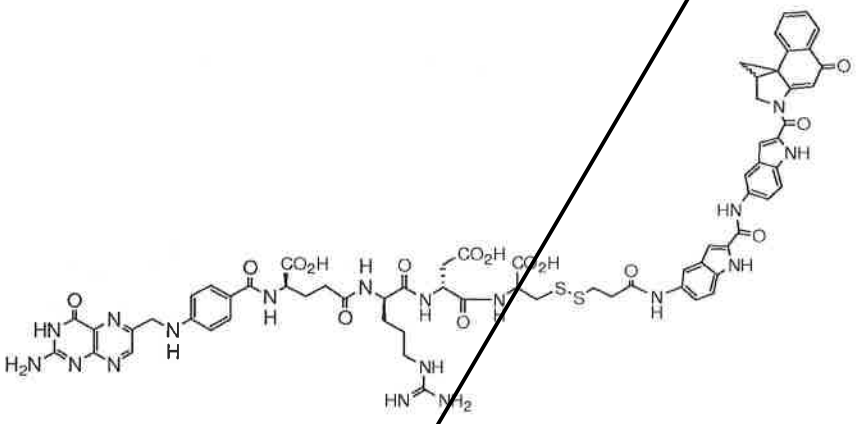
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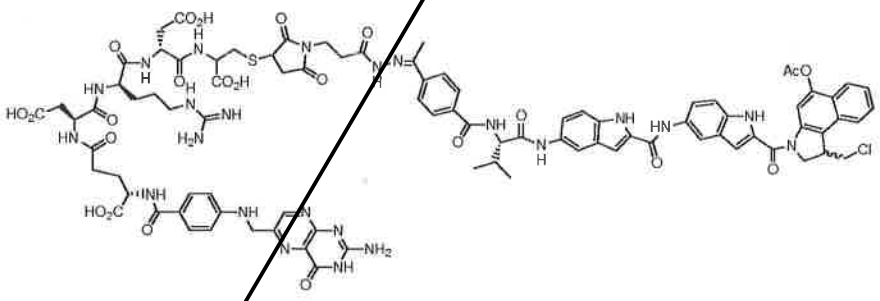
EC0056



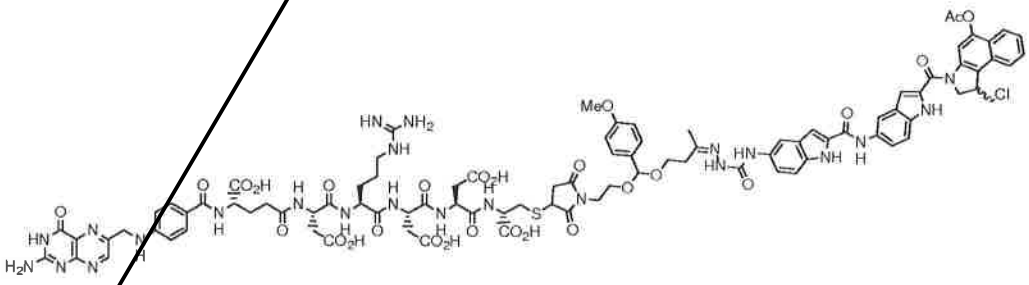
EC0077



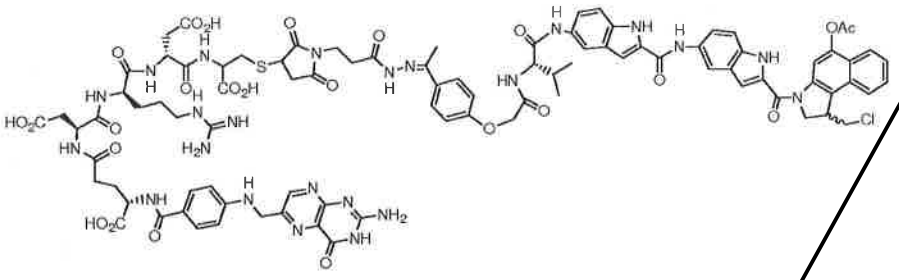
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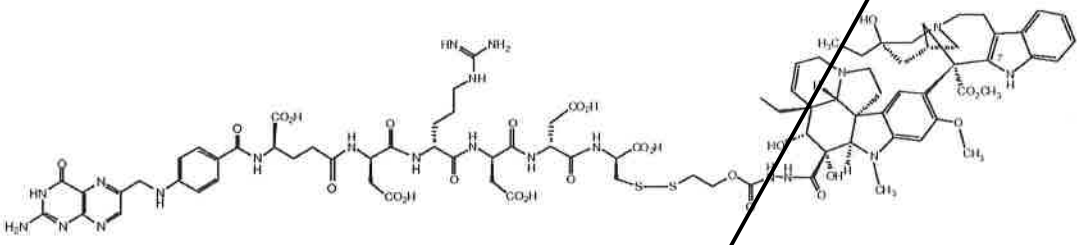
EC0136



EC0137

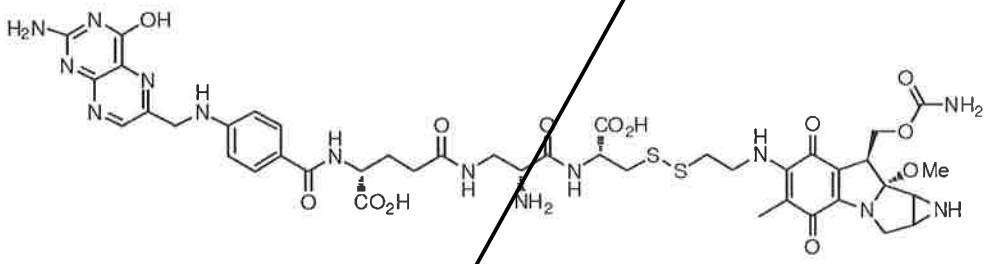


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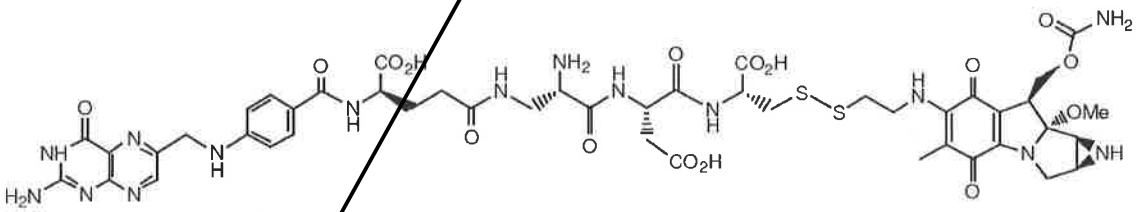
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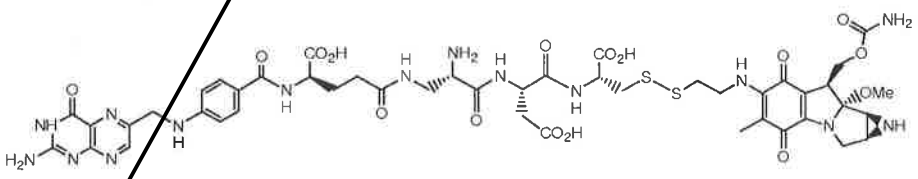
and/or where the compound is not of the following formula



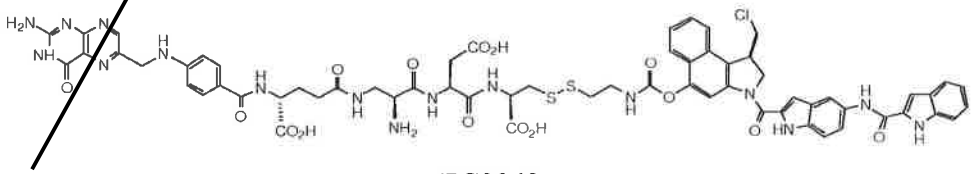
EC0153

and/or where the compound is not any one of or any subgroup or subset of the following formulae



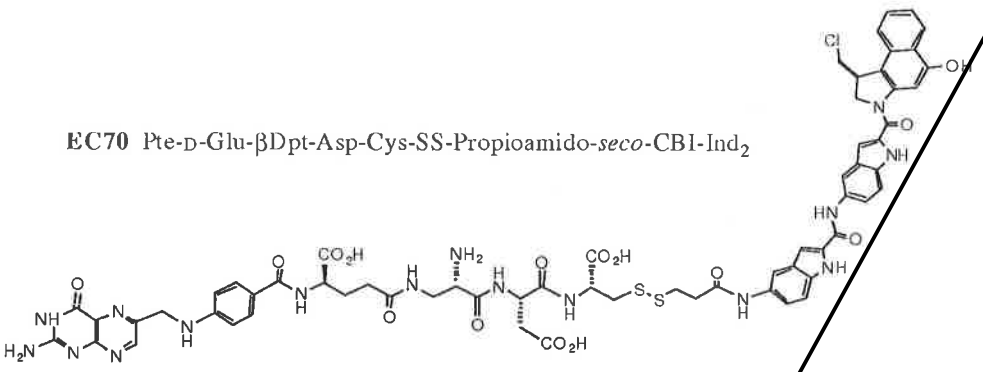


EC0052

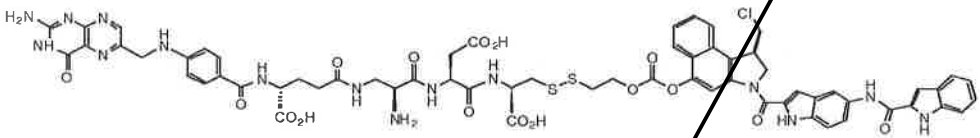


EC0069

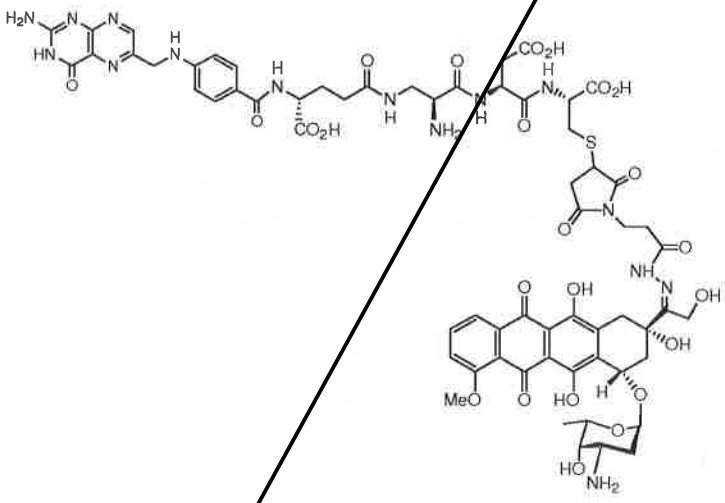
EC70 Pte-D-Glu-βDpt-Asp-Cys-SS-Propioamido-*seco*-CB1-Ind$_2$



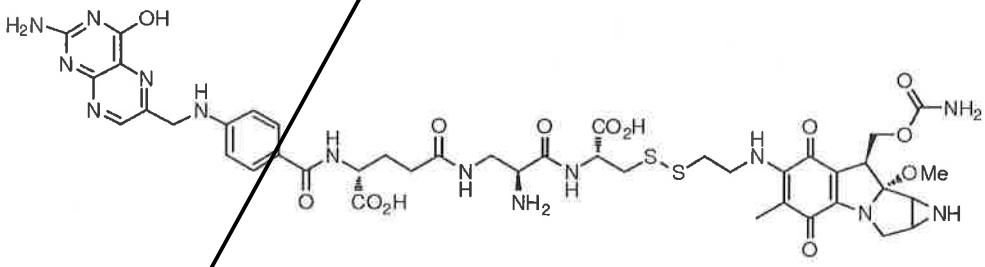
EC0070



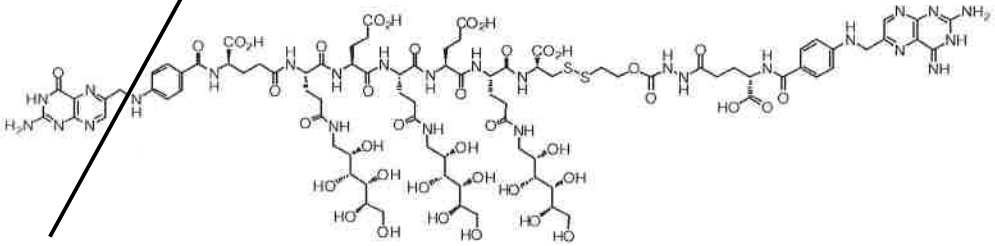
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EC0111

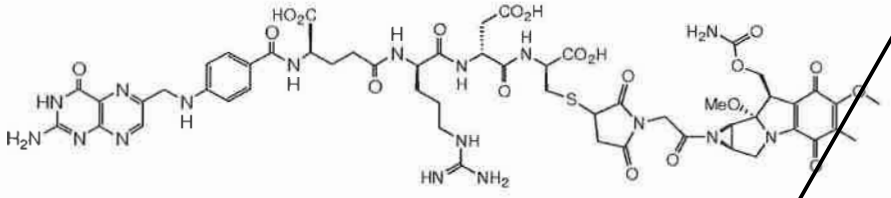


EC0155

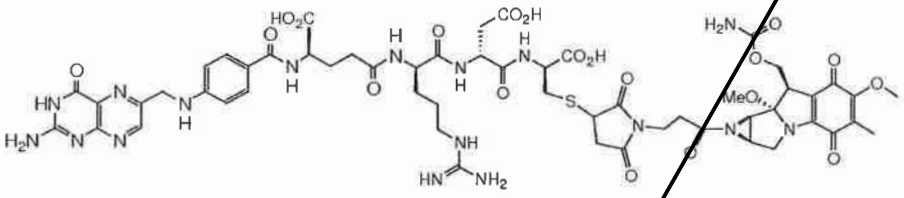


EC0894

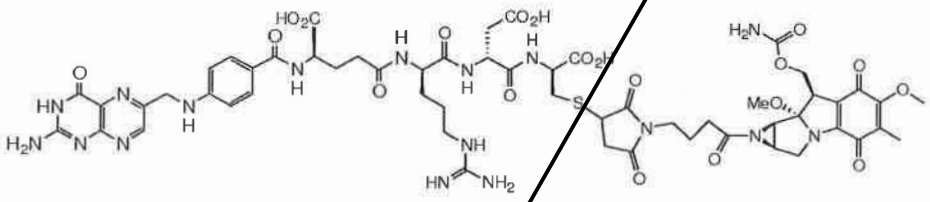
and/or where the compound is not any one of or any subgroup or subset of the following formulae



EC0082

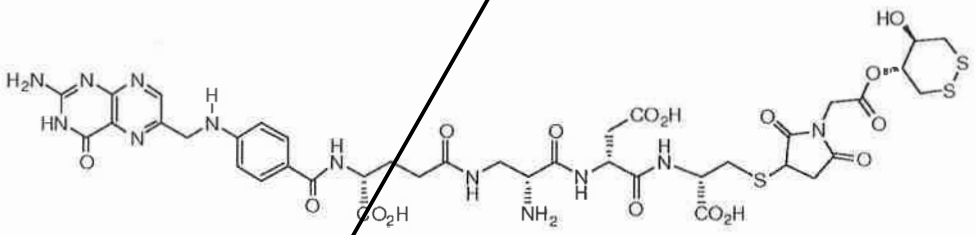


EC0083



EC0084

and/or where the compound is not of the following formula



EC0060

and/or any combination of the foregoing;

or any pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein at least one unnatural amino acid has the D-configuration.

3. The compound of claim 1 wherein at least one unnatural amino acid is selected from D-alanine, D-aspartic acid, D-asparagine, D-cysteine, D-glutamic acid, D-phenylalanine, D-histidine, D-isoleucine, D-lysine, D-leucine, D-methionine, D-proline, D-glutamine, D-arginine, D-serine, D-threonine, D-valine, D-tryptophan, D-tyrosine, and D-ornithine, and any amino acid derivatives thereof.

4. The compound of any one of claim 1 wherein L comprises two or more unnatural amino acids.

5. The compound of claim 1 wherein L comprises three or more unnatural amino acids.

6. The compound of claim 1 wherein L comprises four or more unnatural amino acids.

7. The compound of any one of claims 1 to 6 wherein the ligand is a folate receptor binding ligand.

8. The compound of any one of claim 1 to 6 wherein the ligand is a folate comprising D-glutamyl.

9. The compound of any one of claims 1 to 6 wherein L further comprises one or more disulfides.

10. The compound of any one of claims 1 to 6 wherein L further comprises one or more divalent hydrophilic radicals.

11. The compound of any one of claims 1 to 6 wherein L further comprises one or more divalent polyoxy radicals.

12. The compound of any one of claims 1 to 6 wherein L further comprises one or more divalent polyhydroxy radicals.

13. The compound of claim 12 wherein at least one of the polyhydroxy radicals is of the formula

$$CH_2\text{-}(CH(OH))_n\text{-}CH_2\text{-}OH$$

where n is selected from 1, 2, 3, 4, 5, and 6.

14. The compound of any one of claims 1 to 6 wherein L comprises a divalent polyglutamic acid radical, where at least one glutamic acid forms an amide with an aminopolyhydroxy radical.

15. The compound of 14 wherein at least one of the glutamic acids is D-glutamic acid.

16. The compound of claim 15 wherein at least one of the glutamic acids is unsubstituted D-glutamic acid.

17. The compound of any one of claims 1 to 6 wherein L comprises a divalent poly(D-glutamic acid) radical, where at least one glutamic acid forms an amide with an aminopolyhydroxy radical.

18. The compound of any one of claims 1 to 6 wherein L comprises a divalent radical of the formula (K-L)d, where K is a divalent D-glutamic acid radical, L is a divalent L-glutamic acid radical that forms an amide with an aminopolyhydroxy radical, and d

is 1, 2, 3, or 4.

19. The compound of claim 14 wherein at least one of the aminopolyhydroxy radicals is of the formula

$$NH\text{-}CH_2\text{-}(CH(OH))_m\text{-}CH_2\text{-}OH$$

where m is selected from 1, 2, 3, 4, 5, and 6.

20. The compound of claim 17 wherein at least one of the aminopolyhydroxy radicals is of the formula

$$NH\text{-}CH_2\text{-}(CH(OH))m\text{-}CH2\text{-}OH$$

where m is selected from 1, 2, 3, 4, 5, and 6.

21. The compound of claim 14 wherein at least one of the aminopolyhydroxy radicals is of the formula

$$NH\text{-}CH_2\text{-}(CH(OH))_m\text{-}R$$

where m is selected from 1, 2, 3, 4, 5, and 6; and R is H, alkyl, cycloalkyl, or arylalkyl.

22. The compound of claim 17 wherein at least one of the aminopolyhydroxy radicals is of the formula

$$NH\text{-}CH_2\text{-}(CH(OH))m\text{-}R$$

where m is selected from 1, 2, 3, 4, 5, and 6; and R is H, alkyl, cycloalkyl, or arylalkyl.

23. The compound of any one of claims 1 to 6 wherein L comprises a divalent radical of the formula

$$S\text{-}CH_2CH_2\text{-}O\text{-}C(O).$$

24. The compound of any one of claims 1 to 6 wherein L comprises a divalent radical of the formula

$$S\text{-}S\text{-}CH_2CH_2\text{-}O\text{-}C(O).$$

25. The compound of any one of claims 1 to 6 wherein x is 1.

26. The compound of any one of claims 1 to 6 wherein at least one drug is a cytotoxic agent.

27. The compound of any one of claims 1 to 6 wherein at least one drug is a cancer treating agent.

28. The compound of any one of claims 1 to 6 wherein at least one drug is a tubulysin.

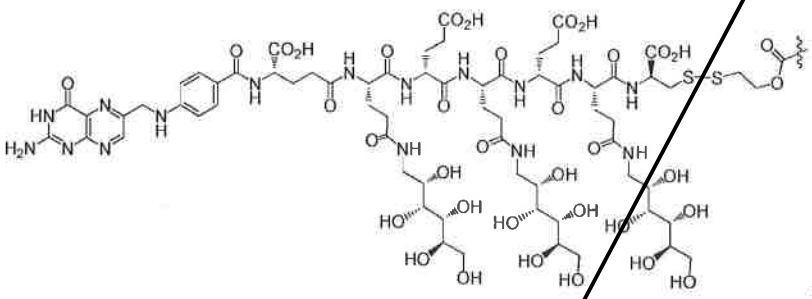
29. The compound of any one of claims 1 to 6 wherein at least one drug is a inflammation-treating agent.

30. The compound of any one of claims 1 to 6 wherein at least one drug is a

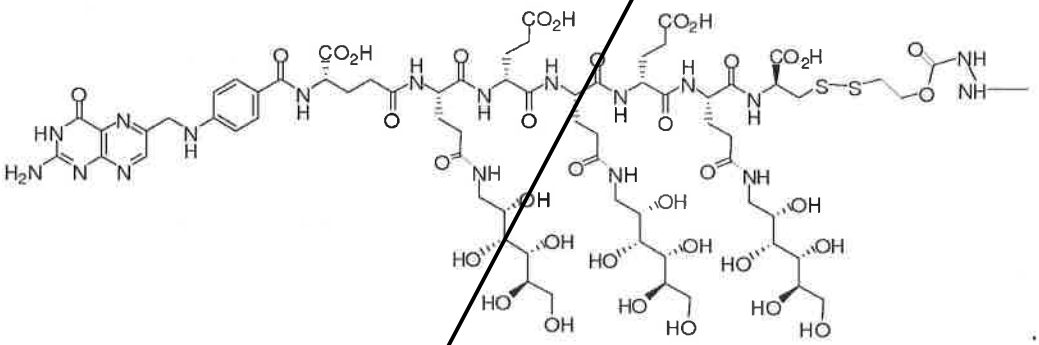
dihydrofolate reductase inhibitor.

31. The compound of any one of claims 1 to 6 wherein at least one drug is an inhibitor of mammalian target of rapamycin (mTOR).

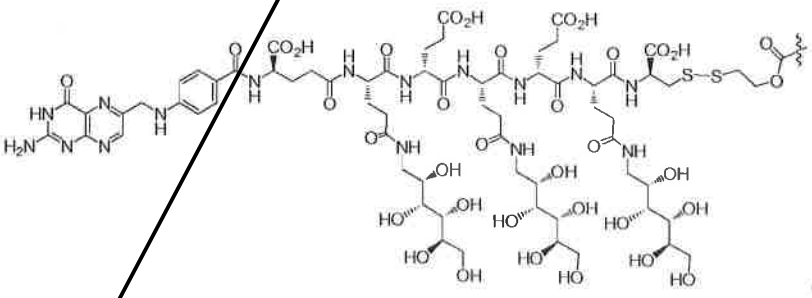
32. The compound of any one of claims 1 to 5 wherein B-L is a radical of the formula



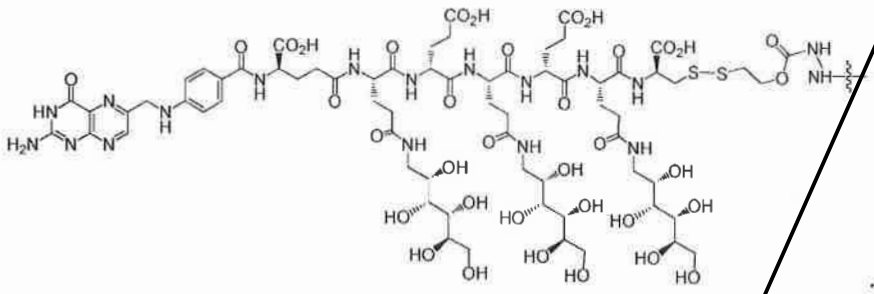
33. The compound of any one of claims 1 to 5 wherein B-L is a radical of the formula



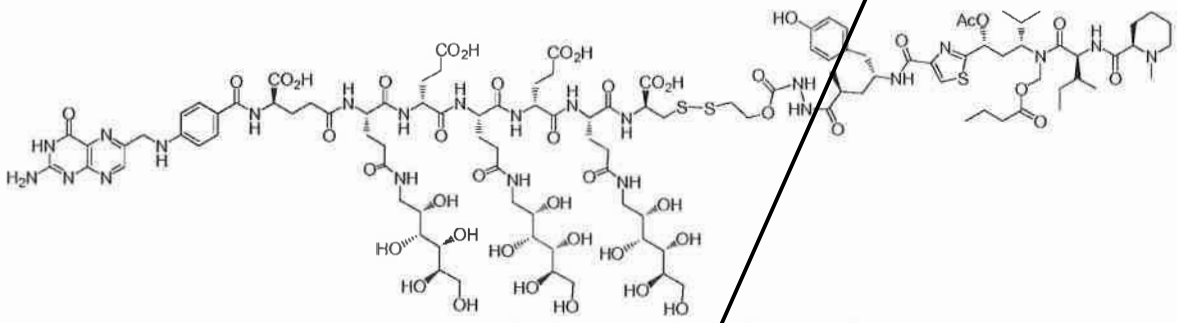
34. The compound of any one of claims 1 to 5 wherein B-L is a radical of the formula



35. The compound of any one of claims 1 to 5 wherein B-L is a radical of the formula

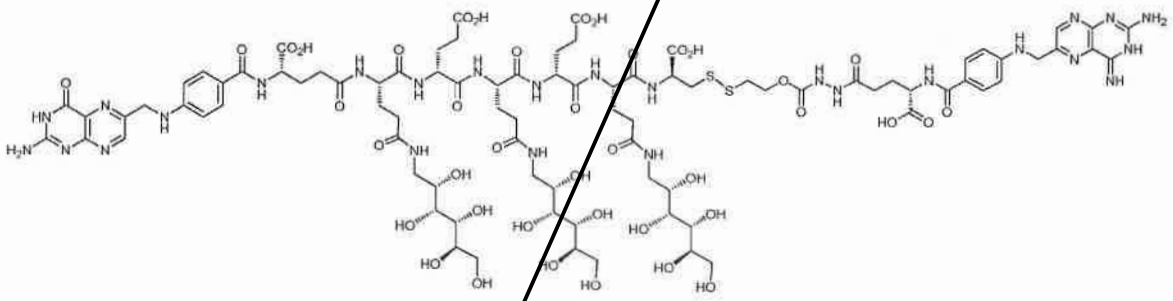


36. The compound of any one of claims 1 to 5 wherein the compound is of the formula EC1456



or a pharmaceutically acceptable salt thereof.

37. The compound of any one of claims 1 to 5 wherein the compound is of the formula EC1669



or a pharmaceutically acceptable salt thereof.

38. A pharmaceutical composition comprising a compound of any one of claims 1 to 6 in combination with one or more carriers, diluents, or excipients, or a combination thereof.

39. A unit dose or unit dosage form composition comprising a therapeutically effective amount of one or more compounds of any one of claims 1 to 6, optionally in combination with one or more carriers, diluents, or excipients, or a combination thereof.

40. A composition for treating cancer or inflammation in a host animal, the composition comprising a therapeutically effective amount of one or more compounds of any one of claims 1 to 6; or a pharmaceutical composition comprising a therapeutically effective amount of one or more compounds of any one of claims 1 to 6, optionally further comprising

one or more carriers, diluents, or excipients, or a combination thereof.

41. Use of one or more compounds of any one of claims 1 to 6, optionally in combination with one or more carriers, diluents, or excipients, or a combination thereof, in the manufacture of a medicament for treating a cancer or inflammation in a host animal.

42. The use of claim 41 wherein the cancer is drug resistant cancer.

43. The use of claim 41 wherein the cancer is an ovarian cancer.

44. The use of claim 41 wherein the cancer is a breast cancer.

45. The use of claim 41 wherein the cancer is a non-small cell lung cancer.

46. The use of claim 41 wherein the cancer is a hepatocellular carcinoma or cancer.

WHAT IS CLAIMED IS:

1. A compound of the formula B-L$(D)_x$, or a pharmaceutically acceptable salt thereof, wherein B is a radical of a cell surface receptor binding ligand, D is a tubulysin, x is 1; and L is a polyvalent releasable linker comprising one or more unnatural amino acids; and where B is covalently attached to L, and L is covalently attached to D.

2. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein at least one unnatural amino acid has the D-configuration.

3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein at least one unnatural amino acid is selected from D-alanine, D-aspartic acid, D-asparagine, D-cysteine, D-glutamic acid, D-phenylalanine, D-histidine, D-isoleucine, D-lysine, D-leucine, D-methionine, D-proline, D-glutamine, D-arginine, D-serine, D-threonine, D-valine, D-tryptophan, D-tyrosine, and D-ornithine.

4. The compound of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein at least one unnatural amino acid is D-glutamic acid.

5. The compound of any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein at least one unnatural amino acid is D-cysteine.

6. The compound of any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, wherein L comprises at least one D-cysteine and at least two D-glutamic acids.

7. The compound of any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, wherein L comprises one D-cysteine and two D-glutamic acids.

8. The compound of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein L comprises two D-glutamic acids.

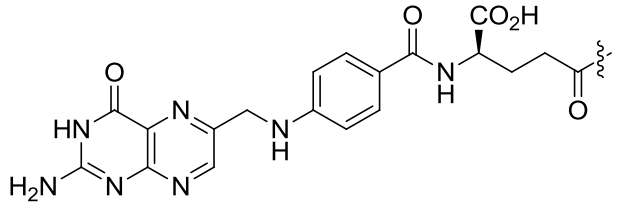
9. The compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof,

wherein B is a folate receptor binding ligand.

10. The compound of any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, wherein B is a folate.

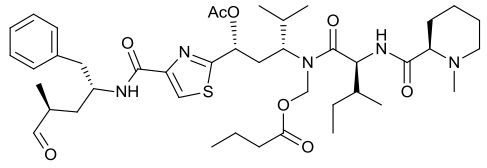
11. The compound of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein B is a D-folate.

12. The compound of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, wherein B is of the formula

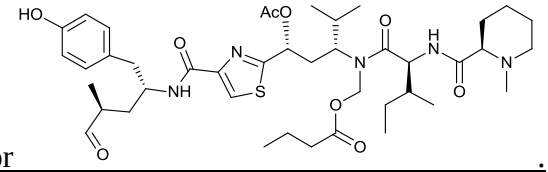


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13. The compound of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein D is a radical of the formula

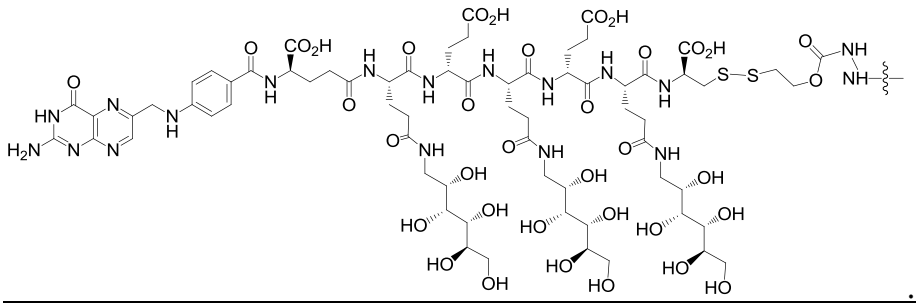


or



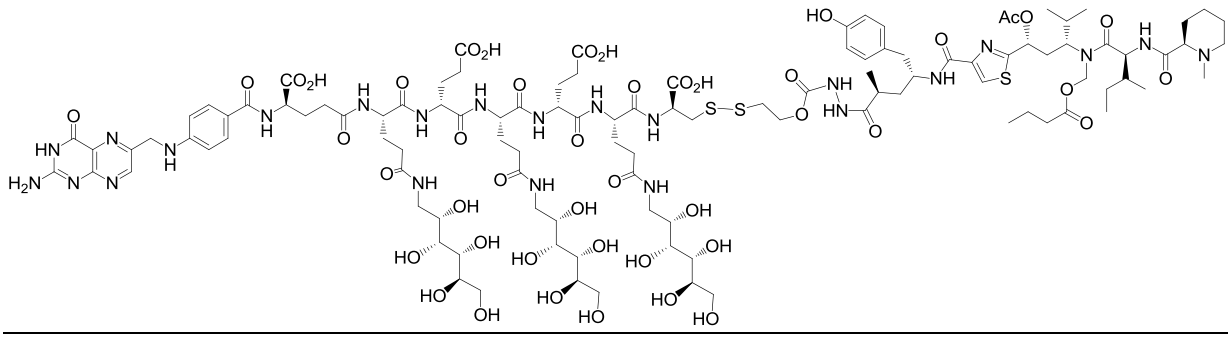
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14. The compound of any one of claims 1 to 13, or a pharmaceutically acceptable salt thereof, wherein B-L is a radical of the formula



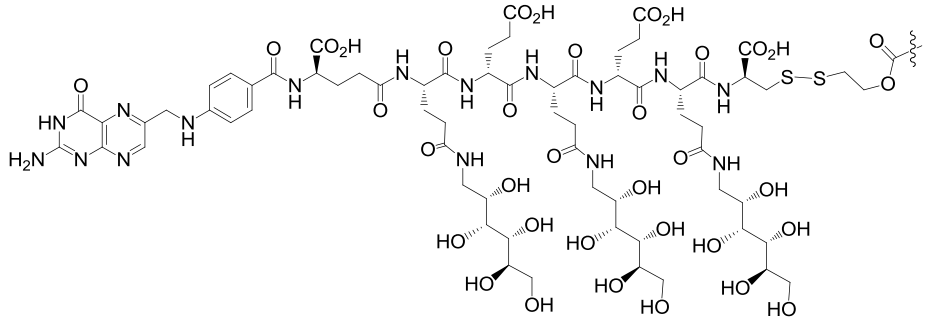
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15. The compound of claim 1 having the formula



or a pharmaceutically acceptable salt thereof.

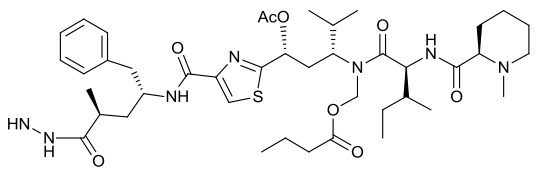
16. A compound of the formula B-L$(D)_x$, or a pharmaceutically acceptable salt thereof, wherein x is 1, D is a drug, and B-L is of the formula



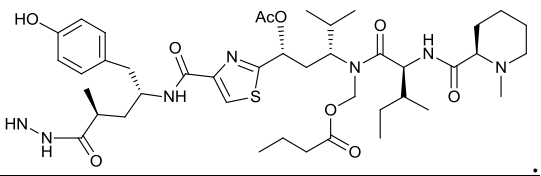
wherein L is covalently attached to D.

17. The compound of claim 16, or a pharmaceutically acceptable salt thereof, wherein D is selected from a cryptophytic, bortezomib, thiobortezomib, a tubulysin, aminopterin, a rapamycin, paclitaxel, docetaxel, doxorubicin, daunorubicin, everolimus, α-amanatin, verucarin, didemnin B, geldanomycin, purvalanol A, ispinesib, budesonide, dasatinib, an epothilone, a maytansine, and a tyrosine kinase inhibitor.

18. The compound of claim 16, or a pharmaceutically acceptable salt thereof, wherein D is a radical of a tubulysin of the formula



or



.

19. A pharmaceutical composition comprising the compound of any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, in combination with one or more carriers, diluents, or excipients, or a combination thereof.

20. Use of a compound according to any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, optionally in combination with one or more carriers, diluents, or excipients, or a combination thereof, in the manufacture of a medicament for treating a cancer in a patient.

21. The use of claim 20, wherein the cancer is a carcinoma, a sarcoma, a lymphoma, Hodgekin's disease, a melanoma, a mesothelioma, Burkitt's lymphoma, a nasopharyngeal carcinoma, a leukemia, or a myeloma.

22. The use of claim 20, wherein the cancer is oral, thyroid, endocrine, skin, gastric, esophageal, laryngeal, pancreatic, colon, bladder, bone, ovarian, cervical, uterine, breast, testicular, prostate, rectal, kidney, liver, or lung cancer.

23. The use of any one of claims 20 to 22, wherein the cancer is ovarian cancer.

24. The use of any one of claims 20 to 22, wherein the cancer is breast cancer.

25. The use of any one of claims 20 to 22, wherein the cancer is non-small cell lung cancer.

26. A unit dose or unit dosage form composition comprising a therapeutically effective amount of one or more compounds of any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, optionally in combination with one or more carriers, diluents, or excipients, or a combination thereof.

27. A composition for treating a cancer in a patient, the composition comprising a therapeutically effective amount of one or more compounds of any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising a

therapeutically effective amount of one or more compounds of any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, optionally further comprising one or more carriers, diluents, or excipients, or a combination thereof.