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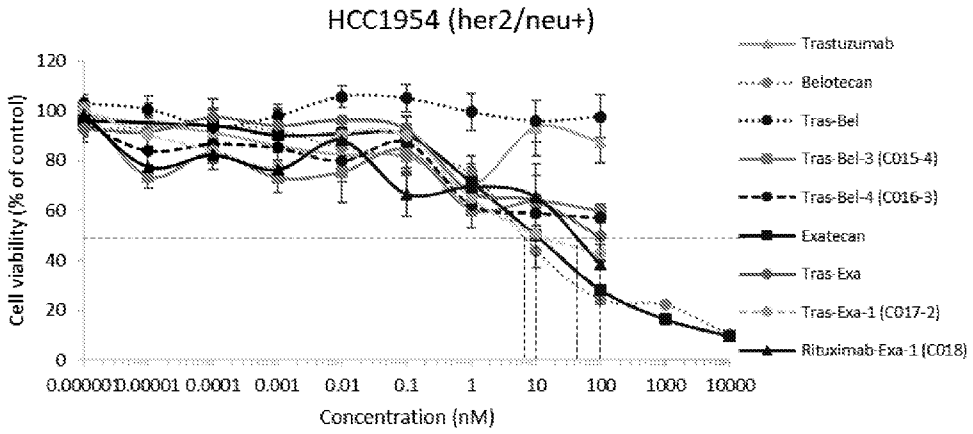

Figure 1A

(57) Abstract: This invention provides prodrug and conjugates thereof, uses, kits and pharmaceutical compositions thereof.

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WATER SOLUBLE PRODRUG, CONJUGATES AND USES THEREOF

FIELD OF THE INVENTION

[001] This invention provides prodrug and conjugates thereof, uses, kits and pharmaceutical compositions thereof.

BACKGROUND OF THE INVENTION

[002] Targeting therapy, with small molecules or biologics has gained more attention in the last decade. One special class of targeting therapy are ADCs (Antibody Drug conjugates). Antibody drug conjugates (ADCs) present a unique opportunity to increase the safety of highly toxic drugs by utilizing the specificity of antibodies to obtain targeted delivery of potent drugs to specific tissues. Much of the success of ADCs is due to technological advances made in the design of the linkage between the antibody and the therapeutic payload.

For ADCs to be selective and potent, the linker technology employed should strive towards three key properties:

(1) High stability in circulation.

(2) High water solubility to aid bioconjugation and avoid formation of inactive ADC aggregates.

(3) Efficient release of a highly cytotoxic payload-linker metabolite.

[003] Although cleavable linkers are generally preferred to non-cleavable linkers due to their range of applicability, there is a greater potential for instability in circulation. The success of cleavable linkers therefore depends on their ability to effectively differentiate between circulatory and target-cell conditions. The poor tumor penetration of large IgG antibodies, combined with any internalization, intracellular trafficking and drug release inefficiencies, leads to the requirement of robust cleavable linker methodologies that maximize delivery of the potent cytotoxins to cancer cells.

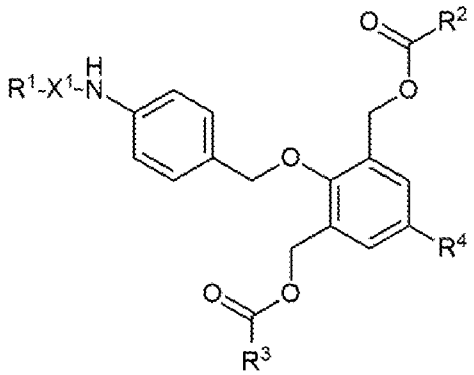
[004] The stability of ADC linkers are not only defined by the cleavable linker technology but also the attachment site on the antibody. Conjugation to a large antibody renders linkers less accessible to chemical and enzymatic triggers, thereby increasing plasma stability and slowing target cell release rates.

[005] This effect can be further amplified when site-selective bioconjugation techniques are employed to attach the linker-payload to less solvent-accessible sites.

[006] Although a lot of work was taken around the conjugation technology as well as the cleavage mechanism, the addition of more than one drug per cleavage site is not well explored. Taking into account that low potent drugs need a higher DAR (Drug Antibody Ratio), the identification of such a module would be beneficial, as it might allow the release of more drugs per cleavage step.

SUMMARY OF THE INVENTION

[007] In one embodiment, this invention provides a compound represented by Formula (I):



(I)

wherein

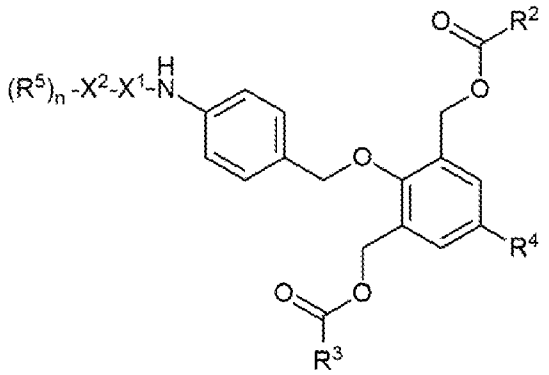
R^1 is a reactive moiety;

R^2 and R^3 are the same or different active pharmaceutical ingredient moiety;

R^4 is a hydrophilic moiety; and

X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety, an oligo or polyethylene glycol or an oligo or polyvinylalcohol or oligo- or poly-glycerol moiety.

[008] In one further embodiment, this invention provides a Conjugate represented by Formula (I(a)):



(I(a))

wherein

R^2 and R^3 are the same or different active pharmaceutical ingredient moiety;

R^4 is a hydrophilic moiety;

R^5 is an antibody or antigen moiety;

X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety, an oligo or polyethylene glycol or an oligo or polyvinylalcohol or oligo- or poly-glycerol moiety;

X^2 is a linker; and

n is a number between 0.01 – 10.

[009] In one further embodiment, this invention provides the compound or the conjugate as described hereinabove, for use in treating cancer

[0010] In one further embodiment, this invention provides a kit comprising:

i) the compound as described hereinabove; and

ii) an antibody or an antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0012] **Figures 1A-1D** depict cytotoxic effects of the conjugates of this invention on various cell viability assays (exposure time: 5 days). **Figure 1A**: HCC1954 cell viability

following 120 h (5 days) treatment with serial dilutions of the conjugates; **Figure 1B:** MDA-MB-231 cell viability following 120 h (5 days) treatment with serial dilutions of the conjugates; **Figure 1C**: Table summarizing the IC_{50} values of the different treatments in HCC1954 cells; and **Figure 1D:** Table summarizing the IC_{50} values of the different treatments in MDA-MB-231 cells. In Figures 1A-1D, data represent mean ±SD; and is representative of 2 individual experiments.

[0013] **Figures 2A-2D** depict cytotoxic effects of the conjugates of this invention on various cell viability assays (exposure time: 6 days). **Figure 2A:** Viability of HCC1954; **Figure 2B:** Viability of JIMT-1; and **Figure 2C:** Viability of MDA-MB-468 cells, following 144 h (6 days) treatment with serial dilutions of Trastuzumab, Exatecan or Tras-Exa-1; and **Figure 2D:** Table summarizing the IC_{50} values of the different treatments, where first two lines are IC50 values, "Tras-Exa/Exa fold change" is the division of Tras-Exa divided by exa values and "-", "+" and "++" denote her2 levels as found in the literature – not detected, low level and high level, respectively (relative levels, as in e.g. Yang, L.; Li, Y.; Bhattacharya, A.; Zhang, Y. S. A recombinant human protein targeting HER2 overcomes drug resistance in HER2-positive breast cancer. Sci. Transl. Med. **2019**, 11, 11). In Figures 2A-2D, data represent mean ±SD; and is representative of 3 individual experiments.

[0014] **Figures 3A-3D** show that Trastuzumab-based ADCs lead to a dose-dependent inhibition of tumor growth. **Figures 3A-3C:** Growth curves of HCC1954 tumors following single intravenous injection of Trastuzumab-based ADCs at three concentrations (n=9 mice/group): 10 mg/kg (**3A**); 3 mg/kg (**3B**) and 1 mg/kg (**3C**). **Figure 3D:** Body weight change, expressed as percent change from the day of tumor cell inoculation. Data are presented as mean ± s.e.m.

[0015] **Figures 4A-4D** show that Trastuzumab-based ADCs prolong the survival of mice in a dose-dependent manner. **Figures 4A-4C**: Kaplan–Meier overall survival curves of HCC1954 tumor-bearing SCID mice treated intravenously with a single injection of Trastuzumab-based ADCs at three concentrations (n=9 mice/group): 10 mg/kg (**4A**); 3 mg/kg (**4B**) and 1 mg/kg (**4C**). **Figure 4D:** Median survival and individual time-point to death. Dashed line marks the pre-defined end point of the study (day 152).

[0016] It will be appreciated that for simplicity and clarity of illustration, elements shown in the figures have not necessarily been drawn to scale. For example, the dimensions

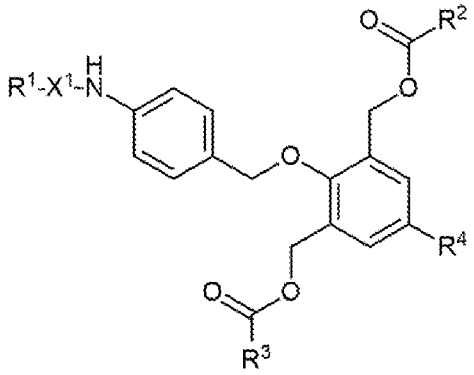
of some of the elements may be exaggerated relative to other elements for clarity. Further, where considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements.

DETAILED DESCRIPTION OF THIS INVENTION

[0017] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that this invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure this invention.

Prodrug

[0018] In one embodiment, this invention provides a compound represented by Formula (I):



(I)

wherein

R^1 is a reactive moiety;

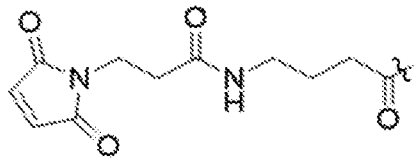
R^2 and R^3 are the same or different active pharmaceutical ingredient moiety;

R^4 is a hydrophilic moiety; and

X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety, an oligo- or poly-ethylene glycol or an oligo- or poly-vinylalcohol or oligo- or poly-glycerol moiety.

.

[0019] In one embodiment, R^1 is a reactive moiety. In another embodiment, a "reactive moiety" in the context of this invention is defined as a chemical moiety (e.g. alkylene group) which may contain functional group or groups (identical or different), wherein at least one such functional group is able to react or interact with an antibody or an antigen in order to conjugate such moiety and the antibody or antigen. In some other embodiments, a thiol of Cys or an amine of Lys from the antibody or an antigen reacts with this moiety in a 1,4 (Michael) addition. In another embodiment, R^1 comprises dihydropyridazine maleimide, bromoacetamide, tetrazine, alkyne or amine moiety or any combination thereof. In another embodiment, R^1 comprises an alkyne moiety. In another embodiment, R^1 comprises a constrained alkyne moiety. In another embodiment, R^1 comprises a cycloalkyne moiety. In another embodiment, non-limiting examples of cyclooctyne moieties include dibenzocyclooctyne (DBCO), bicyclo[6.1.0]nonyne (BCN) and biarylazacyclooctynone (BARAC) moieties. In another embodiment, R^1 is represented by: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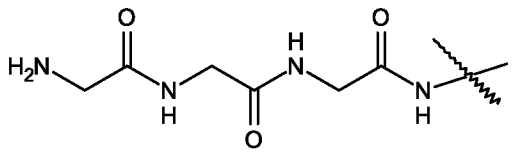
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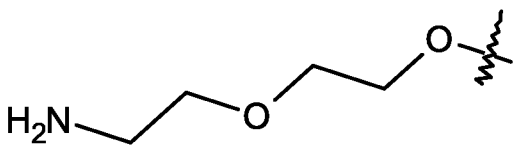
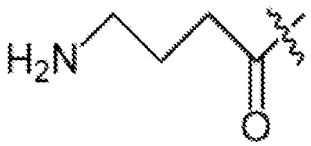
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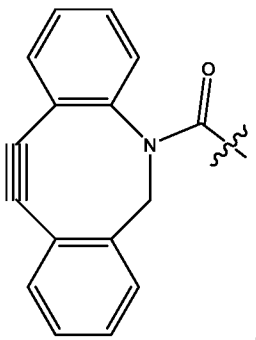
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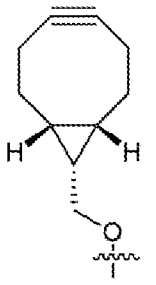
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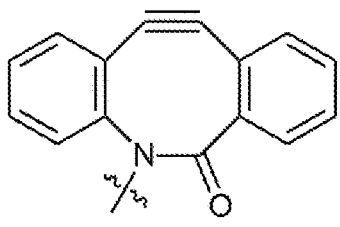
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[0020] In one embodiment, R^2 of Formula (I) is an anti-cancer, immune stimulating (for immune-oncology) or immune dampening (e.g. steroids for immunology) agent. In one further embodiment, R^2 is an agent that is used in immunology, immune-oncology, in

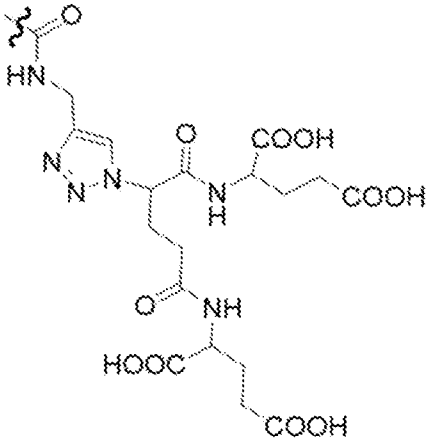
treating cancer-related disease and/or disorder or in treating deficiencies/malfunctions of the immune system. In other embodiments, the conditions/disorders related/associated with cancer and/or deficiencies/malfunctions of the immune system, include at least one of the following non limiting examples: auto-immune diseases, arthritis, ectopic dermatitis, Crohn's disease, colitis, IBD, fibrosis, psoriasis, Lupus, Multiple Sclerosis, neuro-degenerative disease (Parkinson, Alzheimer, ALS, myasthenia gravis, Graves' disease, Ankylosing spondylitis), and respiratory disease (COPD, Asthma). In another embodiment, R^2 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl auristatin E (MMAE) or a doxorubicin moiety. In another embodiment, R^2 is exatecan, auristatin or belotecan.

[0021] In one embodiment, R^3 of Formula (I) is an anti-cancer, immune stimulating (for immune-oncology) or immune dampening (e.g. steroids for immunology) agent. In one further embodiment, R^3 is an agent that is used in immunology, immune-oncology, in treating cancer-related disease and/or disorder or in treating deficiencies/malfunctions of the immune system. In other embodiments, the conditions/disorders related/associated with cancer and/or deficiencies/malfunctions of the immune system, include at least one of the following non limiting examples: auto-immune diseases, arthritis, ectopic dermatitis, Crohn's disease, colitis, IBD, fibrosis, psoriasis, Lupus, Multiple Sclerosis, neuro-degenerative disease (Parkinson, Alzheimer, ALS, myasthenia gravis, Graves' disease, Ankylosing spondylitis), and respiratory disease (COPD, Asthma). In another embodiment, R^3 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl auristatin E (MMAE) or a doxorubicin moiety. In another embodiment, R^3 is exatecan, auristatin, Monomethyl auristatin E (MMAE) or belotecan.

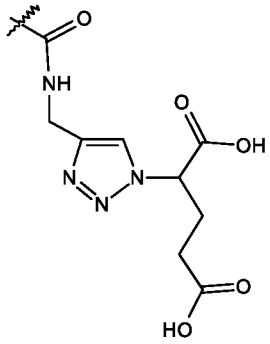
[0022] In one embodiment, R^2 and R^3 of Formula (I) are anticancer, immune stimulating (for immune-oncology) or immune dampening agents (e.g. steroids), or any agents used in immunology, immune-oncology, in treating cancer-related disease and/or disorder or in treating deficiencies/malfunctions of the immune system, where said conditions/disorders related/associated with cancer and/or deficiencies/malfunctions of the immune system are as described hereinabove (see R^2 and/or R^3 embodiments). In another embodiment, R^2 and R^3 are each independently exatecan, belotecan, auristatin, Monomethyl auristatin E (MMAE) camptothecin or a doxorubicin moiety. In another embodiment, R^2 and R^3 are each independently exatecan, auristatin, Monomethyl auristatin E (MMAE) or belotecan

moieties. In another embodiment, R^2 and R^3 are each independently exatecan moieties. In another embodiment, R^2 and R^3 are belotecan moieties.

[0023] In one embodiment, R^4 of Formula (I) comprises an oligo- or poly- carboxylic acid moiety or oligo- or poly-ethylene glycol or oligo- or poly-alcohol or oligo- or poly-vinylalcohol or oligo- or poly-glycerol moiety. In another embodiment, R^4 is represented by:



or



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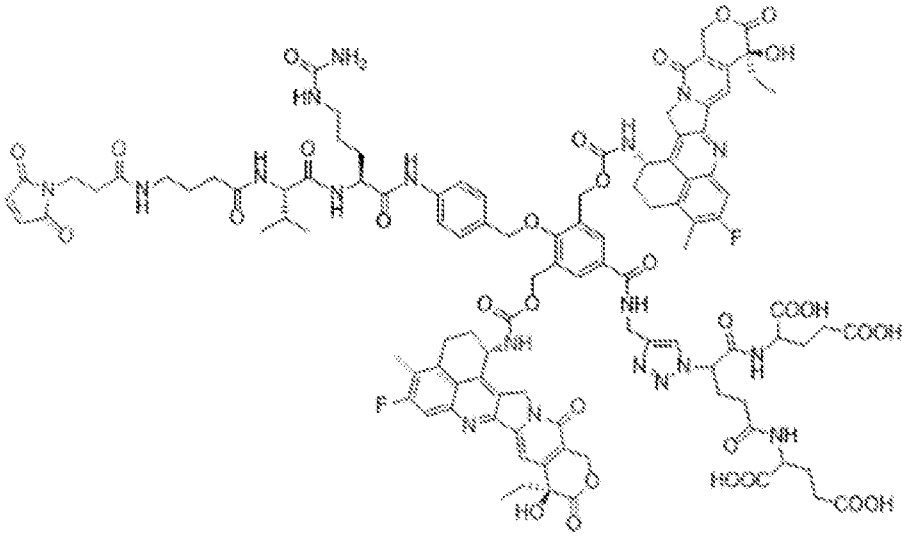
In one embodiment, X^1 of Formula (I) is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety or an oligo- or poly-ethylene glycol or an oligo- or poly-vinylalcohol or oligo- or poly-glycerol moiety. In another embodiment, X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide comprising any amino acid: natural, non-natural (artificial), standard or non—standard, or combination thereof, in any order/sequence. In one other embodiment, X^1 is CGKRK, -Val-Cit-, -Ala-Ala-, AAN, GGFG, -Val-Arg- or -Val-Ala- or any combination thereof. In one other embodiment, X^1 is -Val-Cit-, -Ala-Ala-, AAN, GGFG, -Val-Arg- or -Val-Ala- or any combination thereof. In another embodiment, X^1 comprises a sequence (e.g. Ala-Ala) cleavable by specific proteases, non-limiting examples include Cathepsin B and Legumain. Each possibility represents a separate embodiment of this invention.

[0024] In one embodiment, the compound of Formula (I) is represented by the structure of Formula 1,17, 18, 19 or 20:



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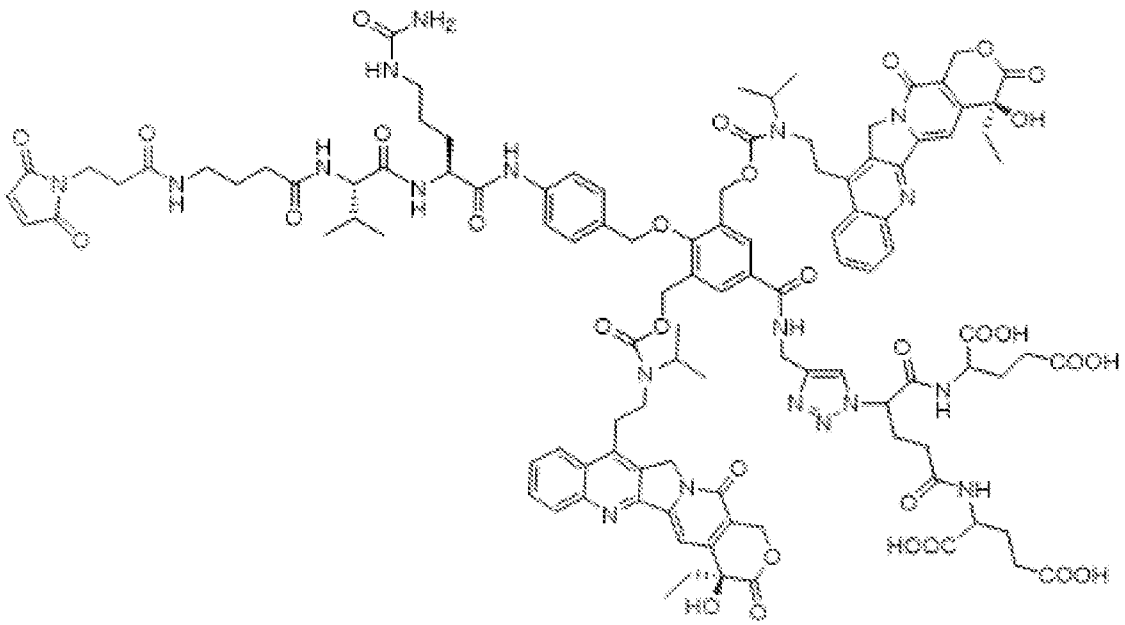
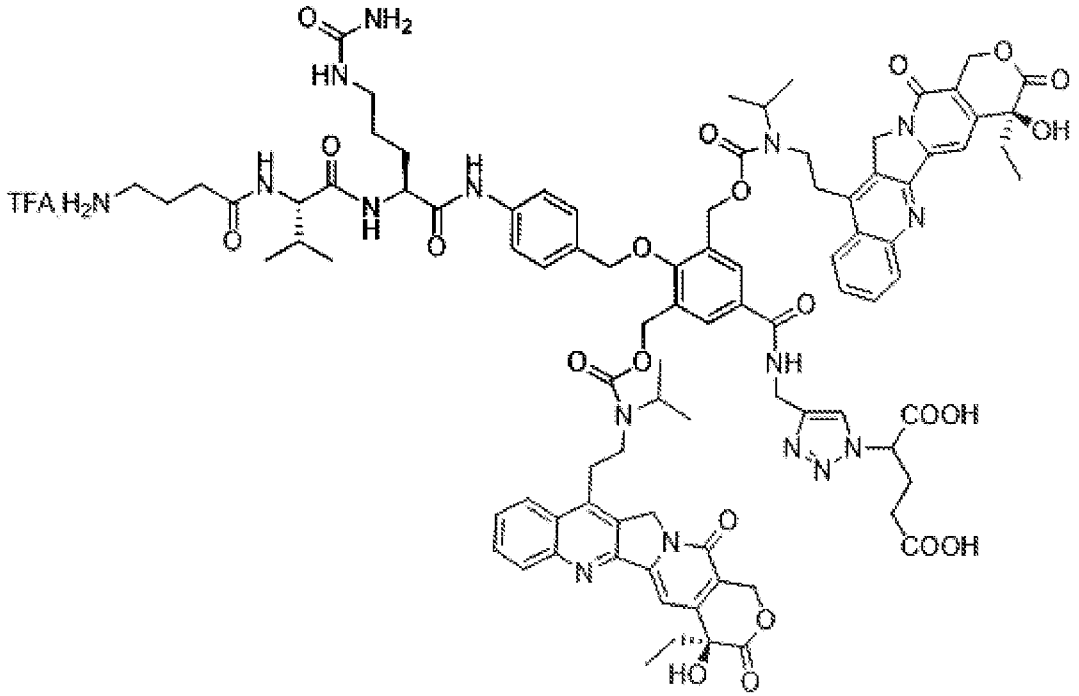
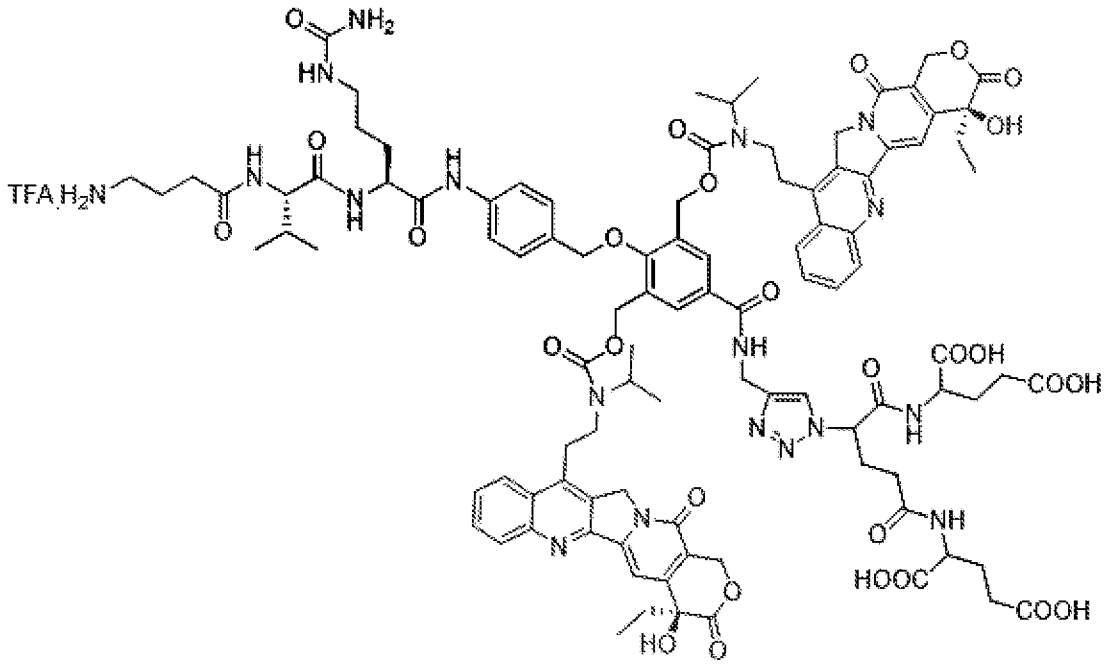


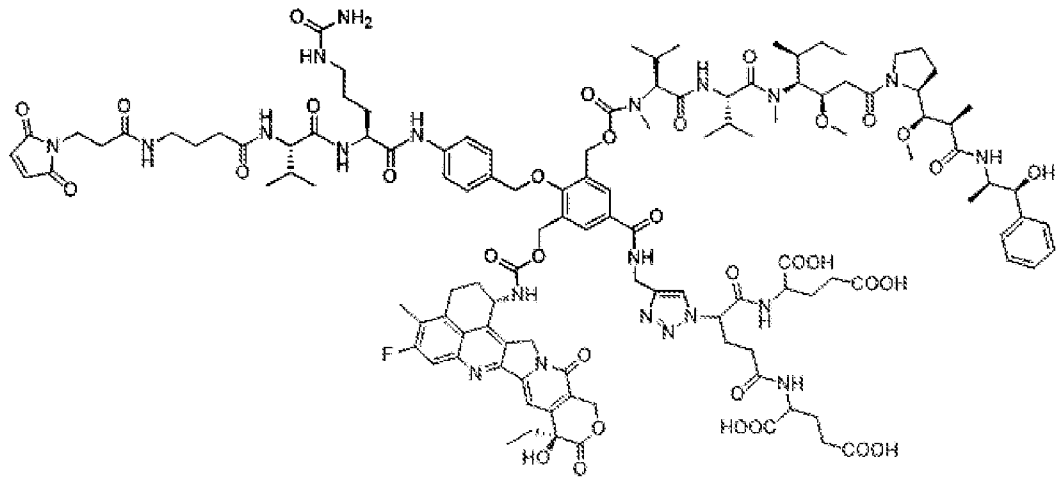
17;



18;



19; or



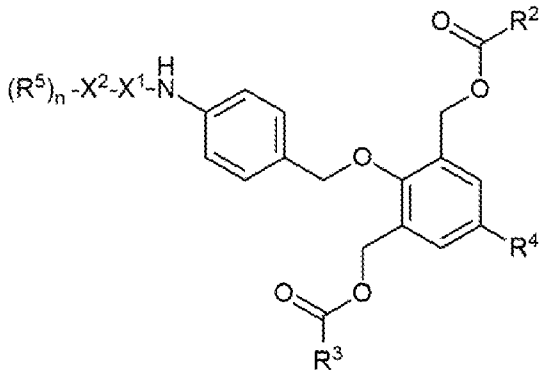
20.

wherein TFA is trifluoroacetic acid.

[0025] In some embodiments, the compound of Formula (I) is a prodrug which is conjugated to an antibody or an antigen via R^1 which reacts and/or interacts with the relevant moieties/portions/functional groups/amino acids of the antibody or antigen, wherein the product of said reaction is the conjugate presented herein below in the following section, e.g. by Formula (I(a), 1(a), 2(a) and/or compound 21. In one specific embodiment, lysine or cysteine of the antibody or antigen reacts via Michael addition with R^1 which is a maleimide moiety. In one further embodiment, R^1 reacts with artificial amino-acid such as azido-tyrosine or with alkynes to form said conjugate. In another embodiment, the antibody/antigen reacts with the compound of Formula (I), and then it interacts with some portion of said compound (e.g. some peptide recognition motif/trigger), an interaction that might trigger the compound to release the covalently-loaded drugs. In one embodiment, the antibody is trastuzumab or rituximab.

Conjugate of the prodrug and an antibody or an antigen

[0026] In one embodiment, this invention provides a conjugate represented by Formula (I(a)):



(I(a))

wherein

R^5 is an antibody or antigen moiety;

X^2 is a linker;

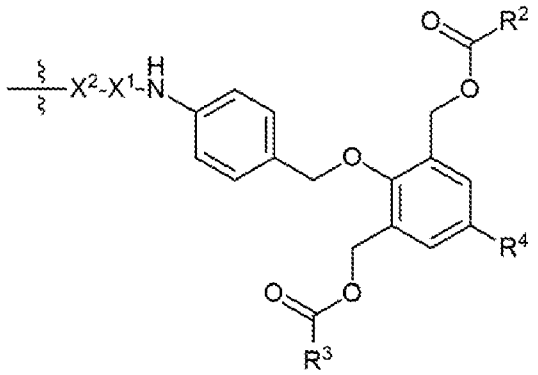
n is a number between 0.01 – 10;

and

R^2-R^4 and X^1 are described hereinabove.

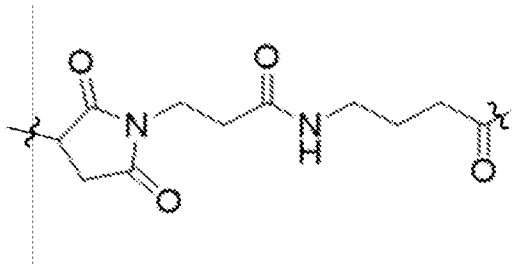
[0027] In one embodiment, this invention provides a conjugate represented by Formula (I(a)), prepared by reacting the prodrug or compound of Formula (I) with an antibody or an antigen, wherein all definitions and structures of the formulae are described hereinabove and/or below.

[0028] In some embodiments, the bond between the antigen/antibody moiety (R^5) and the rest of Formula (I(a)) can be covalent or non-covalent. The term "non covalent bond" refers within the meaning of this invention to any non-covalent interaction/bond, such as van der waals/ interaction/London dispersion forces, hydrogen bonding, halogen bonding, pie-pie (π–π) interactions, ionic bonding (e.g. salt bridge between antibody or antigen of R^5 and the peptide moiety of X^1), etc., as known in the art. In some embodiments, R^5 is connected in more than one position thereof to the rest of Formula (I(a)), in an identical or different interaction/bond at each position. In one specific embodiment, R^5 is connected covalently to one or more compounds of the rest of Formula (I(a)) and also connected/interacts non-covalently with one or more such compounds, at a different position compared to the covalent attachment point. In one embodiment, the "rest of Formula (I(a))" refers to



where the curly bond denotes the bond to R^5 (not presented). Each possibility represents a separate embodiment of this invention.

[0029] In one embodiment, X^2 of Formula (I(a)) comprises succinimide, acetamide, dihydropyridazine, alkene or amine moiety or any combination thereof. In another embodiment, X^2 comprises an alkene moiety. In another embodiment, X^2 comprises a constrained alkene moiety. In another embodiment, X^2 is represented by



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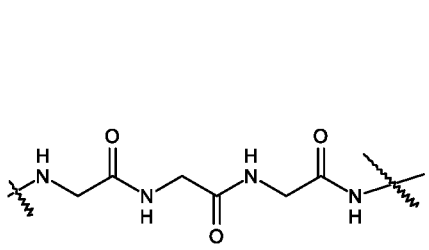

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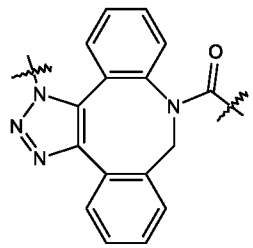

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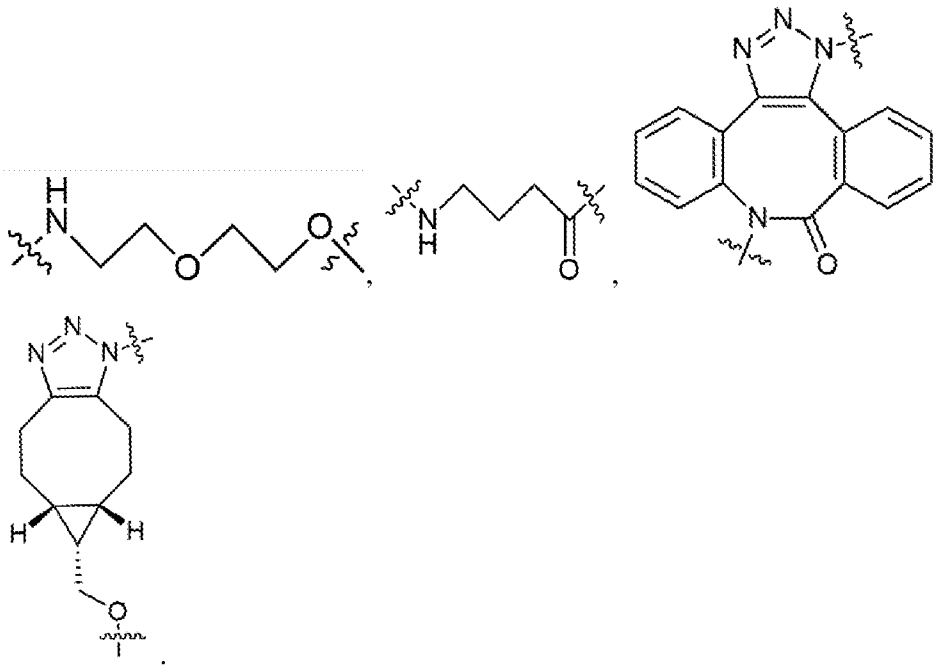
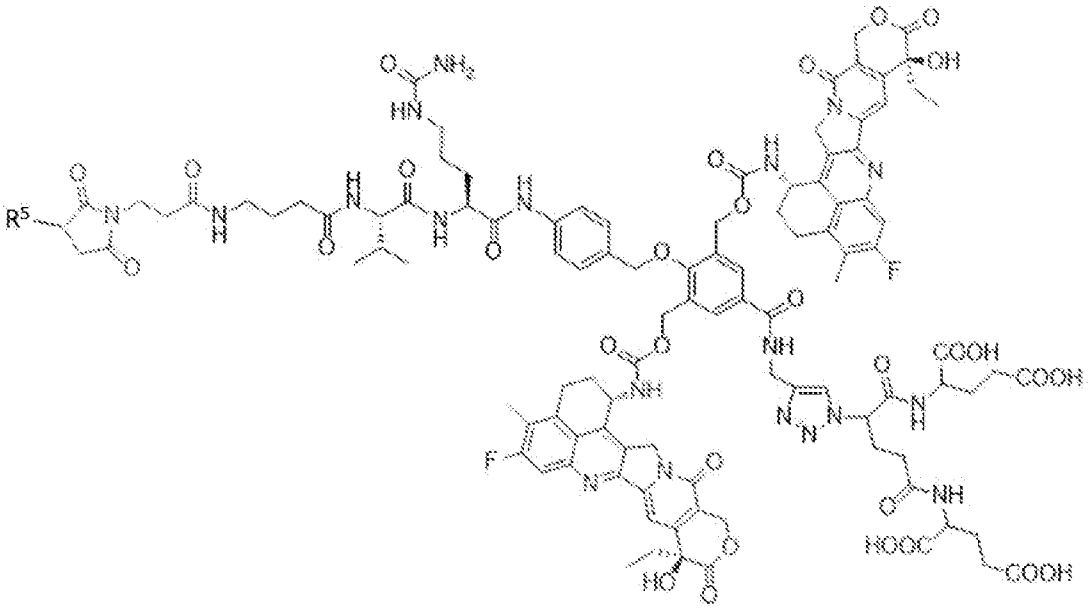


[0030] In some embodiments, n is between 0.01-10. In one embodiment, n is between 0.01-1. In another embodiment, n is between 0.01-2. In another embodiment, n is between 0.01-5. In another embodiment, n is between 0.05-1. In another embodiment, n is between 0.05-2. In another embodiment, n is between 0.1-1. In another embodiment, n is between 0.1-2. In another embodiment, n is between 0.5-1. In another embodiment, n is between 0.01-0.5. In another embodiment, n is between 0.1-0.5. In another embodiment, n is between 0.5-2. Each possibility represents a separate embodiment of this invention.

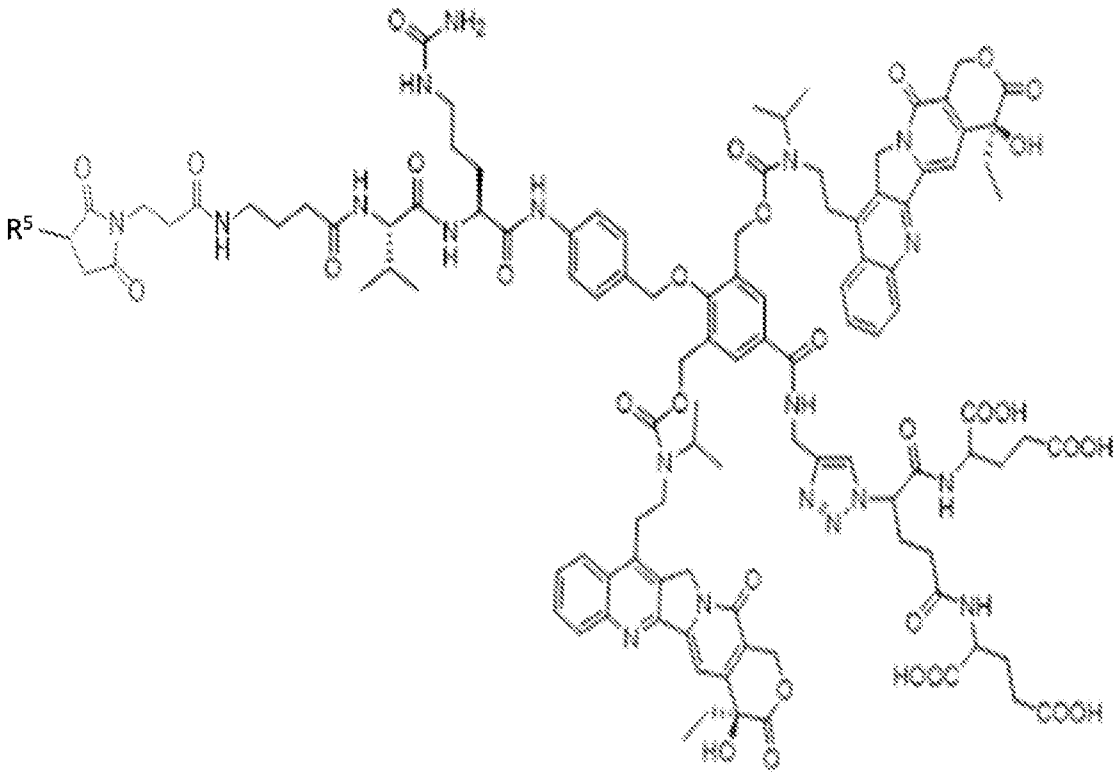
[0031] In one embodiment, R^5 of Formula (I(a)) is an antibody or an antigen. In another embodiment, R^5 is an antibody targeting tumor. In another embodiment, R^5 is an immune cell specific antigen. In other embodiments, R^5 is an anti-EGFR or anti-CD33 antibody. In another embodiment, the antibody of R^5 is a model antibody or a moiety/fragment which "simulates" an antibody, where such model/moiety/fragment is exemplified by, *inter alia*, the CGKRK peptide. In another embodiment, R^5 is any one of the CEACAM family, Folate receptor, ER, PR, Notch receptors, Notch ligands, PSMA, Glypican-3, Mesothelin, MET, EGFR, Erb2, EpCAM, NCAM, EphrinA4, IGF-1R, FAP, Ly6E, Cadherins family, VEGFR-2, RNF43, MUC family, PD-1, PD-L1, FAK, CCR2, CCR4, CXCR1, CXCR2, Nectin-4, Transferrin, TIM-1, LAG-3, Axl, CTLA-4, 4-1BB, MART-1, TIGIT, SLAMF6, OX40, trastuzumab or rituximab. In another embodiment, R^5 is trastuzumab or rituximab

or CGKRK moiety. In another embodiment, R^5 is trastuzumab moiety. In another embodiment, R^5 is rituximab moiety. In another embodiment, R^5 is CGKRK moiety. Each possibility represents a separate embodiment of this invention.

[0032] In one embodiment, the compound of Formula (I(a)) is represented by the structure of Formula 1(a) or 2(a):



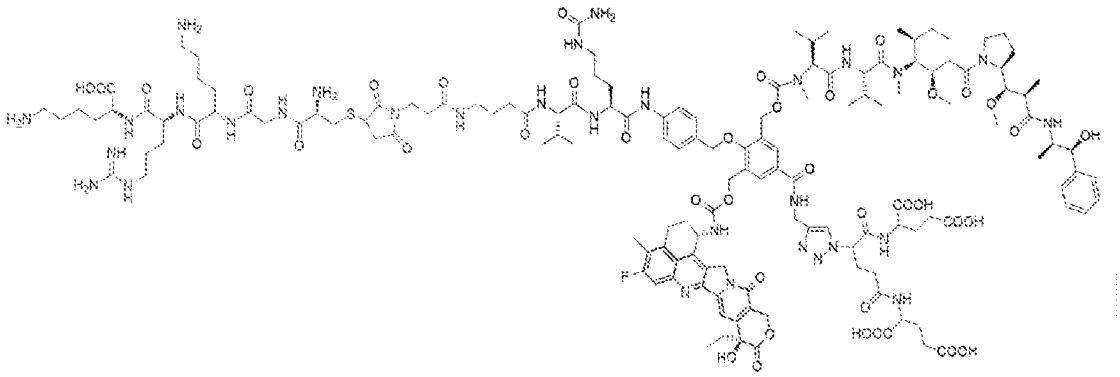
1(a); or



2(a).

Wherein R^5 is as described hereinabove.

[0033] In one embodiment, the compound of Formula (I(a)) is represented by the structure of compound 21:



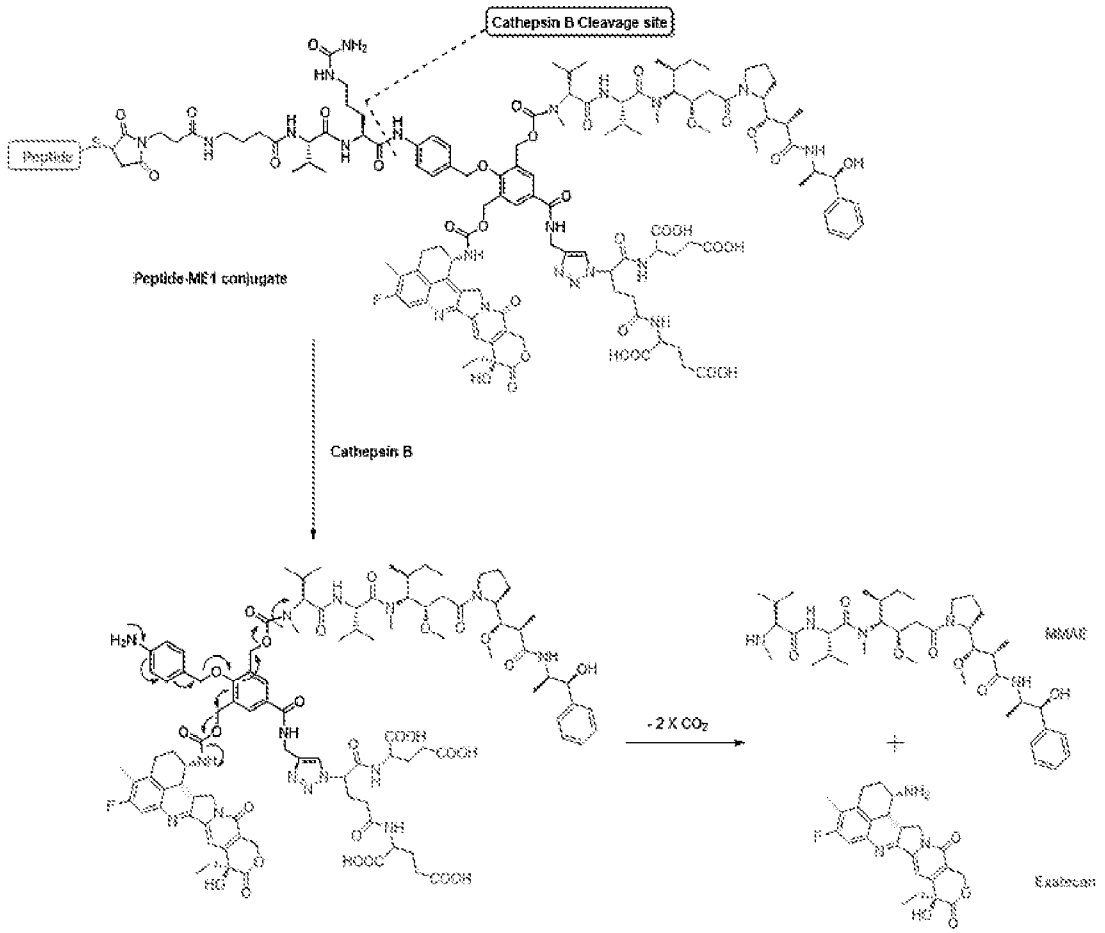
21.

[0034] In some embodiments, the compound represented by the structure of e.g. Formula (I) or 1, 17, 18, 19 or 20 is also termed "prodrug" and it can be conjugated with an antibody or an antigen to form the conjugate. The term "conjugate", in the context of this invention, can be interchangeably termed "antibody-drug conjugate" (ADC).

Proposed mechanism of release

In some embodiments, without being bound by any mechanism or theory, it is contemplated that the drugs are released from the covalent conjugate via reaction with a protease and/or hydrolase and/or other enzyme which leads subsequently to additional cascade of reactions. In one embodiment, an example of said mechanism/cascade is presented in the following below (cathepsin B is the protease presented):

Scheme 1: An example of drugs' release mechanism



Accordingly, the peptide in the above scheme or any other feasible antibody/antigen of R^5 affords targeted delivery of the conjugate to the desired (biological) site, in which it is activated by some trigger, e.g. reaction with a hydrolase/protease as exemplified above.

Pharmaceutical Compositions comprising the prodrug, antibody or antigen and/or the conjugate thereof

[0035] In one embodiment, this invention provides a pharmaceutical composition comprising a therapeutically effective amount of the prodrug or the conjugate thereof as described hereinabove and optionally at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In other embodiment, the composition comprises a combination of the prodrug and an antibody or an antigen, and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In another embodiment, the pharmaceutical composition is in a form selected from the group consisting of tablets (including e.g. film-coated tablets), powders, granules, capsules (including soft capsules), orally disintegrating

tablets, pills, pellets, lozenges, sachets, cachets, patches, elixirs, suspensions, dispersions, emulsions, solutions, syrups, aerosols, ointments, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders, and sustained-release preparations as is well known in the art. In another embodiment, the composition is a solid state composition (e.g. tablet, pill, capsule, pellet, granule, powder etc.). Each possibility represents a separate embodiment of this invention.

[0036] In some embodiment, pharmacologically acceptable carriers, diluents, vehicles or excipients that may be used in the context of this invention include, but are not limited to, surfactants, lubricants, binders, fillers, compression aids, disintegrants, water-soluble polymers, inorganic salts, preservatives, antioxidants, coloring agents, sweetening agents, souring agents, bubbling agents and flavorings. Each possibility represents a separate embodiment of this invention.

[0037] In some embodiments, specific non-limiting examples of suitable carriers, diluents, vehicles or excipients within this invention include e.g. lactose, D-mannitol, starch, cornstarch, crystalline cellulose, light silicic anhydride and titanium oxide. Each possibility represents a separate embodiment of this invention. Suitable surfactants include e.g. lecithin and phosphatidylcholine. Each possibility represents a separate embodiment of this invention. Suitable lubricants include e.g. magnesium stearate, sucrose fatty acid esters, polyethylene glycol, talc and stearic acid. Each possibility represents a separate embodiment of this invention. Suitable binders include e.g. hydroxypropyl cellulose, hydroxypropylmethyl cellulose, crystalline cellulose, a-starch, polyvinylpyrrolidone, gum arabic powder, gelatin, pullulan and low-substitutional hydroxypropyl cellulose. Each possibility represents a separate embodiment of this invention. Suitable disintegrants include e.g. crosslinked povidone (any crosslinked 1-ethenyl-2-pyrrolidinone homopolymer including polyvinylpyrrolidone (PVPP) and 1-vinyl-2-pyrrolidinone homopolymer), crosslinked carmellose sodium, carmellose calcium, carboxymethyl starch sodium, low-substituted hydroxypropyl cellulose, cornstarch and the like. Each possibility represents a separate embodiment of this invention. Suitable water-soluble polymers include e.g. cellulose derivatives such as hydroxypropyl cellulose, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, methyl cellulose and carboxymethyl cellulose sodium, sodium polyacrylate, polyvinyl alcohol, sodium alginate, guar gum, and the like. Each possibility represents a separate embodiment of this invention. Suitable inorganic salts

include e.g. basic inorganic salts of sodium, potassium, magnesium and/or calcium. Each possibility represents a separate embodiment of this invention. Particular embodiments include the basic inorganic salts of magnesium and/or calcium. Basic inorganic salts of sodium include, for example, sodium carbonate, sodium hydrogen carbonate, disodiumhydrogenphosphate, and the like. Each possibility represents a separate embodiment of this invention. Basic inorganic salts of potassium include, for example, potassium carbonate, potassium hydrogen carbonate, and the like. Each possibility represents a separate embodiment of this invention. Basic inorganic salts of magnesium include, for example, heavy magnesium carbonate, magnesium carbonate, magnesium oxide, magnesium hydroxide, magnesium metasilicate aluminate, magnesium silicate, magnesium aluminate, synthetic hydrotalcite, aluminahydroxidemagnesium, and the like. Each possibility represents a separate embodiment of this invention. Basic inorganic salts of calcium include, for example, precipitated calcium carbonate, calcium hydroxide, and the like. Each possibility represents a separate embodiment of this invention.

[0038] Suitable preservatives include e.g. sodium benzoate, benzoic acid, and sorbic acid. Each possibility represents a separate embodiment of this invention. Suitable antioxidants include e.g. sulfites, ascorbic acid and α-tocopherol. Each possibility represents a separate embodiment of this invention. Suitable coloring agents include e.g. food colors such as Food Color Yellow No. 5, Food Color Red No. 2 and Food Color Blue No. 2, and the like. Each possibility represents a separate embodiment of this invention. Suitable sweetening agents include e.g. dipotassium glycyrrhetinate, aspartame, stevia and thaumatin. Each possibility represents a separate embodiment of this invention. Suitable souring agents include e.g. citric acid (citric anhydride), tartaric acid and malic acid. Each possibility represents a separate embodiment of this invention. Suitable bubbling agents include e.g. sodium bicarbonate. Suitable flavorings include synthetic substances or naturally occurring substances, including e.g. lemon, lime, orange, menthol and strawberry. Each possibility represents a separate embodiment of this invention.

[0039] In one embodiment, the compounds (prodrug and/or conjugate thereof) of this invention are useful as pharmaceuticals for medical treatment. This invention thus provides pharmaceutical compositions comprising the prodrug or the conjugate thereof disclosed herein, or a combination of the prodrug and an antibody or an antigen, and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. The compounds of this

invention can be safely administered orally or non-orally. Routes of administration include, but are not limited to, oral, topical, subcutaneous, intraperitoneal, rectal, intravenous, intra-arterial, transdermal, intramuscular, topical, and intranasal. Each possibility represents a separate embodiment of this invention. Additional routes of administration include, but are not limited to, mucosal, nasal, parenteral, gastrointestinal, intraspinal, intrauterine, intraocular, intradermal, intracranial, intratracheal, intravaginal, intracerebroventricular, intracerebral, ophthalmic, buccal, epidural and sublingual. Each possibility represents a separate embodiment of this invention.

[0040] In another embodiment, the tablets and other solid dosage forms of the pharmaceutical compositions described herein may optionally be stored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices and the like. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients. Each possibility represents a separate embodiment of this invention.

Uses of the prodrug and conjugate

[0041] In one embodiment, this invention provides a method of treating cancer or related disorders/conditions, comprising the step of administering to a subject in need thereof the prodrug (e.g. compound of Formula (I)), conjugate thereof (e.g. compound of Formula (I(a))) or a combination of the prodrug and an antibody or an antigen or a pharmaceutical composition comprising the prodrug/conjugate/combination and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In another embodiment, this invention provides a method of treating cancer or related disorders/conditions, comprising the step of administering to a subject in need thereof the conjugate (e.g. compound of Formula (I(a))) or a combination of the prodrug (e.g. compound of Formula I) and an antibody or an antigen or a pharmaceutical composition comprising the conjugate/combination and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In other embodiment, the method is an immunological method. In another

embodiment, the method is an immune-oncological method. Each possibility represents a separate embodiment of this invention.

[0042] In one embodiment, this invention provides a method of treating conditions and/or diseases associated with deficiencies/malfunctions of the immune system, comprising the step of administering to a subject in need thereof the prodrug (e.g. compound of Formula (I), conjugate thereof (e.g. compound of Formula (I(a)) or a combination of the prodrug and an antibody or an antigen or a pharmaceutical composition comprising the prodrug/conjugate/combination and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In another embodiment, this invention provides a method of treating cancer or related disorders/conditions, comprising the step of administering to a subject in need thereof the conjugate (e.g. compound of Formula (I(a))) or a combination of the prodrug (e.g. compound of Formula I) and an antibody or an antigen or a pharmaceutical composition comprising the conjugate/combination and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient. In other embodiment, the method is an immunological method. In another embodiment, the method is an immune-oncological method. Each possibility represents a separate embodiment of this invention.

[0043] In other embodiments, the conditions/disorders related/associated with cancer and/or deficiencies/malfunctions of the immune system, include at least one of the following non limiting examples: auto-immune diseases, arthritis, ectopic dermatitis, Crohn's disease, colitis, IBD, fibrosis, psoriasis, Lupus, Multiple Sclerosis, neuro-degenerative disease (Parkinson, Alzheimer, ALS, myasthenia gravis, Graves' disease, Ankylosing spondylitis), and respiratory disease (COPD, Asthma). In one embodiment, this invention provides a method of treating cancer, comprising administering:

a) the prodrug (e.g. compound of Formula (I); and

b) an antibody or an antigen; or

administering the conjugate of the prodrug and the antibody or antigen, to a subject in need thereof.

[0044] In some embodiments, the cancer is a breast, kidney, brain, skin, ovary, lung, pancreas, colon, head/neck, prostate, endometrium, liver, blood, stomach, fibrosarcoma, bone neoplasms/osteosarcoma or duodenum cancer. In one embodiment, the cancer is a breast cancer. In another embodiment, the administration of the prodrug (e.g. compound of Formula (I) and the antibody or antigen is subsequent or in conjunction. Each possibility represents a separate embodiment of this invention.

[0045] In some embodiments, the subject is a mammal, preferably a human.

[0046] In some embodiments, this invention provides the prodrug (e.g. compound of Formula (I), conjugate thereof (e.g. compound of Formula (I(a)) or a combination of the prodrug and an antibody or an antigen or a pharmaceutical composition comprising the prodrug/conjugate/combination and at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient - for use in the treatment of cancer or the conditions/disorders related/associated with cancer and/or of deficiencies/malfunctions of the immune system, as described within the above method embodiments.

[0047] In one embodiment, this invention provides a kit comprising:

i) the prodrug as described hereinabove (e.g. compound of Formula (I); and

ii) an antibody or an antigen.

In other embodiment, the kit further comprises instructions for use.

Definitions

[0048] It is herein defined that the term "therapeutically effective amount" as used herein refers to an amount of an agent which is effective, upon single or multiple dose administration to the subject in providing a therapeutic benefit to the subject. In additional embodiments, the crystalline forms of the present invention are used for the preparation of a medicament for treating the aforementioned diseases or disorders.

[0049] It is herein defined that the term “treating”, in regards to medical treatment, as used herein - refers to medical care of a medical condition, disease or disorder following its appearance/identification in a subject, or alleviation of the same or inhibition or prevention of the same.

[0050] It is herein defined that the term “DAR” or “Drug-Antibody Ratio” equals the number of drug equivalents per one equivalent of antibody or antigen. In one embodiment, DAR is between 0.5 and 200. In another embodiment, DAR is between 0.5 and 50. In another embodiment, DAR is between 0.5 and 20. In another embodiment, DAR is between 0.5 and 10. In another embodiment, DAR is between 0.5 and 5. In another embodiment, DAR is between 1 and 10. In another embodiment, DAR is between 1 and 20. In another embodiment, DAR is between 1 and 50. In another embodiment, DAR is between 1 and 100. In another embodiment, DAR is between 1 and 200. In another embodiment, DAR is

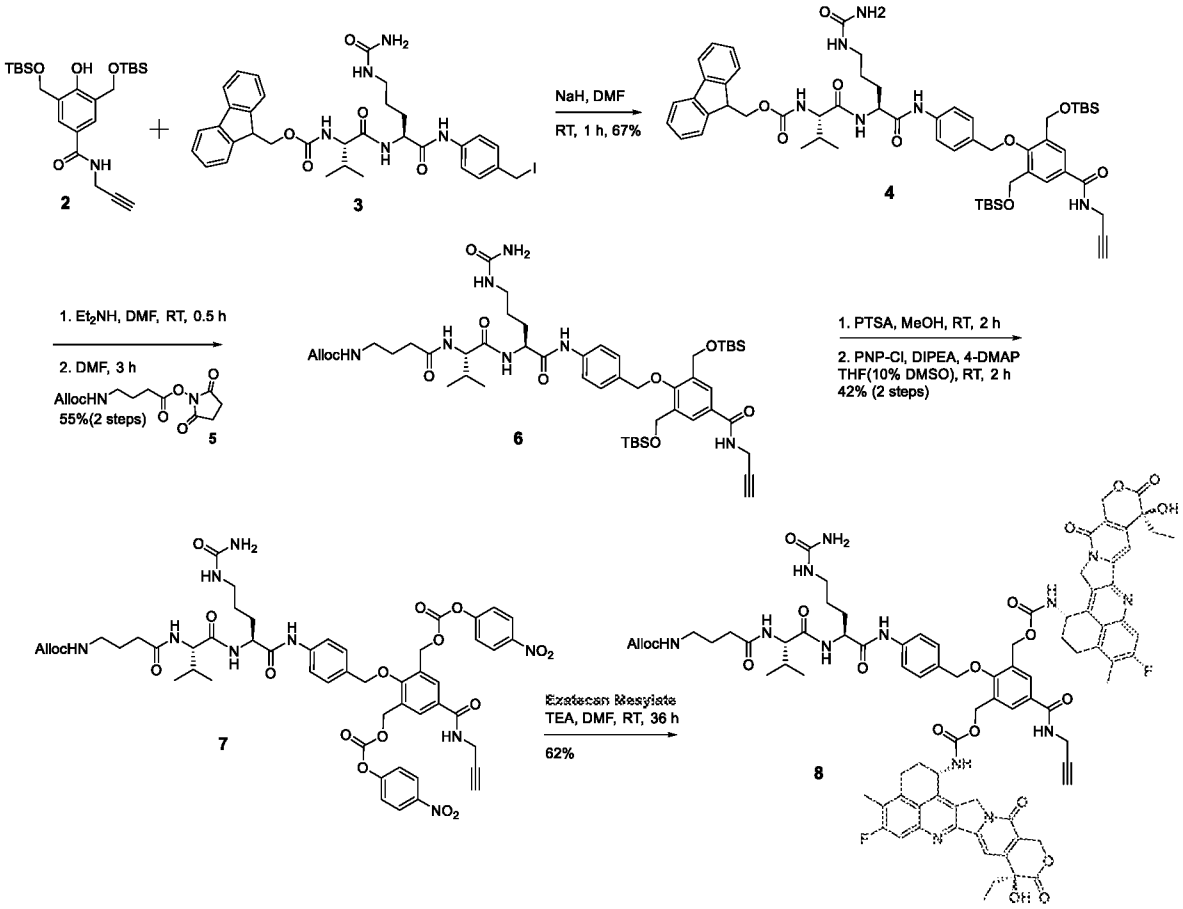
between 5 and 10. In another embodiment, DAR is between 5 and 20. In another embodiment, DAR is between 5 and 50. In another embodiment, DAR is between 10 and 20. In another embodiment, DAR is between 20 and 50. In another embodiment, DAR is between 50 and 100. In another embodiment, DAR is between 50 and 200. In another embodiment, DAR is between 100 and 200. In some embodiments, DAR is explicitly mentioned (e.g. Table 1 within Example 4). In other embodiments, the DAR can be calculated via "n" within the meaning of Formula (I(a)). Few examples for said calculation are given below, where it is known that for the compounds/conjugates of this invention, i.e. Formula (I(a)), per each compound linked to R^5 - there are 2 (two) different or same drug/API moieties R^2 and R^3:

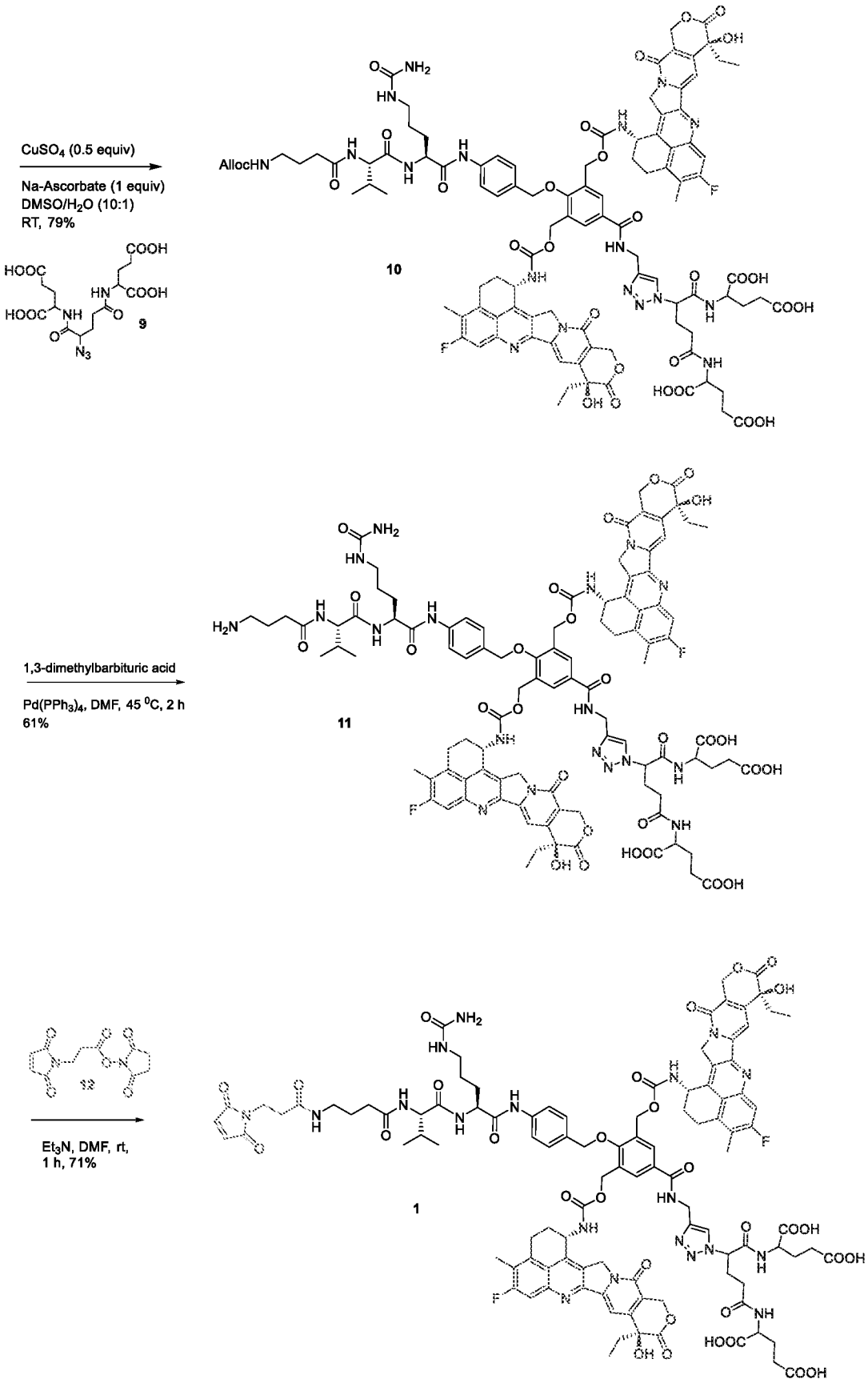
- n=1, thus 2 drug moieties in 1 compound attached to R^5, give DAR of 2/1 or 2.
- n=0.5, thus 2 drug moieties in 1 compound attached to R^5, give DAR of 2/0.5 or 4.
- n=0.33, thus 2 drug moieties in 1 compound attached to R^5, give DAR of 2/0.33 or 6.

Examples

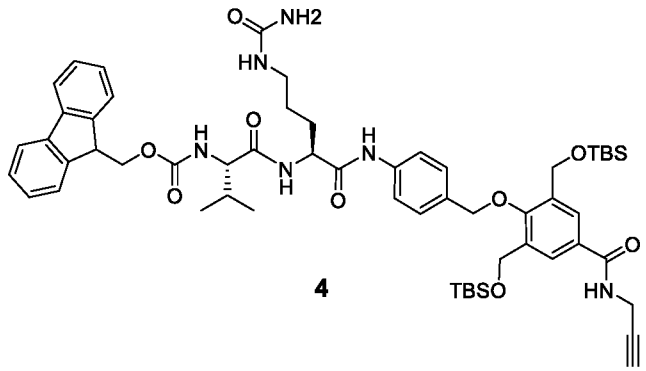
Example 1: Synthesis of compound 1

Scheme 2: overall synthesis of compound 1


TBSO OH OTBS
NH
2
3
NaH, DMF
RT, 1 h, 67%
4
1. Et2NH, DMF, RT, 0.5 h
2. DMF, 3 h
AllocHN
55%(2 steps)
5
6
1. PTSA, MeOH, RT, 2 h
2. PNP-Cl, DIPEA, 4-DMAP
THF(10% DMSO), RT, 2 h
42% (2 steps)
7
Exatecan Mesylate
TEA, DMF, RT, 36 h
62%
8

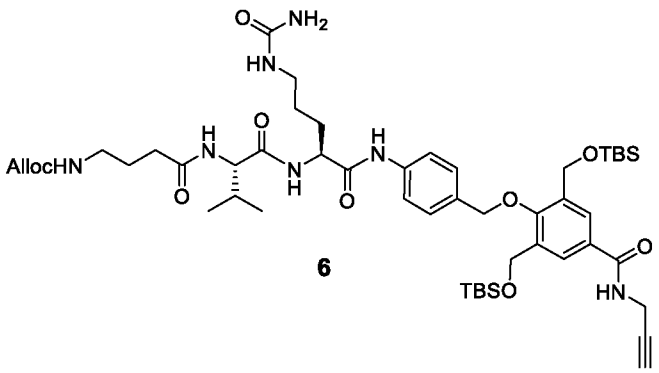

CuSO4 (0.5 equiv)
Na-Ascorbate (1 equiv)
DMSO/H2O (10:1)
RT, 79%
9
10
1,3-dimethylbarbituric acid
Pd(PPh3)4, DMF, 45 °C, 2 h
61%
11
12
Et3N, DMF, rt,
1 h, 71%
1

Experimental Procedure



4

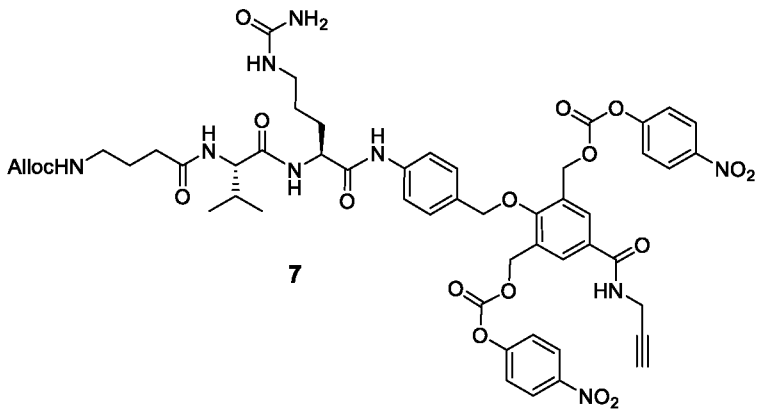
Compound 4: Compound **2** (see E. Danieli, D. Shabat, *Bioorg. Med. Chem.* **2007**, *15*, 7318–7324; R. J. Amir, E. Danieli, D. Shabat, *Chem. – A Eur. J.* **2007**, *13*, 812–821; and A. Gopin, S. Ebner, B. Attali, D. Shabat, *Bioconjug. Chem.* **2006**, *17*, 1432–1440) (1.07 g, 2.32 mmol) was dissolved in dry DMF (5 mL) under argon atmosphere and cooled to 0 °C. Sodium hydride (97 mg, 2.44 mmol) was added and the reaction was allowed to warm to room temperature. After stirring for 15 minutes at room temperature, compound **3** (M. E. Roth-Konforti, C. R. Bauer, D. Shabat, *Angew. Chemie Int. Ed.* **2017**, *56*, 15633–15638) (1.65 g, 2.32 mmol) was added and the reaction was monitored by TLC (MeOH:EtOAc 5:95). Upon completion, the reaction was diluted with EtOAc (20 mL) and NH_4Cl (10 mL). The biphasic mixture was then extracted with EtOAc (3 x 20 mL), washed with brine (20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel to afford the titled compound **4** as an off-white solid (1.61g, 67%). MS (ES+) *m/z* calculated for $C_{57}H_{78}N_6O_9Si_2$: 1046.5, found 1048.9 for $[M+2H]^+$.



6

Compound 6: Compound **4** (1.2 g, 1.15 mmol) was dissolved in DMF (6 ml) and diethylamine (2 ml) was added. The reaction was monitored by TLC. Upon disappearance of starting material, the solvent and diethylamine were removed under reduced pressure. The product was dried under vacuum and directly taken for the next reaction.

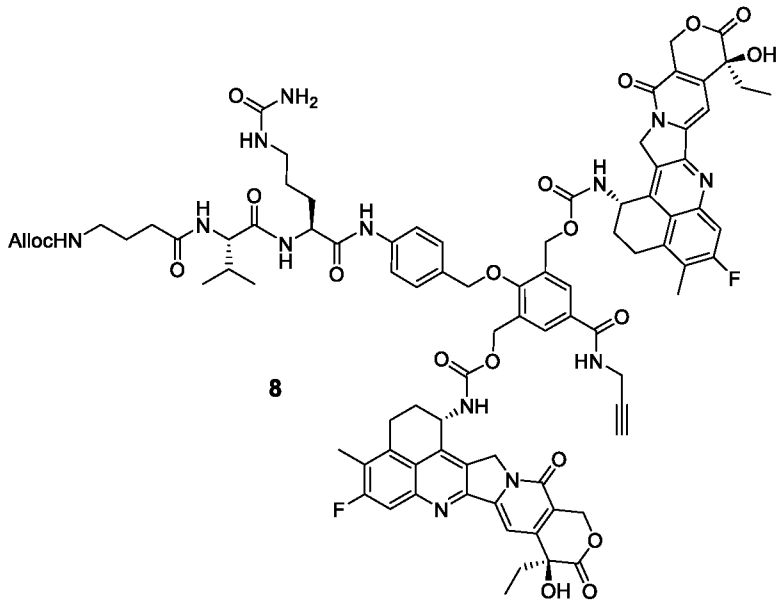
The crude material was dissolved in DMF (5 mL) and Compound **5** (327 mg, 1.15 mmol) was added. The mixture was stirred at room temperature for 3 hours and the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel to afford the titled compound **6** as an off-white solid (630 mg, 55% over 2 steps). MS (ES+) *m/z* calculated for $C_{50}H_{79}N_7O_{10}Si_2$: 993.5, found 995.0 for $[M+2H]^+$.



7

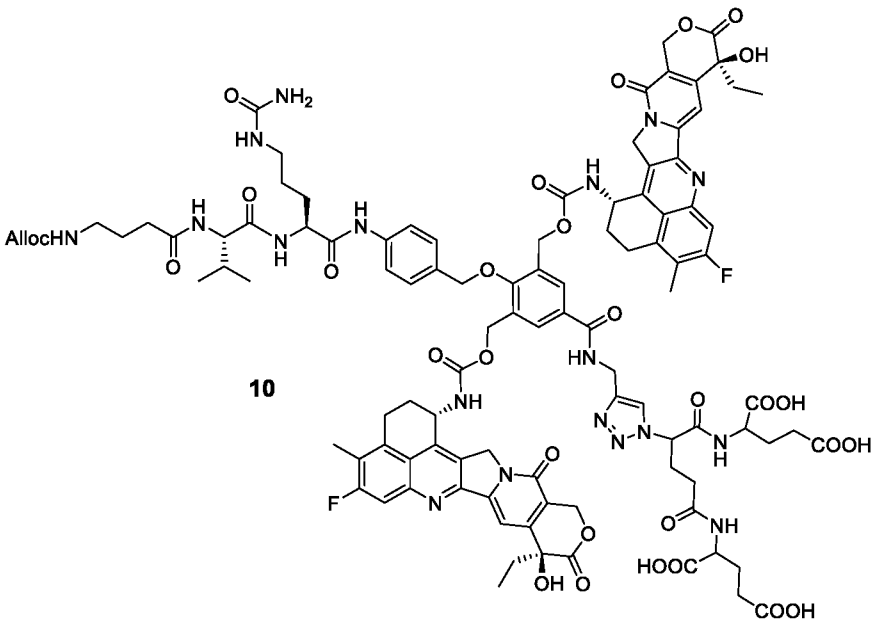
Compound 7: Compound **6** (625 mg, 0.63 mmol) was dissolved in MeOH (4 ml) and 4-touenesulfonic acid monohydrate (PTSA, 12 mg, 0.063 mmol) was added. The reaction was monitored by TLC. Upon completion the reaction mixture was diluted with EtOAc (20 mL) and saturated $NaHCO_3$ (5 mL) was added. The biphasic mixture was then extracted with EtOAc (3 x 20 mL), washed with brine (20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was dissolved in dry THF/DMSO solvent system (8 mL, THF: DMSO 10:1) before DIPEA (0.45 mL, 2.52 mmol) and 4-DMAP (4 mg, 0.03 mmol) were added. The mixture was cooled to 0 °C. 4-Nitrophenyl chloroformate (PNP-Cl, 317 mg, 1.58 mmol) was then added portion wise and the reaction was stirred for 2 h at RT. The progress of the reaction was monitored by TLC. Upon completion, the solution was directly loaded on silica gel and the product was purified by column chromatography to afford compound **7** as off-white solid (289 mg, 42% over 2 steps). MS (ES+) *m/z* calculated for $C_{52}H_{57}N_9O_{18}$: 1095.4, found 1096.9 for

$[M+2H]^+$. Analytical RP-HPLC: Column C18 5μ, 250x4.6 mm. Eluent: ACN/H_2O (H_2O with 0.1% of TFA). Method: 30-100 % ACN gradient. t_R for compound **7**: 15.1 min.



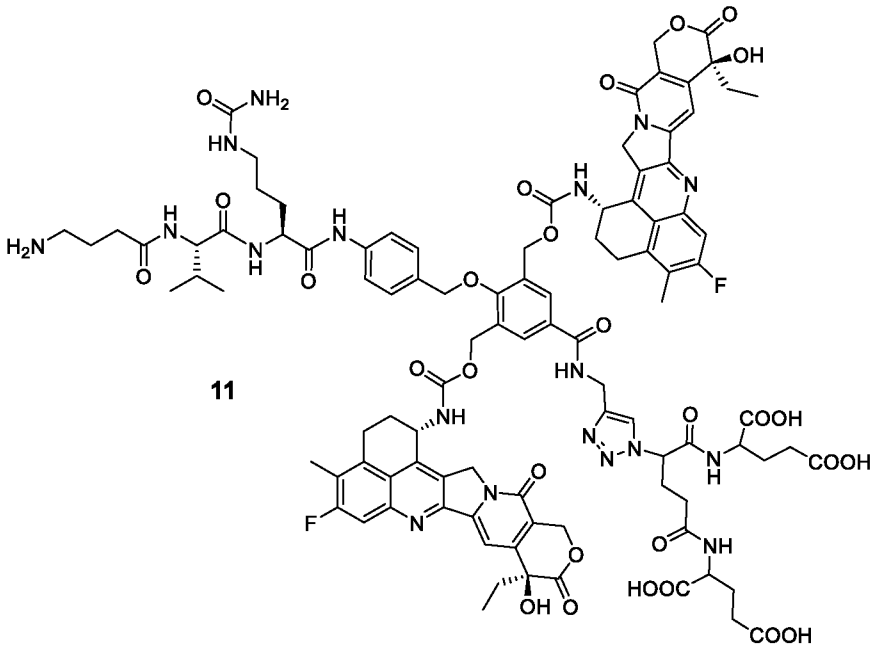
8

Compound 8: compound **7** (80 mg, 0.07 mmol) was dissolved in dry DMF (2 mL) at RT. Exatecan Mesylate (77 mg, 0.14 mmol) was added followed by Et_3N (60 μL, 0.44 mmol. The reaction mixture was stirred for 2 days and the progress of the reaction was monitored by RP-HPLC. Upon disappearance of starting material the product was precipitated by addition of MeOH (5 mL). The precipitate was filtered and washed repeatedly with EtOAc and dried under vacuum to afford compound **8** as yellow solid (76 mg, 62%). MS (ES+) *m/z* calculated for $C_{88}H_{91}F_2N_{13}O_{20}$: 1687.6, found 845.5 for $[M/2+H]^+$. Analytical RP-HPLC: Column C18 5μ, 250x4.6 mm. Eluent: ACN/H_2O (H_2O with 0.1% of TFA). Method: 30-100 % ACN gradient. t_R for compound **8**: 11.2 min.



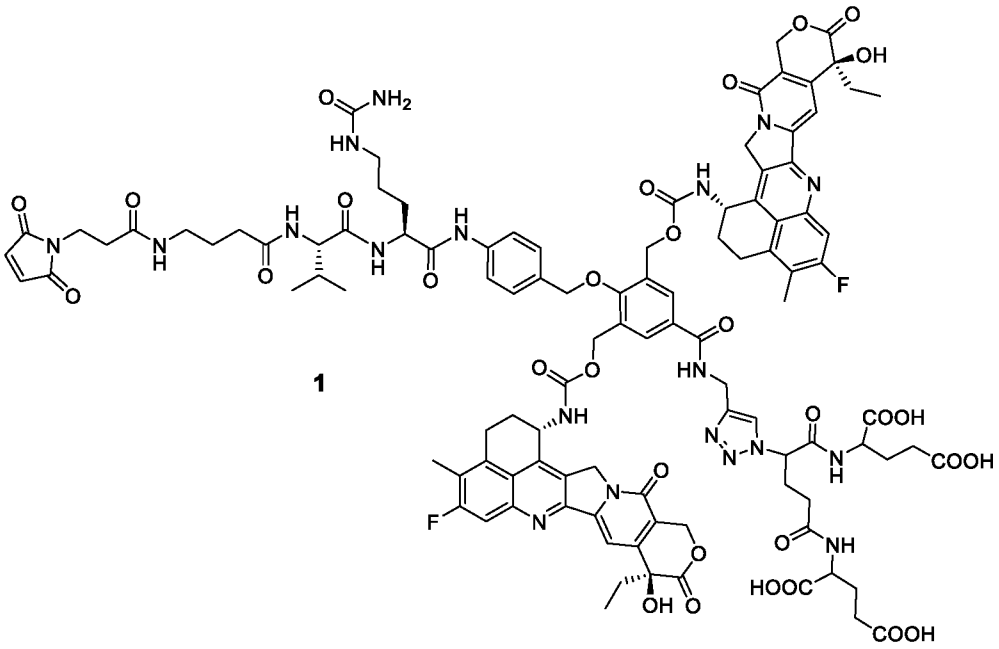
10

Compound 10: compound **8** (65 mg, 0.04 mmol) and azide **9** (86 mg, 0.2 mmol) were dissolved in a solvent system DMSO/H_2O (10:1, 2 mL) followed by the addition of $CuSO_4.5H_2O$ (5 mg, 0.02 mmol) and sodium ascorbate (3.2 mg, 0.03 mmol). The solution was degassed with argon for 30 min and then stirred at room temperature. After 30 min of stirring the progress of the reaction was monitored by RP-HPLC. Upon completion the product was isolated by reverse phase preparative HPLC (30-100% ACN in water with 0.1% TFA, 20 min) to afford compound **10** as yellow solid (64 mg, 79%). MS (ES+) *m/z* calculated for $C_{103}H_{112}F_2N_{18}O_{30}$: 2119.9, found 1061.4 for $[M/2+H]^+$. Analytical RP-HPLC: Column C18 5μ, 250x4.6 mm. Eluent: ACN/H_2O (H_2O with 0.1% of TFA). Method: 30-100 % ACN gradient. t_R for compound **10**: 10.7min.



11

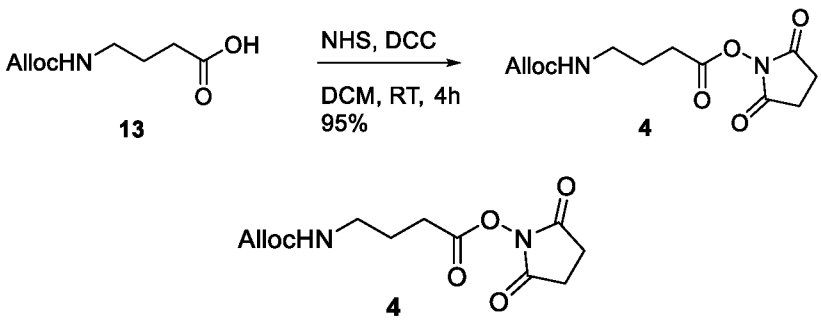
Compound 11: Compound **10** (52 mg, 0.025 mmol) was dissolved in DMF (2 mL) under argon followed by the addition of $Pd(PPh_3)_4$ (15 mg, 0.012) and 1,3-dimethyl barbituric acid (8.0 mg, 0.05 mmol). The mixture was stirred at 45 °C under argon and the progress of the reaction was monitored by RP-HPLC. After 30 min the HPLC chromatogram shows that about 50% conversion has occurred. Thereby another batches of $Pd(PPh_3)_4$ and 1,3-dimethyl barbituric acid were added. Upon completion of the reaction after 2 h the product was isolated by reverse phase preparative HPLC (10-90% ACN in water with 0.1% TFA, 20 min)to afford compound **11** as yellow solid (30 mg, 61%). MS (ES+) *m/z* calculated for $C_{99}H_{108}F_2N_{18}O_{28}$: 2035.7, found 1019.2 for $[M/2+H]^+$. Analytical RP-HPLC: Column C18 5μ, 250x4.6 mm. Eluent: ACN/H_2O (H_2O with 0.1% of TFA). Method: 10-90 % ACN gradient. t_R for compound **11**: 12.3 min.



1

Compound 1: compound **11** (25 mg, 0.012 mmol) and compound **12** (4 mg, 0.014 mmol) was dissolved in dry DMF (1.5 mL). Et_3N was added and the reaction mixture was stirred at room temperature for 1 h. Upon completion, as monitored by RP-HPLC, the product was purified by reverse phase preparative HPLC (10-90% ACN in water with 0.1% TFA, 20 min) to afford the final prodrug **1** as yellow solid (19 mg, 71%). MS (ES+) *m/z* calculated for $C_{106}H_{113}F_2N_{19}O_{31}$: 2186.79, found 1094.8 for $[M/2+H]^+$. Analytical RP-HPLC: Column C18 5μ, 250x4.6 mm. Eluent: ACN/H_2O (H_2O with 0.1% of TFA). Method: 10-90 % ACN gradient. t_R for prodrug **1**: 13.2 min.

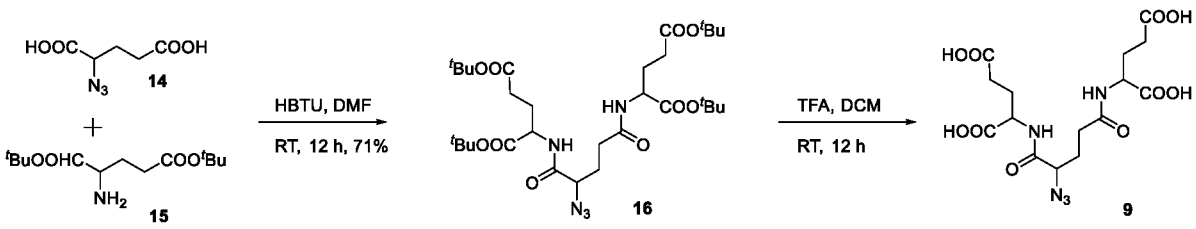
Scheme 3: synthesis of Compound **4**

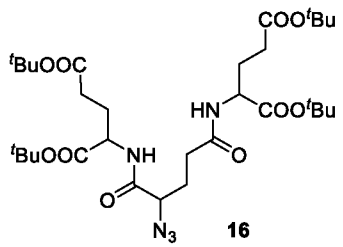


Compound 4: Compound **13** (P. D. Jeffrey, S. W. McCombie, *J. Org. Chem.* 1982, *47*, 587–590) (750 mg, 4.0 mmol) was dissolved in DCM (8 mL) and cooled to 0 °C. N-hydroxysuccinimide (692 mg, 6.0 mmol) was added followed by N,N'-

Dicyclohexylcarbodiimide (908 mg, 4.4 mmol). The reaction was allowed to warm up to room temperature and stirring was continued for 4 h. Upon completion the turbid mixture was filtered, washed with DCM. The combined organic solution was concentrated and the product was purified by silica gel column chromatography to afford compound **4** (1.08 g, 95%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95-5.85 (m, 1H), 5.28 (dd, *J* = 17.2, 1.1 Hz, 1H), 5.19 (dd, *J* = 10.4, 1.1 Hz, 1H), 4.54 (d, *J* = 5.2 Hz, 2H), 3.28 (q, *J* = 6.5 Hz, 2H), 2.82 (s, 4H), 2.66 (t, *J* = 7.3 Hz, 2H), 1.99-1.92(m, 2H). MS (ES+) *m/z* calculated for $C_{12}H_{16}N_2O_6$:284.1, found 285.3 for $[M+H]^+$.

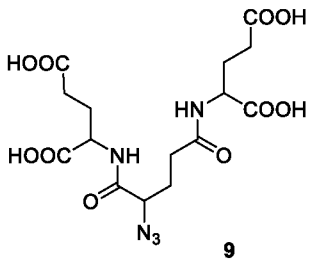
Scheme 4: synthesis of compound **9**:





Compound 16: Compound **16** is prepared according to the literature procedure (E. Roussakis, Z. Li, N. H. Nowell, A. J. Nichols, C. L. Evans, *Angew. Chemie Int. Ed.* **2015**, *54*, 14728–14731). The L-azidoglutamic acid **14** (J. Bachl, J. Mayr, F. J. Sayago, C. Cativiela, D. Díaz Díaz, *Chem. Commun.* **2015**, *51*, 5294–5297) (222 mg, 1.3 mmol) was dissolved in dry DMF (10 mL) under an argon atmosphere. HBTU (1.23 g, 3.25 mmol) was added to the solution and the mixture was stirred for 5 min at room temperature. DIPEA (2.30 mL, 13.0 mmol) was added to the solution in a single portion, followed immediately by L-glutamic acid ditertiary butyl ester **15** (760 mg, 2.6 mmol). The reaction mixture was stirred overnight, after which the solvent was removed in vacuum.

The crude product was purified by by silica gel column chromatography to afford compound **16** (597 mg, 71% with respect to **14**) as gummy liquid. MS (ES+) *m/z* calculated for $C_{31}H_{53}N_5O_{10}$: 655.4, found 656.7 for $[M+H]^+$.

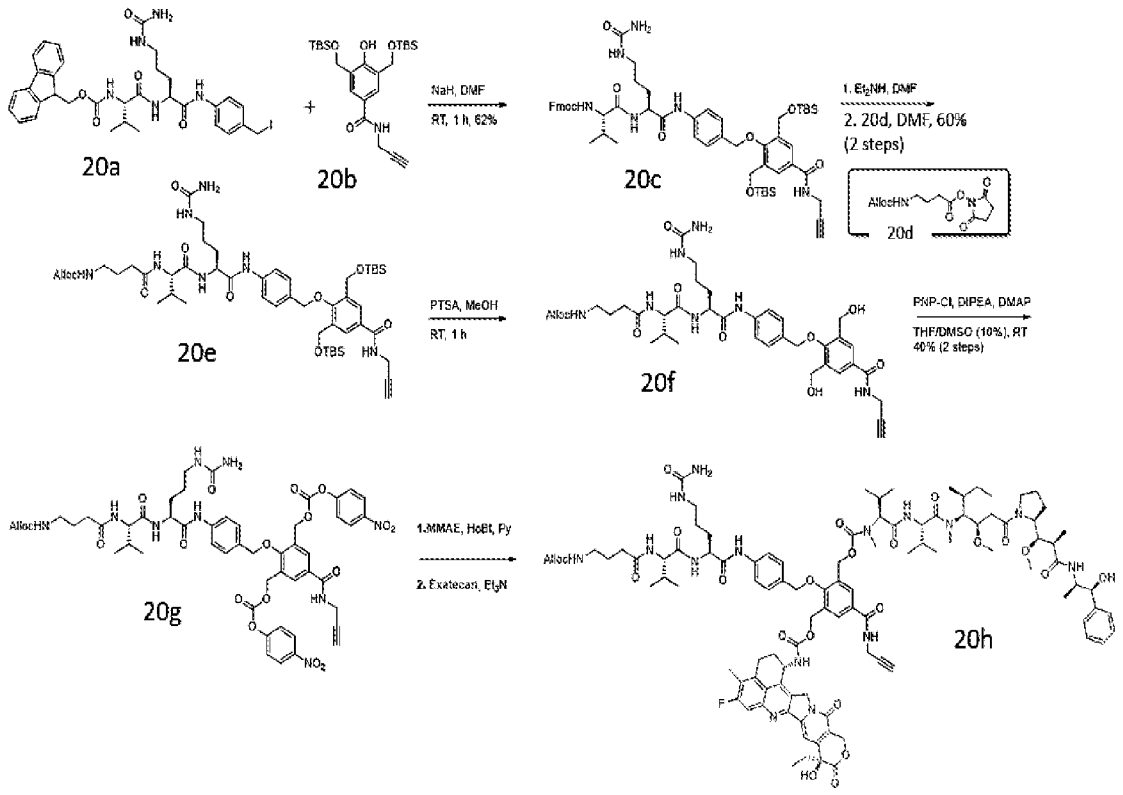


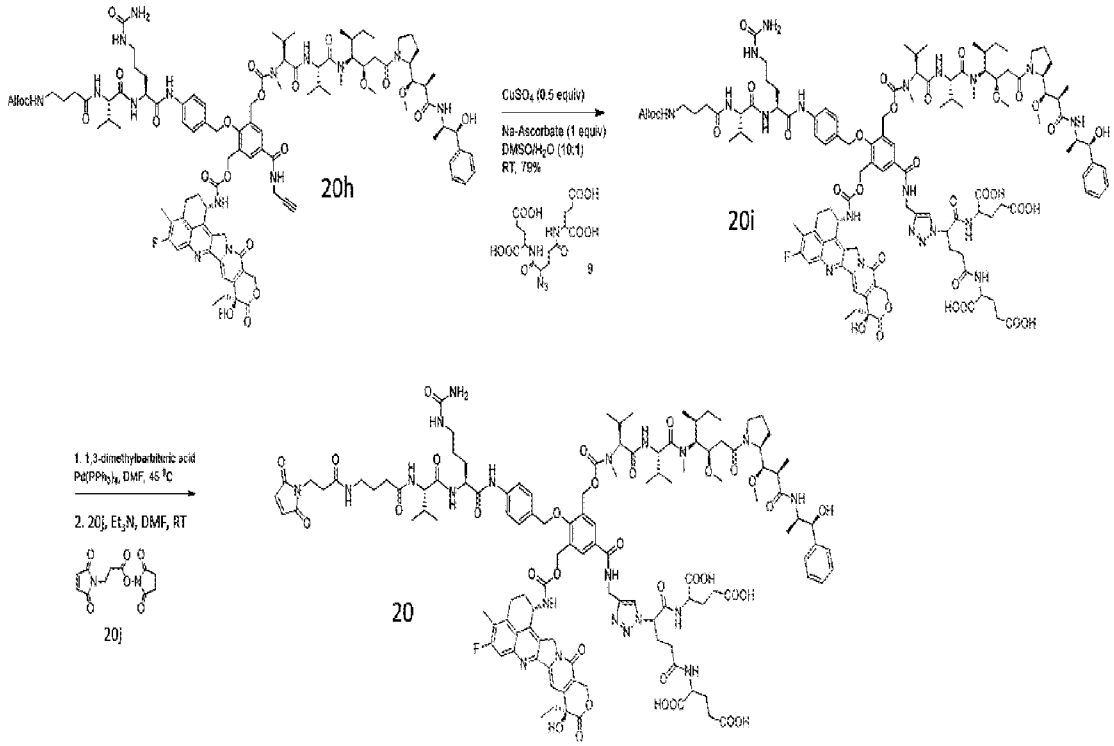
9

Compound 9: Compound **16** (550 mg, mmol) was dissolved in DCM (3 ml) and TFA (3 ml) was added. The reaction was stirred overnight. Upon completion, as monitored by TLC, the solvent and TFA was removed under reduced pressure. The product formation was confirmed my MS and the crude material was directly taken for the click reaction. MS (ES+) *m/z* calculated for $C_{15}H_{21}N_5O_{10}$: 431.1, found 432.3 for $[M+H]^+$.

Example 2: Synthesis of compound 20

Scheme 5: overall synthesis of compound 20

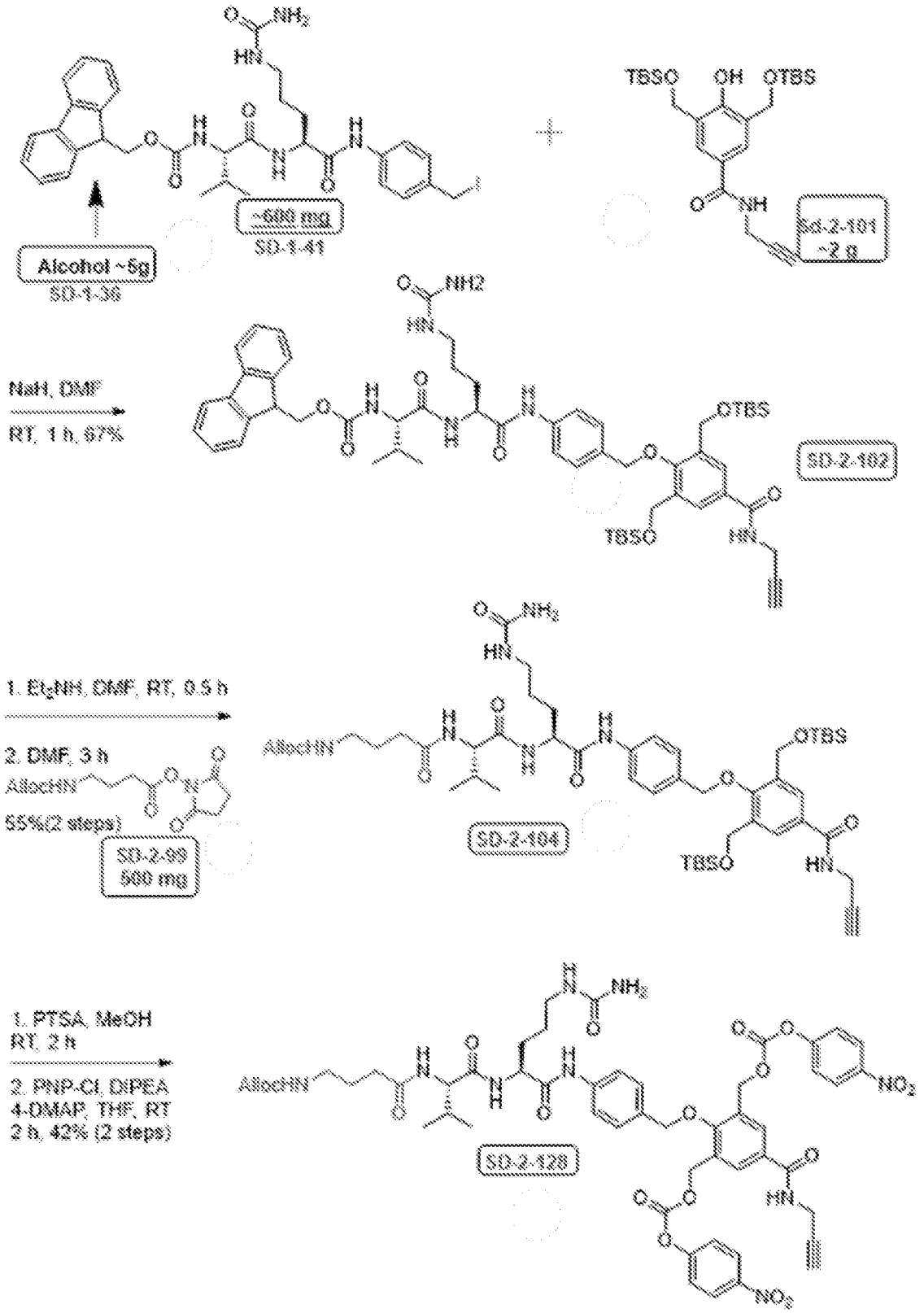


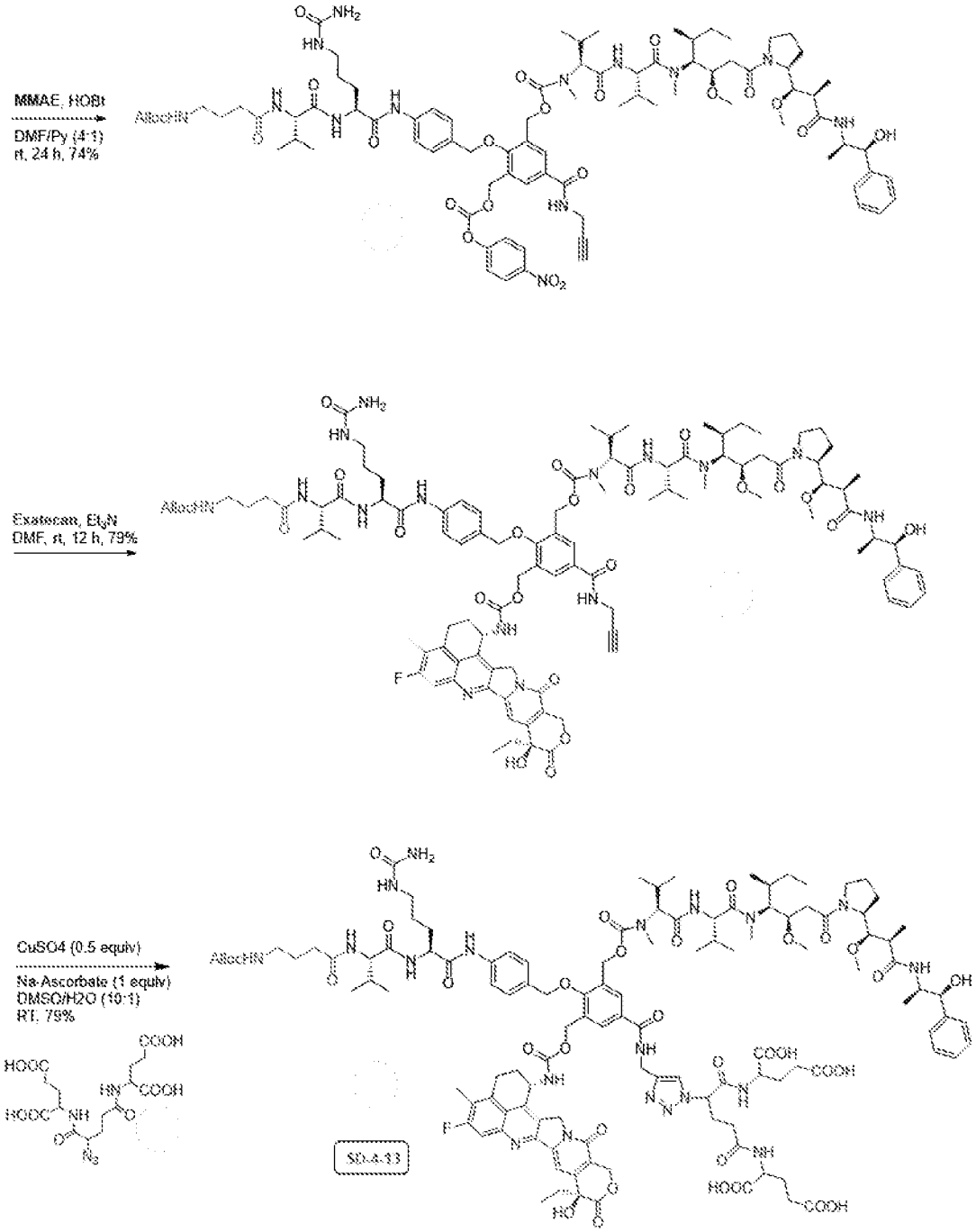


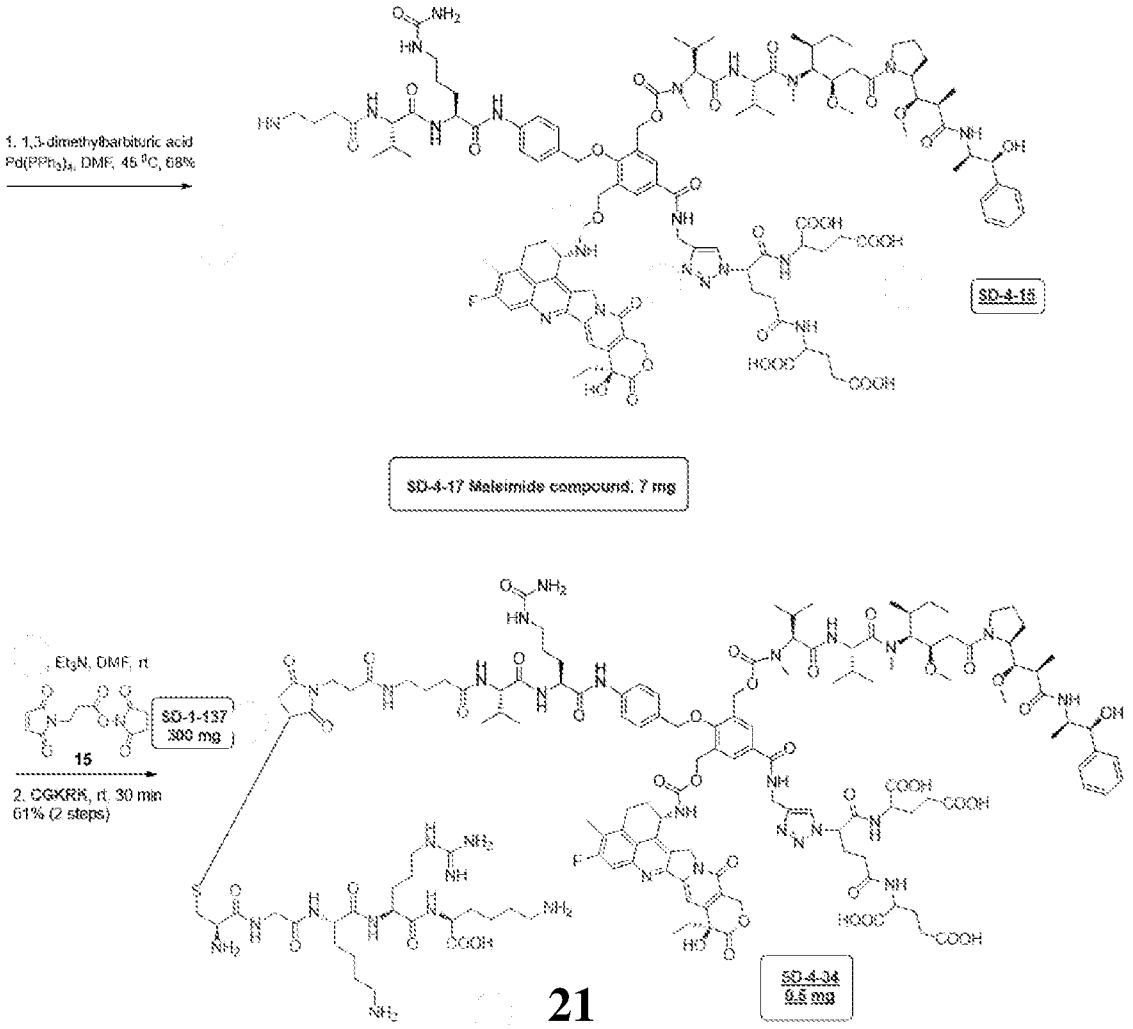
Compound 20 was synthesized according to the above scheme.

Example 3: Synthesis of compound 21

Scheme 6: overall synthesis of compound 21




MMAE, HOBt
DMF/Py (4:1)
rt, 24 h, 74%
AllocHN
Exatecan, Et3N
DMF, rt, 12 h, 79%
CuSO4 (0.5 equiv)
Na-Ascorbate (1 equiv)
DMSO/H2O (10:1)
RT, 79%
SD-4-13



21

Compound 21 was synthesized according to the above scheme and was confirmed by mass spectrometry.

Example 4: Trastuzumab-Exatecan/Belotecan conjugates inhibit the proliferation of HER2-positive HCC1954 cells

Materials and Methods

Cell lines

[0051] HCC1954 and MDA-MB-468 human mammary adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). HCC1954 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml Penicillin, 100 µg/ml Streptomycin, 12.5 U/ml Nystatin, 2 mM L-glutamine and 100 µg/ml sodium pyruvate. MDA-MD-468 cells were cultured in RPMI supplemented with 10% fetal bovine serum

(FBS), 100 IU/ml Penicillin, 100 µg/ml Streptomycin, 12.5 U/ml Nystatin, 2 mM L-glutamine. Cells were grown at 37°C in 5% CO_2.

Cell viability assay

Human mammary HCC1954 and MDA-MB231 cells were plated onto 24-well culture plates (5,000 cells/well and 10,000 cells/well, respectively) and incubated for 24 h. Cells were then exposed to serial dilutions of Exatecan or Belotecan, free or conjugated to trastuzumab. Cell viability was evaluated following 5-6 days incubation using MTT (3 µg/ml, Sigma). MTT absorbance was measured at 570 nm using SpectraMax M5e multidetection reader.

Results

Trastuzumab-Exatecan/Belotecan conjugates inhibit the proliferation of HER2-positive HCC1954 cells

Several batches of trastuzumab conjugated to exatecan or belotecan were obtained from ITL (**Table 1**). The different conjugates were evaluated for their inhibitory effect on the proliferation of two human mammary adenocarcinoma cells lines, HCC1954 (HER2-positive) and MDA-MB-468 cells (HER2-negative), compared to the free drugs. Belotecan and Exatecan exhibited similar IC_{50} values in both cell lines tested (7 and 10 nM in HCC1954; 5 and 2.5 nM in MDA-MB-468). However, in HCC1954 cells the cytotoxicity of the conjugates resembled to that of the free drugs (apart from Tras-Bel PPB-4435), whereas in MDA-MB-468 cells the conjugates were slightly less active. Trastuzumab had no inhibitory effect on the proliferation of both cells lines (**Figures 1A-1D**).

Table 1. Trastuzumab-Ecatecan/Belotexan conjugates

Batch	ADC		Antibudy conc. (mg/ml)	DAR	ADC Mw (Da)	ADC conc. (µM)
C015-1	Trastuzumab-Bel-3	NBE00778	2.16	5.14	155393.47	13.87
C015-2	Trastuzumab-Bel-3	NBE00778	2.65	4.59	154334.83	17.14
C015-3	Trastuzumab-Bel-3	NBE00778	1.31	7.36	159666.53	8.23
C015-4	Trastuzumab-Bel-3	NBE00778	1.59	7.94	160782.91	9.89
C016-1	Trastuzumab-Bel-4	NBE00778	2.24	2.19	150278.14	14.89
C016-2	Trastuzumab-Bel-4	NBE00778	1.83	5.06	156539.91	11.67
C016-3	Trastuzumab-Bel-4	NBE00778	0.97	7.22	161252.6	6
C016-4	Trastuzumab-Bel-4	NBE00778	1.11	6.89	160532.6	6.94
C017-1	Trastuzumab-Exa-1	DOK00401	3.07	6.81	160385.3	19.14
C017-2	Trastuzumab-Exa-1	DOK00401	2.44	7.84	162636.67	15.03
C017-3	Trastuzumab-Exa-1	DOK00401	3.7	7.78	162505.52	22.77
C018	Rituximab-Exa-1	DOK00401	1.3	7.5	161893.5	8.02
	Trastuzumab-Bel	PPB-4435	2.38	4.3	153776.64	15.5
	Trastuzumab-Exa	PPB-5624	3.3	8	160898.4	20.51

Tras-Exa-1 was selected for further evaluation due to its enhanced cytotoxicity. To evaluate whether increased exposure time of the cells to the conjugates will result in enhanced activity compared to free Exatecan, cells were exposed to the drug for an additional 24 h (6 days total). Moreover, to further demonstrate the advantage of the conjugate in HER2 positive cells, another HER2+ breast cancer cell line, JIMT-1, was evaluated. In correlation with the previous experiment, HCC1954 cells were significantly more sensitive to Tras-Exa-1 compared to MDA-MB-468. Tras-Exa-1 did not show any advantage compared to free Exatecan in JIMT-1 cells (**Figures 2A-2D**).

Example 5: Trastuzumab-Exatecan/Belotecan conjugates tumor growth compared to commercial Enhertu®

Materials and Methods

Cell line

HCC1954 human mammary adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml Penicillin, 100 µg/ml Streptomycin, 12.5 U/ml Nystatin, 2 mM L-glutamine and 100 µg/ml sodium pyruvate. Cells were grown at 37°C in 5% CO_2.

***In vivo* study**

All animals were housed in the Tel Aviv University Specific Pathogen Free (SPF) animal facility. The experiments were approved by the animal care and use committee (IACUC) of Tel Aviv University (approval no. 01-19-088) and conducted in accordance with NIH guidelines.

HCC1954 cells ($1x10^6$) were injected into the mammary fat pad of 6 weeks old female severe combined immune deficient (SCID) mice. Tumor growth was monitored by caliper and the volume was defined as 0.52 x width x $length^2$. When tumor volume reached approximately 95 mm^3, mice were randomized into groups (n=9) and intravenously injected with a single dose of Trastuzumab-Deruxtecan (Enhertu®; DAR=8), Trastuzumab-Exatecan (Tras-Exa; DAR=4) or Trastuzumab-Belotecan (Tras-Bel; DAR=4), at three different concentrations (1, 3 or 10 mg/kg). As controls, mice were treated either with 10 mg/kg of Rituximab- deruxtecan (Ritux-DXd; DAR=8) or with a vehicle control (10 mM Histidine, 3% Trehalose, 100 mM NaCl, pH 5.5). All treatments were administered at a volume of 200 µl/20 g mouse. Mice were euthanized when tumor volume reached 1000 mm^3, when tumors became necrotic and ulcerated, or when mice lost 15% of their body weight.

Statistical analyses

Data is presented as mean ± standard error of mean (SEM), unless stated otherwise. The statistical significance in overall survival was determined with a log-rank (Mantel-Cox) test using Graphpad Prism 9 software. Statistical significance was defined as $p < 0.05$.

Results

Trastuzumab-based antibody-drug conjugates (ADCs), linked to exatecan ("Tras-Exa"; DAR=4) or belotecan ("Tras-Bel"; DAR=4) were evaluated for their *in vivo* antitumor efficacy. To this end, mice bearing HCC1954 (HER2+) human mammary adenocarcinoma xenografts were treated with a single dose of Tras-Exa or Tras-Bel at three concentrations (1, 3 and 10 mg/kg) and monitored for tumor growth and survival. The therapeutic efficacy of our ADCs was compared to the commercially-available Enhertu® (Tras-deruxtecan; DAR=8), which was also administered at three concentrations (1, 3 and 10 mg/kg), and to an isotype control of rituximab-deruxtecan ("Ritux-DXd", DAR=8), which was given only at the highest dosage of 10 mg/kg. An

additional control group was treated with the vehicle. A dose-dependent inhibition of tumor growth was observed, with the most potent antitumor effect observed at 10 mg/kg for the three trastuzumab-based ADCs (**Figures 3A-3C**). Interestingly, a single dose of Tras-Exa or Enhertu® at the highest concentration (10 mg/kg) resulted in a complete tumor regression. Conversely, 4 out of 9 (44%) of the Tras-Bel-treated mice (10 mg/kg) relapsed 60 days following treatment (**Figure 3A**). Furthermore, a moderate anti-tumor activity was observed at 3 mg/kg for all ADCs, with Enhertu® demonstrating stronger tumor growth inhibition amongst the different ADCs (**Figure 3B**). Overall, Tras-Exa and Enhertu® showed a superior anti-tumor activity compared to Tras-Bel ADC at 3 and 10 mg/kg, but with no statistically significant difference in efficacy between these two ADCs for both concentrations. In contrast, treatment with the lowest dose (1 mg/kg) had no impact on tumor growth compared to the controls in either ADC (**Figure 3C**).

Of note, following treatments, mice of all experimental groups, including the vehicle and isotype control, exhibited transient body weight loss, which was recovered after 5 days (**Figure 3D**). Overall, our trastuzumb-based ADCs as well as Enhertu® were well tolerated in all concentrations evaluated, similarly to vehicle or isotype control, with net positive weight gain over the course of the study (**Figure 3D**).

In line with the data described above, Kaplan-Meier analyses demonstrated a dose-dependent survival benefit of the different ADCs evaluated. At the end of the study (day 152), 100% of mice treated with 10 mg/kg of Tras-Exa or Enhertu® and 89% of mice treated with Tras-Bel at the same concentration were alive; while none of the control-treated mice survived past day 109 (vehicle) and 116 (Ritux-DXd) (**Figures 4A and 4D**). Moreover, treatment at 3 mg/kg significantly prolonged the median survival of mice treated with Tras-Exa (116 days) or Enhertu® (137 days) compared to vehicle control (60 days) and Ritux-DXd (71 days) (**Figures 4B, 4D and Table 2**). Tras-Bel (3 mg/kg) had no significant effect on mice survival compared to the controls, with a median survival of 84 days (**Figures 4B and 4D and Table 2**). At the lowest dosage of 1 mg/kg, neither ADC showed any therapeutic efficacy in comparison to vehicle and isotype controls (**Figures 4C and 4D and Table 2**).

Table 2. Statistical significance of survival curves between different treatment groups, as analyzed using the log-rank (Mantel-Cox) test. $P < 0.05$; ns = not significant.

Comparison of survival curves	Log-rank (Mantel-Cox) test
Vehicle *vs.* Ritux-Dxd 10 mg/kg	ns
Vehicle *vs.* Enhertu 10 mg/kg	<0.0001
Vehicle *vs.* Enhertu 3 mg/kg	<0.0001
Vehicle *vs.* Enhertu 1 mg/kg	ns
Vehicle *vs.* Tras-Exa 10 mg/kg	<0.0001
Vehicle *vs.* Tras-Exa 3 mg/kg	0.0007
Vehicle *vs.* Tras-Exa 1 mg/kg	ns
Vehicle *vs.* Tras-Bel 10 mg/kg	<0.0001
Vehicle *vs.* Tras-Bel 3 mg/kg	ns
Vehicle *vs.* Tras-Bel 1 mg/kg	ns
Ritux-Dxd 10 mg/kg *vs.* Enhertu 10 mg/kg	<0.0001
Ritux-Dxd 10 mg/kg *vs.* Enhertu 3 mg/kg	0.0001
Ritux-Dxd 10 mg/kg *vs.* Enhertu 1 mg/kg	ns
Ritux-Dxd 10 mg/kg *vs.* Tras-Exa 10 mg/kg	<0.0001
Ritux-Dxd 10 mg/kg *vs.* Tras-Exa 3 mg/kg	0.0017
Ritux-Dxd 10 mg/kg *vs.* Tras-Exa 1 mg/kg	ns
Ritux-Dxd 10 mg/kg *vs.* Tras-Bel 10 mg/kg	<0.0001
Ritux-Dxd 10 mg/kg *vs.* Tras-Bel 3 mg/kg	ns
Ritux-Dxd 10 mg/kg *vs.* Tras-Bel 1 mg/kg	ns
Enhertu 10 mg/kg *vs.* Enhertu 3 mg/kg	0.0101
Enhertu 10 mg/kg *vs.* Enhertu 1 mg/kg	<0.0001
Enhertu 3 mg/kg *vs.* Enhertu 1 mg/kg	0.0002
Tras-Exa 10 mg/kg *vs.* Tras-Exa 3 mg/kg	0.0008
Tras-Exa 10 mg/kg *vs.* Tras-Exa 1 mg/kg	<0.0001
Tras-Exa 3 mg/kg *vs.* Tras-Exa 1 mg/kg	0.003
Tras-Bel 10 mg/kg *vs.* Tras-Bel 3 mg/kg	<0.0001
Tras-Bel 10 mg/kg *vs.* Tras-Bel 1 mg/kg	<0.0001
Tras-Bel 3 mg/kg *vs.* Tras-Bel 1 mg/kg	0.0016

Enhertu 10 mg/kg vs. Tras-Exa 10 mg/kg	ns
Enhertu 10 mg/kg vs. Tras-Bel 10 mg/kg	ns
Tras-Exa 10 mg/kg vs. Tras-Bel 10 mg/kg	ns
Enhertu 3 mg/kg vs. Tras-Exa 3 mg/kg	ns
Enhertu 3 mg/kg vs. Tras-Bel 3 mg/kg	0.0012
Tras-Exa 3 mg/kg vs. Tras-Bel 3 mg/kg	0.0076
Enhertu 1 mg/kg vs. Tras-Exa 1 mg/kg	ns
Enhertu 1 mg/kg vs. Tras-Bel 1 mg/kg	ns
Tras-Exa 1 mg/kg vs. Tras-Bel 1 mg/kg	ns

Example 6: Antibodies Conjugation Techniques

Linkage by transglutaminase was carried out with wild type microbial transglutaminase (WT Transglutaminase purchased from Zedira, Germany) to the light chain of trastuzumab-T by standard methods, following a procedure that is also described in Dickgiesser et al., Bioconjugate Chem. (2020), vol. 31(4), p. 1070-1076. To this end, 1 eq antibody in a buffer with 150 mM NaCl, 25 mM Tris (pH 8.0) was mixed with a solution of the respective payload (10x or 20x surplus to the concentration of the antibody, depending on the number of linkage sites) and 6 U/ml of transglutaminase. The mixture was incubated for 16 h in a thermomixer at 37 °C and 450 rpm.

For linkage by maleimide chemistry, a 5 mg/mL solution of the respective antibody component in PBS (pH 7.4) was prepared (~33 µM antibody). The antibody was reduced with TCEP((tris(2-carboxyethyl)phosphine); ratio of antibody component to TCEP 1:2 to 1:6, depending on the desired DAR; TCEP was used as 2 mM stock, pH 7.0) or in some cases with DTT (dithiothreitol; 20 mM). After incubation for 0.5-2 hours (depending on the number of cysteines to be activated) in a 37 °C water bath, the reaction was allowed to cool down to room temperature. The solution was desalted to conjugation buffer (10 mM sodium phosphate, pH 6.0, 2 Mm EDTA, N2 degassed) on a Sephadex G25 column and adjusted to an antibody concentration of 0.2 mg/mL in conjugation buffer. Conjugation was initiated by adding the reduced antibody to a solution of the appropriate maleimide-activated linker-payload construct at a suitable ratio (e.g. 1:4 to 1:8 ratio antibody to linker-payload construct for DAR = 4; e.g. 1:20 ratio antibody to linker-payload construct for DAR= 8). The reaction was incubated for 1 h at 22 °C with slow

rocking, then the conjugation was checked by LCMS. If necessary, the reaction was continued until the desired DAR was reached.

The conjugated sample was purified by hydrophobic interaction chromatography (HIC) on a 15 PHE (Phenyl) column (GE Healthcare) or HiTrap HP or FF Butyl Sepharose column (GE Healthcare). Elution fractions were concentrated, and buffer exchanged to PBS pH 7.4 or 10 mM potassium phosphate, 200 mM NaCl, 10 mM histidine, 50 mM trehalose, pH 7.0. The identity and purity of each prepared ADC was confirmed by LC-MS and SDS-PAGE. The DAR for each prepared ADC was calculated and confirmed from HIC (hydrophobic interaction chromatography) data and mass spectrometry data.

The stability of each prepared ADC was tested by a freeze-thawing experiment. Specifically, the ADC in histidine buffer was shock-frozen in liquid nitrogen to -80^{0}C and stored at this temperature for several weeks up to a few months. After putting the sample to room temperature until the sample had been completely thawed, the sample was subjected to SE-HPLC (size exclusion-high performance liquid chromatography) analysis to test for compound degradation, and the activity of the thawed ADC was examined. Specifically, target antigen binding was examined by Octet binding assay and payload-mediated cytotoxicity was tested by cell titer glow assay on positive and negative cell lines. The results for the freeze-thawed ADC was compared to the corresponding ADC without freeze-thawing. In each case, the ADC with solubility tag was found to be stable in this freeze-thawing procedure.

Endotoxin was determined by the PTS (Portable Test System) cartridge method (Nexgen) according to the manufacturer's instructions under standard conditions. For each prepared ADC, it was found that the endotoxin level was < 5.0 endotoxin units (EU)/mg.

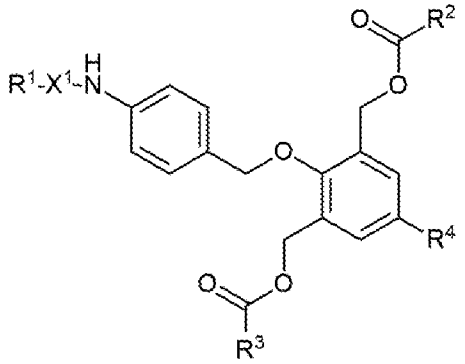
While certain embodiments of the invention have been illustrated and described, it is to be clear that the invention is not limited to the embodiments described herein. Numerous modifications, changes, variations, substitutions and equivalents will be apparent to those skilled in the art without departing from the spirit and scope of the present invention as described by the claims, which follow.

CLAIMS

What is claimed is:

Claims

1. A compound represented by Formula (I):



(I)

wherein

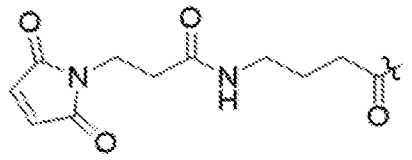
R^1 is a reactive moiety comprising maleimide, bromoacetamide, tetrazine, alkyne, amine moiety or any combination thereof;

R^2 and R^3 are the same or different active pharmaceutical ingredient moiety;

R^4 comprises an oligo- or poly- carboxylic acid moiety or oligo- or poly-ethylene glycol or oligo- or poly-alcohol or oligo- or poly-vinylclcohol or oligo- or poly-glycerol moiety; and

X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety, an oligo or polyethylene glycol or an oligo or polyvinylalcohol or oligo- or poly-glycerol moiety.

2. The compound of claim 1, wherein R^1 is represented by



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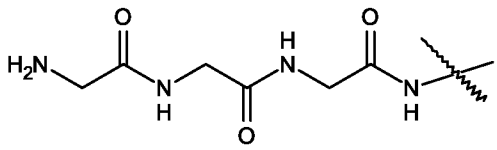

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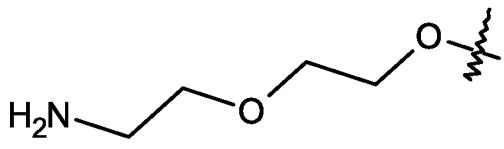

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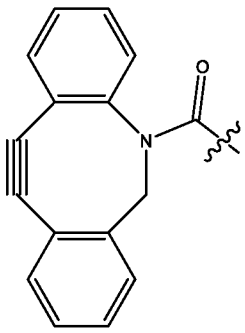

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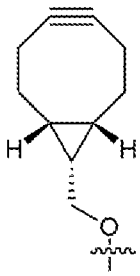

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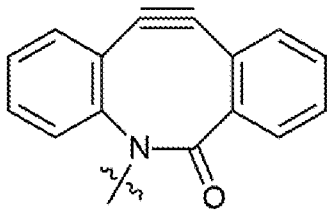


.

3. The compound according to any one of the preceding claims, wherein R^2 is an anti-cancer, immune stimulating or immune dampening agent moiety.

4. The compound of claim 3, wherein R^2 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

5. The compound of claim 4, wherein R^2 is exatecan, auristatin, Monomethyl *auristatin* E (MMAE) or belotecan moiety.

6. The compound according to any one of the preceding claims, wherein R^3 is an anti-cancer, immune stimulating or immune dampening agent moiety.

7. The compound of claim 6, wherein R^3 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

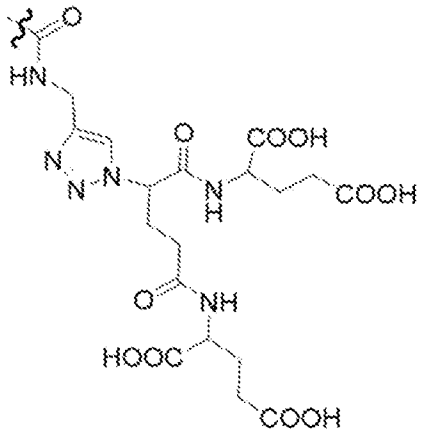
8. The compound of claim 7, wherein R^3 is exatecan, auristatin, Monomethyl *auristatin* E (MMAE) or belotecan moiety

9. The compound according to any one of the preceding claims, wherein R^2 and R^3 are anticancer, immune stimulating or immune dampening agent moieties.

10. The compound of claim 9, wherein R^2 and R^3 are each independently exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

11. The compound of claim 10, wherein R^2 and R^3 are exatecan, auristatin or belotecan moieties.

12. The compound according to any one of the preceding claims, , wherein R^4 is represented by:



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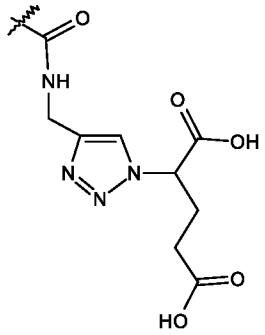
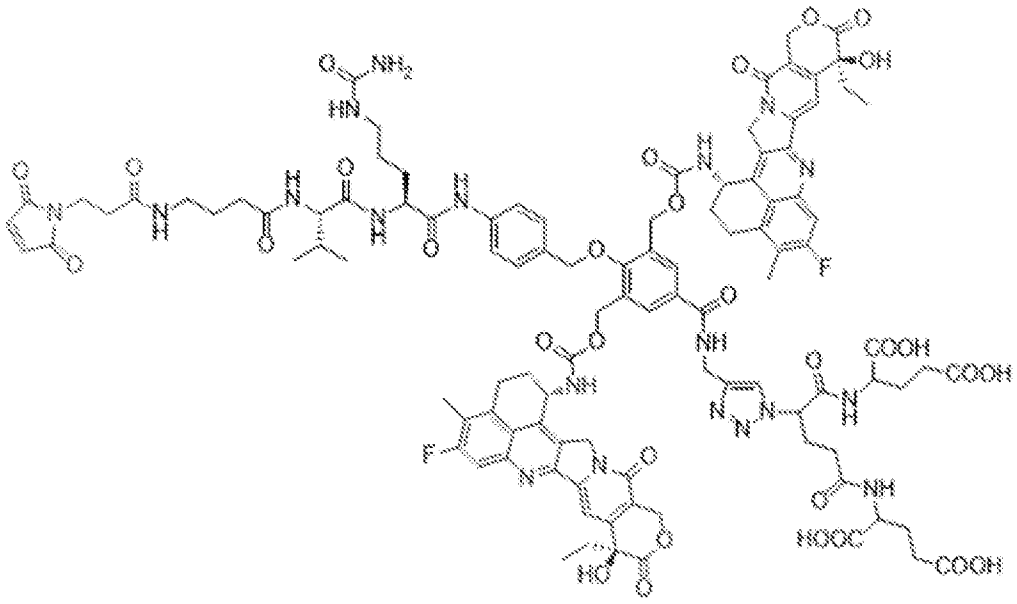


.

13. The compound according to any one of the preceding claims, wherein X^1 is -Val-Cit-, -Ala-Ala-, AAN, GGFG, -Val-Ala- or -Val-Arg-.

14. The compound according to any one of the preceding claims, represented by the structure of Formula 1, 17, 18, 19, or 20:



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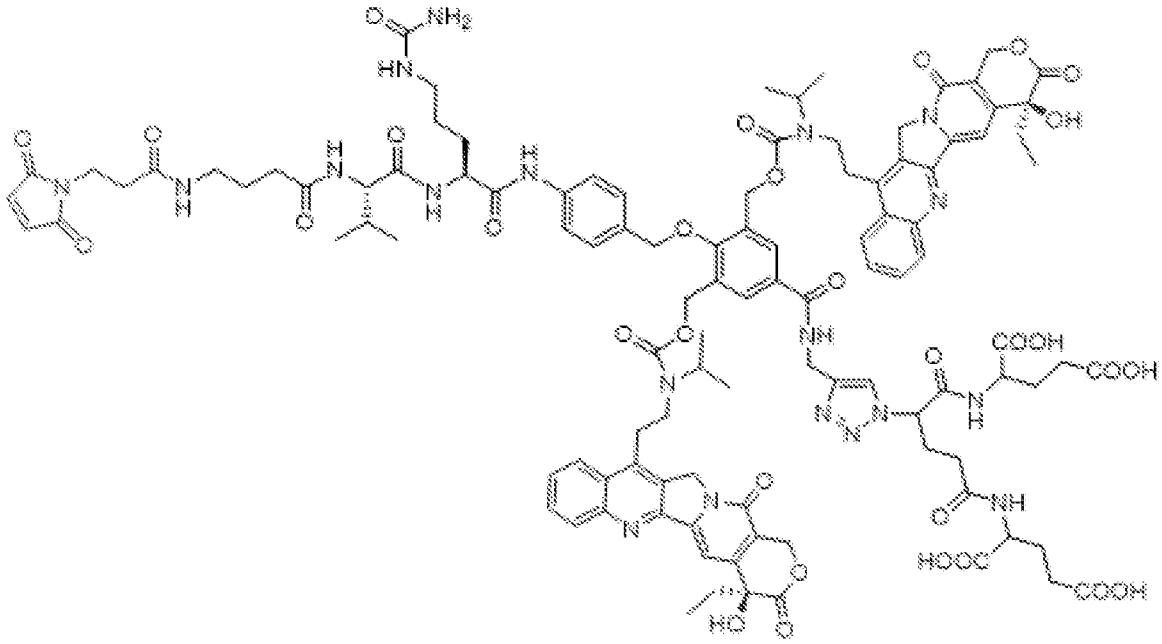
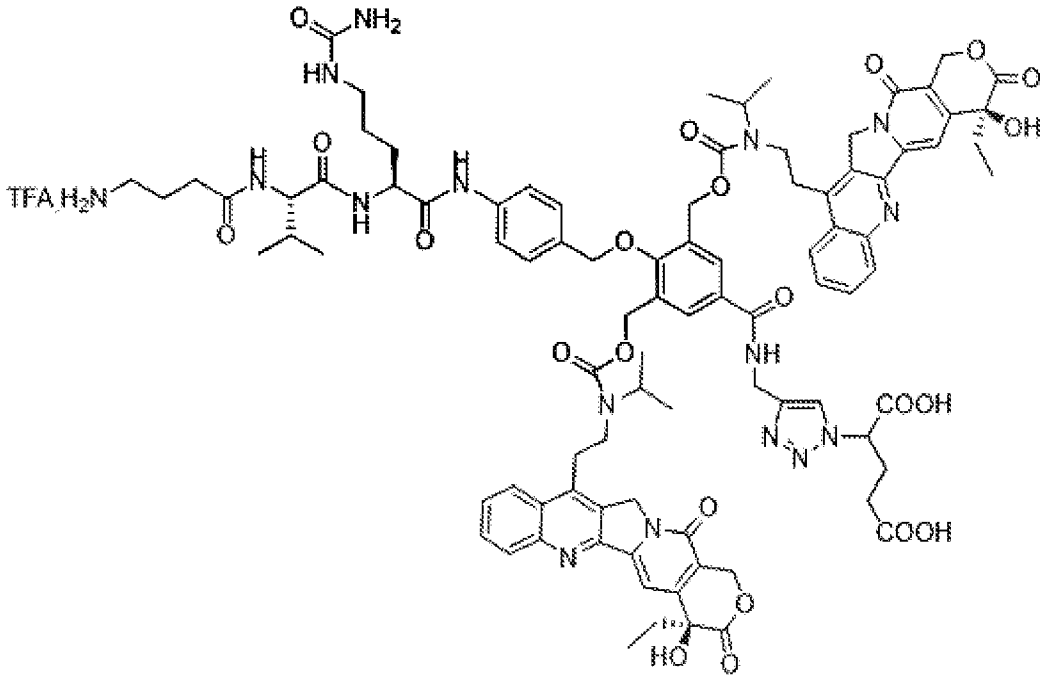
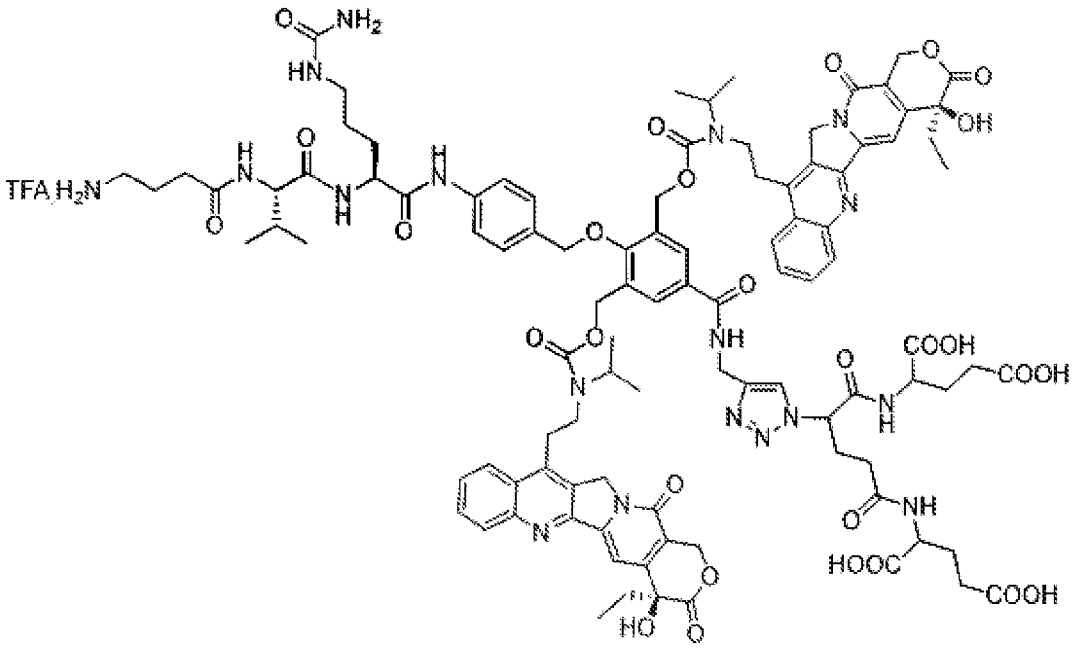


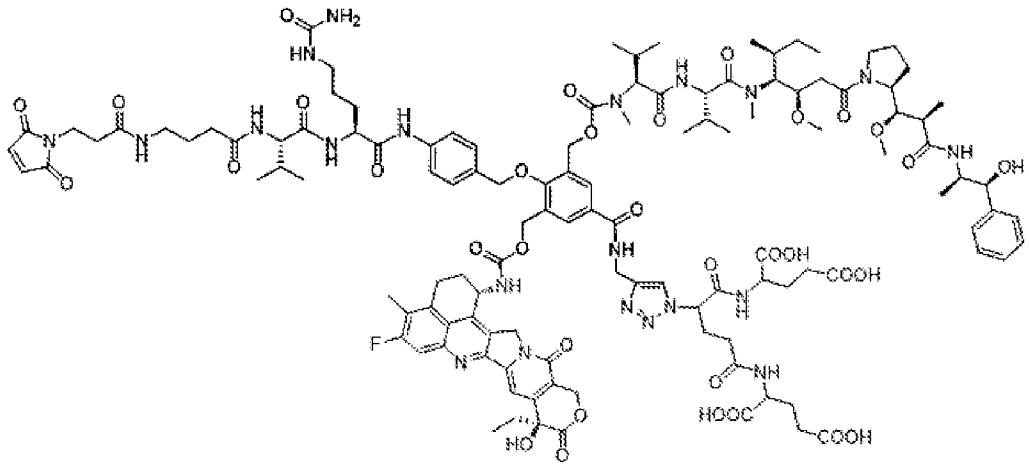
17;



18;



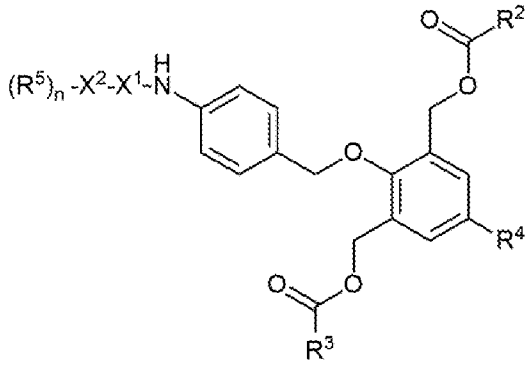
19; or



20,

wherein TFA is trifluoroacetic acid.

15. A Conjugate represented by Formula (I(a)):



(**I(a)**)

wherein

R^2 and R^3 are the same or different active pharmaceutical ingredient moiety;

R^4 comprises an oligo- or poly- carboxylic acid moiety or oligo- or poly-ethylene glycol or oligo- or poly-alcohol or oligo- or poly-vinylclcohol or oligo- or poly-glycerol moiety;

R^5 is an antibody or antigen moiety;

X^1 is a mono-, di-, tri-, tetra-, oligo- or polypeptide moiety, an oligo or polyethylene glycol or an oligo or polyvinylalcohol or oligo- or poly-glycerol moiety;

X^2 is a linker comprising succinimide, acetamide, dihydropyridazine, alkene or amine moiety or any combination thereof; and

n is a number between 0.01 – 10.

16. The conjugate of claim 15, wherein R^2 is an anti-cancer, immune stimulating or immune dampening agent moiety.

17. The conjugate of claim 16, wherein R^2 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

18. The conjugate of claim 17, wherein R^2 is exatecan, auristatin, Monomethyl *auristatin* E (MMAE) or belotecan moiety.

19. The conjugate according to any one of claims 15-18, wherein R^3 is an anti-cancer, immune stimulating or immune dampening agent moiety.

20. The conjugate of claim 19, wherein R^3 is an exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

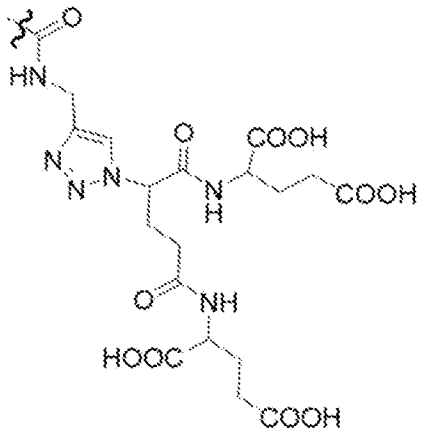
21. The conjugate of claim 20, wherein R^3 is exatecan, auristatin, Monomethyl *auristatin* E (MMAE) or belotecan moiety.

22. The conjugate according to any one of claims 15-21, wherein R^2 and R^3 are anticancer, immune stimulating or immune dampening agent moieties.

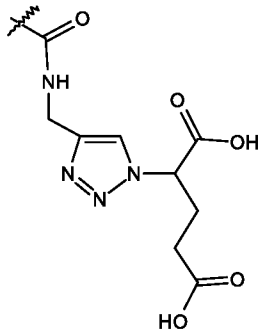
23. The conjugate of claim 22, wherein R^2 and R^3 are each independently exatecan, belotecan, camptothecin, auristatin, Monomethyl *auristatin* E (MMAE) or a doxorubicin moiety.

24. The conjugate of claim 23, wherein R^2 and R^3 are exatecan, auristatin, Monomethyl *auristatin* E (MMAE) or belotecan moieties.

25. The conjugate according to any one of claim 15-24, wherein R^4 is represented by:



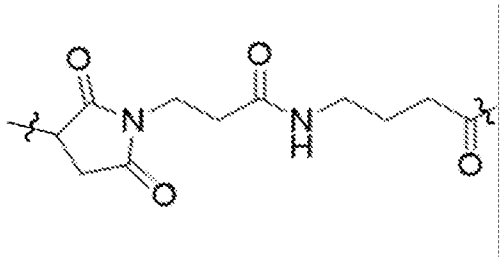
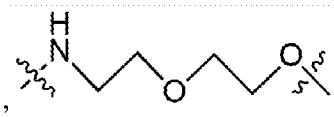
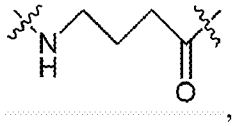
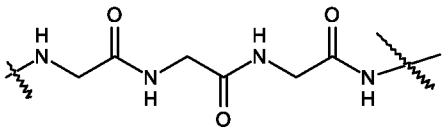
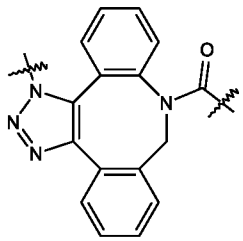
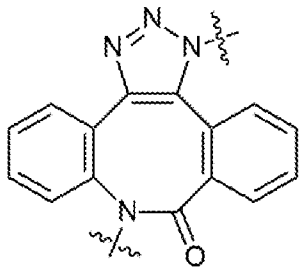
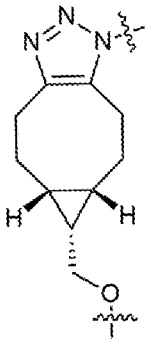
or



.

26. The conjugate according to any one of claims 15-25, wherein X^1 is -Val-Cit-, -Ala-Ala-, AAN, GGFG, -Val-Ala- or -Val-Arg-.

27. The conjugate according to any one of claims 15-26, wherein X^2 is represented by

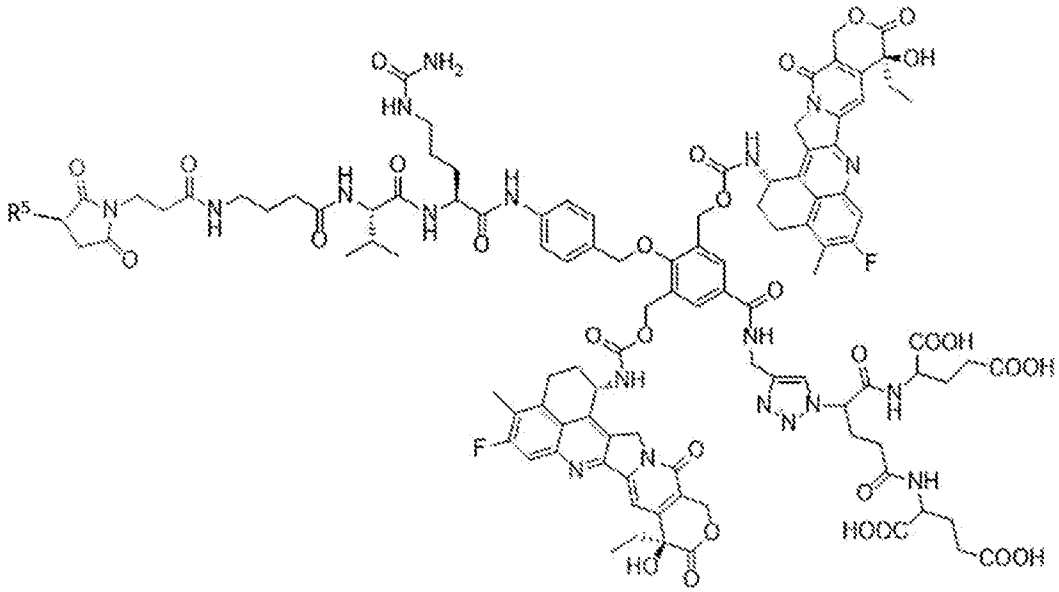








28. The conjugate according to any one of claims 15-27, wherein R^5 is an anti-EGFR or anti-CD33 antibody.

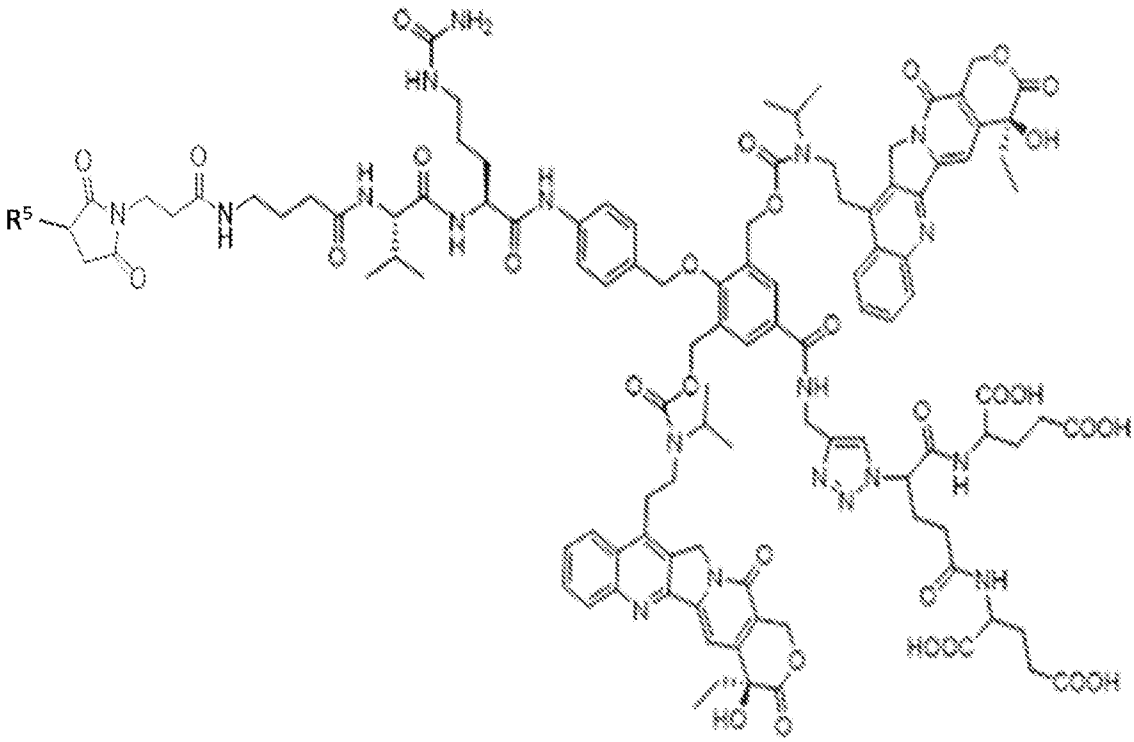
29. The conjugate according to any one of claims 15-28, wherein R^5 is trastuzumab or rituximab or CGKRK moiety.

30. The conjugate of claim 29, wherein R^5 is trastuzumab moiety.

31. The conjugate according to any one of claims 15-30, represented by the structure of 1(a) or 2(a):



1(a) **; or**

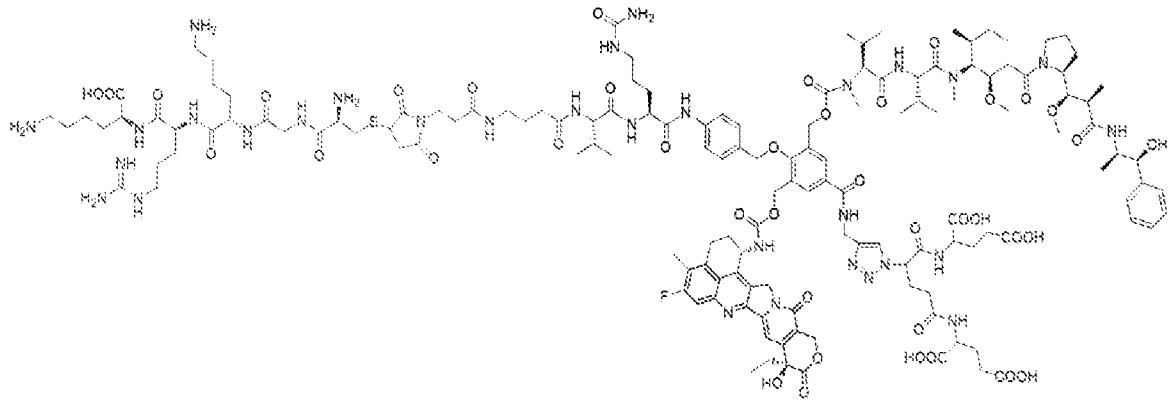


2(a).

32. The conjugate of claim 31, wherein R^5 is trastuzumab or rituximab moiety.

33. The conjugate of claim 32, wherein R^5 is trastuzumab moiety.

34. The conjugate according to any one of claims 15-29, represented by the structure of compound 21:



.

35. The conjugate according to any one of claims 15-34, wherein the drug to antibody ratio (DAR) is between 0.5 and 200.

36. The conjugate according to any one of claims 15-35, wherein n is between 0.1-1.

37. The compound according to any one of claims 1-14, or the conjugate according to any one of claims 15-36, for use in treating cancer

38. The compound or conjugate of claim 37, wherein the cancer is a breast, kidney, brain, skin, ovary, lung, pancreas, colon, head/neck, prostate, endometrium, liver, blood, stomach, fibrosarcoma, bone neoplasms, osteosarcoma or duodenum cancer.

39. A kit comprising:

i) the compound according to any one of claims 1-14; and

ii) an antibody or an antigen.

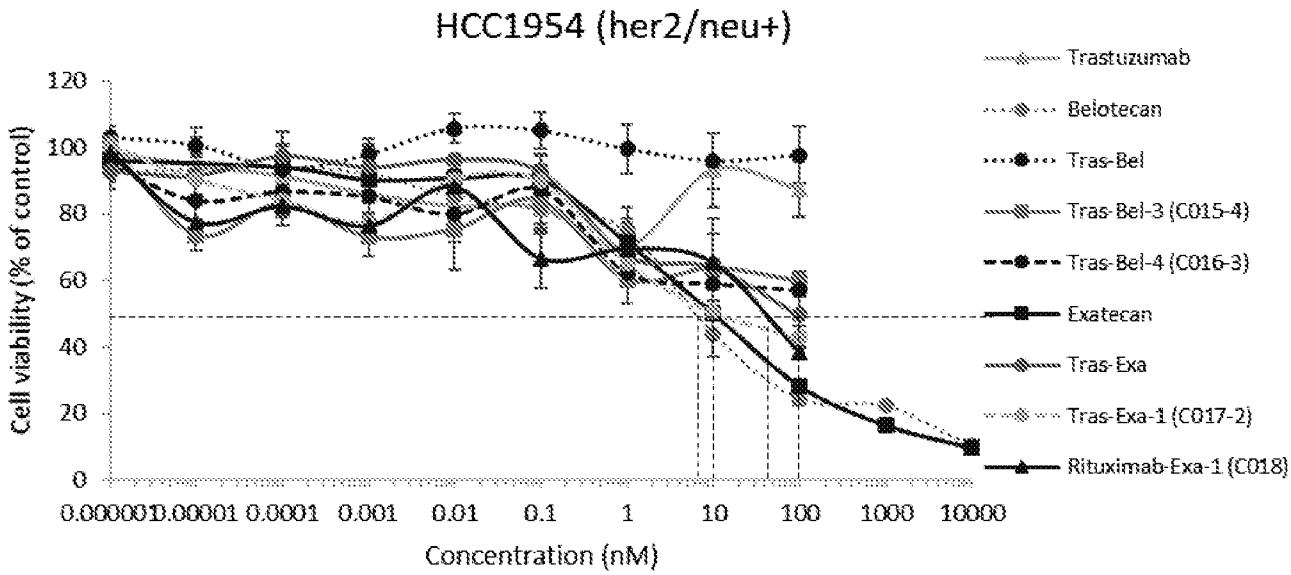


Figure 1A

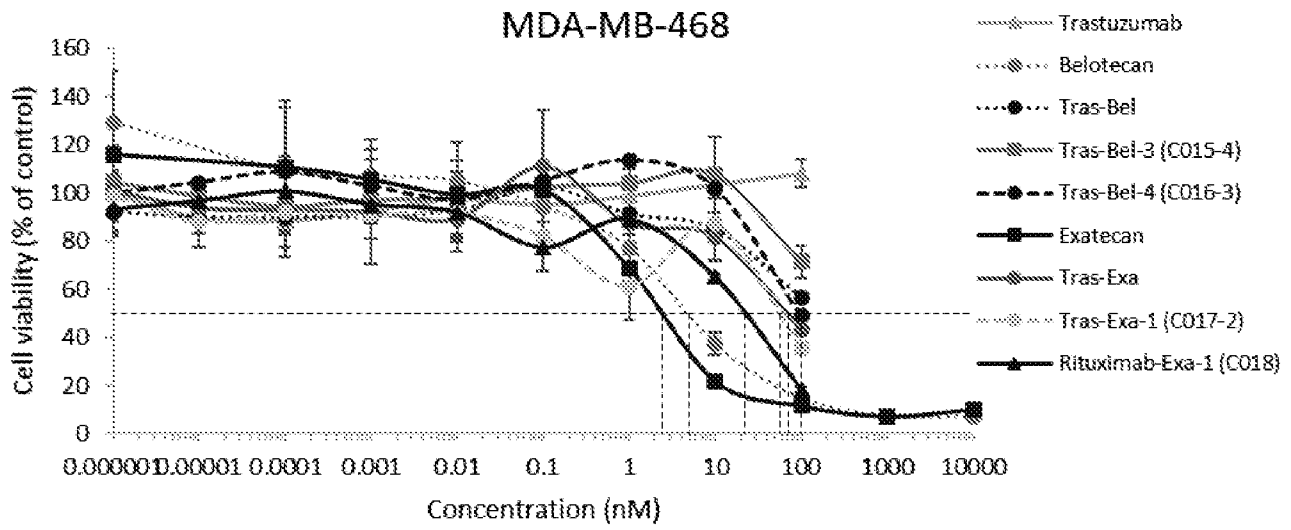


Figure 1B

Treatment	IC50 (nM)
Trastuzumab	N/A
Bel	7
Tras-Bel	N/A
Tras-Bel-3 (C015-4)	N/A
Tras-Bel-4 (C016-3)	N/A
Exa	10
Tras-Exa	100
Tras-Exa-1 (C017-2)	10
Rituximab-Exa-1 (C018)	40

Figure 1C

Treatment	IC50 (nM)
Trastuzumab	N/A
Bel	5
Tras-Bel	N/A
Tras-Bel-3 (C015-4)	N/A
Tras-Bel-4 (C016-3)	N/A
Exa	2.5
Tras-Exa	70
Tras-Exa-1 (C017-2)	60
Rituximab-Exa-1 (C018)	20

Figure 1D

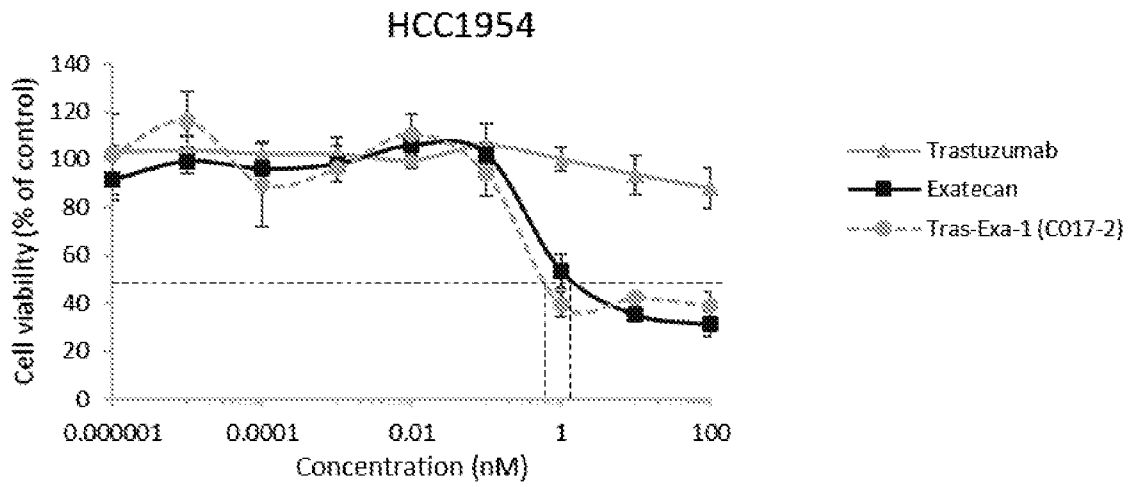


Figure 2A

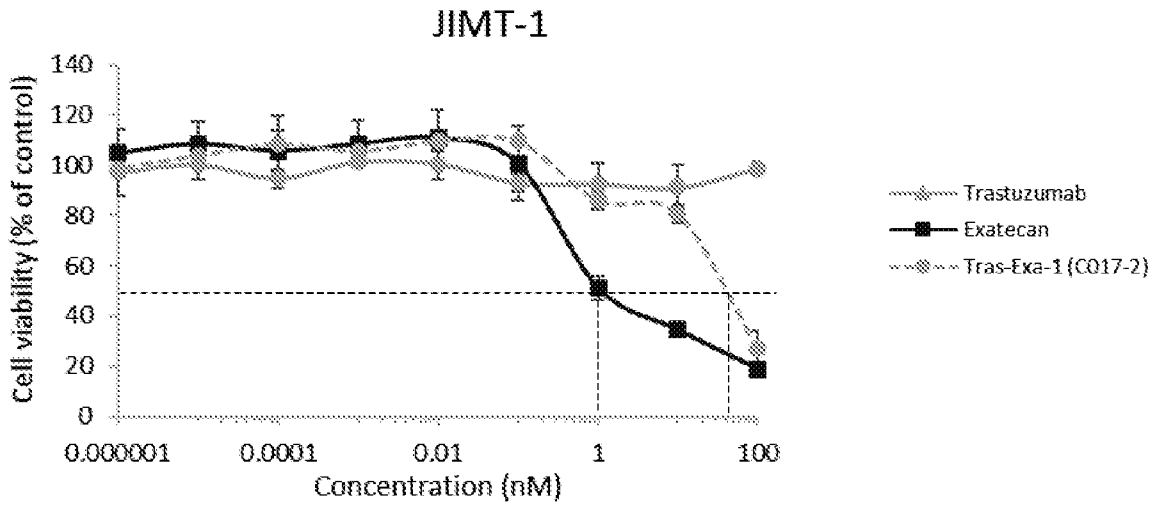


Figure 2B

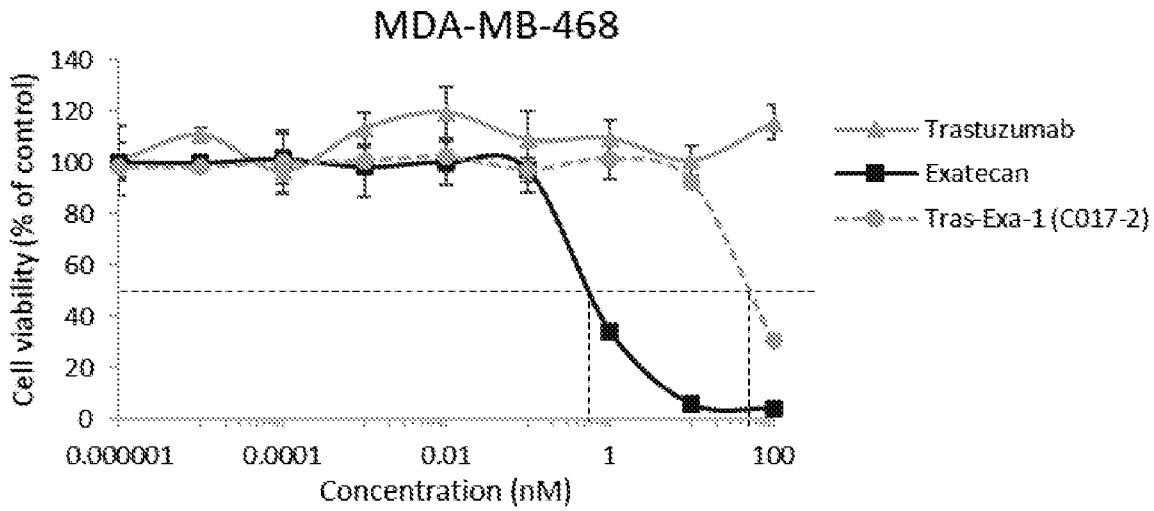


Figure 2C

	HCC1954	JIMT-1	MDA-MB-468
Trastuzumab	N/A	N/A	N/A
Exatecan	1.5	1	0.6
Trastuzumab-Exa-1 (C017-2)	0.6	40	50
Tras-Exa/Exa fold change	**0.4**	**40**	**83**
her2 levels	**++**	**+**	**-**

Figure 2D

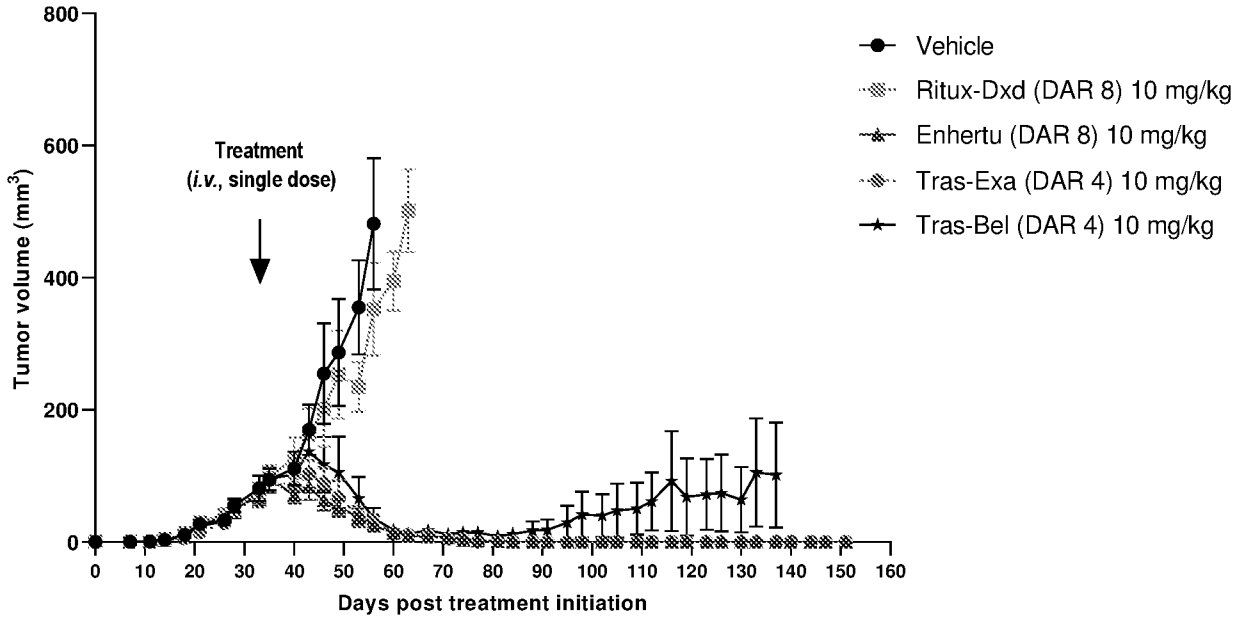


Figure 3A

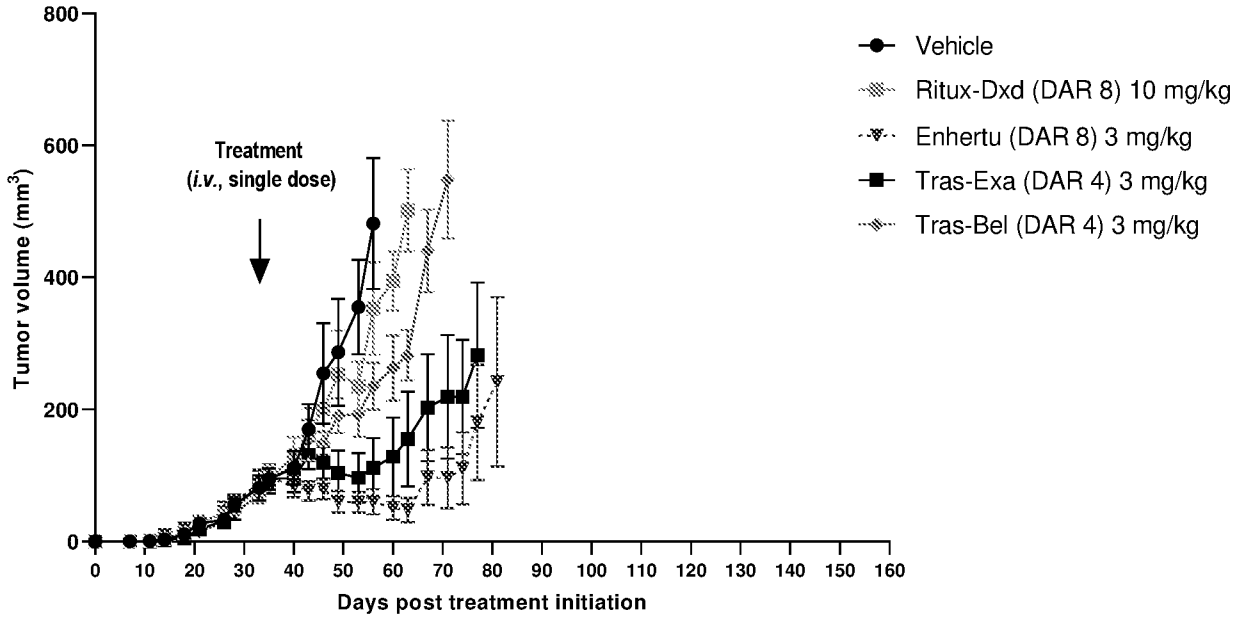


Figure 3B

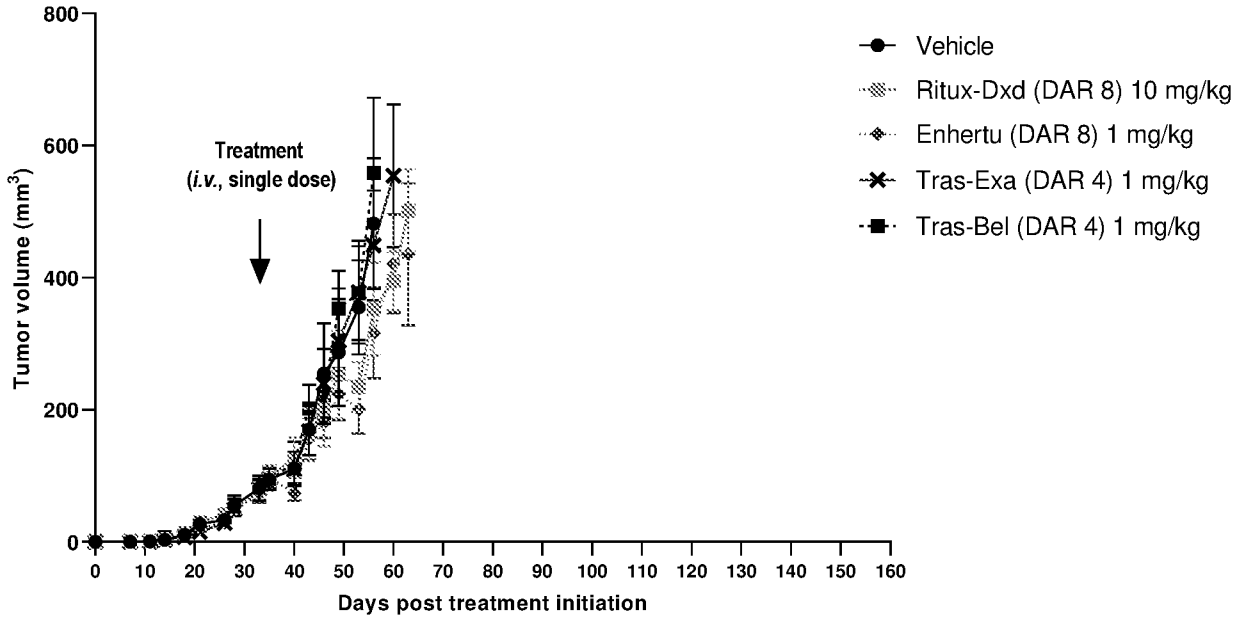


Figure 3C

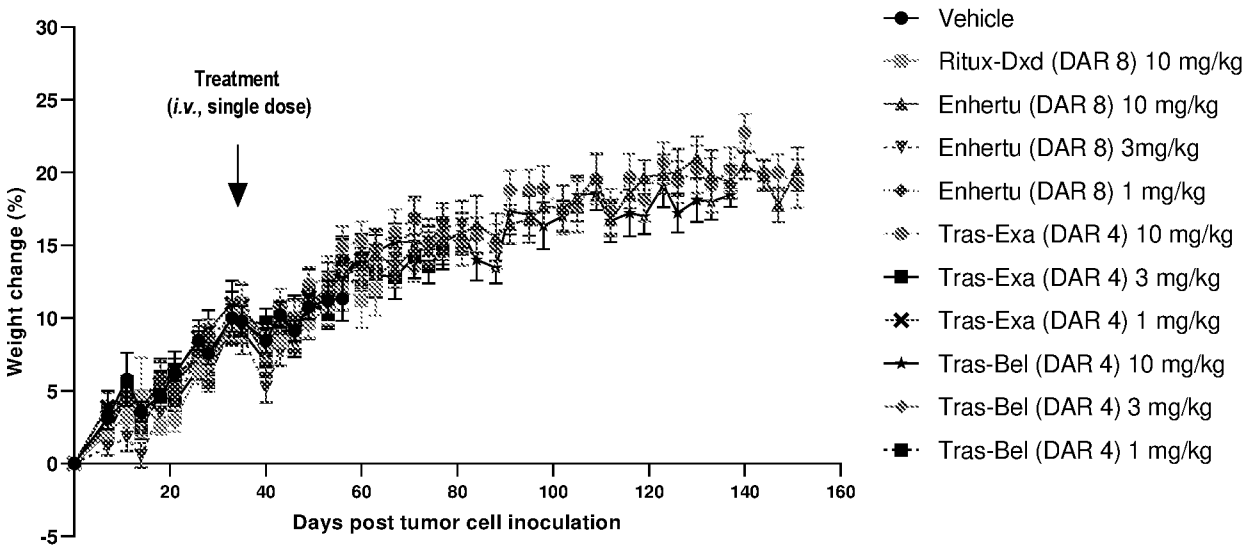


Figure 3D

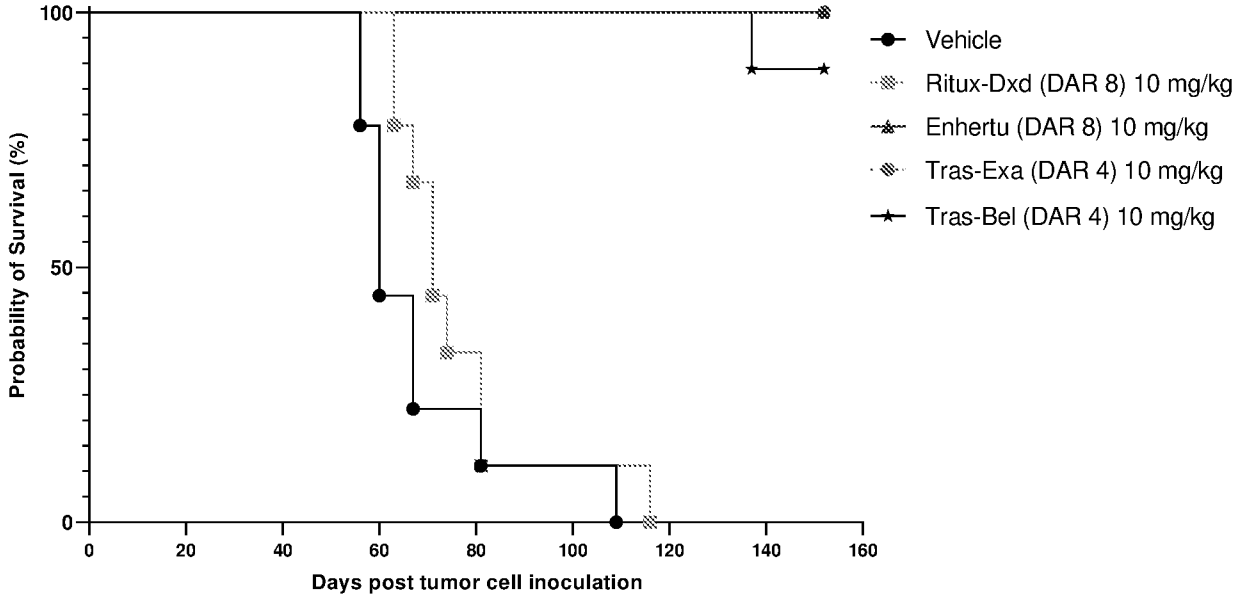


Figure 4A

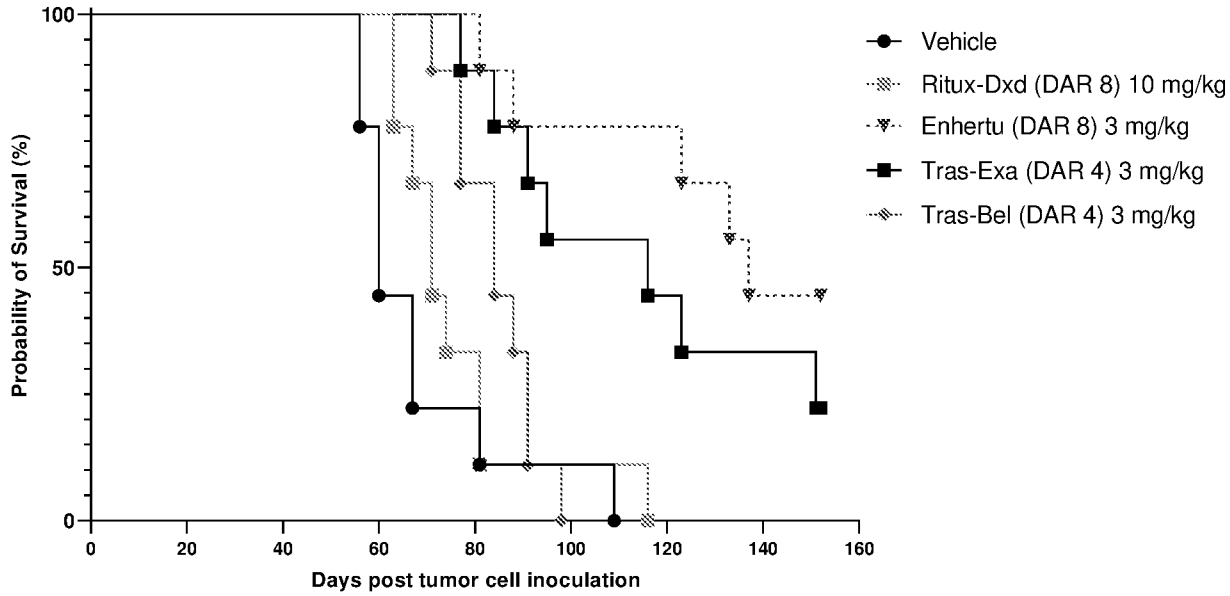


Figure 4B

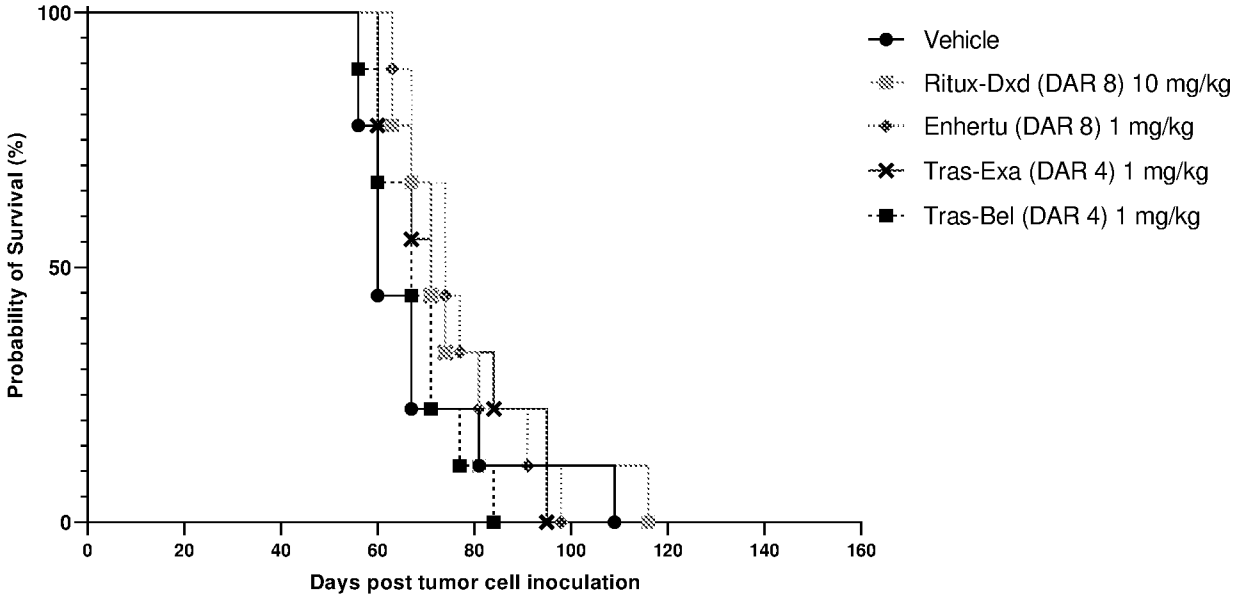


Figure 4C

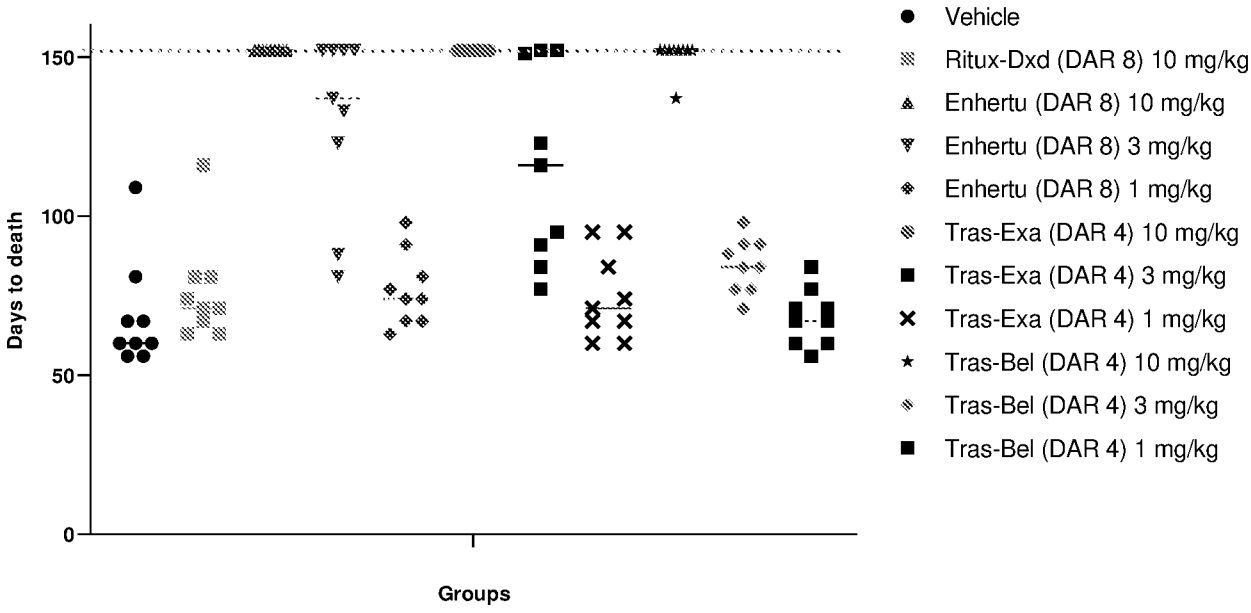


Figure 4D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2022/050782

A. CLASSIFICATION OF SUBJECT MATTER

C07C 211/46(2022.01)i; ***C07C 69/00***(2022.01)i; ***C07K 16/32***(2022.01)i; ***C07K 16/00***(2022.01)i; ***A61P 35/00***(2022.01)i
CPC:C07C 211/46; C07K 16/32; C07K 16/00; A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C 211/46; C07C 69/00; C07K 16/32; C07K 16/00; A61P 35/00
CPC:C07C 211/46; C07K 16/32; C07K 16/00; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: Google Patents, REGISTRY, Google Scholar Search terms used: "benzyloxyphenyl", " conjugate", "antibody", "drug", ADC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Suho Park, et al. "Introduction of para-hydroxy benzyl spacer greatly expands the utility of ortho-hydroxy-protected aryl sulfate system: application to nonphenolic payloads". Bioconjugate Chem. 2019, 30, 1969?1978. 19 June 2019. (2019/06/19) The whole document.	1-39
A	WO 2016/040856 A2 (Genentech Inc [US])17 March 2016 (2016-03-17) The whole document.	1-39
A	WO 2020/086858 A1 (Genentech Inc [US])30 April 2020 (2020-04-30) The whole document.	1-39
A	Xiangjie Luo, et al. "Hypoxia-Activated Prodrug Enabling Synchronous Chemotherapy and HIF-1alpha Downregulation for Tumor Treatment". Bioconjugate Chem. 2021, 32, 5, 983–990. (2021/04/13) The whole document.	1-39

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2022	**31 October 2022**

Name and mailing address of the ISA/IL
Israel Patent Office
Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel
Israel
Telephone No. **972-73-3927258**
Email: **pctoffice@justice.gov.il**

Authorized officer
GARBER Nathan
Telephone No.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2022/050782

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