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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2023 (19.05.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/083919 A1

(51) International Patent Classification:
A61K 47/68 (2017.01)

(21) International Application Number:
PCT/EP2022/081371

(22) International Filing Date:
09 November 2022 (09.11.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
21207284.7 09 November 2021 (09.11.2021) EP

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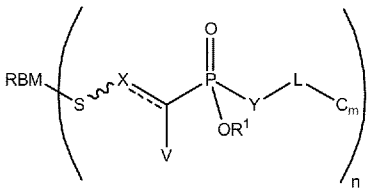
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ,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

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

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

(54) Title: CONJUGATES COMPRISING A PHOSPHORUS (V) AND A CAMPTOTHECIN MOIETY

(57) Abstract: The present invention relates to a conjugate having the formula (I) wherein a receptor binding molecule (RBM) is connected with a camptothecin moiety (C). The present invention also relates to intermediates for producing the same, methods of preparing the same, pharmaceutical compositions comprising the same, as well as uses thereof.



(I)

WO 2023/083919 A1

CONJUGATES COMPRISING A PHOSPHORUS (V) AND A CAMPTOTHECIN MOIETY

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims the benefit of priority of European Patent Application No. 21207284.7 filed 9 November 2021, the content of which is hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[002] The present invention relates to conjugates of a receptor binding molecule with a camptothecin moiety, intermediates for producing the same, methods of preparing the same, pharmaceutical compositions comprising the same, as well as uses thereof.

BACKGROUND

[003] Antibody-drug conjugates (ADCs) are biotherapeutics that combine cytotoxic molecules with the targeting property of antibodies to specifically kill cancer cells. A class of drugs, which has been explored for their use in ADCs, are camptothecin and derivatives thereof. Camptothecin and derivatives thereof have attracted considerable interest as they act as inhibitors of topoisomerase I. Exemplary ADCs of camptothecin an derivatives thereof have been described by, e.g., Han et al., *"The Potential of Topoisomerase Inhibitor-Based Antibody-Drug Conjugates"*, Pharmaceutics 2022, 14, 1701, https://doi.org/10.3390/pharmaceutics14081707; Conilh et al., *"Exatecan Antibody Drug Conjugates Based on a Hydrophilic Polysarcosine Drug-Linker Platform"*, Pharmaceuticals 2021, 14, 247, https://doi.org/10.3390/ph14030247; WO 2020/245229; WO 2019/236954; Burke et al., *"Design, Synthesis, and Biological Evaluation of Antibody-Drug Conjugates Comprised of Potent Camptothecin Analogs"*, Bioconjugate Chem. 2009, 20, 1242-1250, doi: 10.1021/bc9001097; and Viricel et al., *"Monodisperse polysarcosine-based highly-loaded antibody-drug conjugates"*, Chemical Science, 2019, 10, 4048-4053, doi: 10.1039/c9sc00285e).

[004] An ADC of a camptothecin derivative which has gained a lot of attention is the ADC of the anti-Her2 antibody Trastuzumab with deruxtecan. This ADC has been approved for medical use, and is also known as DS-8201a and is marketed under the tradename Enhertu.

This ADC is described by Ogitani et al., *"DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor Efficacy with Differentiation from T-DM1"*, Clinical Cancer Research (22)20, October 15, 2016, pp. 5097-5108 (DOI: 10.1158/1078-0432.CCR-15-2822).

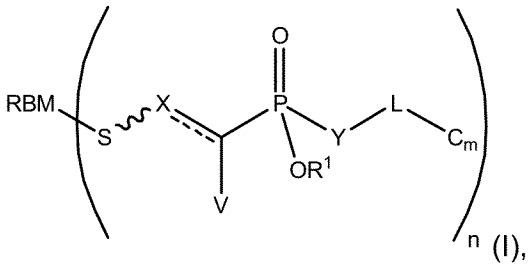
[005] Enhertu has been initially approved for the treatment of solid tumors, namely Her2+ breast cancer and colorectal cancer. Most recently, Enhertu has been found to switch the paradigm for the targeted treatment of Her2-positive breast cancer, since it shows highly promising results in patients even with a low expression level of Her2, that were previously thought to not be eligible for a targeted Her2-treatment (see, e.g., Siddiqui et al., *"Enhertu (Fam-trastuzumab-deruxtecan-nxki) – Revolutionizing treatment paradigm for HER2-Low breast cancer"*, Annals of Medicine and Surgery 82 (2022) 104665; https://doi.org.10.1016/j.amsu.2022.104665). While Enhertu is an approved and marketed ADC, certain drawbacks still remain. In particular, it has turned out that Enhertu exhibits a comparably low serum stability. Further, Enhertu non-target related toxicities are still commonly observed problems in therapeutic applications. Many reasons for this can be related to shortcomings in the linker system between the payload and the antibody (Mckertish et al., *"Advances and Limitations of Antibody Drug Conjugates for Cancer"*, Biomedicines 2021, 9, 872; https://doi.org/10.3390/biomedicines9080872). For instance, ADC uptake into non-targeted cells due to membrane interaction with the hydrophobic linker-payload structure, or the formation of aggregates in form of higher molecular weight species caused by hydrophobicity of the payload are likely to cause non-target related toxicities in the patient. Moreover, premature release of the payload from the ADC and transfer to serum proteins can additionally lead to off-target side effects. The combination of those effects can lead to life threatening side effects, such as Interstitial Lung disease or reduction of white blood cells, in particular neutrophiles, both of which are the most common severe side effects that are described for Enhertu.

[006] Accordingly, there is still a need for further conjugates comprising a camptothecin moiety as drug, in particular conjugates which exhibit an improved serum stability or show other improvements over Enhertu.

SUMMARY

[007] This need is addressed by the subject-matter as defined in the claims and in the embodiments described herein.

[008] Accordingly, the present invention relates to a conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

RBM is a receptor binding molecule;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

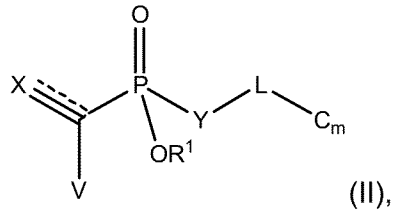
L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

[009] The present invention also relates to a compound having the formula (II):



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is R_3-C when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

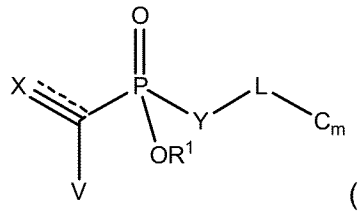
L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10.

[0010] The present invention also relates to a method of preparing a conjugate of formula (I), said method comprising:

reacting a compound of formula (II)



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is R_3-C when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

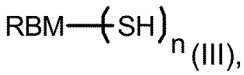
R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10;

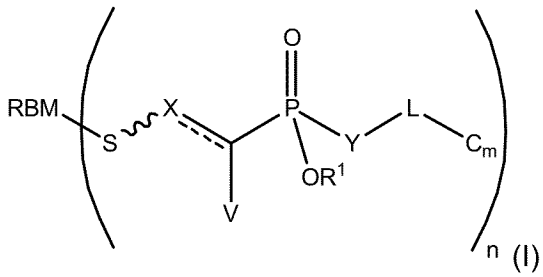
with a thiol-containing molecule of formula (III)



(III),

wherein RBM is a receptor binding molecule; and
n is an integer ranging from 1 to 20;

resulting in a compound of formula (I)



(I)

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a double bond when  in a compound of formula (II) is a triple bond; or

 is a bond when  in a compound of formula (II) is a double bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

[0011] The present invention also relates to a conjugate of formula (I) obtainable or being obtained by a method of the invention.

[0012] The present invention also relates to a pharmaceutical composition comprising a conjugate of the invention.

[0013] The present invention also relates to a conjugate of the invention for use in a method of treating a disease. The disease may be cancer. The cancer may be a solid tumor.

[0014] The present invention also relates to a pharmaceutical composition of the invention for use in a method of treating a disease. The disease may be cancer. The cancer may be a solid tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 shows an analytical HPLC chromatogram of the compound methyl 4-azido-2-(dodecaethyleneglycol)benzoate. The horizontal axis depicts the retention time in minutes.

[0016] Figure 2 shows an analytical HPLC chromatogram of the compound methyl 4-azido-2-(dodecaethyleneglycol)benzoate. The horizontal axis depicts the retention time in minutes.

[0017] Figure 3 shows an analytical HPLC chromatogram of the compound P5(PEG12)-COOH.

[0018] Figure 4 shows an analytical HPLC chromatogram of the compound P5(PEG24)-OSu. The horizontal axis depicts the retention time in minutes.

[0019] Figure 5 shows an analytical HPLC chromatogram of the compound P5(PEG12,PEG24)-COOH. The horizontal axis depicts the retention time in minutes.

[0020] Figure 6 shows an analytical HPLC chromatogram of the compound P5(PEG24,PEG24)-COOH. The horizontal axis depicts the retention time in minutes.

[0021] Figure 7 shows an analytical HPLC chromatogram of the compound NH_2-VC-PAB-Exatecan TFA salt.

[0022] Figure 8 shows an analytical HPLC chromatogram of the compound NH_2-VC-PAB-Exatecan TFA salt.

[0023] Figure 9 shows an analytical HPLC chromatogram of isomer A of the compound NH_2-VA-Exatecan.

[0024] Figure 10 shows an analytical HPLC chromatogram of isomer B of the compound NH_2-VA-Exatecan.

[0025] Figure 11 shows an analytical HPLC chromatogram of the compound P5(PEG2)-VC-PAB-Exatecan.

[0026] Figure 12 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VC-PAB-Exatecan.

[0027] Figure 13 shows an analytical HPLC chromatogram of the compound P5(PEG24)-VC-PAB-Exatecan.

[0028] Figure 14 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-PAB-Exatecan.

[0029] Figure 15 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-Exatecan from Isomer A.

[0030] Figure 16 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-Exatecan from Isomer B.

[0031] Figure 17 shows an analytical HPLC chromatogram of the compound P5(PEG12)-Exatecan.

[0032] Figure 18 shows an analytical SEC chromatogram of Trastuzumab. SEC means size-exclusion chromatography.

[0033] Figure 19 shows an analytical HIC chromatogram of Trastuzumab. HIC means hydrophobic interaction chromatography.

[0034] Figure 20 shows an analytical SEC chromatogram of Brentuximab.

[0035] Figure 21 shows an analytical HIC chromatogram of Brentuximab.

[0036] Figure 22 shows an analytical SEC chromatogram of Palivizumab.

[0037] Figure 23 shows an analytical HIC chromatogram of Palivizumab.

[0038] Figure 24 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VC-PAB-Exatecan.

[0039] Figure 25 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VC-PAB-Exatecan.

[0040] Figure 26 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan.

[0041] Figure 27 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan.

[0042] Figure 28 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[0043] Figure 29 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[0044] Figure 30 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[0045] Figure 31 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[0046] Figure 32 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer A).

[0047] Figure 33 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer A).

[0048] Figure 34 shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer B).

[0049] Figure 35 shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer B).

[0050] Figure 36 shows an analytical SEC chromatogram of Brentuximab-P5(PEG12)-VC-PAB-Exatecan.

[0051] Figure 37 shows an analytical HIC chromatogram of Brentuximab-P5(PEG12)-VC-PAB-Exatecan.

[0052] Figure 38 shows an analytical SEC chromatogram of Brentuximab-P5(PEG24)-VC-PAB-Exatecan.

[0053] Figure 39 shows an analytical HIC chromatogram of Brentuximab-P5(PEG24)-VC-PAB-Exatecan.

[0054] Figure 40 shows an analytical SEC chromatogram of Palivizumab-P5(PEG24)-VC-PAB-Exatecan.

[0055] Figure 41 shows an analytical HIC chromatogram of Palivizumab-P5(PEG24)-VC-PAB-Exatecan.

[0056] Figure 42 shows MS spectra of glycosylated, reduced Trastuzumab after the reaction with different equivalents of TCEP (top) and 15 eq. of linker-payload (P5(PEG24)-VC-PAB-Exatecan). Calculation of the DAR from those spectra in dependency on the TCEP amounts is shown in the bottom graph.

[0057] Figure 43 shows the In vitro cytoxicity of Trastuzumab (anti-Her2) ADCs linked to different Exatecan-based linker-payload constructs on antigen positive cell lines (HCC-78, top and SKBR3, bottom left) and an antigen negative cell line (MDA-MB-468, bottom right).

[0058] Figure 44 shows the in vitro cytoxicity of a Trastuzumab (anti-Her2) ADC (Tras-P5(PEG24)-VC-PAB-Exatecan) and a non-binding isotype control (Pali-P5(PEG24)-VC-PAB-Exatecan) on an antigen positive cell line (HCC-78).

[0059] Figure 45 shows the in vitro cytoxicity of a Brentuximab (anti-CD30) ADC (Bren-P5(PEG12)-VC-PAB-Exatecan) on two antigen positive cell lines (L-540, left and SU-DHL-1, right).

[0060] Figure 46 shows the in vitro cytoxicity of a Brentuximab (anti-CD30) ADC (Bren-P5(PEG24)-VC-PAB-Exatecan) on a panel of antigen positive cell lines (SR-786, SU-DHL-1, HH, HBLM-2, L-540, MOTN-1) and a non-targeted control cell line (HL-60).

[0061] Figure 47 shows the Evaluation of the bystander effect of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan in direct comparison to Enhertu. In vitro cytoxicity of the ADCs on an antigen positive cell line (SKBR3, top left) and an antigen negative cell line (MDA-MB-468, top right). Transfer of the supernatant after incubation of SKBR-3 with the ADCs to MDA-MB-468 has been performed in order to evaluate bystander killing (MDA-MB-468, bottom).

[0062] Figure 48 shows the relative quantification of Histone H2A.X phosphorylation (top left), activated Caspase 3 (top right) and activated PARP (bottom left) and cell viability (bottom right) after treatment of SKBR-3 cells with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan, Enhertu, unconjugated Exatecan or unconjugated Camptothecin after 1, 2 or 3 days versus untreated.

[0063] Figure 49 shows the drug to antibody ratio of Enhertu and P5(PEG24)-VC-PAB-Exatecan after incubation in rat serum at 37°C for 0, 1, 3 and 7 days. Drug to antibody ratio has been measured by MS after pulldown of the ADC from serum.

[0064] Figure 50 shows the cytotoxicity of the ADCs Trastuzumab-P5(PEG12)-VC-PAB-Exatecan (top), Trastuzumab-P5(PEG24)-VC-PAB-Exatecan (mid) and Enhertu (bottom) measured after 0,1,3 and 7 days of incubation with rat serum at 37°C on a Her2-negative cell line MDA-MB-468 (left) and a Her2-positive cell line SKBR3 (right).

[0065] Figure 51 shows the cytotoxicity of the ADCs Trastuzumab-P5(PEG12)-VC-PAB-Exatecan (top), Trastuzumab-P5(PEG24)-VC-PAB-Exatecan (mid) and Enhertu (bottom) measured after 0,1,3 and 7 days of incubation with human serum at 37°C on a Her2-negative cell line MDA-MB-468 (left) and a Her2-positive cell line SKBR3 (right).

[0066] Figure 52 shows the quantification of the amount of total antibody in blood circulation after treatment of female Spraque-Dawley rats with Brentuximab-P5(PEG12)-VC-PAB-Exatecan-DAR8 via ELISA.

[0067] Figure 53 shows melting curves of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan and Enhertu determined using nano differential scanning fluorimetry (nanoDSF).

[0068] Figure 54 shows a graph for determining the equilibrium binding constants (K_D) for the binding of Enhertu and Trastuzumab-P5(PEG24)-VC-PAB-Exatecan to extracellular Her2, and the obtained values for the equilibrium binding constants (K_D).

[0069] Figure 55 shows the percentage of aggregates formed when incubating ADC Trastuzumab-P5(PEG24)-VC-PAB-Exatecan having a drug to antibody ratio of 8 (denoted as "DAR8") and Enhertu at 37°C and 4°C in the dark after 0, 1, 2 and 4 weeks.

[0070] Figure 56 shows the percent specific killing measured in a calcein release-based antibody-dependent cellular cyctotoxicity (ADCC) assay with Her2-positive target cells SKBR-3, SKOV-3 and N87 when using unconjugated Trastuzumab, Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8, Enhertu and an isotype control.

[0071] Figure 57 shows the results of pHrodo-based investigation of internalization using unconjugated Trastuzumab, Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu with Her2-positive SKOV-3 cells and Her2-negative MDA-MB-468 cells.

[0072] Figure 58 shows the results of in vitro cytotoxicity measurements carried out using Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu with Her2-positive cells SKBR-3, N87, HCC-1569, HCC-78, OE-19, SK-GT-2 and SKOV-3.

[0073] Figure 59 shows the results for the in vitro bystander capacity measured after incubation with Her2-positive SKBR3 cells with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu, and supernatant transfer to Her2-negative cells Karpas-299 and DU-145.

[0074] Figure 60 shows the results of measurements of the in vitro bystander capacity of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu in a co-culture of Her2-positive SKBR-3 cells and Her2-negative MDA-MB-468 cells.

[0075] Figure 61 shows the results of cytotoxicity measurements carried out on human umbilical vein endothelial cells, human bronchial endothelial cells, liver sinusoidal endothelial cells, Schwann cells, human renal proximal tubular epithelial cells, normal human dermal fibroblasts, human corneal epithelial cells and THLE-3 (hepatocytes) using Tras-P5(PEG24)-VC-PAB-Exatecan DAR8, Enhertu and Palivizumab-P5(PEG24)-VC-PAB-Exatecan DAR8. Shown is the cyctotoxicity of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Palivizumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu.

[0076] Figure 62 shows the results of the in vivo pharmacokinetics experiments carried out with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 in female SCID mice that have been treated with 20 mg/kg of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 or Enhertu as reference.

[0077] Figure 63 shows the mean tumor volume of CB17-Scid mice determined in a solid tumor model after treatment with H8-P5(PEG24)-VC-PAB-Exatecan DAR8.

[0078] Figure 64 shows the body weight of CB17-Scid mice after treatment with H8-P5(PEG24)-VC-PAB-Exatecan DAR8.

[0079] Figure 65 shows the results of the in vivo pharmacokinetics experiments (PK-experiments) obtained in female SD rats that have been treated with 10 mg/kg of H8-P5(PEG24)-VC-PAB-Exatecan DAR8 or the unmodified H8 antibody.

[0080] Figure 66 shows the HIC and SEC chromatograms of Trastuzumab P5(PEG24)-VC-PAB-Exatecan DAR4 having an average DAR of 4.

[0081] Figure 67 shows the results of in vivo pharmacokinetics experiments (PK-experiments) obtained in female SD rats that have been treated with 10 mg/kg of Tras-P5(PEG24)-VC-PAB-Exatecan having an average DAR of 4.

[0082] Figure 68 shows results of an in vivo evaluation of trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 having a drug to antibody ratio of 8 (DAR8) in direct comparison to Enhertu. Reported are initial results after several days of observation of the tumor growth.

DETAILED DESCRIPTION

[0083] The present invention is described in detail in the following and will also be further illustrated by the appended examples and figures.

Definitions

[0084] Unless otherwise indicated, the term "alkyl" by itself or as part of another term in general refers to a substituted or unsubstituted straight chain or branched, saturated hydrocarbon having the indicated number of carbon atoms; e.g., "-(C_1-C_8)alkyl" or "-(C_1-C_{10})alkyl" refer to an alkyl group having from 1 to 8 or 1 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkyl group may have from 1 to 8 carbon atoms. Representative straight chain -(C_1-C_8)alkyl groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl and -n-octyl; branched -(C_1-C_8)alkyl groups include, but are not limited to, -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, and -2-methylbutyl. In some aspects, an alkyl group may be unsubstituted. Optionally, an alkyl group may be substituted, such as e.g. with one or more groups.

[0085] Unless otherwise indicated, the term "alkylene" by itself or as part of another term, in general refers to a substituted or unsubstituted branched or straight chain, saturated hydrocarbon radical of the stated number of carbon atoms, preferably 1-10 carbon atoms (-(C_1-C_{10})alkylene-) or preferably 1 to 8 carbon atoms (-(C_1-C_8)alkylene-), and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. When the number of carbon atoms is not indicated, the alkylene group may have from 1 to 8 carbon atoms. Typical alkylene radicals include, but are not limited to: methylene (-CH_2-), 1,2-ethylene (-CH_2CH_2-), 1,3-n-propylene (-$CH_2CH_2CH_2$-), and 1,4-n-butylene (-$CH_2CH_2CH_2CH_2$-). In some aspects, an alkylene group may be unsubstituted. Optionally, an alkylene group may be substituted, such as e.g. with one or more groups.

[0086] Unless otherwise indicated, the term "alkenyl" by itself or as part of another term in general refers to a substituted or unsubstituted straight chain or branched, unsaturated hydrocarbon having a double bond and the indicated number of carbon atoms; e.g., "-(C_2-C_8)alkenyl" or "-(C_2-C_{10})alkenyl" refer to an alkenyl group having from 2 to 8 or 2 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkenyl group may have from 2 to 8 carbon atoms. Representative -(C_2-C_8)alkenyl groups include, but are not limited to, -ethenyl, -1-propenyl, -2-propenyl, -1-butenyl, -2-butenyl, -isobutenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, and -2,3-dimethyl-2-butenyl.

In some aspects, an alkenyl group may be unsubstituted. Optionally, an alkenyl group may be substituted, such as e.g. with one or more groups.

[0087] Unless otherwise indicated, the term "alkenylene" by itself of as part of another term, in general refers to a substituted or unsubstituted unsaturated branched or straight chain hydrocarbon radical of the stated number of carbon atoms, preferably 2-10 carbon atoms (-(C_2-C_{10})alkenylene-) or preferably 2 to 8 carbon atoms (-(C_2-C_8)alkenylene-), and having a double bond, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. When the number of carbon atoms is not indicated, the alkenylene group may have from 2 to 8 carbon atoms. Typical alkenylene radicals include, but are not limited to: -ethenylene-, -1-propenylene-, 2-propenylene-, -1-butenylene-, -2-butenylene-, -isobutenylene-, -1-pentenylene-, -2-pentenylene-, -3-methyl-1-butenylene-, -2-methyl-2-butenylene-, and -2,3-dimethyl-2-butenylene-. In some aspects, an alkenylene group may be unsubstituted. Optionally, an alkenylene group may be substituted, such as e.g. with one or more groups.

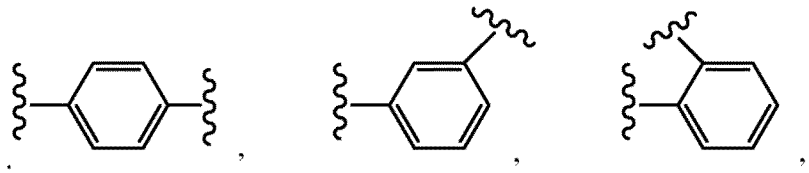
[0088] Unless otherwise indicated, the term "alkynyl" by itself or as part of another term in general refers to a substituted or unsubstituted straight chain or branched, unsaturated hydrocarbon having a triple bond and the indicated number of carbon atoms; e.g., "-(C_2-C_8)alkynyl" or "-(C_2-C_{10})alkynyl" refer to an alkynyl group having from 2 to 8 or 2 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkynyl group may have from 2 to 8 carbon atoms. Representative -(C_2-C_8)alkynyl groups include, but are not limited to, -acetylenyl, -1-propynyl, -2-propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl and -3-methyl-1-butynyl. In some aspects, an alkynyl group may be unsubstituted. Optionally, an alkynyl group may be substituted, such as e.g. with one or more groups.

[0089] Unless otherwise indicated, the term "alkynylene" by itself of as part of another term, in general refers to a substituted or unsubstituted, branched or straight chain, unsaturated hydrocarbon radical of the stated number of carbon atoms, preferably 2-10 carbon atoms (-(C_2-C_{10})alkynylene-) or preferably 2 to 8 carbon atoms (-(C_2-C_8)alkynylene-), and having a triple bond, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. When the number of carbon atoms is not indicated, the alkynylene group may have from 2 to 8 carbon atoms. Typical alkynylene radicals include, but are not limited to: -ethynylene-, -1-propynylene-, -2-propynylene-, -1-butynylene-, -2-butynylene-, -1-pentynylene-, -2-pentynylene- and -3-methyl-1-butynylene-. In some aspects, an alkynylene group may be

unsubstituted. Optionally, an alkynylene group may be substituted, such as e.g. with one or more groups.

[0090] Unless otherwise indicated, the term "aryl," by itself or as part of another term, in general means a substituted or unsubstituted monovalent carbocyclic aromatic hydrocarbon radical of 6 to 20 carbon atoms (preferably 6 to 14 carbon atoms, more preferably 6 to 10 carbon atoms, in very preferred embodiments 6 carbon atoms) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Some aryl groups are represented in the exemplary structures as "Ar". Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, and biphenyl. An exemplary aryl group is a phenyl group. In some aspects, an aryl group may be unsubstituted. Optionally, an aryl group may be substituted, such as e.g. with one or more groups.

[0091] Unless otherwise indicated, the term "arylene", by itself or as part of another term, in general is an aryl group as defined above wherein one of the hydrogen atoms of the aryl group is replaced with a bond (i.e., it is divalent) and can be in the para, meta, or ortho orientations as shown in the following structures, with phenyl as the exemplary group:



In selected embodiments, e.g., when a parallel connector unit comprises an arylene, the arylene is an aryl group as defined above wherein two or more of the hydrogen atoms of the aryl group are replaced with a bond (i.e., the arylene can be trivalent). In some aspects, an arylene group may be unsubstituted. Optionally, an alkynylene group may be substituted, such as e.g. with one or more groups.

[0092] Unless otherwise indicated, the term "heterocycle" or “heterocyclic ring”, by itself or as part of another term, in general refers to a monovalent substituted or unsubstituted aromatic or non-aromatic monocyclic or bicyclic ring system having the indicated number of carbon atoms (e.g., “(C_3-C_8)heterocycle” or “(C_3-C_{10})heterocycle” refer to a heterocycle having from 3 to 8 or from 3 to 10 carbon atoms, respectively) and one to four heteroatom ring members independently selected from N, O, P or S, and derived by removal of one hydrogen atom

from a ring atom of a parent ring system. One or more N, C or S atoms in the heterocycle can be oxidized. The ring that includes the heteroatom can be aromatic or nonaromatic. Unless otherwise noted, the heterocycle is attached to its pendant group at any heteroatom or carbon atom that results in a stable structure. Representative examples of a (C_3-C_8)heterocycle include, but are not limited to, pyrrolidinyl, azetidinyl, piperidinyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, pyrrolyl, thiophenyl (thiophene), furanyl, thiazolyl, imidazolyl, pyrazolyl, pyrimidinyl, pyridinyl, pyrazinyl, pyridazinyl, isothiazolyl, and isoxazolyl. In some aspects, a heterocycle group may be unsubstituted. Optionally, a heterocycle group may be substituted, such as e.g. with one or more groups.

[0093] Unless otherwise indicated, the term "heterocyclo" or “heterocyclic ring”, by itself or as part of another term, in general refers to a heterocycle group as defined above and having the indicated number of carbon atoms (e.g., (C_3-C_8)heterocycle or (C_3-C_{10})heterocycle) wherein one of the hydrogen atoms of the heterocycle group is replaced with a bond (i.e., it is divalent). In selected embodiments, e.g., when a parallel connector unit comprises a heterocyclo, the heterocyclo is a heterocycle group as defined above wherein two or more of the hydrogen atoms of the heterocycle group are replaced with a bond (i.e., the heterocyclo can be trivalent). In some aspects, a heterocyclo or heterocyclic ring may be unsubstituted. Optionally, a heterocyclo or heterocyclic ring may be substituted, such as e.g. with one or more groups.

[0094] Unless otherwise indicated, the term "carbocycle" or “carbocyclic ring” by itself or as part of another term, in general refers to a monovalent, substituted or unsubstituted aromatic or non-aromatic monocyclic or bicyclic carbocyclic ring system having the indicated number of carbon atoms (e.g., “(C_3-C_8)carbocycle” or “(C_3-C_{10})carbocycle” refer to a carbocycle having from 3 to 8 or from 3 to 10 carbon atoms, respectively) derived by the removal of one hydrogen atom from a ring atom of a parent ring system. As illustrative but non-limiting examples the carbocycle may be a 3-, 4-, 5-, 6-, 7- or 8-membered carbocycle. Representative (C_3-C_8)carbocycles include, but are not limited to, phenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, cycloheptyl, 1,3- cycloheptadienyl, 1,3,5-cycloheptatrienyl, cyclooctyl, and cyclooctadienyl. In some aspects, a carbocycle may be unsubstituted. Optionally, a carbocycle may be substituted, such as e.g. with one or more groups.

[0095] Unless otherwise indicated, the term "carbocyclo" or “carbocyclic ring”, by itself or as part of another term, in general refers to a carbocycle group as defined above having the indicated number of carbon atoms (e.g., “(C_3-C_8)carbocyclo” or “(C_3-C_{10})carbocyclo” refer to a carbocyclo or carbocyclic ring having from 3 to 8 or from 3 to 10 carbon atoms, respectively), wherein another of the hydrogen atoms of the carbocycle groups is replaced with a bond (i.e., it is divalent). In selected embodiments, e.g., when a parallel connector unit comprises a carbocyclo or carbocyclic ring, the carbocyclo or carbocyclic ring is a carbocycle group as defined above, wherein two or more of the hydrogen atoms of the carbocycle group are replaced with a bond (i.e., the carbocyclo or carbocyclic ring can be trivalent). In some aspects, a carbocyclo or carbocyclic ring may be unsubstituted. Optionally, a heterocyclo or heterocyclic ring may be substituted, such as e.g. with one or more groups.

[0096] Unless otherwise indicated, the term "heteroalkyl", by itself or in combination with another term, may mean, unless otherwise stated, a stable straight or branched chain hydrocarbon, or combinations thereof, fully saturated or containing from 1 to 3 degrees of unsaturation, consisting of the stated number of carbon atoms (e.g., (C_1-C_8)heteroalkyl or (C_1-C_{10})heteroalkyl) and from one to ten, preferably one to three, heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include -CH_2-CH_2-O-CH_3, -CH_2-CH_2-NH-CH_3, -CH_2-CH_2-N(CH_3)-CH_3, -CH_2-S-CH_2-CH_3, -CH_2-CH_2-S(O)-CH_3, -NH-CH_2-CH_2-NH-C(O)-CH_2-CH_3, -CH_2-CH_2-S(O)$_2$-CH_3, -CH=CH-O-CH_3, -Si(CH_3)$_3$, -CH_2-CH=N-O-CH_3, and -CH=CH-N(CH_3)-CH_3. Up to two heteroatoms may be consecutive, such as, for example, -CH_2-NH-OCH_3 and -CH_2-O-Si(CH_3)$_3$. In preferred embodiments, a (C_1-C_4)heteroalkyl or heteroalkylene has 1 to 4 carbon atoms and 1 or 2 heteroatoms and a (C_1-C_3)heteroalkyl or heteroalkylene has 1 to 3 carbon atoms and 1 or 2 heteroatoms. In some aspects, a heteroalkyl or heteroalkylene is saturated. In some aspects, a heteroalkyl or heteroalkylene may be unsubstituted. Optionally, a heteroalkyl or heteroalkylene may be substituted, such as e.g. with one or more groups.

[0097] Unless otherwise indicated, the term "heteroalkylene" by itself or as part of another substituent means a divalent group derived from heteroalkyl (as described above) having the indicated number of carbon atoms (e.g., (C_1-C_8)heteroalkylene or (C_1-C_{10})heteroalkylene), as

exemplified by -CH_2-CH_2-S-CH_2-CH_2- and -CH_2-S-CH_2-CH_2-NH-CH_2-. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini. Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied. In selected embodiments, e.g., when a parallel connector unit comprises a heteroalkylene, the heteroalkylene is a heteroalkyl group defined above wherein two or more of the hydrogen atoms of the heteroalkyl group are replaced with a bond (i.e., the heteroalkylene can be trivalent). In some aspects, a heteroalkyl or heteroalkylene may be saturated. In some aspects, a heteroalkylene is unsubstituted. Optionally, a heteroalkylene may be substituted, such as e.g. with one or more groups.

[0098] The term "halogen", unless defined otherwise, in general refers to elements of the 7th main group; preferably fluorine, chlorine, bromine and iodine; more preferably fluorine, chlorine and bromine; even more preferably, fluorine and chlorine.

[0099] The term "substituted", "optionally substituted", "optionally may be substituted" or the like, unless otherwise indicated, in general means that one or more hydrogen atoms can be each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O^-, -OR, -SR, -S^-, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, -NRC(=O)R, -C(=O)R, -C(=O)NR_2, -SO_3^-, -SO_3H, -S(=O)$_2$R, -OS(=O)$_2$OR, -S(=O)$_2$NR, -S(=O)R, -OP(=O)(OR)$_2$, -P(=O)(OR)$_2$, -PO_4^{3-}, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR_2, -C(=S)NR_2, or -C(=NR)NR_2, where each X is independently a halogen: -F, -Cl, -Br, or -I; and each R is independently -H, -(C_1-C_{20})alkyl (such as e.g. -(C_1-C_{10})alkyl or -(C_1-C_8)alkyl), -(C_6-C_{20})aryl, (such as e.g. -(C_6-C_{10})aryl or, preferably, -C_6-aryl), -(C_3-C_{14})heterocycle (such as e.g. -(C_3-C_{10})heterocycle or -(C_3-C_8)heterocycle), a protecting group, or a prodrug moiety. Typical substituents also include (=O).

[00100] The term "aliphatic or aromatic residue", as used herein, in general refers to an aliphatic substituent, such as e.g. but not limited to an alkyl residue, which, however, can be optionally substituted by further aliphatic and/or aromatic substituents. As non-limiting examples an aliphatic residue can be a nucleic acid, an enzyme, a co-enzyme, a nucleotide, an oligonucleotide, a monosaccharide, a polysaccharide, a polymer, a fluorophore, optionally substituted benzene, etc., as long as the direct link of such a molecule to the core structure (in case of R^1, e.g., the link to the oxygen atom bound to the phosphorus) is aliphatic. An aromatic residue is a substituent, wherein the direct link to the core structure is part of an aromatic system, e.g., an optionally substituted phenyl or triazolyl or pyridyl or nucleotide; as

non-limiting example if the direct link of the nucleotide to the core structure is for example via a phenyl-residue. The term “aromatic residue”, as used herein, also includes a heteroaromatic residue.

[00101] The term “peptide”, unless otherwise indicated, in general refers to an organic compound comprising two or more amino acids covalently joined by peptide bonds (amide bond). Peptides may be referred to with respect to the number of constituent amino acids, i.e., a dipeptide contains two amino acid residues, a tripeptide contains three, etc. Peptides containing ten or fewer amino acids may be referred to as oligopeptides, while those with more than ten amino acid residues, e.g. with up to about 30 amino acid residues, are polypeptides.

[00102] The term “amino acid”, as used herein, in general refers to an organic compound having a -CH(NH_3)-COOH group. In one embodiment, the term “amino acid” refers to a naturally occurring amino acid. As illustrative examples, naturally occurring amino acids include arginine, lysine, aspartic acid, glutamic acid, glutamine, asparagine, histidine, serine, threonine, tyrosine, cysteine, methionine, tryptophan, alanine, isoleucine, leucine, phenylalanine, valine, proline and glycine. However, the term in its broader meaning also encompasses non-naturally occurring amino acids.

[00103] Amino acids and peptides according to the disclosure can also be modified at functional groups. Non limiting examples are saccharides, e.g., N-Acetylgalactosamine (GalNAc), or protecting groups, e.g., Fluorenylmethoxycarbonyl (Fmoc)-modifications or esters.

[00104] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules, preferably comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains which are typically inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region can comprise e.g. three domains CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is

typically composed of three CDRs and up to four FRs arranged from amino-terminus to carboxy-terminus e.g. in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00105] As used herein, the term "Complementarity Determining Regions" (CDRs; e.g., CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable domain the presence of which are necessary for antigen binding. Each variable domain typically has three CDR regions identified as CDR1, CDR2 and CDR3. Each complementarity determining region may comprise amino acid residues from a "complementarity determining region" as defined by Kabat (e.g. about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; and/or those residues from a "hypervariable loop" (e.g. about residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26- 32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain). In some instances, a complementarity determining region can include amino acids from both a CDR region defined according to Kabat and a hypervariable loop.

[00106] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes". There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these maybe further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. A preferred class of immunoglobulins for use in the present invention is IgG.

[00107] The heavy-chain constant domains that correspond to the different classes of antibodies are called [alpha], [delta], [epsilon], [gamma], and [mu], respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. As used herein antibodies are conventionally known antibodies and functional fragments thereof.

[00108] A “functional fragment”, or “antigen-binding antibody fragment” of an antibody/immunoglobulin, or “antigen-binding fragment of an antibody”, or an “antibody fragment”, or a “fragment of an antibody” in general relates to a fragment of an antibody/immunoglobulin (e.g., a variable region of an IgG) that retains the antigen-binding region. An “antigen-binding region” of an antibody typically is found in one or more hyper variable region(s) of an antibody, e.g., the CDR1, -2, and/or –3 regions; however, the variable “framework” regions can also play an important role in antigen binding, such as by providing a scaffold for the CDRs. Preferably, the “antigen-binding region” comprises at least

amino acid residues 4 to 103 of the variable light (VL) chain and 5 to 109 of the variable heavy (VH) chain, more preferably amino acid residues 3 to 107 of VL and 4 to 111 of VH, and particularly preferred are the complete VL and VH chains (amino acid positions 1 to 109 of VL and 1 to 113 of VH; numbering according to WO 97/08320).

[00109] “Functional fragments”, “antigen-binding antibody fragments”, “antigen-binding fragments of an antibody”, or “antibody fragments” or “fragments of an antibody” of the disclosure may include, but are not limited to, those which contain at least one disulfide bond that can be reacted with a reducing agent as described herein. Examples of suitable fragments include Fab, Fab', Fab'-SH, $F(ab')_2$, and Fv fragments; diabodies; single domain antibodies (DAbs), linear antibodies; single-chain antibody molecules (scFv); and multispecific, such as bi- and tri-specific, antibodies formed from antibody fragments. An antibody other than a "multi-specific" or "multi-functional" antibody is understood to have each of its binding sites identical. The $F(ab')_2$ or Fab may be engineered to minimize or completely remove the intermolecular disulfide interactions that occur between the CH1 and CL domains.

[00110] The term "Fc region" herein is in general used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index.

[00111] Variants of the antibodies or antigen-binding antibody fragments contemplated herein are molecules in which the binding activity of the antibody or antigen-binding antibody fragment is maintained.

[00112] “Binding proteins” or “proteinaceous binding molecules with antibody-like binding properties”, as used herein, are generally known to a person skilled in the art. Illustrative, non-limiting examples include affibodies, adnectins, anticalins, DARPins, and avimers.

[00113] A “human” antibody or antigen-binding fragment thereof is in general defined as one that is not chimeric (e.g., not “humanized”) and not from (either in whole or in part) a non-human species. A human antibody or antigen-binding fragment thereof can be derived from a human or can be a synthetic human antibody. A “synthetic human antibody” is defined herein as an antibody having a sequence derived, in whole or in part, in silico from synthetic sequences that are based on the analysis of known human antibody sequences. In silico design of a human antibody sequence or fragment thereof can be achieved, for example, by analyzing a database of human antibody or antibody fragment sequences and devising a polypeptide sequence utilizing the data obtained there from. Another example of a human antibody or antigen-binding fragment thereof is one that is encoded by a nucleic acid isolated from a library of antibody sequences of human origin (e.g., such library being based on antibodies taken from a human natural source).

[00114] A “humanized antibody” or humanized antigen-binding fragment thereof is in general defined herein as one that is (i) derived from a non-human source (e.g., a transgenic mouse which bears a heterologous immune system), which antibody is based on a human germline sequence; (ii) where amino acids of the framework regions of a non-human antibody are partially exchanged to human amino acid sequences by genetic engineering or (iii) CDR-grafted, wherein the CDRs of the variable domain are from a non-human origin, while one or more frameworks of the variable domain are of human origin and the constant domain (if any) is of human origin.

[00115] A “chimeric antibody” or antigen-binding fragment thereof is in general defined herein as one, wherein the variable domains are derived from a non-human origin and some or all constant domains are derived from a human origin.

[00116] The term "monoclonal antibody" as used herein in general refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the term "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. The term "monoclonal” is not to be

construed as to require production of the antibody by any particular method. The term monoclonal antibody specifically includes chimeric, humanized and human antibodies.

[00117] An "isolated" antibody is in general one that has been identified and separated from a component of the cell that expressed it. Contaminant components of the cell are materials that would interfere with diagnostic or therapeutic uses of the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes.

[00118] As used herein, an antibody “binds specifically to”, is “specific to/for” or “specifically recognizes” an antigen of interest, e.g. a tumor-associated polypeptide antigen target, is in general one that binds the antigen with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting a cell or tissue expressing the antigen, and does not significantly cross-react with other proteins or does not significantly cross-react with proteins other than orthologs and variants (e.g. mutant forms, splice variants, or proteolytically truncated forms) of the aforementioned antigen target. The term "specifically recognizes" or "binds specifically to" or is "specific to/for" a particular polypeptide or an epitope on a particular polypeptide target as used herein can be exhibited, for example, by an antibody, or antigen-binding fragment thereof, having a monovalent K_D for the antigen of less than about 10^{-4} M, alternatively less than about 10^{-5} M, alternatively less than about 10^{-6} M, alternatively less than about 10^{-7} M, alternatively less than about 10^{-8} M, alternatively less than about 10^{-9} M, alternatively less than about 10^{-10} M, alternatively less than about 10^{-11} M, alternatively less than about 10^{-12} M, or less. An antibody “binds specifically to,” is “specific to/for” or “specifically recognizes” an antigen if such antibody is able to discriminate between such antigen and one or more reference antigen(s). In its most general form, “specific binding”, “binds specifically to”, is “specific to/for” or “specifically recognizes” is referring to the ability of the antibody to discriminate between the antigen of interest and an unrelated antigen, as determined, for example, in accordance with one of the following methods. Such methods comprise, but are not limited to surface plasmon resonance (SPR), Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogen peroxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. This means the difference positive/negative is more than 5-fold, 10-fold, 50-fold, and preferably more than 100-fold. Typically, determination of binding specificity is performed by

using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like.

[00119] "Binding affinity" or “affinity” in general refers to the strength of the total sum of non-covalent interactions between a single binding site of a molecule and its binding partner. Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1 : 1 interaction between members of a binding pair (e.g. an antibody and an antigen). The dissociation constant “K_D” is commonly used to describe the affinity between a molecule (such as an antibody) and its binding partner (such as an antigen) i.e. how tightly a ligand binds to a particular protein. Ligand-protein affinities are influenced by non-covalent intermolecular interactions between the two molecules. Affinity can be measured by common methods known in the art, including those described herein. In one embodiment, the "K_D" or "K_D value" according to this invention is measured by using surface plasmon resonance assays using suitable devices including but not limited to Biacore instruments like Biacore T100, Biacore T200, Biacore 2000, Biacore 4000, a Biacore 3000 (GE Healthcare Biacore, Inc.), or a ProteOn XPR36 instrument (Bio-Rad Laboratories, Inc.).

[00120] The term “antibody drug conjugate” or abbreviated ADC is well known to a person skilled in the art, and, as used herein, in general refers to the linkage of an antibody or an antigen binding fragment thereof with a drug, such as a chemotherapeutic agent, a toxin, an immunotherapeutic agent, an imaging probe, and the like.

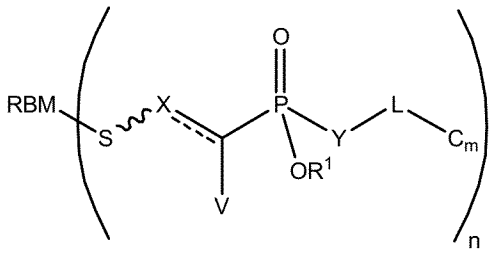
[00121] The present disclosure also relates to a “pharmaceutically acceptable salt”. Any pharmaceutically acceptable salt can be used. In particular, the term “pharmaceutically acceptable salt” refers to a salt of a conjugate or compound of the invention that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts have low toxicity and may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include, but are not limited to: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-

methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, purely by way of example, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of nontoxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. A counterion or anionic counterion can be used in a quaternary amine to maintain electronic neutrality. Exemplary counterions include halide ions (e.g., F^-, Cl^-, Br^-, I^-), NO_3^-, ClO_4^-, OH^-, $H_2PO_4^-$, HSO_4^-, sulfonate ions (*e.g.,* methanesulfonate, trifluoromethanesulfonate, p–toluenesulfonate, benzenesulfonate, 10–camphor sulfonate, naphthalene–2–sulfonate, naphthalene–1–sulfonic acid–5–sulfonate, and the like), and carboxylate ions (e.g., acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).

[00122] As used herein, the term “solvate” may refer to an aggregate that comprises one or more molecules of a conjugate or compound described herein with one or more molecules of solvent. The solvent may be water, in which case the solvate may be a hydrate. Alternatively, the solvent may be an organic solvent. Thus, the conjugates or compounds of the present disclosure may exist as a hydrate, including a monohydrate, dihydrate, hemihydrate, sesquihydrate, trihydrate, tetrahydrate and the like, as well as the corresponding solvated forms. The compounds of the invention may be true solvates, while in other cases, the compounds of the invention may merely retain adventitious water or be a mixture of water plus some adventitious solvent.

Conjugate of Formula (I)

[00123] As set out above, the present invention relates to a conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

RBM is a receptor binding molecule;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

[00124] Conjugates of formula (I) comprise a receptor binding molecule which is connected to a camptothecin moiety via a phosphorus (V) moiety (the phosphorus (V) moiety sometimes is also denoted as "P5") and a linker. It has been found that conjugates of formula (I) have numerous advantages, as shown in the following.

[00125] As an initial advantage, the conjugates of formula (I) exhibit good hydrophilicity and show low aggregation in solution as well as during the conjugation process to the antibody, exemplified by high yields of the ADCs with no or only minor aggregates being formed (**Example 2** and **Figures 18 to 41**). Further, conjugates of formula (I) show a good cytotoxicity, which is selective for the cell line which is targeted b the antibody. The selectivity exceeds the one of the commercial product Enhertu (**Example 4** and **Figures 43 to 46**). Conjugates of formula (I) also show a favorable bystander effect, which is equal to or even better compared to Enhertu (**Example 5** and **Figure 47**, as well as **Example 16** and **Figure 59**, and **Example 17** and **Figure 60**). Also, conjugates of formula (I) show good DNA damage in cancer cells (**Example 6** and **Figure 48**). In particular, conjugates of formula (I) show an excellent serum stability, which exceeds the stability of the commercial product Enhertu (**Example 7** and **Figure 49**). As another advantage compared to Enhertu, conjugates of formula (I) maintain their efficacy and selectivity after incubation for certain times in human and rodent sera in vitro (**Example 8** and Figure **50** and **51**). This effect, in combination with the excellent stability in presence of serum, may be helpful to reduce side effects during treatment of a patient with the conjugate. Conjugates of formula (I) also show favorable pharmacokinetic properties in vivo (**Example 9** and **Figure 52**, and **Example 23** and **Figure 67**). In particular, in vivo pharmacokinetic experiments carried out with conjugates of formula (I) have demonstrated similar clearance compared to Enhertu. Further, pharmacokinetic experiments have demonstrated, as further advantage, that conjugates of formula (I) exhibit a significantly higher stability compared to Enhertu in vivo (**Example 19** and **Figure 62**). Further in vivo pharmacokinetic experiments have revealed that conjugates of formula (I) clear with kinetics very similar to the unmodified antibody, even at high load of the camptothecin drug; also, long-term stability of conjugates of formula (I) during circulation in vivo has been demonstrated (**Example 21** and **Figure 65**). Conjugates of formula (I) also have similar thermal stability compared to Enhertu (**Example 10** and **Figure 53**). Also, conjugates of formula (I) and Enhertu exhibit similar binding properties to the extracellular target (**Example 11** and **Figure 54**). As a further advantage, conjugates of formula (I) also show reduced aggregation compared to Enhertu over an incubation time of up to several weeks in aqueous medium (**Example 12** and **Figure 55)**. Conjugates of formula (I), as another advantage, also show enhanced antibody-dependent cellular cytotoxicity (ADCC)

compared to Enhertu (**Example 13** and **Figure 56**). The inventors have further observed that conjugates of formula (I), compared to Enhertu, exhibit similar internalization into target-positive (Her2+) cells, while undesired internalization into target-negative cells is decreased (**Example 14** and **Figure 57**). Conjugates of formula (I) also show better in vitro efficacy compared to Enhertu for cell lines which do not highly overexpress the target (**Example 16** and **Figure 58**). Accordingly, the conjugates described herein have improved properties compared to Enhertu in terms of efficacy, in particular in cells with low expression of the target. The inventors have also found that conjugates of formula (I), as an advantage, exhibit less undesired toxicity towards different cells of healthy human tissue when compared to Enhertu (**Example 18** and **Figure 61**). Excellent efficacy of conjugates of formula (I) for the treatment of tumors has been demonstrated in vivo (**Example 20**, **Figure 63** and **Figure 64**) In particular, dose dependency and superior in vivo efficacy over Enhertu has been demonstrated (**Example 24** and **Figure 68**). Also, conjugates of formula (I) can be prepared with various ratios of the camptothecin moiety to the receptor binding molecule (see **Examples 2** and **3** and **Figure 42**, and **Example 22** and **Figure 66**). In sum, the inventors have surprisingly found that conjugates of formula (I) exhibit excellent properties which make them useful as pharmaceuticals, including an enhanced serum stability and other advantages such as, e.g., a favorable bystander effect, good pharmacokinetic properties in vivo, long-term stability in vivo, reduced aggregation, enhanced ADCC, decreased undesired internalization into target-negative cells, better efficacy for cell lines with low expression of the target, reduced undesired toxicity towards cells of healthy human tissue, and excellent efficacy for the treatment of tumors in vivo. It is noted that conjugates, which comprise a phosphorus (V) moiety, are e.g. described in WO 2018/041985 A1 and WO 2019/170710, which are hereby incorporated by reference.

[00126] Preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H. Preferably R^4, when present is H or (C_1-C_8)alkyl; more preferably R^4, when present, is H. Preferably R^5, when present is H or (C_1-C_8)alkyl; more preferably R^5, when present, is H. Preferably R^6, when present is H or (C_1-C_8)alkyl; more preferably R^6, when present, is H. Preferably R^7, when present is H or (C_1-C_8)alkyl; more preferably R^7, when present, is H.

[00127] Preferably,  is a double bond; V is absent; X is R_3-C; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H.

[00128] More preferably,  represents a double bond; V is absent; X represents R_3-C, and R_3 represents H or $(C_1\text{-}C_8)$alkyl. Preferably, R^3 represents H or $(C_1\text{-}C_6)$alkyl, more preferably H or $(C_1\text{-}C_4)$alkyl, still more preferably H or $(C_1\text{-}C_2)$alkyl. In preferred embodiments, R_3 is H.

[00129] In some embodiments,  may be a bond; V is H or $(C_1\text{-}C_8)$alkyl, preferably V is H; X is $R_3-\overset{R_4}{\underset{|}{C}}$; R_3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; more preferably R^3 is H or $(C_1\text{-}C_8)$alkyl, more preferably R^3 is H; R^4 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably, R^4 is H or $(C_1\text{-}C_8)$alkyl, preferably R^4 is H.

[00130] In some embodiments,  may represent a bond; V may be H or $(C_1\text{-}C_8)$alkyl; X may represent $R_3-\overset{R_4}{\underset{|}{C}}$; and R_3 and R_4 may independently represent H or $(C_1\text{-}C_8)$alkyl. Preferably, R_3 and R_4 independently represent H or $(C_1\text{-}C_6)$alkyl, more preferably H or $(C_1\text{-}C_4)$alkyl, still more preferably H or $(C_1\text{-}C_2)$alkyl. Preferably, R_3 and R_4 are the same; even more preferably, R_3, R_4 and V are the same. More preferably, R_3 and R_4 are both H. Preferably, V is H or $(C_1\text{-}C_6)$alkyl, more preferably H or $(C_1\text{-}C_4)$alkyl, still more preferably H or $(C_1\text{-}C_2)$alkyl. Even more preferably, V is H. In preferred embodiments, R_3, R_4 and V are each H.

[00131] The integer m ranges from 1 to 10. Accordingly, the integer m may be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Preferably, the integer m ranges from 1 to 4. More preferably, the integer m is 1 or 2. Even more preferably, the integer m is 1.

[00132] The integer n ranges from 1 to 20. Accordingly, the integer n may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Preferably, the integer n ranges from 1 to 10. More preferably, the integer n ranges from 2 to 10. Still more preferably, the integer n ranges from 4 to 10. Still more preferably, the integer n ranges from 6 to 10. Still more preferably, the integer n is 6, 7, 8, 9 or 10. Still more preferably, the integer n ranges from 7 to 10. Still more preferably, the integer n is 7, 8 or 9. Still more preferably, the integer n is 7 or 8. Even more preferably, the integer n is 8.

[00133] The integer n ranges from 1 to 20. Preferably, the integer n ranges from 1 to 10. More preferably, the integer n ranges from 2 to 8. Still more preferably, the integer n is 2, 3, 4, 5 or 6. Still more preferably, the integer n ranges from 3 to 6. Still more preferably, the integer n is 3, 4 or 5. Still more preferably, the integer n is 4 or 5. Even more preferably, the integer n is 4.

[00134] Preferably, m is an integer ranging from 1 to 4, more preferably 1 or 2, still more preferably 1; and preferably n is an integer ranging from 1 to 20, more preferably from 1 to 10, still more preferably from 2 to 10, still more preferably from 4 to 10, still more preferably from 6 to 10, still more preferably n is 6, 7, 8, 9 or 10, still more preferably n ranges from from 7 to 10, still more preferably n is 7, 8 or 9, still more preferably n is 7 or 8, even more preferably n is 8.

[00135] Preferably, m is an integer ranging from 1 to 4, preferably 1 or 2, more preferably 1; and preferably n is an integer ranging from 1 to 20, more preferably from 1 to 10, still more preferably from 2 to 8; still more preferably n is 2, 3, 4, 5 or 6; still more preferably n ranges from 3 to 6; still more preferably n is 3, 4 or 5; still more preferably n is 4 or 5, even more preferably n is 4.

[00136] Preferably, m is 1; and preferably n is an integer ranging from 1 to 20, more preferably from 1 to 10, still more preferably from 2 to 10, still more preferably from 4 to 10, still more preferably from 6 to 10, still more preferably n is 6, 7, 8, 9 or 10, still more preferably n ranges from 7 to 10, still more preferably n is 7, 8 or 9, still more preferably n is 7 or 8, even more preferably n is 8. Accordingly, preferably, m is 1 and n is an integer ranging from 1 to 20. More preferably, m is 1 and n is an integer ranging from 1 to 10. Still more preferably, m is 1 and n is an integer ranging from 2 to 10. Still more preferably, m is 1 and n is an integer ranging from 4 to 10. Still more preferably, m is 1 and n is an integer ranging from 6 to 10. Still more preferably, m is 1 and n is 6, 7, 8, 9 or 10. Still more preferably, m is 1 and n is an integer ranging from 7 to 10. Still more preferably, m is 1 and n is 7, 8 or 9. Still more preferably, m is 1 and n is 7 or 8. Even more preferably, m is 1 and n is 8.

[00137] Preferably, m is 1; and preferably n is an integer ranging from 1 to 20, more preferably from 1 to 10, still more preferably from 2 to 8; still more preferably n is 2, 3, 4, 5 or 6, still more preferably n ranges from 3 to 6; still more preferably n is 3, 4 or 5; still more

preferably n is 4 or 5, even more preferably n is 4. Accordingly, preferably, m is 1 and n is an integer ranging from 1 to 20. More preferably, m is 1 and n is an integer ranging from 1 to 10. Still more preferably, m is 1 and n is an integer ranging from 2 to 8. Still more preferably, m is 1 and n is 2, 3, 4, 5 or 6. Still more preferably, m is 1 and n ranges from 3 to 6. Still more preferably, m is 1 and n is 3, 4 or 5. Still more preferably, m is 1 and n is 4 or 5. Even more preferably, m is 1 and n is 4.

[00138] In some embodiments, the number of camptothecin moieties C per receptor binding molecule may be from 1 to 20. Preferably, the number of camptothecin moieties C per receptor binding molecule is from 1 to 14. More preferably, the number of camptothecin moieties C per receptor binding molecule is from 2 to 14. More preferably, the number of camptothecin moieties C per receptor binding molecule is from 4 to 14. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 5 to 12. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 6 to 12. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 7 to 10. Even more preferably, the number of camptothecin moieties C per receptor binding molecule is 8.

[00139] In some embodiments, the number of camptothecin moieties C per receptor binding molecule may be from 1 to 20. Preferably, the number of camptothecin moieties C per receptor binding molecule is from 1 to 14. More preferably, the number of camptothecin moieties C per receptor binding molecule is from 1 to 12. More preferably, the number of camptothecin moieties C per receptor binding molecule is from 2 to 10. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 2 to 8. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 2 to 6. Still more preferably, the number of camptothecin moieties C per receptor binding molecule is from 3 to 5. Even more preferably, the number of camptothecin moieties C per receptor binding molecule is 4.

Receptor Binding Molecule (RBM)

[00140] RBM is a receptor binding molecule. The term “receptor binding molecule” in general refers to any molecule which is capable to bind to a receptor. As illustrative but non-limiting example, the receptor, to which a receptor binding molecule may bind, may be expressed on a cell surface. As illustrative but non-limiting example, the cell which expresses the receptor, may be a cancer cell. A person skilled in the art knows to select a suitable receptor binding molecule.

[00141] The receptor may be a tumor associated surface antigen. Accordingly, the receptor binding molecule may be capable to specifically bind to a tumour associated surface antigen. The term “tumour associated surface antigen” as used herein in general refers to an antigen that is or can be presented on a surface that is located on or within tumour cells. These antigens can be presented on the cell surface with an extracellular part, which is often combined with a transmembrane and cytoplasmic part of the molecule. These antigens can in some embodiments be presented only by tumour cells and not by normal, i.e. non-tumour cells. Tumour antigens can be exclusively expressed on tumour cells or may represent a tumour specific mutation compared to non-tumour cells. In such an embodiment a respective antigen may be referred to as a tumour-specific antigen. Some antigens are presented by both tumour cells and non-tumour cells, which may be referred to as tumour-associated antigens. These tumour-associated antigens can be overexpressed on tumour cells when compared to non-tumour cells or are accessible for antibody binding in tumour cells due to the less compact structure of the tumour tissue compared to non-tumour tissue. In some embodiments the tumour associated surface antigen is located on the vasculature of a tumour. Illustrative but non-limiting examples of a tumour associated surface antigen include CD19, CD30, Her2 or PMSA. Tumor associated surface antigens, are known to a person skilled in the art. In particular, those which have been found useful for the development of ADCs are described, e.g., in the review article of Criscitello et al., *“Antibody-drug conjugates in solid tumors: a look into novel targets”*, Journal of Hematology and Oncology, (2021) 14:20 (https://doi.org/10.1186/s13045-021-01035-z).

[00142] The receptor binding molecule may be selected from the group consisting of an antibody, an antibody fragment, and a proteinaceous binding molecule with antibody-like binding properties.

[00143] Preferably, the receptor binding molecule is an antibody. More preferably, the antibody is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, and a single domain antibody, such as a camelid or shark single domain antibody. Still more preferably, the antibody is a monoclonal antibody. Preferably, the antibody is capable to specifically bind to a tumour associated surface antigen. In some embodiments, the antibody may be Brentuximab. In some embodiments, the antibody may be Trastuzumab.

[00144] The receptor binding molecule may be an antibody fragment. Preferably, the antibody fragment is a divalent antibody fragment. More preferably, the divalent antibody fragment is selected from the group consisting of a $(Fab)_2$'-fragment, a divalent single-chain Fv fragment, a dual affinity re-targeting (DART) antibody, and a diabody. Alternatively, preferably the antibody fragment is a monovalent antibody fragment. More preferably the monovalent antibody fragment is selected from the group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv). It is also possible that the monovalent antibody fragment is a fragment of a single domain camelid or shark single domain antibody. Preferably, the antibody fragment is capable to specifically bind to a tumour associated surface antigen.

[00145] The receptor binding molecule may be a proteinaceous binding molecule with antibody-like binding properties. Examples of proteinaceous binding molecules with antibody-like binding properties that can be used as receptor binding molecule include, but are not limited to, an aptamer, a mutein based on a polypeptide of the lipocalin family, a glubody, a protein based on the ankyrin scaffold, a protein based on the crystalline scaffold, an adnectin, an avimer, a EGF-like domain, a Kringle-domain, a fibronectin type I domain, a fibronectin type II domain, a fibronectin type III domain, a PAN domain, a G1a domain, a SRCR domain, a Kunitz/Bovine pancreatic trypsin Inhibitor domain, tendamistat, a Kazal-type serine protease inhibitor domain, a Trefoil (P-type) domain, a von Willebrand factor type C domain, an Anaphylatoxin-like domain, a CUB domain, a thyroglobulin type I repeat, LDL-receptor class A domain, a Sushi domain, a Link domain, a Thrombospondin type I domain, an immunoglobulin domain or a an immunoglobulin-like domain (for example, domain antibodies or camel heavy chain antibodies), a C-type lectin domain, a MAM domain, a von Willebrand factor type A domain, a Somatomedin B domain, a WAP-type four disulfide core domain, a F5/8 type C domain, a Hemopexin domain, an SH2 domain, an SH3 domain, a Laminin-type EGF-like domain, a C2 domain, "Kappabodies" (Ill. et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" Protein Eng 10:949-57 (1997)), "Minibodies" (Martin et al. "The affinity-selection of a minibody polypeptide inhibitor of human interleukin-6" EMBO J 13:5303-9 (1994)), "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" EMBO J 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" Int J Cancer Suppl 7:51-52 (1992), a nanobody, a adnectin, a tetranectin, a microbody, an affilin, an affibody or an ankyrin, a crystallin, a knottin, ubiquitin, a zinc-finger protein, an autofluorescent protein, an ankyrin or ankyrin repeat protein or a leucine-rich repeat protein, an avimer (Silverman, Lu Q,

Bakker A, To W, Duguay A, Alba BM, Smith R, Rivas A, Li P, Le H, Whitehorn E, Moore KW, Swimmer C, Perlroth V, Vogt M, Kolkman J, Stemmer WP 2005, Nat Biotech, Dec;23(12):1556-61, E-Publication in Nat Biotech. 2005 Nov 20 edition); as well as multivalent avimer proteins evolved by exon shuffling of a family of human receptor domains as also described in Silverman J, Lu Q, Bakker A, To W, Duguay A, Alba BM, Smith R, Rivas A, Li P, Le H, Whitehorn E, Moore KW, Swimmer C, Perlroth V, Vogt M, Kolkman J, Stemmer WP, Nat Biotech, Dec;23(12):1556-61, E-Publication in Nat. Biotechnology. 2005 Nov 20 edition. Preferably the proteinaceous binding molecule with antibody-like binding properties is selected from the group consisting of a mutein based on a polypeptide of the lipocalin family, a glubody, a protein based on the ankyrin scaffold, a protein based on the crystalline scaffold, an adnectin, an avimer, a DARPin, and an affibody. Preferably, the proteinaceous binding molecule with antibody-like binding properties is capable to specifically bind to a tumour associated surface antigen.

Group Y

[00146] The group Y is selected from the group consisting of NR^5, S, O, and CR^6R^7. R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^5 is H or $(C_1\text{-}C_8)$alkyl; more preferably R^5 is H. R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^6 is H or $(C_1\text{-}C_8)$alkyl; more preferably R^6 is H. R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^7 is H or $(C_1\text{-}C_8)$alkyl, more preferably R^7 is H.

[00147] Preferably, Y is selected from the group consisting of NH, S, O and CH_2. More preferably, Y is NH, S or O. In some embodiments, Y is CH_2. In some embodiments, Y is O. In some embodiments, Y is S.

[00148] In very preferred embodiments, Y is NH.

Group R^1

[00149] R^1 is an optionally substituted aliphatic residue or optionally substituted aromatic residue.

[00150] R^1 may represent optionally substituted $(C_1\text{-}C_8)$alkyl.

[00151] R^1 may represent $(C_1\text{-}C_8)$alkyl optionally substituted with at least one of F, Cl, Br, I, $-NO_2$, $-N((C_1\text{-}C_8)alkyl)H$, $-NH_2$, $-N_3$, $-N((C_1\text{-}C_8)alkyl)_2$, =O, $(C_3\text{-}C_8)$cycloalkyl, –S-S-$((C_1\text{-}C_8)$alkyl), $(C_2\text{-}C_8)$alkenyl or $(C_2\text{-}C_8)$alkynyl.

[00152] R^1 may represent optionally substituted phenyl.

[00153] R^1 may represent phenyl optionally independently substituted with at least one of $(C_1\text{-}C_8)$alkyl, F, Cl, I, Br, $-NO_2$, $-N((C_1\text{-}C_8)alkyl)H$, $-NH_2$ or $-N((C_1\text{-}C_8)alkyl)_2$.

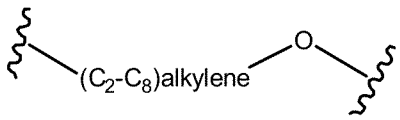
[00154] R^1 may represent an optionally substituted 5- or 6-membered heteroaromatic ring such as e.g. pyridyl.

[00155] R^1 may represent $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$alkyl substituted with –S-S-$(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$alkyl substituted with optionally substituted phenyl; or phenyl; or phenyl substituted with $–NO_2$.

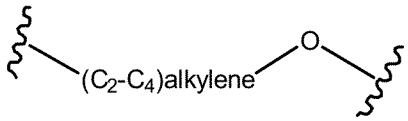
[00156] R^1 may represent methyl, ethyl, propyl or butyl, preferably methyl or ethyl, more preferably ethyl.

First Polyalkylene Glycol Unit R^F

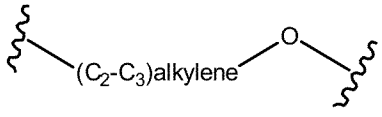
[00157] Preferably, R^1 is a first polyalkylene glycol unit R^F. The term “first polyalkylene glycol unit”, as used herein, refers to a polyalkylene glycol unit bound to the O atom, which is attached to the phosphorus of the phosphorus (V) moiety. The first polyalkylene glycol unit R^F comprises at least one alkylene glycol subunit. Preferably, the first polyalkylene glycol unit R^F comprises one or more alkylene glycol subunits having the following structure:



. More preferably, the first polyalkylene glycol unit R^F comprises one or more alkylene glycol subunits having the following structure:



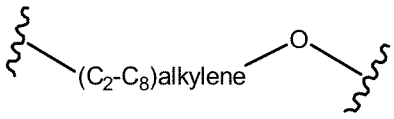
. Accordingly, the first polyalkylene glycol unit R^F may be a polytetramethylene glycol unit, a polypropylene glycol unit, or a polyethylene glycol unit. Still more preferably, the first polyalkylene glycol) unit R^F comprises one or more alkylene glycol subunits having the following structure:

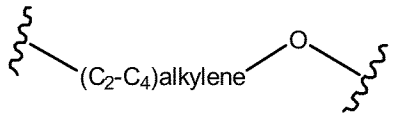


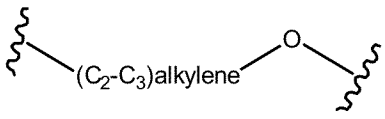
.

[00158] Preferably, the first polyalkylene glycol unit R^F comprises of from 1 to 100 alkylene glycol subunits as described herein. More preferably, the first polyalkylene glycol unit R^F comprises of from 2 to 50 alkylene glycol subunits as described herein. Still more preferably, the first polyalkylene glycol unit R^F comprises of from 3 to 45 alkylene glycol subunits as described herein. Still more preferably, the first polyalkylene glycol unit R^F comprises of from 4 to 40 alkylene glycol subunits as described herein. Still more preferably, the first polyalkylene glycol unit R^F comprises of from 6 to 35 alkylene glycol subunits as described herein. Even more preferably, the first polyalkylene glycol unit R^F comprises of from 8 to 30 alkylene glycol subunits as described herein.

[00159] Preferably, the first polyalkylene glycol unit R^F comprises of from 1 to 20 alkylene glycol subunits as described herein. More preferably, the first polyalkylene glycol unit R^F comprises of from 2 to 12 alkylene glycol subunits as described herein. Still more preferably, the first polyalkylene glycol unit R^F comprises of from 3 to 11 alkylene glycol subunits as described herein.

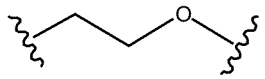
[00160] The first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

. Preferably, the first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

. More preferably, the first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

. In very preferred embodiments, the first polyalkylene glycol unit R^F may be a polyethylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably

of from 6 to 35, even more preferably of from 8 to 30 subunits each having the structure:



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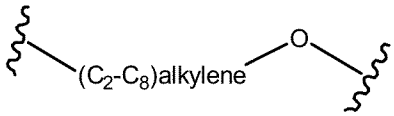
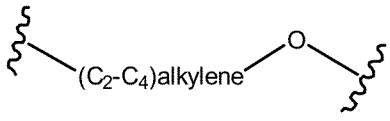
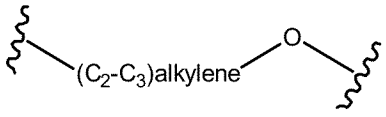
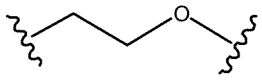
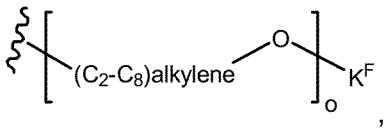
[00161] The first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits having the structure:  . Preferably, the first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits having the structure:



. More preferably, the first polyalkylene glycol unit R^F may be a polyalkylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11subunits having the structure:  . In very preferred embodiments, the first polyalkylene glycol unit R^F may be a polyethylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits each having the structure:  .

[00162] Preferably, the first polyalkylene glycol unit R^F is:



,

wherein:

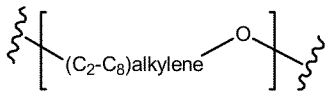
 indicates the position of the O attached to the phosphorus;

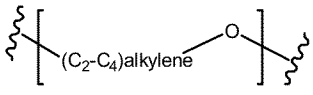
K^F is H or a first capping group; preferably K^F is selected from the group consisting of -H (hydrogen), $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-NH$(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-N$((C_1\text{-}C_3)\text{alkyl})_2$; more preferably K^F is H; and

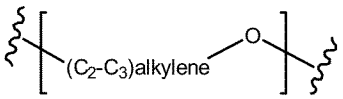
o is an integer ranging from 1 to 100.

[00163] The "first capping group", when referred to herein, may be any moiety which is capable to function as a terminal group of the first polyalkylene glycol unit. Examples for first capping groups, which can be used in the present disclosure, include $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$. In some embodiments, the first capping group may be $-(C_1\text{-}C_{10})$alkyl, in particular methyl.

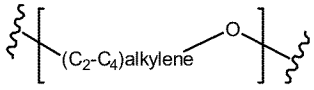
[00164] Preferably, K^F is H (hydrogen).

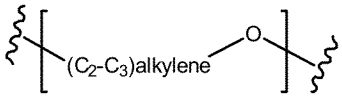
[00165] The integer o denotes the number of repeating units 

in the first polyalkylene glycol unit. The integer o may range from 1 to 100. Preferably, o ranges from 2 to 50. More preferably, o ranges from 3 to 45. Still more preferably, o ranges from 4 to 40. Still more preferably, o ranges from 6 to 35. Even more preferably, o ranges from 8 to 30. In preferred embodiments, o is 12 or about 12. Even more preferably, o ranges from 16 to 30. Even more preferably, o ranges from 20 to 28. Even more preferably, o is 22, 23, 24, 25 or 26. Even more preferably, o is 23, 24 or 25. In preferred embodiments, o is 24 or about 24. Preferably, the repeating unit is 

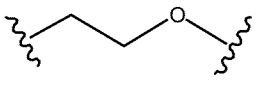
. More preferably, the repeating unit is 

.

[00166] In the first polyalkylene glycol unit, the integer o may range from 1 to 20. Preferably, o ranges from 2 to 12. More preferably, o ranges from 3 to 11. Preferably, the repeating unit is 

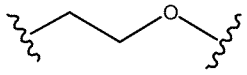
. More preferably, the repeating unit is 

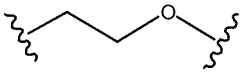
.

[00167] Preferably, the first polyalkylene glycol unit R^F comprises ethylene glycol subunits each having the following structure: 

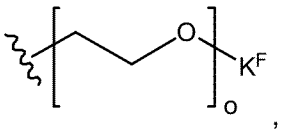
, i.e. this subunit is denoted an "ethylene glycol subunit". Accordingly, preferably the first polyalkylene glycol unit is a first

polyethylene glycol unit. The first polyethylene glycol unit comprises at least one ethylene glycol subunit.

[00168] Preferably, the first polyalkylene glycol unit R^F may be a first polyethylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 ethylene glycol subunits each having the structure:  .

[00169] Preferably, the first polyalkylene glycol unit R^F may be a first polyethylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 ethylene glycol subunits each having the structure:  .

[00170] Preferably, the first polyalkylene glycol unit R^F is a first polyethylene glycol unit having the structure:

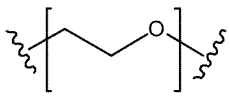
 ,

wherein:

 indicates the position of the O attached to the phosphorus;

K^F is H (hydrogen) or a first capping group as described herein; preferably K^F is selected from the group consisting of -H (hydrogen), $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$; more preferably K^F is H; and

o is an integer ranging from 1 to 100.

[00171] The integer o denotes the number of repeating units  in the first polyethylene glycol unit. The integer o may range from 1 to 100. Preferably, o ranges from 2 to 50. More preferably, o ranges from 3 to 45. Still more preferably, o ranges from 4 to 40. Still more preferably, o ranges from 6 to 35. Even more preferably, o ranges from 8 to 30.

In preferred embodiments, o is 12 or about 12. Even more preferably, o ranges from 16 to 30. Even more preferably, o ranges from 20 to 28. Even more preferably, o is 22, 23, 24, 25 or 26. Even more preferably, o is 23, 24 or 25. In preferred embodiments, o is 24 or about 24.

[00172] In the first polyethylene glycol unit, the integer o may range from 1 to 20. Preferably, o ranges from 2 to 12. More preferably, o ranges from 3 to 11.

[00173] In general, in the first polyalkylene glycol unit R^F, (preferably, first polyethylene glycol unit), polydisperse polyalkylene glycols (preferably, polydisperse polyethylene glycols), monodisperse polyalkylene glycols (preferably, monodisperse polyethylene glycol), and discrete polyalkylene glycols (preferably, discrete polyethylene glycols) can be used. Polydisperse polyalkylene glycols (preferably, polydisperse polyethylene glycols) are a heterogenous mixture of sizes and molecular weights, whereas monodisperse polyalkylene glycols (preferably, monodisperse polyethylene glycols) are typically purified from heterogenous mixtures and therefore provide a single chain length and molecular weight. Preferred first polyalkylene glycols units are discrete polyalkylene glycols (preferably, discrete polyethylene glycols), i.e. compounds that are synthesized in step-wise fashion and not via a polymerization process. Discrete polyalkylene glycols (preferably, discrete polyethylene glycols) provide a single molecule with defined and specified chain length.

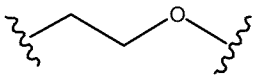
[00174] The first polyalkylene glycol unit (preferably, first polyethylene glycol unit) provided herein comprises one or multiple polyalkylene glycol chains (preferably, polyethylene glycol chains). The polyalkylene glycol chains (preferably, polyethylene glycol chains) can be linked together, for example, in a linear, branched or star shaped configuration. Optionally, at least one of the polyalkylene glycol chains (preferably, polyethyleneglycol chains) may be derivatized at one end for covalent attachment to the oxygen atom bound to the phosphorus.

[00175] The first polyalkylene glycol unit (preferably, first polyethylene glycol unit) will be attached to the conjugate (or intermediate thereof) at the oxygen atom which is bound to the phosphorus. The other terminus (or termini) of the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) will be free and untethered and may take the form of a hydrogen, methoxy, carboxylic acid, alcohol or other suitable functional group, such as e.g. any first capping group as described herein. The methoxy, carboxylic acid, alcohol or other suitable functional group acts as a cap for the terminal polyalkylene glycol subunit

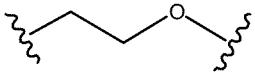
(preferably, polyethylene glycol subunit) of the first polyalkylene glycol unit (preferably, first polyethylene glycol unit). By untethered, it is meant that the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) will not be attached at that untethered site to a camptothecin moiety (C), to a receptor binding molecule, or to a component of the linker (L) linking a camptothecin moiety and/or a receptor binding molecule. For those embodiments wherein the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) comprises more than one polyalkylene glycol chain (preferably, polyethylene glycol chain), the multiple polyalkylene glycol chains (preferably, polyethylene glycol chains) may be the same or different chemical moieties (e.g., polyalkylene glycols, in particular polyethylene glycols, of different molecular weight or number of subunits). The multiple first polyalkylene glycol chains (preferably, first polyethylene glycol chains) are attached to the oxygen atom bound to the phosphorus at a single attachment site. The skilled artisan will understand that the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) in addition to comprising repeating polyalkylene glycol subunits (preferably, polyethylene glycol subunits) may also contain non-polyalkylene glycol material (preferably, non-polyethylene glycol material) (e.g., to facilitate coupling of multiple polyalkylene glycol chains (preferably, polyethylene glycol chains) to each other or to facilitate coupling to the oxygen atom bound to the phosphorus. Non-polyalkylene glycol material (preferably, non-polyethelyne glycol material) refers to the atoms in the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) that are not part of the repeating alkylene glycol subunits (preferably, $-CH_2CH_2O-$ subunits). In embodiments provided herein, the first polyalkyleneglycol unit (preferably, first polyethyleneglycol unit) can comprise two monomeric polyalkylene glycol chains (preferably, polyethylene glycol chains) linked to each other via non-polyalkylene glycol (non-polyethylene glycol) elements. In other embodiments provided herein, the first polyalkylene glycol unit (preferably, first polyethylene glycol unit) can comprise two linear polyalkylene glycol chains (preferably, polyethylene glycol chains) attached to a central core that is attached to the oxygen atom bound to the phosphorus (i.e., the polyalkylene glycol unit (preferably, polyethyleneglycol unit) is branched).

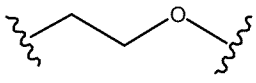
[00176] There are a number of polyalkylene glycol (preferably, polyethylene glycol) attachment methods available to those skilled in the art, [see, e.g., EP 0 401 384 (coupling PEG to G-CSF); U.S. Pat. No. 5,757,078 (PEGylation of EPO peptides); U.S. Pat. No. 5,672,662 (Polyethylene glycol) and related polymers mono substituted with propionic or butanoic acids and functional derivatives thereof for biotechnical applications); U.S. Pat. No. 6,077,939 (PEGylation of an N- terminal .alpha.-carbon of a peptide); and Veronese (2001) Biomaterials 22:405-417 (Review article on peptide and protein PEGylation)].

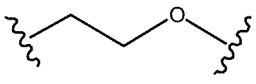
[00177] In preferred embodiments, the first polyalkylene glycol unit, more preferably the first polyethylene glycol unit, is directly attached to the oxygen atom bound to the phosphorus. In these embodiments, the first polyalkylene glycol unit, preferably first polyethylene glycol unit, does not comprise a functional group for attachment to the oxygen atom bound to the phosphorous, i.e. the oxygen atom is directly bound to a carbon atom of the first polyalkylene glycol unit, preferably to a CH_2 of the first polyethylene glycol unit.

[00178] In one group of embodiments, the first polyalkylene glycol unit comprises at least 1 alkylene glycol subunit, preferably at least 2 alkylene glycol subunits, more preferably at least 3 alkylene glycol subunits, still more preferably at least 4 alkylene glycol subunits, still more preferably at least 6 alkylene glycol subunits, even more preferably at least 8 alkylene glycol subunits. In some such embodiments, the first polyalkylene glycol unit comprises no more than about 100 alkylene glycol subunits, preferably no more than about 50 alkylene glycol units, more preferably no more than about 45 alkylene glycol subunits, more preferably no more than about 40 alkylene glycol subunits, more preferably no more than about 35 subunits, even more preferably no more than about 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit.

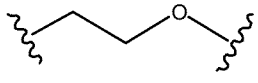
[00179] In one group of embodiments, the first polyalkylene glycol unit comprises one or more linear polyalkylene glycol chains each having at least 1 alkyleneglycol subunit, preferably at least 2 alkyleneglycol subunits, more preferably at least 3 alkyleneglycol subunits, still more preferably at least 4 alkyleneglycol subunits, still more preferably at least 6 alkylene glycol subunits, even more preferably at least 8 alkylene glycol subunits. In preferred embodiments, the first polyalkylene glycol unit comprises a combined total of at least 1 alkylene glycol subunit, preferably at least 2 alkylene glycol subunits, more preferably at least 3, still more preferably at least 4, still more preferably at least 6, or even more preferably at least 8 alkylene glycol subunits. In some such embodiments, the first polyalkylene glycol unit comprises no more than a combined total of about 100 alkylene glycol subunits, preferably no more than a combined total of about 50 alkylene glycol

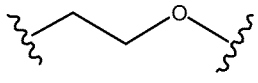
subunits, more preferably no more than a combined total of about 45 subunits, still more preferably no more than a combined total of about 40 subunits, still more preferably no more than a combined total of about 35 subunits, even more preferably no more than a combined total of about 30 subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit comprising one or more linear polyethylene glycol chains.

[00180] In another group of embodiments, the first polyalkylene glycol unit comprises a combined total of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit.

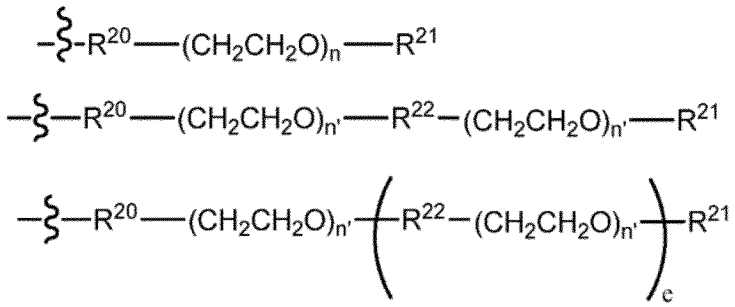
[00181] In another group of embodiments, the first polyalkylene glycol unit comprises one or more linear polyalkylene glycol chains having a combined total of from 1 to 100, preferably 2 to 50, more preferably 3 to 45, still more preferably 4 to 40, still more preferably 6 to 35, even more preferably 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit comprising one or more linear polyethylene glycol chains.

[00182] In another group of embodiments, the first polyalkylene glycol unit is a linear single polyalkylene glycol chain having at least 1 subunit, preferably at least 2 subunits, more

preferably at least 3 subunits, still more preferably at least 6 subunits, even more preferably at least 8 subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit which is a linear single polyethylene glycol chain. Optionally, in any one of these embodiments the linear single polyalkylene glycol chain may be derivatized.

[00183] In another group of embodiments, the polyalkylene glycol unit is a linear single polyalkylene glycol chain having from 1 to 100, preferably 2 to 50, more preferably 3 to 45, more preferably 4 to 40, more preferably 6 to 35, more preferably 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the first polyalkylene glycol unit is a first polyethylene glycol unit which is a linear single polyethylene glycol chain. Optionally, in any one of these embodiments the linear single polyalkylene glycol chain may be derivatized.

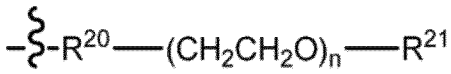
[00184] Exemplary linear polyethylene glycol units that can be used as first polyalkylene glycol unit, in particular as a first polyethylene glycol unit, in any one of the embodiments provided herein are as follows:



wherein the wavy line indicates the site of attachment to the oxygen atom bound to the phosphorus;

R^{20} is a PEG attachment unit; preferably, R^{20} is absent;
R^{21} is a PEG capping unit (herein, R^{21} is also denoted as "K^F");
R^{22} is a PEG coupling unit (i.e. for coupling multiple PEG subunit chains together;
n is independently selected from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, more preferably from 4 to 40, still more preferably from 6 to 35, even more preferably from 8 to 30;
e is 2 to 5;
each n' is independently selected from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, more preferably from 4 to 40, still more preferably from 6 to 35, even more preferably from 8 to 30. In preferred embodiments, there are at least 1, preferably at least 2, more preferably at least 3, more preferably at least 4, more preferably at least 6, even more preferably at least 8 ethylene glycol subunits in the polyethylene glycol unit. In some embodiments, there are no more than 100, preferably no more than 50, more preferably no more than 45, more preferably no more than 40, more preferably no more then 35, even more preferably no more than 30 ethylene glycol subunits in the polyethylene glycol unit. When R^{20} is absent, a (CH_2CH_2O) subunit is directly bound to the oxygen atom, which is attached to the phosphorus.

[00185] Preferably, the linear polyethylene glycol unit is



,

wherein the wavy line indicates the site of attachment to the oxygen atom bound to the phosphorus; R^{20}, R^{21} (also denoted herein as "K^F") and n are as defined herein; more preferably R^{20} is absent. In preferred embodiments, n is 12 or about 12. In preferred embodiments, n is 24 or about 24. Preferably, R^{21} is H.

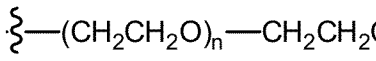
[00186] The polyethylene glycol attachment unit R^{20}, when present, is part of the first polyethylene glycol unit and acts to link the first polyethylene glycol unit to the oxygen atom bound to the phosphorus. In this regard, the oxygen atom bound to the phosphorus forms a bond with the first polyethylene glycol unit. In exemplary embodiments, the PEG attachment unit R^{20}, when present, is selected from the group consisting of *-(C_1-C_{10})alkyl-$^{\#}$, *-arylene-$^{\#}$, *-(C_1-C_{10})alkyl-O-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)O-$^{\#}$, *-(C_1-C_{10})alkyl-NH-$^{\#}$, *-(C_1-C_{10})alkyl-S-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)-NH-$^{\#}$, *-(C_1-C_{10})alkyl-NH-C(O)-$^{\#}$, and *-CH_2-CH_2SO_2-(C_1-

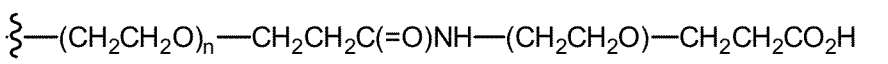
C_{10})alkyl-$^{\#}$; wherein * denotes the attachment point to the oxygen bound to the phosphorus, and # denotes the attachment point to the ethylene glycol unit.

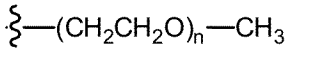
[00187] The PEG coupling unit R^{22}, when present, is part of the polyethylene glycol unit and is non-PEG material that acts to connect two or more chains of repeating -CH_2CH_2O- subunits. In exemplary embodiments, the PEG coupling unit R^{22}, when present, is independently selected from the group consisting of *-(C_1-C_{10})alkyl-C(O)-NH-$^{\#}$, *-(C_1-C_{10})alkyl-NH-C(O)-$^{\#}$, *-(C_2-C_{10})alkyl-NH-$^{\#}$, *-(C_2-C_{10})alkyl-O-$^{\#}$, *-(C_1-C_{10})alkyl-S-$^{\#}$, or *-(C_2-C_{10})alkyl-NH-$^{\#}$; wherein * denotes the attachment point to an oxygen atom of an ethylene glycol subunit, and # denotes the attachment point to a carbon atom of another ethylene glycol subunit.

[00188] The group R^{21}, also denoted herein as "K^F", in exemplary embodiments is H (hydrogen), or may be a first capping group, as described herein; preferably, R^{21} is independently selected from the group consisting of -H, -PO_3H, -(C_1-C_{10})alkyl, -(C_1-C_{10})alkyl-SO_3H, -(C_2-C_{10})alkyl-CO_2H, -(C_2-C_{10})alkyl-OH, -(C_2-C_{10})alkyl-NH_2, -(C_2-C_{10})alkyl-NH(C_1-C_3)alkyl and -(C_2-C_{10})alkyl-N((C_1-C_3)alkyl)$_2$. In some embodiments R^{21} may be -(C_1-C_{10})alkyl, in particular methyl. More preferably, R^{21} is H.

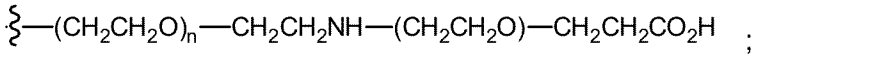
[00189] Illustrative linear first polyethylene glycol units, which can be used as first polyalkylene glycol units in any one of the embodiments provided herein, are as follows.







; and



wherein the wavy line indicates the site of attachment to the oxygen atom which is bound to the phosphorus; and each n is from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, still more preferably from 4 to 40, still more preferably from 6 to 35, even more

preferably from 8 to 30. In some embodiments, n is about 12. In some embodiments, n is about 24.

[00190] In some embodiments, the first polyalkylene glycol unit is from about 300 daltons to about 5 kilodaltons; from about 300 daltons, to about 4 kilodaltons; from about 300 daltons, to about 3 kilodaltons; from about 300 daltons, to about 2 kilodaltons; or from about 300 daltons, to about 1 kilodalton. In some such aspects, the first polyalkylene glycol unit may have at least 6 alkylene glycol subunits or at least 8 alkylene glycol subunits. In some such aspects, the first polyalkylene glycol unit may have at least 6 alkylene glycol subunits or at least 8 alkylene glycol subunits but no more than 100 alkylene glycol subunits, preferably no more than 50 alkylene glycol subunits. In some embodiments, the first polyalkylene glycol unit is a first polyethylene glycol unit being from about 300 daltons to about 5 kilodaltons; from about 300 daltons, to about 4 kilodaltons; from about 300 daltons, to about 3 kilodaltons; from about 300 daltons, to about 2 kilodaltons; or from about 300 daltons, to about 1 kilodalton. In some such aspects, the first polyethylene glycol unit may have at least 6 ethylene glycol subunits or at least 8 ethylene glycol subunits. In some such aspects, the first polyethylene glycol unit has at least 6 ethylene glycol subunits or at least 8 ethylene glycol subunits but no more than 100 ethylene glycol subunits, preferably no more than 50 ethylene glycol subunits.

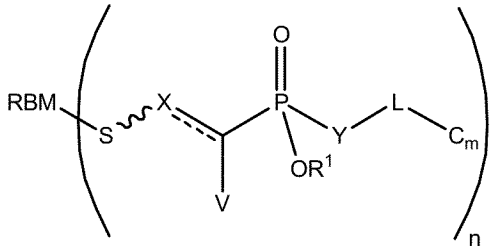
[00191] In some embodiments, when R^1 is a first polyalkylene glycol unit R^F, there are no other alkylene glycol subunits present in the conjugate of formula (I) (i.e., no alkylene glycol subunits are present in any of the other components of the conjugate, such as e.g. in the linker L as provided herein). In other aspects, when R^1 is a first polyalkylene glycol unit, there are no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2 or no more than 1 other alkylene glycol subunits present in the conjugate of formula (I) (i.e., no more than 8, 7, 6, 5, 4, 3, 2, or 1 other alkylene glycol subunits are present in other components of the conjugate, such as e.g. in the linker L as provided herein).

[00192] Preferably, in other embodiments, when R^1 is a first polyalkylene glycol unit R^F, the conjugate further comprises a second polyalkylene glycol unit R^S, as described herein. Preferably, when R^1 is a first polyethylene glycol unit and the conjugate further comprises a second polyalkylene glycol unit R^S, the second polyalkylene glycol unit is a second polyethyleneglycol unit, as described herein.

[00193] It will be appreciated that when referring to alkylene glycol subunits, in particular ethylene glycol subunits, and depending on context, the number of subunits can represent an average number, e.g., when referring to a population of conjugates or intermediate compounds, and using polydisperse polyalkylene glycols, in particular polydisperse polyethylene glycols.

“L”: Linker

[00194] The present disclosure provides conjugates, where a receptor binding molecule, as described herein, is linked to a camptothecin moiety. In accordance with the present disclosure, the receptor binding molecule may be linked, via the group Y and covalent attachment by a linker L, to the camptothecin moiety. As used herein, a "linker" L is any chemical moiety that is capable of linking a group Y, such as e.g. NH, to another moiety, such as a camptothecin moiety. In this regard, it is again referred to the formula (I) described herein:



(I),

Accordingly, the camptothecin moiety C can be linked to Y through a linker L. In formula (I) RBM, , V, X, Y, R^1, L, C, m and n are as defined herein. The linker L serves to connect the Y with the camptothecin moiety (C). The linker L is any chemical moiety that is capable of linking Y to the camptothecin moiety C. In particular, the linker L attaches Y to the camptothecin moiety C through covalent bond(s). The linker reagent is a bifunctional or multifunctional moiety which can be used to link a camptothecin moiety C and Y to form conjugates of formula (I). The terms “linker reagent”, “cross-linking reagent”, “linker derived from a cross-linking reagent” and “linker” may be used interchangeably throughout the present disclosure.

[00195] Linkers can be susceptible to cleavage (cleavable linker) such as enzymatic cleavage, acid-induced cleavage, photo-induced cleavage and disulfide bond cleavage. Enzymatic cleavage includes, but is not limited to, protease-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, glycosidase-induced cleavage, phosphatase-

induced cleavage, and sulfatase-induced cleavage, preferably at conditions under which the camptothecin moiety and/or the receptor binding molecule remains active. Alternatively, linkers can be substantially resistant to cleavage (e.g., stable linker or non-cleavable linker). In some aspects, the linker may be a procharged linker, a hydrophilic linker, a PEG-based linker, or a dicarboxylic acid based linker. Accordingly, in some embodiments of any one of the antibody drug conjugates disclosed herein the linker (L) is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, a PEG-based linker, a procharged linker, a peptidic linker and a dicarboxylic acid based linker. Preferably, the linker L is a cleavable linker. In some embodiments, the linker L is a non-cleavable linker.

[00196] Preferably, as described herein, the linker L is cleavable. In some embodiments, L is a linker susceptible to enzymatic cleavage. In some embodiments, L is an acid-labile linker, a photo-labile linker, a peptidase cleavable linker, a protease cleavable linker, an esterase cleavable linker, a glycosidase cleavable linker, a phosphatase cleavable linker, a sulfatase cleavable linker, a disulfide bond reducible linker, a hydrophilic linker, a procharged linker, a PEG-based linker, or a dicarboxylic acid based linker. Preferably, the linker L is cleavable by a protease, a glucuronidase, a sulfatase, a phosphatase, an esterase, or by disulfide reduction. Preferably, the linker is a peptidase cleavable linker. Other preferred linkers are cleavable by a protease.

[00197] A non-cleavable linker is any chemical moiety capable of linking a camptothecin moiety to Y in a stable, covalent manner and does not fall off under the categories listed herein for cleavable linkers. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, protease-induced cleavage, glycosidase-induced cleavage, phosphatase-induced cleavage, esterase-induced cleavage and disulfide bond cleavage. Furthermore, non-cleavable refers to the ability of the chemical bond in the linker or adjoining to the linker to withstand cleavage induced by an acid, photo labile-cleaving agent, a peptidase, a protease, a glycosidase, a phosphatase, an esterase, or a chemical or physiological compound that cleaves a disulfide bond, at conditions under which the camptothecin moiety or the receptor binding molecule does not lose its activity.

[00198] Acid-labile linkers are linkers cleavable at acidic pH. For example, certain intracellular compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid-labile linkers.

[00199] Some linkers can be cleaved by peptidases, i.e. peptidase cleavable linkers. In this regard, certain peptides are readily cleaved inside or outside cells, see e.g. Trout et al., 79 Proc. Natl. Acad. Sci. USA, 626-629 (1982) and Umemoto et al. 43 Int. J. Cancer, 677-684 (1989). Peptides are composed of α-amino acids and peptidic bonds, which chemically are amide bonds between the carboxylate of one amino acid and the amino group of a second amino acid.

[00200] Some linkers can be cleaved by esterases, i.e. esterase cleavable linkers. In this regard, certain esters can be cleaved by esterases present inside or outside of cells. Esters are formed by the condensation of a carboxylic acid and an alcohol. Simple esters are esters produced with simple alcohols, such as aliphatic alcohols, and small cyclic and small aromatic alcohols.

[00201] Procharged linkers are derived from charged cross-linking reagents that retain their charge after incorporation into an antibody drug conjugate. Examples of procharged linkers can be found in US 2009/0274713.

[00202] Preferably, as described herein, the linker L is cleavable. As illustrative examples, the linker may be cleavable by a protease, a glucuronidase, a sulfatase, a phosphatase, an esterase, or by disulfide reduction. Preferably, the linker L is cleavable by a protease. More preferably, the linker is cleavable by a cathepsin, such as, in particular, cathepsin B. The linker may comprise a dipeptide moiety, such as e.g. a valine-citrulline moiety or a valine-alanine moiety, which can be cleaved by a cathepsin such as cathepsin B. Accordingly, in some embodiments the linker comprises a valine-citrulline moiety. In some embodiments the linker comprises a valine-alanine moiety. The linker may comprise a cleavage site. The term “cleavage site” may refer to a chemical moiety which is recognized by an enzyme, followed by cleavage, e.g. by way of hydrolysis. As an illustrative example, a cleavage site is a sequence of amino acids, which is recognized by a protease or a peptidase, and hydrolyzed by said protease or peptidase. In some embodiments, the cleavage site is a dipeptide. In some embodiments, the cleavage site is a valine-citrulline moiety. In some embodiments, the cleavage site is a valine-alanine moiety.

Second Spacer Unit

[00203] In preferred embodiments, the linker (L) comprises a second spacer unit -A- which is bound to the -Y-. The second spacer unit serves to connect a -Y- to another part of the linker, when present, or to a camptothecin moiety (-C). As readily appreciated by a

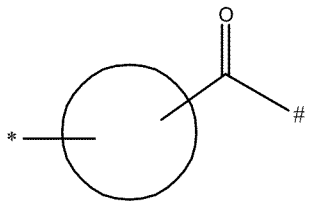
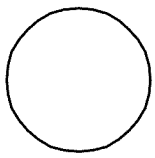
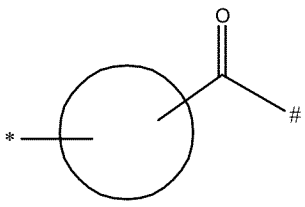
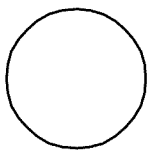
person skilled in the art, this depends on whether another part of the linker is present or not. The second spacer unit (–A–) may be any chemical group or moiety which is capable to connect a -Y- to another part of the linker, when present, or to a campthothecin moiety (-C), depending on whether another part of the linker is present or not. In this regard, the -Y-, as described herein, is bonded to the second spacer unit (–A–). The second spacer unit (–A–) may comprise or may be a functional group that is capable to form a bond to another part of the linker, when present, or to the camptothecin moiety (-C). Again, this depends on whether another part of the linker is present or not. Preferably, the functional group, which is capable to form a bond to another part of the linker, or to a camptothecin moiety (-C), is a carbonyl group which is depicted as, e.g.,  or -C(O)-.

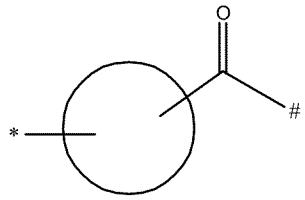
[00204] The second spacer unit may be any spacer known to a person skilled in the art, for example, a straight or branched hydrocarbon-based moiety. The second spacer unit can also comprise cyclic moieties, such as e.g., but not limited to, aromatic moieties. If the second spacer unit is a hydrocarbon-based moiety, the main chain of the second spacer moiety may comprise only carbon atoms but can also contain heteroatoms such as oxygen (O), nitrogen (N) or sulfur (S) atoms, and/or can contain carbonyl groups (C=O). The second spacer unit may comprise or may be, for example, a (C_1-C_{20}) carbon atom chain. In typical embodiments of hydrocarbon-based second spacer units, the spacing moiety comprises between 1 to about 150, 1 to about 100, 1 to about 75, 1 to about 50, or 1 to about 40, or 1 to about 30, or 1 to about 20, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19 main chain atoms. A person skilled in the art knows to select suitable second spacer units.

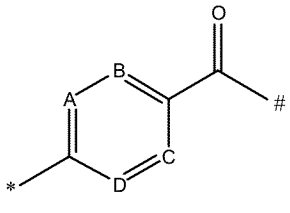
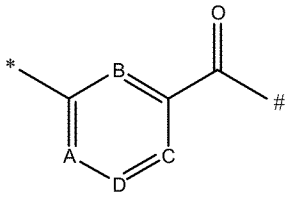
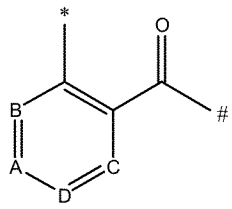
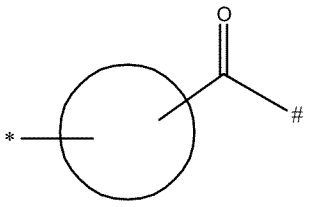
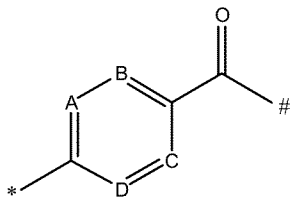
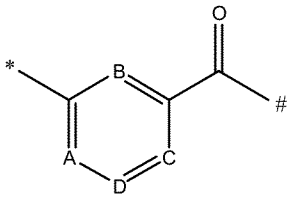
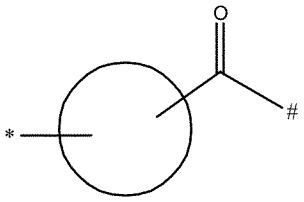
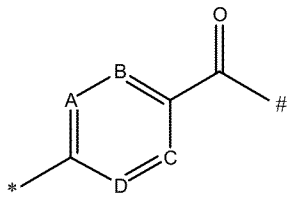
[00205] In some embodiments, the second spacer unit (–A–), when present, is selected from the group consisting of *-(C_1-C_{10})alkylene-C(O)-$^{\#}$, *-(C_3-C_8)carbocyclo-C(O)-$^{\#}$, *-arylene-C(O)-$^{\#}$, *-(C_1-C_{10})alkylene-arylene-C(O)-$^{\#}$, *-arylene-(C_1-C_{10})alkylene-C(O)-$^{\#}$, *-(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-C(O)-$^{\#}$, *-(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-C(O)-$^{\#}$, *-(C_3-C_8)heterocyclo-C(O)-$^{\#}$, *-(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-C(O)-$^{\#}$, and *-(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-C(O)-$^{\#}$; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Preferably, the second spacer unit (–A–), when present, is selected from the group consisting of *-(C_3-C_8)carbocyclo-C(O)-$^{\#}$, *-arylene-C(O)-$^{\#}$, and *-(C_3-C_8)heterocyclo-C(O)-$^{\#}$; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part

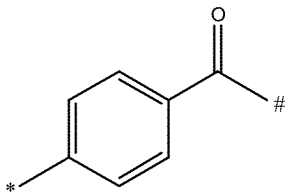
of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

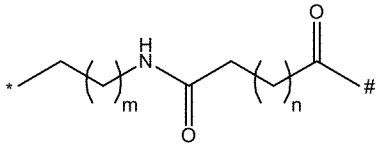
[00206] In other embodiments, the second spacer unit (–A–), when present, may be selected from the group consisting of *-(C_1-C_{10})alkylene-#, *-(C_3-C_8)carbocyclo-#, *-arylene-#, *-(C_1-C_{10})alkylene-arylene-#, *-arylene-(C_1-C_{10})alkylene-#, *-(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-#, *-(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-#, *-(C_3-C_8)heterocyclo-#, *-(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-#, and *-(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-#; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Preferably, the second spacer unit (–A–), when present, may be selected from the group consisting of *-(C_3-C_8)carbocyclo-#, *-arylene-#, and *-(C_3-C_8)heterocyclo-#; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

[00207] Preferably, the second spacer unit -A- is  , wherein  is a five- or six-membered carbocyclic ring; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. The carbocyclic ring may be aromatic or non-aromatic. Preferably, the second spacer unit -A- is  , wherein  is a five- or six-membered heterocyclic ring comprising 1, 2, or 3 heteroatoms independently selected from the group consisting of N, O and S; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. The heterocyclic ring may be aromatic or non-aromatic.

[00208] More preferably,  is selected from the group consisting of

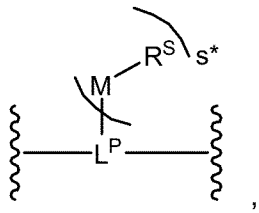
 ,  , and  , wherein each of A, B, C and D is independently selected from N (nitrogen) and C-H; preferably, at least one of A, B, C and D is C-H; more preferably, at least two of A, B, C and D are C-H; still more preferably, at least three of A, B, C and D are C-H, even more preferably, each of A, B, C and D are C-H; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Still more preferably,  is  or  , wherein each of A, B, C and D is independently selected from N (nitrogen) and C-H; preferably, at least one of A, B, C and D is C-H; more preferably, at least two of A, B, C and D are C-H; still more preferably, at least three of A, B, C and D are C-H, even more preferably, each of A, B, C and D are C-H; wherein * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Even more preferably,  is  , wherein each of A, B, C and D is independently selected from N (nitrogen) and C-H; preferably, at least one of A, B, C and D is C-H; more preferably, at least two of A, B, C and D are C-H; still more preferably, at least three of A, B, C and D are C-H, even more preferably, each of A, B, C and D are C-H; wherein * denotes the attachment point to the -Y-; and # denotes the attachment point to

another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. In very preferred embodiments, the scond spacer unit A is  wherein * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

[00209] In other embodiments, the second spacer unit (-A-) may be  ; and m and n are each, independently, an integer of e.g. from 0 to 20, 0 to 15, 1 to 10, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 1 to 2, or 1, preferably m is 1 and n is 1; * indicates the position of the -Y-, and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Such second spacer unit may be optionally substituted, e.g. with one or two (C_1-C_8)alkyl, in particular at the carbon adjacent to the asterisk (*).

Group Z

[00210] In preferred embodiments, the second spacer unit -A- is a group Z, the group Z having the following structure:



wherein:

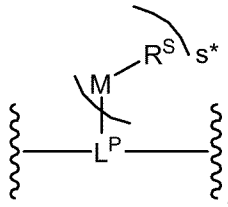
L^P is a parallel connector unit;

R^S is, each independently, a second polyalkylene glycol unit;

M is, each independently, a bond or a moiety that binds R^S with L^P;

s* is an integer ranging from 1 to 4; and

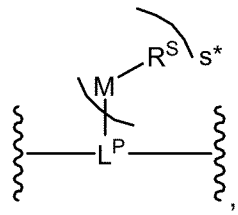
the wavy lines indicate the attachment point to the -Y- and to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

[00211] As indicated in the formula  , the second polyalkylene glycol unit R^S is bonded to the parallel connector unit L^P via a suitable moiety M. In some embodiments, M is a bond. In some embodiments, M may be any moiety which is capable to bind a polyalkylene glycol unit with the parallel connector unit L^P. As illustrative examples, M may be, each independently, selected from the group consisting of -NH-, -O-, S, -C(O)-O-, -C(O)-NH- and -(C_1-C_{10})alkylene. Preferably, M is, each independently, selected from the group consisting of -NH-, -O- and -S-. More preferably, each M is -O-.

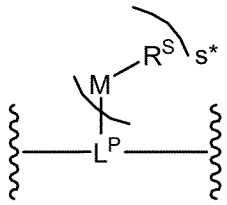
[00212] The integer s* may have a range from 1 to 4. Preferably, the integer s* has a range of from 1 to 3. More preferably, the integer s* is 1 or 2. Even more preferably, the integer s* is 1. The integer s* denotes the number of groups -M-R^S which are attached to the parallel connector unit L^P.

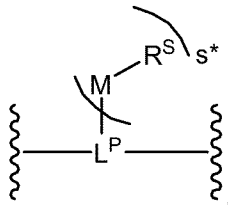
[00213] The parallel connector unit (L^P) serves to connect a -Y- to another part of the linker (L) and, via M, to one or more second polyalkylene glycol unit(s), as indicated by the integer s*. Accordingly, when present, L^P may be any chemical group or moiety which is capable to connect a -Y- to another part of the linker and, via M, to a second polyalkylene glycol unit. Alternatively, the parallel connector unit (L^P) may link the Y to the camptothecin moiety (C), in case no other part of the linker is present, and via M, to the second polyalkylene glycol unit. In this regard, the Y, as described herein, is bonded to the parallel connector unit (L^P). The parallel connector unit (L^P) may comprise or may be a functional group that is capable to form a bond to another part of the linker (L), or to a camptothecin moiety (C), depending on whether another part of the linker (L) is present or not. Preferably, the functional group, which is capable to form a bond to another part of the linker (L), or to a camptothecin moiety (-C), is a carbonyl group which is depicted as, e.g.,  , or -C(O)-, or –(C=O)-.

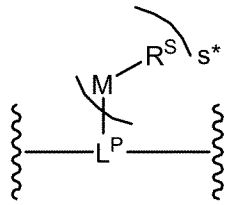
[00214] The parallel connector unit (L^P) may be, for example, a straight or branched hydrocarbon-based moiety. The parallel connector unit (L^P) can also comprise cyclic moieties. If the parallel connector unit (L^P) is a hydrocarbon-based moiety, the main chain of the second spacer moiety may comprise only carbon atoms but can also contain heteroatoms such as oxygen (O), nitrogen (N) or sulfur (S) atoms, and/or can contain carbonyl groups (C=O). The parallel connector unit (L^P) may comprise or may be, for example, a (C_1-C_{20}) carbon atom chain. In typical embodiments of hydrocarbon-based parallel connector unit (L^P), the linking moiety comprises between 1 to about 150, 1 to about 100, 1 to about 75, 1 to about 50, or 1 to about 40, or 1 to about 30, or 1 to about 20, including 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19 main chain atoms. The parallel connector unit L^P is capable to bind to a second polyalkylene glycol unit R^S via M. A person skilled in the art knows to select suitable parallel connector units (L^P).

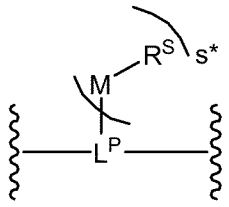
[00215] In some embodiments, the group Z 

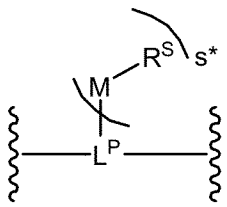
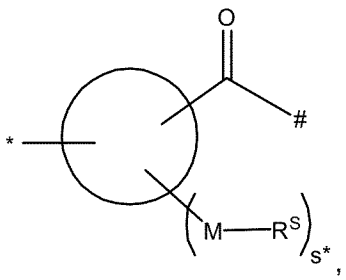
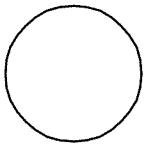
, when present, is selected from the group consisting of *-(C_1-C_{10})alkylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 to 3, more preferably 1 or 2, still more preferably 1 group(s) -M-R^S; *-(C_3-C_8)carbocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-arylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-(C_1-C_{10})alkylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_3-C_8)heterocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; and *-(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the

linker is present or not. Preferably, the group Z  , when present, is selected from the group consisting of *-(C_3-C_8)carbocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; and *-(C_3-C_8)heterocyclo-C(O)-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

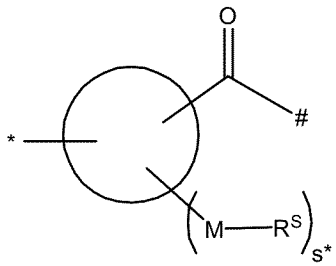
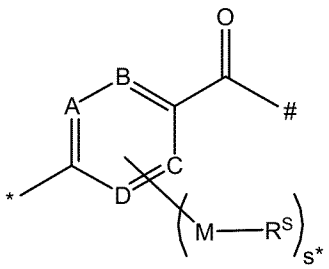
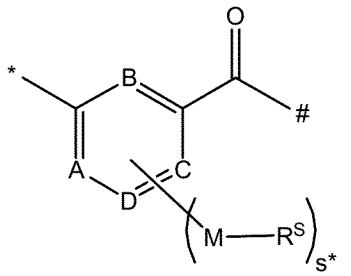
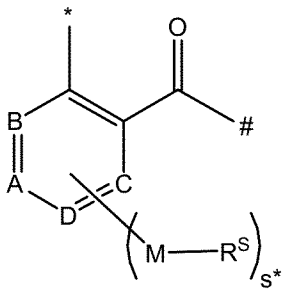
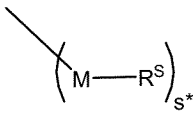
[00216] In other embodiments, the group Z  , when present, may be selected from the group consisting of *-(C_1-C_{10})alkylene-# substituted, each independently, with 1 to 4, preferably 1 to 3, more preferably 1 or 2, still more preferably 1 group(s) -M-R^S; *-(C_3-C_8)carbocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-arylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-(C_1-C_{10})alkylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_3-C_8)heterocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; and *-(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the

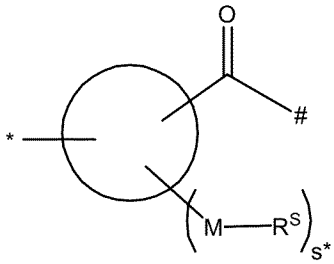
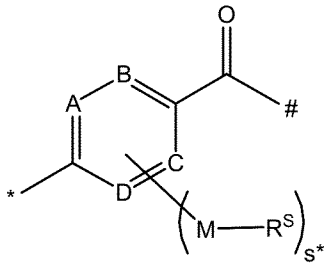
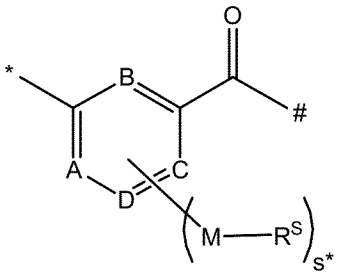
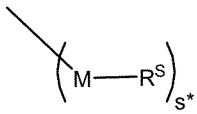
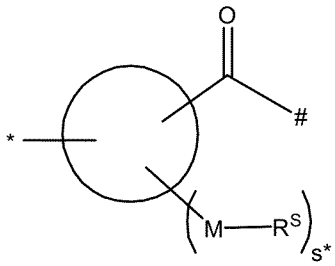
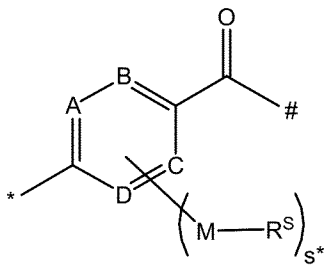
linker is present or not. Preferably, the group Z  , when present, may be selected from the group consisting of *-(C_3-C_8)carbocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; *-arylene-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; and *-(C_3-C_8)heterocyclo-# substituted, each independently, with 1 to 4, preferably 1 or 2, more preferably 1 group(s) -M-R^S; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

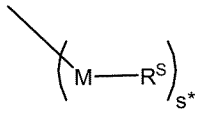
[00217] In some embodiments, the L^P in the group Z  may be one or more amino acid, which comprises a suitable moiety M so that a second polyalkylene glycol unit can be attached; preferably s* is 1. The amino acid may be a natural or non-natural amino acid. For example, the amino acid may be selected from the group consisting of lysine, glutamic acid, aspartic acid, serine, tyrosine, threonine, cysteine, selenocysteine, glycine, and homoalanine. In particular, the amino acid may be selected from the group consisting of tyrosine, serine, threonine, glutamic acid, lysine and glycine. Other suitable moieties L^P may be selected from the group consisting of amino alcohols, amino aldehydes, and polyamines. Suitable amino acids and further groups for attaching a polyalkylene glycol unit are described, e.g. in WO 2015/057699.

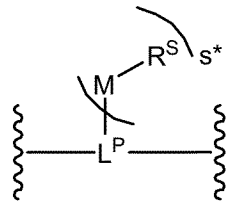
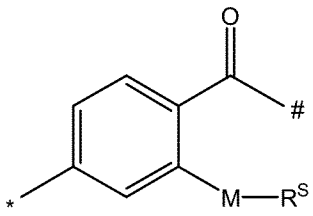
[00218] Preferably, the group Z  is  , wherein  is a five- or six-membered carbocyclic ring; the carbocyclic ring may be

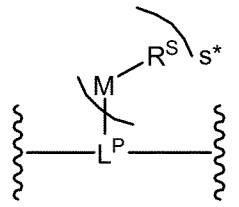
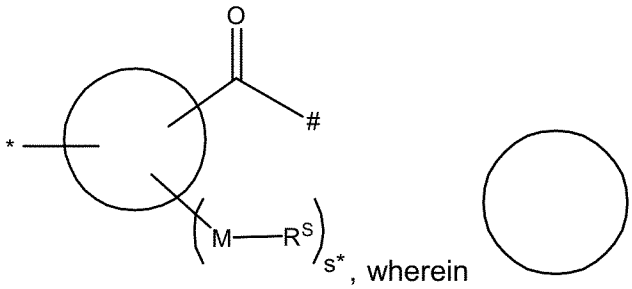
aromatic or non-aromatic; M is, each independently, as defined herein; preferably, each M is -O-; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; s* is an integer ranging from 1 to 3, preferably s* is 1 or 2, more preferably s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

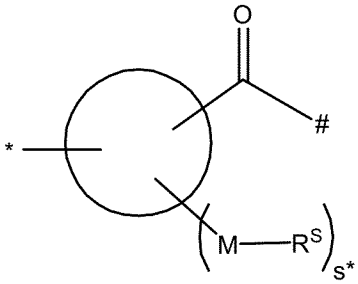
[00219] More preferably,  is selected from the group consisting of  ,  , and  , wherein each of A, B, C and D is C-H; R^S is, each independently, a second poly(alkylene)glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker (e.g., an amino acid unit -W_w-), when present, or to a camptothecin moiety (-C), depending on whether another part of

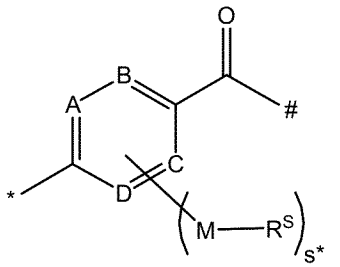
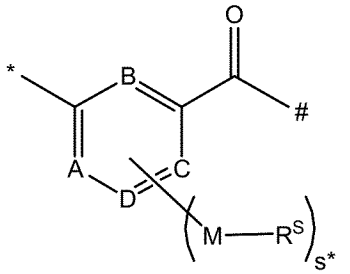
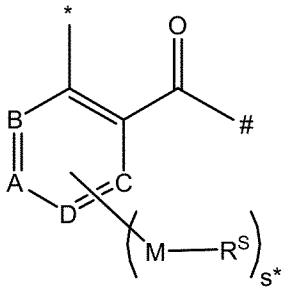
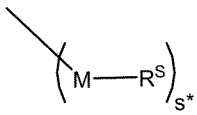
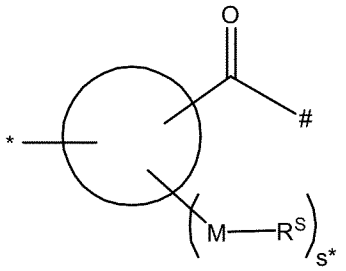
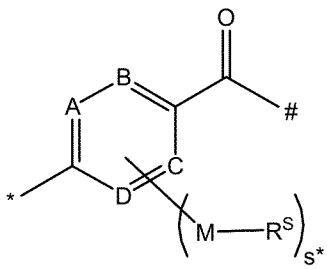
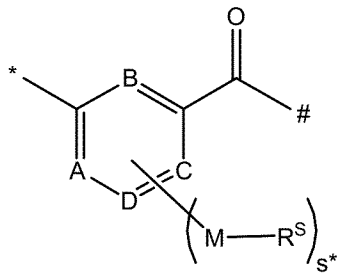
the linker is present or not. Still more preferably,  is  or  , wherein each of A, B, C and D is C-H; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. Still more preferably,  is  , wherein each of A, B, C and D is C-H; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by

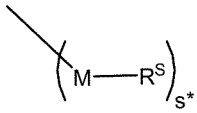
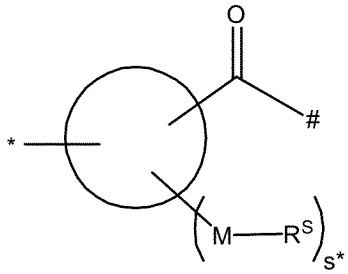
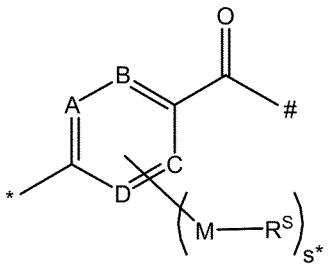
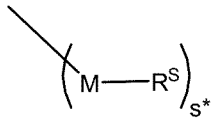
the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

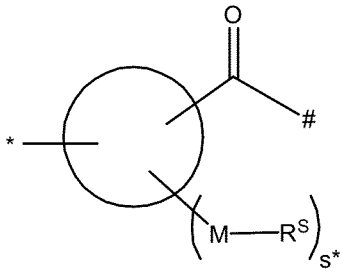
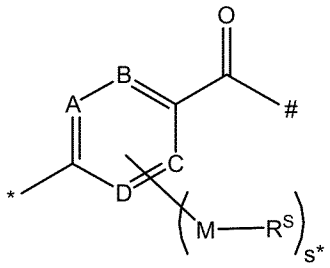
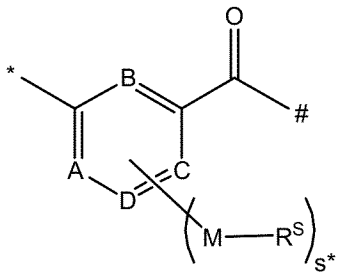
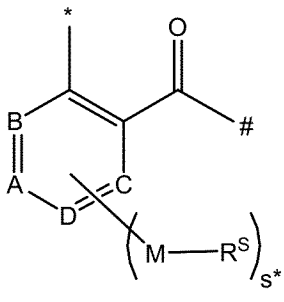
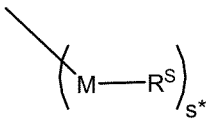
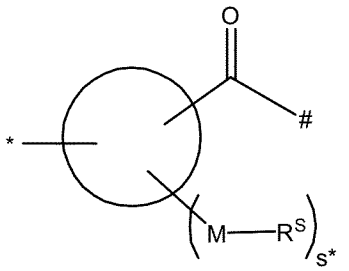
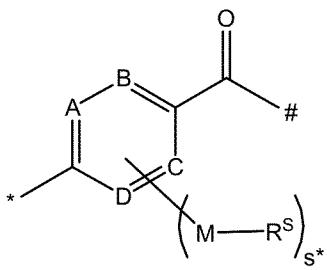
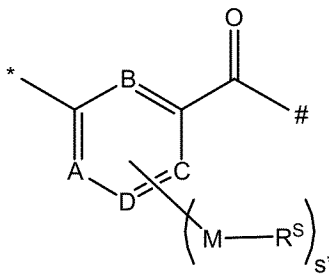
In very preferred embodiments, the group Z  is  wherein R^S is a second polyalkylene glycol unit as described herein; preferably, R^S is a second polyethylene glycol unit as defined herein; M is as described herein; preferably M is -O-; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

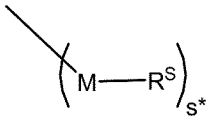
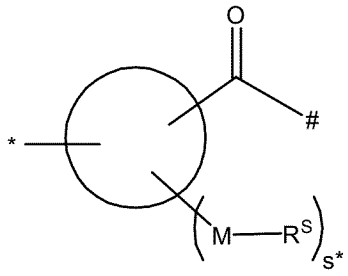
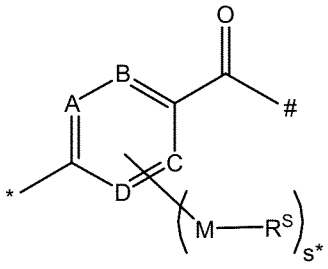
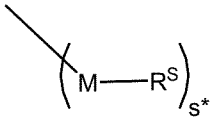
[00220] In some embodiments, the group Z  is  , wherein is a five- or six-membered heterocyclic ring comprising 1 or 2 heteroatoms independently selected from the group consisting of N, O or S; the heterocyclic ring may be aromatic or non-aromatic; M is, each independently, as defined herein; preferably each M is -O-; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; s* is 1 or 2 (in particular, in case of a six-membered heterocyclic ring), preferably s* is 1 (in particular, in case of a five-membered or six-membered heterocyclic ring); * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.



[00221] In some embodiments, is selected from the group consisting of  ,  , and  , wherein three of A, B, C and D are, independently, C-H, and one of A, B, C and D is, independently, N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. In some embodiments,  is  or  , wherein three of A, B, C and D are, independently, C-H, and one of A, B, C and D is, independently,

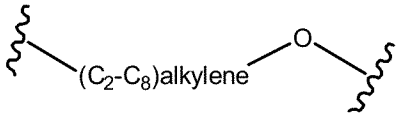
N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. In some embodiments,  is  , wherein three of A, B, C and D are, independently, C-H, and one of A, B, C and D is, independently, N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

[00222] In some embodiments,  is selected from the group consisting of  ,  , and  , wherein two of A, B, C and D are, independently, C-H, and two of A, B, C and D are, independently, N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. In some embodiments,  is  or  , wherein two of A, B, C and D are, independently, C-H, and two of A, B, C and D are, independently, N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably,

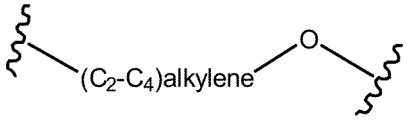
each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not. In some embodiments,  is  , wherein two of A, B, C and D are, independently, C-H, and two of A, B, C and D are, independently, N; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; the integer s* is 1 or 2, preferably s* is 1; as indicated by the  , in two C-H, independently, the H is replaced with -M-R^S when s* is 2, or in one C-H the H is replaced with -M-R^S when s* is 1; * denotes the attachment point to the -Y-; and # denotes the attachment point to another part of the linker, when present, or to a camptothecin moiety (-C), depending on whether another part of the linker is present or not.

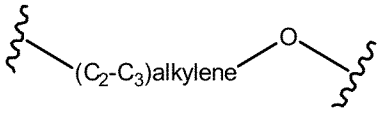
Second Polyalkylene Glycol Unit R^S

[00223] The term "second polyalkylene glycol unit", as used herein, refers to a polyalkylene glycol unit bound to the parallel connector unit (L^P), which is present in the group Z,via M. The second polyalkylene glycol unit comprises at least one alkylene glycol subunit. Preferably, the second polyalkylene glycol unit R^S comprises one or more alkylene glycol subunits having the following structure:



. More preferably, the second polyalkylene glycol unit R^S comprises one or more alkylene glycol subunits having the following structure:



. Accordingly, the second polyalkylene glycol unit R^S may be a poly(tetramethyleneglycol) unit, a poly(propyleneglycol) unit, or a poly(ethylenglycol) unit. Still more preferably, the second polyalkylene glycol unit comprises one or more alkylene glycol subunits having the following structure: 

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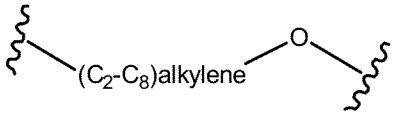
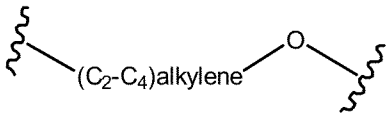
[00224] Preferably, the second polyalkylene glycol unit R^S, each independently, comprises of from 1 to 100 alkylene glycol subunits as described herein. More preferably, the second polyalklyene glycol unit R^S, each independently, comprises of from 2 to 50 alkylene glycol subunits. Still more preferably, the second polyalkylene glycol unit comprises, each independently, of from 3 to 45 alkylene glycol subunits as described herein. Still more preferably, the second polyalkylene glycol unit, each independently, comprises of from 4 to 40 alkylene glycol subunits as described herein. Still more preferably, the second polyalkylene glycol unit, each independently, comprises of from 6 to 35 alkylene glycol subunits as described herein. Even more preferably, the second polyalkylene glycol unit, each independently comprises of from 8 to 30 alkylene glycol subunits as described herein.

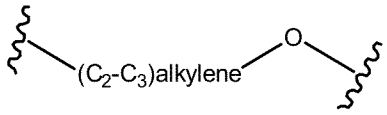
[00225] Preferably, the second polyalkylene glycol unit R^S, each independently, comprises of from 1 to 20 alkylene glycol subunits as described herein. More preferably, the second polyalklyene glycol unit R^S comprises, each independently, of from 2 to 12 alkylene glycol subunits. Still more preferably, the second polyalkylene glycol unit comprises, each independently, of from 3 to 11 alkylene glycol subunits as described herein.

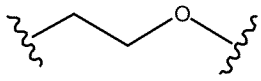
[00226] The second polyalkylene glycol unit R^S may be, each independently, a polyalkylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure:



. Preferably, the second polyalkylene glycol unit R^S may be,

each independently, a polyalkylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

. More preferably, the second polyalkylene glycol unit R^S may be, each independently, a polyalkylene glycol unit comprising of from 1 to 100, more preferably of from 2 to 50, still more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

. In very preferred embodiments, the second polyalkylene glycol unit R^S may be, each independently, a polyethylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

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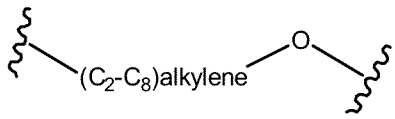
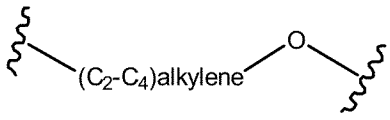
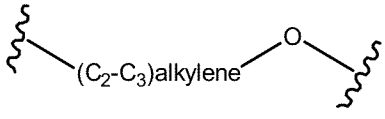
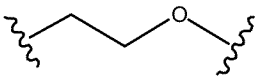
[00227] The second polyalkylene glycol unit R^S may be, each independently, a polyalkylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits having the structure: 

. Preferably, the second polyalkylene glycol unit R^S may be, each independently, a polyalkylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits having the structure: 

. More preferably, the second polyalkylene glycol unit R^S may be, each independently, a polyalkylene glycol unit comprising of from 1 to 20, more preferably of from 2 to 12, still more preferably of from 3 to 11 subunits having the structure: 

. In very preferred embodiments, the second polyalkylene glycol unit R^S may be, each independently, a polyethylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably 3 to 11 subunits having the structure: 

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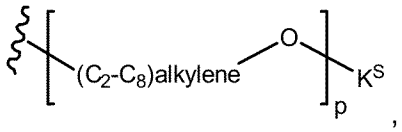
[00228] Preferably, the second polyalkylene glycol unit R^S is, each independently,



,

wherein:

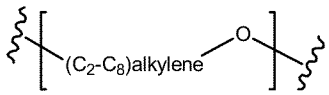
 indicates the position of the M in group Z;

K^S is H or a second capping group; preferably K^S is selected from the group consisting of -H (hydrogen), $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-NH$(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-N$((C_1\text{-}C_3)\text{alkyl})_2$; more preferably K^S is H; and

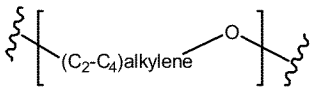
p is an integer ranging from 1 to 100.

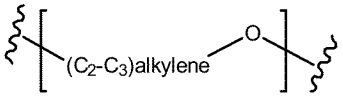
[00229] The "second capping group", when referred to herein, may be any moiety which is capable to function as a terminal group of the second polyalkylene glycol unit. Examples for second capping groups, which can be used in the present disclosure, include -PO_3H, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-NH$(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-N$((C_1\text{-}C_3)\text{alkyl})_2$. In some embodiments, the first capping group may be $-(C_1\text{-}C_{10})$alkyl, in particular methyl.

[00230] Preferably, K^S is H (hydrogen).

[00231] The integer p denotes the number of repeating units 

in the second polyalkylene glycol unit. The integer p may range from 1 to 100. Preferably, p ranges from 2 to 50. More preferably, p ranges from 3 to 45. More preferably, p ranges from 4 to 40. Still more preferably, p ranges from 6 to 35. Even more preferably, p ranges from 8 to 30. In preferred embodiments, p is 12 or about 12. Even more preferably, p ranges from 16 to 30. Even more preferably, p ranges from 20 to 28. Even more preferably, p is 22, 23, 24, 25 or 26. Even more preferably, p is 23, 24 or 25. In preferred embodiments, p is 24 or

about 24. Preferably, the repeating unit is 

. More preferably, the repeating unit is 

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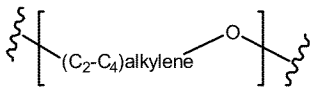
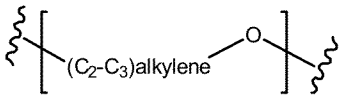
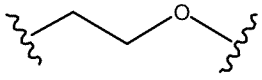
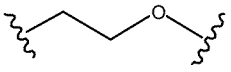
[00232] In the second polyalkylene glycol unit, the integer p may range from 1 to 20. Preferably, p ranges from 2 to 12. More preferably, p ranges from 3 to 11. Preferably, the repeating unit is 

. More preferably, the repeating unit is 

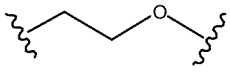
.

[00233] Preferably, the second polyalkylene glycol unit R^S comprises ethylene glycol subunits each having the following structure: 

, i.e. this subunit is denoted an “ethylene glycol subunit”. Accordingly, preferably the second polyalkylene glycol unit is a second polyethylene glycol unit. The second polyethylene glycol unit comprises at least one ethylene glycol subunit.

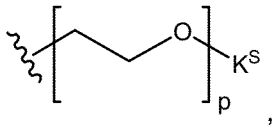
[00234] Preferably, the second polyalkylene glycol unit R^S may be, each independently, a second polyethylene glycol unit comprising of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 subunits having the structure: 

.

[00235] Preferably, the second polyalkylene glycol unit R^S may be, each independently, a second polyethylene glycol unit comprising of from 1 to 20, preferably of from 2 to 12, more preferably of from 3 to 11 subunits having the structure: 

.

[00236] Preferably, the second polyalkylene glycol unit R^S is, each independently, a second polyethylene glycol unit having the structure:

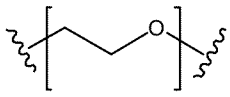


wherein:

 indicates the position of the M in group Z;

K^S is H (hydrogen) or a second capping group as described herein; preferably K^S is selected from the group consisting of -H (hydrogen) , $-PO_3H$, $-(C_1-C_{10})$alkyl, $-(C_1-C_{10})$alkyl-SO_3H, $-(C_2-C_{10})$alkyl-CO_2H, $-(C_2-C_{10})$alkyl-OH, $-(C_2-C_{10})$alkyl-NH_2, $-(C_2-C_{10})$alkyl-$NH(C_1-C_3)$alkyl and $-(C_2-C_{10})$alkyl-$N((C_1-C_3)alkyl)_2$; more preferably K^S is H; and

p is an integer ranging from 1 to 100.

[00237] The integer p denotes the number of repeating units  in the second polyethylene glycol unit. The integer p may range from 1 to 100. Preferably, p ranges from 2 to 50. More preferably, p ranges from 3 to 45. Still more preferably, p ranges from 4 to 40. Still more preferably, p ranges from 6 to 35. Even more preferably, p ranges from 8 to 30. In preferred embodiments, p is 12 or about 12. Even more preferably, p ranges from 16 to 30. Even more preferably, p ranges from 20 to 28. Even more preferably, p is 22, 23, 24, 25 or 26. Even more preferably, p is 23, 24 or 25. In preferred embodiments, p is 24 or about 24.

[00238] In the second polyethylene glycol unit, the integer p may range from 1 to 20. Preferably, p ranges from 2 to 12. More preferably, p ranges from 3 to 11.

[00239] In general, in the second polyalkylene glycol unit R^F, (preferably, second polyethylene glycol unit), polydisperse polyalkylene glycols (preferably, polydisperse polyethylene glycols), monodisperse polyalkylene glycols (preferably, monodisperse polyethylene glycol), and discrete polyalkylene glycols (preferably, discrete polyethylene glycols) can be used. Polydisperse polyalkylene glycols (preferably, polydisperse polyethylene glycols) are a heterogenous mixture of sizes and molecular weights, whereas monodisperse polyalkylene glycols (preferably, monodisperse polyethylene glycols) are typically purified from heterogenous mixtures and therefore provide a single chain length and molecular weight. Preferred second polyalkylene glycols units are discrete polyalkylene

glycols (preferably, discrete polyethylene glycols), i.e. compounds that are synthesized in step-wise fashion and not via a polymerization process. Discrete polyalkylene glycols (preferably, discrete polyethylene glycols) provide a single molecule with defined and specified chain length.

[00240] The second polyalkylene glycol unit (preferably, second polyethylene glycol unit) provided herein comprises one or multiple polyalkylene glycol chains (preferably, polyethylene glycol chains). The polyalkylene glycol chains (preferably, polyethylene glycol chains) can be linked together, for example, in a linear, branched or star shaped configuration. Optionally, at least one of the polyalkylene glycol chains (preferably, polyethyleneglycol chains) may be derivatized at one end for covalent attachment to the M in group Z .

[00241] The second polyalkylene glycol unit (preferably, second polyethylene glycol unit) will be attached to the conjugate (or intermediate thereof) at the M in group Z. The other terminus (or termini) of the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) will be free and untethered and may take the form of a hydrogen, methoxy, carboxylic acid, alcohol or other suitable functional group, such as e.g. any second capping group as described herein. The methoxy, carboxylic acid, alcohol or other suitable functional group acts as a cap for the terminal polyalkylene glycol subunit (preferably, polyethylene glycol subunit) of the second polyalkylene glycol unit (preferably, second polyethylene glycol unit). By untethered, it is meant that the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) will not be attached at that untethered site to a camptothecin moiety (C), to a receptor binding molecule, or to a component of the linker (L) linking a camptothecin moiety and/or a receptor binding molecule. For those embodiments wherein the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) comprises more than one polyalkylene glycol chain (preferably, polyethylene glycol chain), the multiple polyalkylene glycol chains (preferably, polyethylene glycol chains) may be the same or different chemical moieties (e.g., polyalkylene glycols, in particular polyethylene glycols, of different molecular weight or number of subunits). The multiple second polyalkylene glycol chains (preferably, second polyethylene glycol chains) are attached to the M in group Z at a single attachment site. The skilled artisan will understand that the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) in addition to comprising repeating polyalkylene glycol subunits (preferably, polyethylene glycol subunits) may also contain non-polyalkylene glycol material (preferably, non-polyethylene glycol material) (e.g., to facilitate coupling of multiple polyalkylene glycol chains (preferably, polyethylene glycol chains) to

each other or to facilitate coupling to the M in group Z. Non-polyalkylene glycol material (preferably, non-polyethelyne glycol material) refers to the atoms in the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) that are not part of the repeating alkylene glycol subunits (preferably, $-CH_2CH_2O-$ subunits). In embodiments provided herein, the second polyalkyleneglycol unit (preferably, second polyethyleneglycol unit) can comprise two monomeric polyalkylene glycol chains (preferably, polyethylene glycol chains) linked to each other via non-polyalkylene glycol (preferably, non-polyethylene glycol) elements. In other embodiments provided herein, the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) can comprise two linear polyalkylene glycol chains (preferably, polyethylene glycol chains) attached to a central core that is attached to the M in group Z (i.e., the polyalkylene glycol unit (preferably, polyethyleneglycol unit) is branched).

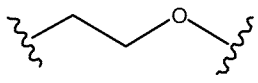
[00242] There are a number of polyalkylene glycol (preferably, polyethylene glycol) attachment methods available to those skilled in the art, [see, e.g., EP 0 401 384 (coupling PEG to G-CSF); U.S. Pat. No. 5,757,078 (PEGylation of EPO peptides); U.S. Pat. No. 5,672,662 (Polyethylene glycol) and related polymers mono substituted with propionic or butanoic acids and functional derivatives thereof for biotechnical applications); U.S. Pat. No. 6,077,939 (PEGylation of an N- terminal .alpha.-carbon of a peptide); and Veronese (2001) Biomaterials 22:405-417 (Review article on peptide and protein PEGylation)].

[00243] For example, polyalkylene glycol (preferably, polyethylene glycol) may be covalently bound to amino acid residues via a reactive group. Reactive groups are those to which an activated polyalkylene glycol molecule (preferably, polyethylene glycol molecule) may be bound (e.g., a free amino or carboxyl group). For example, N-terminal amino acid residues and lysine (K) residues have a free amino group; and C-terminal amino acid residues have a free carboxyl group. Sulfhydryl groups (e.g., as found on cysteine residues) may also be used as a reactive group for attaching polyalkylene glycol (preferably, polyethylene glycol). In addition, enzyme-assisted methods for introducing activated groups (e.g., hydrazide, aldehyde, and aromatic-amino groups) specifically at the C-terminus of a polypeptide have been described (see Schwarz, et al. (1990) Methods Enzymol. 184:160; Rose, et al. (1991) Bioconjugate Chem. 2: 154; and Gaertner, et al. (1994) J. Biol. Chem. 269:7224].

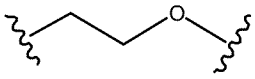
[00244] In some embodiments, at least one of the polyalkylene glycol chains (preferably, polyethylene glycol chains) that make up the second polyalkylene glycol unit (preferably, second polyethylene glycol unit) may be functionalized so that it can attach to the

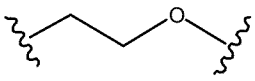
M in group Z, or to the parallel connector unit L^P in group Z when M is a bond. Functionalization can be, for example, via an amine, thiol, NHS ester, alkyne, azide, carbonyl, or other functional group. The polyalkylene glycol unit (preferably, polyethylene glycol unit) can further comprise non-polyalkylene glycol material (preferably, non-polyethylene glycol material, i.e., material not comprised of $-CH_2CH_2O-$) to facilitate coupling to the M in group Z or to the parallel connector unit, when M is a bond, or to facilitate coupling of two or more polyalkylene glycol chains (preferably, polyethylene glycol chains).

[00245] In preferred embodiments, the second polyalkylene glycol unit, more preferably the second polyethylene glycol unit, is directly attached to the M in group Z. In these embodiments, the second polyalkylene glycol unit, preferably second polyethylene glycol unit, does not comprise a functional group for attachment to the M in group Z, i.e. the M is directly bound to a carbon atom of the second polyalkylene glycol unit, more preferably to a CH_2 of the second polyethylene glycol unit. Preferably, in any one of these embodiments M is not a bond.

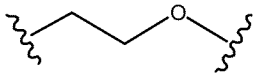
[00246] In one group of embodiments, the second polyalkylene glycol unit comprises at least 1 alkylene glycol subunit, preferably at least 2 alkylene glycol subunits, more preferably at least 3 alkylene glycol subunits, still more preferably at least 4 alkylene glycol subunits, still more preferably at least 6 alkylene glycol subunits, even more preferably at least 8 alkylene glycol subunits. In some such embodiments, the seond polyalkylene glycol unit comprises no more than about 100 alkylene glycol subunits, preferably no more than about 50 alkylene glycol units, more preferably no more than about 45 alkylene glycol subunits, more preferably no more than about 40 alkylene glycol subunits, more preferably no more than about 35 subunits, even more preferably no more than about 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit.

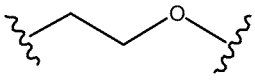
[00247] In one group of embodiments, the second polyalkylene glycol unit comprises one or more linear polyalkylene glycol chains each having at least 1 alkyleneglycol subunit, preferably at least 2 alkyleneglycol subunits, more preferably at least 3 alkyleneglycol

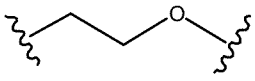
subunits, still more preferably at least 4 alkyleneglycol subunits, still more preferably at least 6 alkylene glycol subunits, even more preferably at least 8 alkylene glycol subunits. In preferred embodiments, the second polyalkylene glycol unit comprises a combined total of at least 1 alkylene glycol subunits, preferably at least 2 alkylene glycol subunits, more preferably at least 3, still more preferably at least 4, still more preferably at least 6, or even more preferably at least 8 alkylene glycol subunits. In some such embodiments, the second polyalkylene glycol unit comprises no more than a combined total of about 100 alkylene glycol subunits, preferably no more than a combined total of about 50 alkylene glycol subunits, more preferably no more than a combined total of about 45 subunits, still more preferably no more than a combined total of about 40 subunits, still more preferably no more than a combined total of about 35 subunits, even more preferably no more than a combined total of about 30 subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit comprising one or more linear polyethylene glycol chains.

[00248] In another group of embodiments, the second polyalkylene glycol unit comprises a combined total of from 1 to 100, preferably of from 2 to 50, more preferably of from 3 to 45, still more preferably of from 4 to 40, still more preferably of from 6 to 35, even more preferably of from 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit.

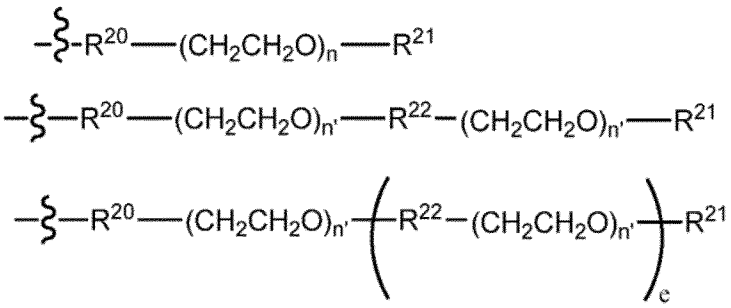
[00249] In another group of embodiments, the second polyalkylene glycol unit comprises one or more linear polyalkylene glycol chains having a combined total of from 1 to 100, preferably 2 to 50, more preferably 3 to 45, still more preferably 4 to 40, still more preferably 6 to 35, even more preferably 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an

ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit comprising one or more linear polyethylene glycol chains.

[00250] In another group of embodiments, the second polyalkylene glycol unit is a linear single polyalkylene glycol chain having at least 1 subunit, preferably at least 2 subunits, more preferably at least 3 subunits, still more preferably at least 6 subunits, even more preferably at least 8 subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit which is a linear single polyethylene glycol chain. Optionally, in any one of these embodiments the linear single polyalkylene glycol chain may be derivatized.

[00251] In another group of embodiments, the second polyalkylene glycol unit is a linear single polyalkylene glycol chain having from 1 to 100, preferably 2 to 50, more preferably 3 to 45, more preferably 4 to 40, more preferably 6 to 35, more preferably 8 to 30 alkylene glycol subunits. In any one of these embodiments, the alkylene glycol subunit may be any alkylene glycol subunit as described herein. Preferably, in any one of these embodiments, each alkylene glycol subunit is an ethylene glycol subunit having the following structure:  . Preferably, when each alkylene glycol subunit is an ethylene glycol subunit, in any one of these embodiments the second polyalkylene glycol unit is a second polyethylene glycol unit which is a linear single polyethylene glycol chain. Optionally, in any one of these embodiments the linear single polyalkylene glycol chain may be derivatized.

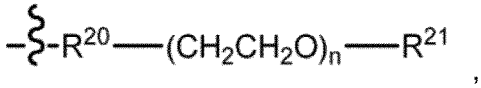
[00252] Exemplary linear polyethylene glycol units that can be used as second polyalkylene glycol unit, in particular as a second polyethylene glycol unit, in any one of the embodiments provided herein are as follows:



wherein the wavy line indicates the site of attachment to the M in group Z;

R^{20} is a PEG attachment unit; preferably, R^{20} is absent; more preferably, M is not a bond;
R^{21} is a PEG capping unit (herein, R^{21} is also denoted as "K^S");
R^{22} is a PEG coupling unit (i.e. for coupling multiple PEG subunit chains together;
n is independently selected from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, more preferably from 4 to 40, still more preferably from 6 to 35, even more preferably from 8 to 30;
e is 2 to 5;
each n' is independently selected from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, more preferably from 4 to 40, still more preferably from 6 to 35, even more preferably from 8 to 30. In preferred embodiments, there are at least 1, preferably at least 2, more preferably at least 3, more preferably at least 4, more preferably at least 6, even more preferably at least 8 ethylene glycol subunits in the polyethylene glycol unit. In some embodiments, there are no more than 100, preferably no more than 50, more preferably no more than 45, more preferably no more than 40, more preferably no more then 35, even more preferably no more than 30 ethylene glycol subunits in the polyethylene glycol unit. When R^{20} is absent, a (CH_2CH_2O) subunit is directly bound to the M in group Z; more preferably, in such embodiments M is not a bond.

[00253] Preferably, the linear polyethylene glycol unit is



,

wherein the wavy line indicates the site of attachment to the M in group Z; R^{20}, R^{21} (also denoted herein as "K^S") and n are as defined herein; more preferably R^{20} is absent; still more

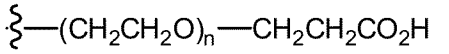
preferably, M is not a bond. In preferred embodiments, n is 12 or about 12. In preferred embodiments, n is 24 or about 24. Preferably, R^{21} is H.

[00254] The polyethylene glycol attachment unit R^{20}, when present, is part of the second polyethylene glycol unit and acts to link the second polyethylene glycol unit to the M. In these embodiments, preferably M is not a bond and forms a bond with the second polyethylene glycol unit. In exemplary embodiments, the PEG attachment unit R^{20}, when present, is selected from the group consisting of *-C(O)-$^{\#}$, *-S(O)-$^{\#}$, *-C(O)O-$^{\#}$, *-C(O)-(C_1-C_{10})alkyl-$^{\#}$, *-C(O)-(C_1-C_{10})alkyl-O-$^{\#}$, *-C(O)-(C_1-C_{10})alkyl-CO_2-$^{\#}$, *-C(O)-(C_1-C_{10})alkyl-NH-$^{\#}$, *-C(O)-(C_1-C_{10})alkyl-S-$^{\#}$; *-C(O)-(C_1-C_{10})alkyl-C(O)-NH-$^{\#}$; *-C(O)-(C_1-C_{10})alkyl-NH-C(O)-$^{\#}$; -(C_1-C_{10})alkyl-$^{\#}$, *-(C_1-C_{10})alkyl-O-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)O-$^{\#}$, *-(C_1-C_{10})alkyl-NH-$^{\#}$, *-(C_1-C_{10})alkyl-S-$^{\#}$, *-(C_1-C_{10})alkyl-C(O)-NH-$^{\#}$, *-(C_1-C_{10})alkyl-NH-C(O)-$^{\#}$, and *-CH_2-CH_2SO_2-(C_1-C_{10})alkyl-$^{\#}$, *-CH_2-C(O)-(C_1-C_{10})alkyl-$^{\#}$; wherein * denotes the attachment point to the M in group Z, and # denotes the attachment point to an ethylene glycol unit.

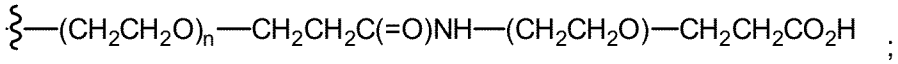
[00255] The PEG coupling unit R^{22}, when present, is part of the second polyethylene glycol unit and is non-PEG material that acts to connect two or more chains of repeating -CH_2CH_2O- subunits. In exemplary embodiments, the PEG coupling unit R^{22}, when present, is independently selected from the group consisting of *-(C_1-C_{10})alkyl-C(O)-NH-$^{\#}$, *-(C_1-C_{10})alkyl-NH-C(O)-$^{\#}$, *-(C_2-C_{10})alkyl-NH-$^{\#}$, *-(C_2-C_{10})alkyl-O-$^{\#}$, *-(C_1-C_{10})alkyl-S-$^{\#}$, or *-(C_2-C_{10})alkyl-NH-$^{\#}$; wherein * denotes the attachment point to an oxygen atom of an ethylene glycol subunit, and # denotes the attachment point to a carbon atom of another ethylene glycol subunit.

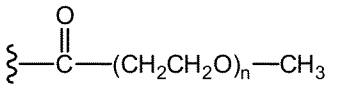
[00256] The group R^{21}, also denoted herein as "K^S", in exemplary embodiments is H (hydrogen), or may be a capping group, as described herein; preferably, R^{21} is independently selected from the group consisting of -H, -PO_3H, -(C_1-C_{10})alkyl, -(C_1-C_{10})alkyl-SO_3H, -(C_2-C_{10})alkyl-CO_2H, -(C_2-C_{10})alkyl-OH, -(C_2-C_{10})alkyl-NH_2, -(C_2-C_{10})alkyl-NH(C_1-C_3)alkyl and -(C_2-C_{10})alkyl-N((C_1-C_3)alkyl)$_2$. In some embodiments R^{21} may be -(C_1-C_{10})alkyl, in particular methyl. More preferably R^{21} is H.

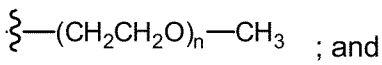
[00257] Illustrative linear second polyethylene glycol units, which can be used as second polyalkylene glycol units in any one of the embodiments provided herein, are as follows.



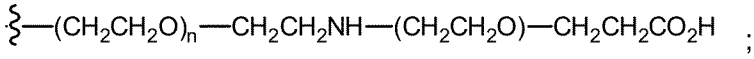
;







; and



wherein the wavy line indicates the site of attachment to the M in group Z; preferably, M is not a bond; and each n is from 1 to 100, preferably from 2 to 50, more preferably from 3 to 45, still more preferably from 4 to 40, still more preferably from 6 to 35, even more preferably from 8 to 30. In some embodiments, n is about 12. In some embodiments, n is about 24.

[00258] In some embodiments, the second polyalkylene glycol unit is from about 300 daltons to about 5 kilodaltons; from about 300 daltons, to about 4 kilodaltons; from about 300 daltons, to about 3 kilodaltons; from about 300 daltons, to about 2 kilodaltons; or from about 300 daltons, to about 1 kilodalton. In some such aspects, the second polyalkylene glycol unit has at least 6 alkylene glycol subunits or at least 8 alkylene glycol subunits. In some such aspects, the second polyalkylene glycol unit may have at least 6 alkylene glycol subunits or at least 8 alkylene glycol subunits but no more than 100 alkylene glycol subunits, preferably no more than 50 alkylene glycol subunits. In some embodiments, the second polyalkylene glycol unit is a second polyethylene glycol unit being from about 300 daltons to about 5 kilodaltons; from about 300 daltons, to about 4 kilodaltons; from about 300 daltons, to about 3 kilodaltons; from about 300 daltons, to about 2 kilodaltons; or from about 300 daltons, to about 1 kilodalton. In some such aspects, the second polyethylene glycol unit may have at least 6 ethylene glycol subunits or at least 8 ethylene glycol subunits. In some such aspects, the second polyethylene glycol unit may have at least 6 ethylene glycol subunits or at least 8 ethylene glycol subunits but no more than 100 ethylene glycol subunits, preferably no more than 50 ethylene glycol subunits.

[00259] In some embodiments, when a second polyalkylene glycol unit R^S is present, there are no other alkylene glycol subunits present in the conjugate of formula (I) (i.e., no alkylene glycol subunits are present in any of the other components of the conjugate, such

as e.g. in the group R^1 or in another part of the linker L as provided herein). In other aspects, when a second polyalkylene glycol unit R^S is present, there are no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2 or no more than 1 other alkylene glycol subunits present in the conjugate of formula (I) (i.e., no more than 8, 7, 6, 5, 4, 3, 2, or 1 other alkylene glycol subunits are present in other components of the conjugate, such as e.g. in the group R^1 or in another part of the linker L as provided herein).

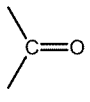
[00260] Preferably, in other embodiments, when a second polyalkylene glycol unit R^S is present, the conjugate further comprises a first polyalkylene glycol unit R^F as R^1, as described herein. Preferably, when R^S is a second polyethylene glycol unit and the conjugate further comprises a first polyalkylene glycol unit R^F, the first polyalkylene glycol unit is a first polyethyleneglycol unit, as described herein.

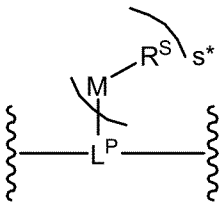
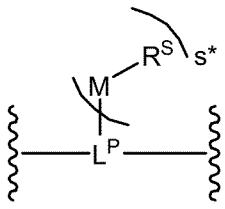
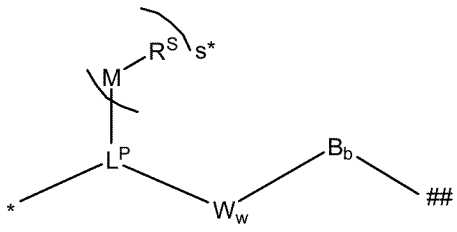
[00261] It will be appreciated that when referring to alkylene glycol subunits, in particular ethylene glycol subunits, and depending on context, the number of subunits can represent an average number, e.g., when referring to a population of conjugates or intermediate compounds, and using polydisperse polyalkylene glycols, in particular polydisperse polyethylene glycols.

*Linker *–A_a–W_w–B_b–##*

[00262] In some embodiments, the Linker L has the formula: *–A_a–W_w–B_b–##, wherein: –A– is a second spacer unit, as described herein; a is 0 or 1; each –W– is independently an amino acid; w is independently an integer ranging from 0 to 12; –B– is a first spacer unit; and b is 0 or 1; * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety. Herein, the notation "W_w", or –"W_w–", or the like, i.e. the combination of W and the associated integer w, is also denoted as "amino acid unit". Examples for suitable second spacer units, amino acid units and first spacer units are described, e.g., in WO 2004/010957 A2.

[00263] In the linker having the structure *–A_a–W_w–B_b–##, the second spacer unit serves to connect a -Y- to the amino acid unit -W_w-. The second spacer unit (–A–) may be any second spacer unit as described herein. When present, the second spacer unit (-A-) may be any chemical group or moiety which is capable to link a -Y- to the amino acid unit. Alternatively, the second spacer unit may link the -Y- to the first spacer unit, in case no amino acid unit is present. Alternatively, the second spacer unit may link the -Y- to the

camptothecin moiety (-C), in case no first spacer unit and no amino acid unit are present. In this regard, the -Y-, as described herein, is bonded to the second spacer unit (–A–). The second spacer unit (–A–) may comprise or may be a functional group that is capable to form a bond to an amino acid unit (–W_w–), or to a first spacer unit (–B–), or to a camptothecin moiety (-C), depending on whether an amino acid unit (–W_w–) and/or a first spacer unit (–B–) is present or not. Preferably, the functional group, which is capable to form a bond to an amino acid unit (–W_w–), in particular to the N terminus of the amino acid unit, or to a first spacer unit (–B–), or to a camptothecin moiety (-C), is a carbonyl group which is depicted as, e.g.,  or -C(O)-. The integer a associated with the second spacer unit may be 0 or 1. Preferably, the integer a is 1. Alternatively, in other embodiments the second spacer unit is absent (a = 0).

[00264] In the linker *–A_a–W_w–B_b–$^{##}$, the second spacer unit -A-, when present, may be any second spacer unit as described herein. In preferred embodiments of the linker *–A_a–W_w–B_b–$^{##}$, the second spacer unit -A-, when present (a = 1), may be a group Z having the structure  , wherein  is as defined herein. Accordingly, in preferred embodiments, the linker (L) may have the structure  , wherein L^P, R^S, s*, M, W, w, B and b are as defined herein; * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00265] The amino acid unit (–W_w–), when present, may link the second spacer unit A to the first spacer unit B in case the first spacer unit is present. Alternatively, the amino acid unit may link the second spacer unit to the camptothecin moiety (C) in case the first spacer unit is absent. Alternatively, the amino acid unit may link the Y to the first spacer unit in case the second spacer unit is absent. Alternatively, the amino acid unit may link the Y to the camptothecin moiety in case the first spacer unit and the second spacer unit are absent.

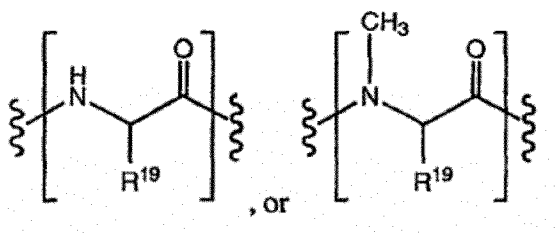
[00266] The amino acid unit -W_w- may be a dipeptide (w = 2), a tripeptide (w = 3), a tetrapeptide (w = 4), a pentapeptide (w = 5), a hexapeptide (w = 6), a heptapeptide (w = 7), an octapeptide (w = 8), a nonapeptide (w = 9), a decapeptide (w = 10), an undecapeptide (w = 11) or a dodecapeptide (w = 12).

[00267] In some embodiments, the amino acid unit can comprise natural amino acids. In some embodiments, the amino acid unit can comprise non-natural amino acids.

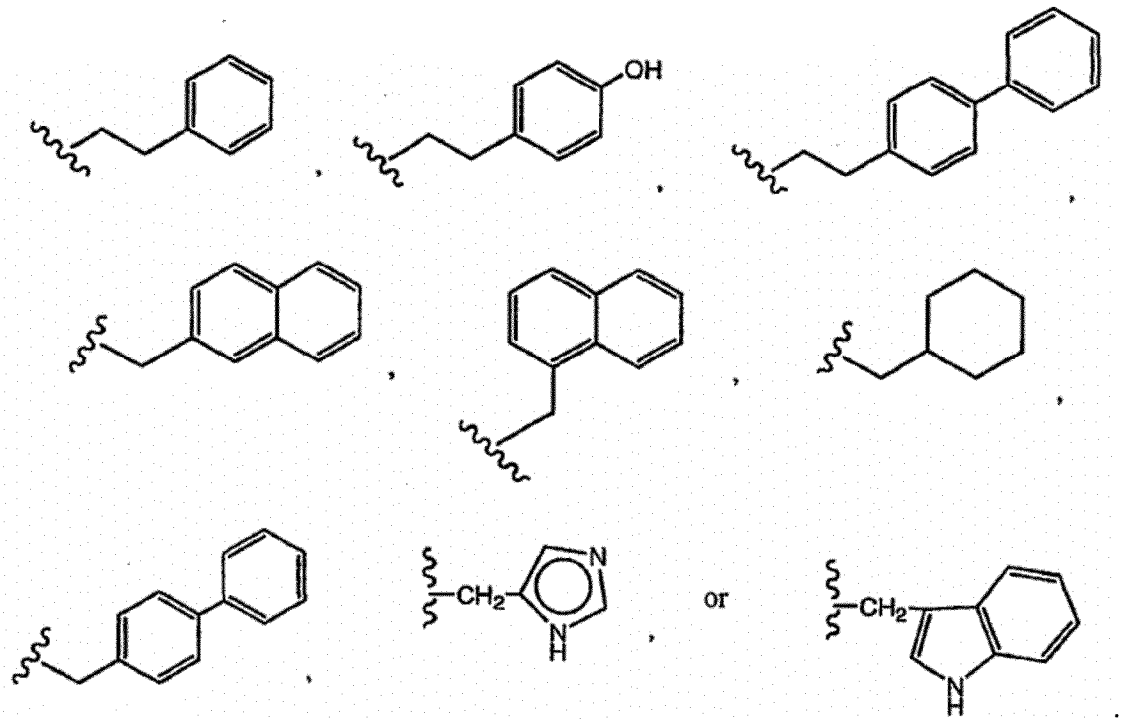
[00268] In any one of the embodiments described herein, each amino acid of the amino acid unit, except for amino acids which are not chiral such as e.g. glycine, may be independently in the L configuration or in the D configuration. Preferably, in any one of the embodiments described herein each amino acid of the amino acid unit, except for amino acids which are not chiral such as e.g. glycine, is in the L configuration (i.e., in the naturally occurring configuration).

[00269] Preferably, when a second spacer unit (-A-) is present, in any one of the embodiments described herein the N terminus of the amino acid unit -W_w- is bound to the second spacer unit (A), more preferably via a carbonyl group of the second spacer unit. Preferably, in any one of the embodiments described herein, the C terminus of the amino acid unit -W_w- is bound to a first spacer unit (B) in case a first spacer unit is present. Alternatively, in any one of the embodiments described herein, the C terminus of the amino acid unit -W_w- may be bound to the camptothecin moiety (-C) in case a first spacer unit is absent. In other embodiments, the N-terminus of the amino acid unit -W_w- may be bound to the first spacer unit (B), when present, and the C-terminus may be bound to the second spacer unit A, when present.

[00270] In some embodiments, w may be 1 or 2. Preferably, the amino acid unit W_w is a dipeptide (w = 2). In the dipeptide each amino acid independently may have the formula denoted below in the square brackets,:

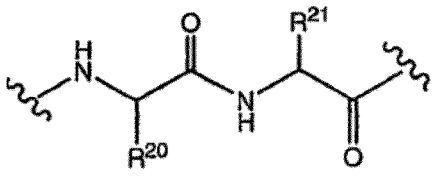


wherein R^{19} is hydrogen, methyl, isopropyl, isobutyl, *sec*-butyl, benzyl, *p*-hydroxybenzyl, -CH_2OH, -$CH(OH)CH_3$, -$CH_2CH_2SCH_3$, -CH_2CONH_2, -CH_2COOH, -$CH_2CH_2CONH_2$, -CH_2CH_2COOH, -$(CH_2)_3NHC(=NH)NH_2$, -$(CH_2)_3NH_2$, -$(CH_2)_3NHCOCH_3$, -$(CH_2)_3NHCHO$, -$(CH_2)_4NHC(=NH)NH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_4NHCOCH_3$, -$(CH_2)_4NHCHO$, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NHCONH_2$, -$CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



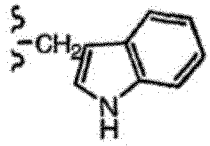
[00271] The amino acid unit can be enzymatically cleaved by one or more enzymes, including but not limited to a tumor-associated protease, preferably a cathepsin, more preferably cathepsin B, to liberate the camptothecin moiety (-C), which in one embodiment is protonated *in vivo* upon release to provide a free camptothecin moiety (C). Illustrative -W_w- units are represented by formula (**VII**).

[00272] Accordingly, the -W_w- unit may be a dipeptide of formula (**VII**):



(**VII**)

wherein R^{20} and R^{21} are as follows:

R^{20}	R^{21}
benzyl	$(CH_2)_4NH_2$;
methyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_4NH_2$;
isopropyl	$(CH_2)_3NHCONH_2$;
benzyl	$(CH_2)_3NHCONH_2$;
isobutyl	$(CH_2)_3NHCONH_2$;
sec-butyl	$(CH_2)_3NHCONH_2$;
	$(CH_2)_3NHCONH_2$;
benzyl	methyl; and
benzyl	$(CH_2)_3NHC(=NH)NH_2$;

[00273] Exemplary amino acid units include, but are not limited to, units of formula (**VII**) where: R^{20} is benzyl and R^{21} is $-(CH_2)_4NH_2$ (Phe-Lys); R^{20} is isopropyl and R^{21} is $-(CH_2)_4NH_2$ (Val-Lys); R^{20} is isopropyl and R^{21} is $-(CH_2)_3NHCONH_2$ (Val-Cit).

[00274] Useful $-W_w-$ units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease. In one embodiment, a $-W_w-$ unit is that whose cleavage is catalyzed by cathepsin B, C and/or D, or a plasmin protease ("tumor-associated proteases"). Preferably, the $-W_w-$ unit is cleaved by cathepsin B. Suitable linkers, which can be cleaved by a protease, are described, e.g., in G.M. Dubowchik et al., *"Cathepsin B-Labile Dipeptide Linkers for Lysosomal Release of Doxorubicin from Internalizing Immunoconjugates; Model Studies of Enzymatic Drug Release and Antigen-Specific In Vitro Anticancer Activity"*, Bioconjugate Chem., Vol. 13, No. 4, 2002, 855-869; S.C. Jeffrey et al., *"Dipeptide-based highly potent doxorubicin antibody conjugate"*, Bioorg. Med. Chem. Lett. 16 (2006), 358-362; and M.S. Kung Sutherland et al., *"SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML"*, Blood, 22 August 2013, volume 122, number 8, 1455-1463.

[00275] When R^{19}, R^{20} or R^{21} is other than hydrogen, the carbon atom to which R^{19}, R^{20} or R^{21} is attached is chiral. Each carbon atom to which R^{19}, R^{20} or R^{21} is attached may be independently in the (S) or (R) configuration. Preferably, each carbon atom to which R^{19}, R^{20} or R^{21} is attached, when chiral, is in the (S) configuration.

[00276] In one preferred embodiment, the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). In another preferred embodiment, the amino acid unit is valine-alanine (i.e. Val-

Ala or VA). In another preferred embodiment, the amino acid unit is alanine-alanine (i.e. Ala-Ala or AA). In another preferred embodiment, the amino acid unit is phenylalanine-lysine (i.e. Phe-Lys or FK). Such linkers are illustrative examples for a linker which can be cleaved by a protease, such as e.g. cathepsin B.

[00277] The notation of peptides used herein throughout this specification follows the conventional nomenclature. Accordingly, the N-terminus of a peptide is written on the left, and the C-terminus of the peptide is written on the right. As an illustrative but non-limiting example, in the dipeptide valine-citrulline (i.e. Val-Cit or VC), the valine has the N-terminus, and the citrulline has the C-terminus. Preferably, in any one of the embodiments described herein, when a second spacer unit (-A-) is present, the N-terminus of a peptide, such as e.g. of a dipeptide (as illustrative non-limiting example: Val-Cit), is bound to the second spacer unit (-A-), more preferably via a carbonyl group of the second spacer unit, and the C-terminus of the peptide is bound to a first spacer unit (-B-), in case a first spacer unit (-B-) is present, or to the camptothecin moiety (-C) in case a first spacer unit (-B-) is absent.

[00278] In yet another embodiment, the amino acid unit is N-methylvaline-citrulline. In yet another embodiment, the amino acid unit is selected from the group consisting of 5-aminovaleric acid, homophenylalanine-lysine, tetraisoquinolinecarboxylate-lysine, cyclohexylalanine-lysine, isonepecotic acid-lysine, betaalanine-lysine, and isonepecotic acid.

[00279] Preferably, the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, the amino acid unit is valine-citrulline (i.e. Val-Cit or VC).

[00280] In some embodiments, the amino acid unit is selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). Preferably, the amino acid unit is selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). More preferably, the amino acid unit is valine-glutamine (i.e. Val-Gln or VQ) or

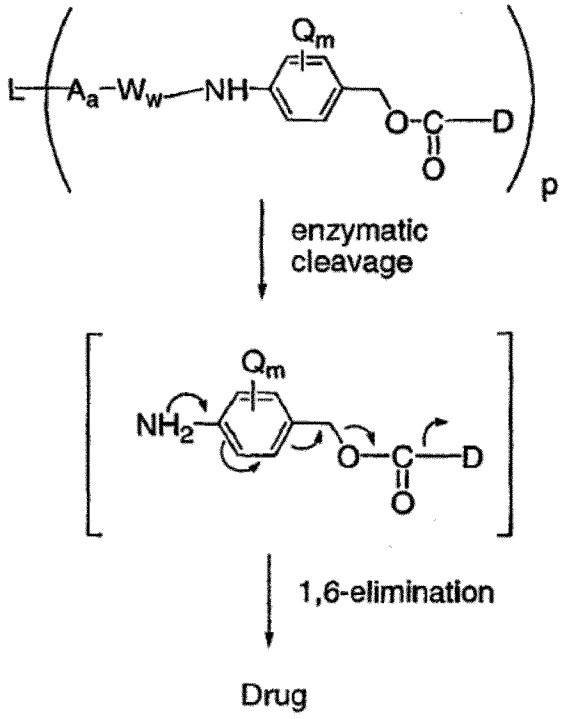
leucine-glutamine (i.e. Leu-Gln or LQ). Linkers which comprise amino acid units according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B). The amino acid unjts of these embodiments and further suitable amino acid units are disclosed, e.g., in Salomon et al., *"Optimizing Lysosomal Activation of Antibody-Drug Conjugates (ADCs) by Incorporation of Novel Cleavable Dipeptide Linkers"*, *Mol. Pharmaceutics* 2019, 16, 12, 4817–4825.

[00281] The first spacer unit (B), when present, may link an amino acid unit (W_w) to the camptothecin moiety when an amino acid unit is present. Alternatively, the first spacer unit (B) may link the second spacer unit (A) to the camptothecin moiety (C) when the amino acid unit is absent. The first spacer unit may link the camptothecin moiety to the Y when both the amino acid unit and second spacer unit are absent.

[00282] The integer b may be 0 or 1. In preferred embodiments, the integer b is 1. Alternatively, in other embodiments, the integer b is 0, and the first spacer unit is absent.

[00283] The first spacer unit (–B–) may be of two general types: self-immolative and non-self-immolative. A non-self-immolative first spacer unit is one in which part or all of the first spacer unit remains bound to the camptothecin moiety (C) after cleavage, particularly enzymatic, of an amino acid unit (–W_w–) of the linker (L). Alternatively, an exemplary compound containing a self-immolative first spacer unit can release a drug moiety -D without the need for a separate hydrolysis step. In an exemplary embodiment, a self-immolative first spacer unit is a PAB group that is linked to -W_w- via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group. Without being bound by any particular theory or mechanism, Scheme 2 depicts a possible mechanism of drug release of a PAB group which is attached directly to -D via a carbamate or carbonate group espoused by Toki et al. (2002) J Org. Chem. 67:1866-1872.

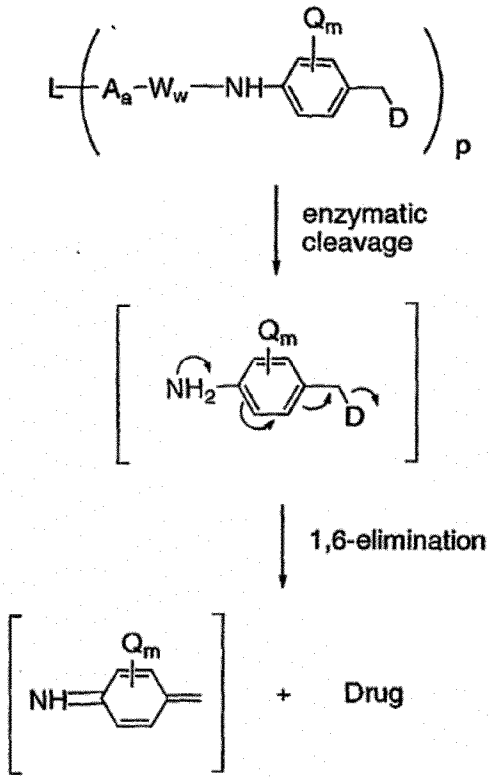
Scheme 2



wherein Q is -(C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -halogen, -nitro or -cyano; m is an integer ranging from 0 to 4, preferably m is 0, 1 or 2, more preferably m is 0 or 1, still more preferably m is 0; and p ranges from 1 to 20.

[00284] Without being bound by any particular theory or mechanism, Scheme 3 depicts a possible mechanism of drug release of a PAB group which is attached directly to a drug moiety -D via an ether or amine linkage.

Scheme 3

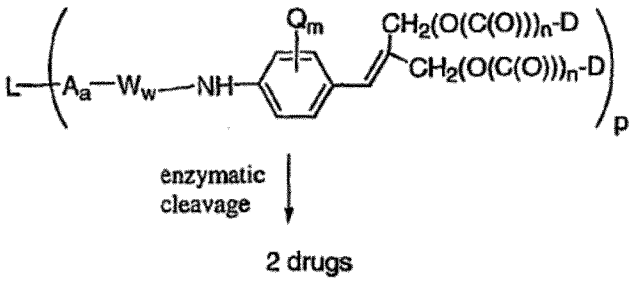


wherein Q is -(C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -halogen,- nitro or -cyano; m is an integer ranging from 0 to 4, preferably m is 0, 1 or 2, more preferably m is 0 or 1, still more preferably m is 0; and p ranges from 1 to 20.

[00285] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., Chemistry Biology, 1995, 2, 223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, et al., J. Amer. Chem. Soc., 1972, 94, 5815) and 2-aminophenylpropionic acid amides (Amsberry, et al., J. Org. Chem., 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the alpha-position of glycine (Kingsbury, et al., J. Med. Chem., 1984, 27, 1447) are also examples of self-immolative spacer useful in exemplary compounds.

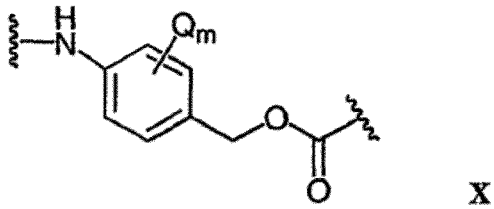
[00286] In one embodiment, the first spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted in Scheme 4, which can be used to incorporate and release multiple drugs (D).

Scheme 4



wherein Q is -(C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -halogen, -nitro or -cyano; m is an integer ranging from 0 to 4; preferably m is 0, 1 or 2; more preferably m is 0 or 1; still more preferably m is 0; and p ranges from 1 to 10; n is 0 or 1; and p ranges from 1 to 20.

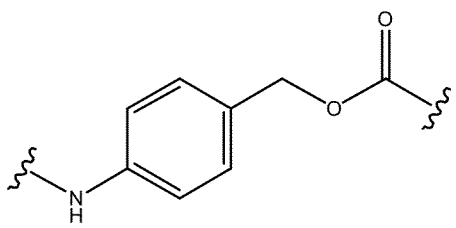
[00287] In preferred embodiments, the first spacer unit is represented by formula (**X**):



X

wherein Q is -(C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -halogen, -nitro or -cyano; and m is an integer ranging from 0 to 4; preferably m is 0, 1 or 2; more preferably m is 0 or 1; in very preferred embodiments m is 0. Preferably, when an amino acid unit is present, in formula (**X**), the NH group is bound to a C-terminus of the amino acid unit. Preferably, in formula (**X**), the C(O) group is bound to the camptothecin moiety (C).

[00288] In very preferred embodiments, the first spacer unit is a PAB group having the following structure:



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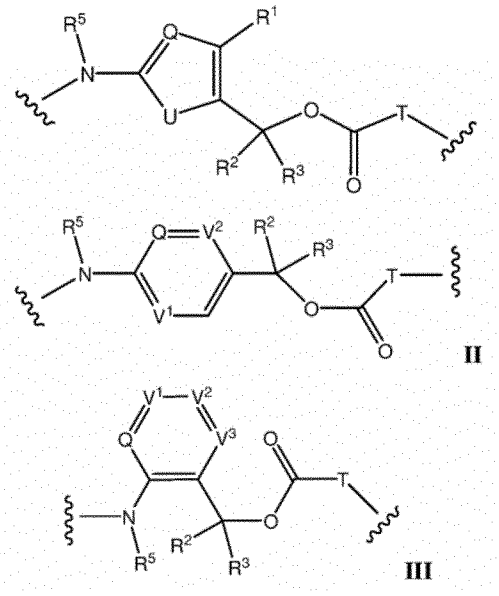
Preferably, when an amino acid unit is present, the NH group is bound to an amino acid unit (-W_w-), more preferably to a C-terminus of the amino acid unit. Preferably, the C(O) group is bound to the camptothecin moiety (C).

[00289] In some embodiments, the first spacer group (-B-) is a heterocyclic "self-immolating moiety" of Formulas **I**, **II** or **III** bound to the camptothecin moiety and incorporates an amide group that upon hydrolysis by an intracellular protease initiates a reaction that ultimately cleaves the first spacer unit (-B-) from the camptothecin moiety such that the drug is released from the conjugate in an active form. The linker moiety further comprises an amino acid unit (-W_w-) adjacent to the first spacer group (-B-) that is a substrate for an intracellular enzyme, for example an intracellular protease such as a cathepsin (e.g., cathepsin B), that cleaves the peptide at the amide bond shared with the first spacer group (-B-). Heterocyclic self-immolating moieties are described, e.g., in WO 2019/236954.

[00290] In some embodiments, the first spacer unit (-B-) is a heterocyclic self-immolating group selected from Formulas I, II and III:



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wherein the wavy lines indicate the covalent attachment sites to the amino acid unit -W_w- and the camptothecin moiety, and wherein U is O, S or NR^6; Q is CR^4 or N; V^1, V^2 and V^3 are independently CR^4 or N provided that for formula **II** and **III** at least one of Q, V^1 and V^2 is N; T is O pending from a camptothecin moiety (-C); R^1, R^2, R^3 and R^4 are independently selected from the group consisting of H, F, Cl, Br, I, OH, $-N(R^5)_2$, $-N(R^5)_3^+$, -(C_1-C_8)alkylhalide, carboxylate, sulfate, sulfamate, sulfonate, $-SO_2R^5$, $-S(=O)R^5$, $-SR^5$, $-SO_2N(R^5)_2$, $-C(=O)R^5$, -

CO_2R^5, -C(=O)N(R^5)$_2$, -CN, -N_3, -NO_2, -(C_1-C_8)alkoxy, -(C_1-C_8)halosubstituted alkyl, polyethyleneoxy, phosphonate, phosphate, -(C_1-C_8)alkyl, -(C_1-C_8)substituted alkyl, -(C_2-C_8)alkenyl, -(C_2-C_8)substituted alkenyl, -(C_2-C_8)alkynyl, -(C_2-C_8)substituted alkynyl, -(C_6-C_{20})aryl, -(C_6-C_{20})substituted aryl, -(C_3-C_{20})heterocycle, and -(C_3-C_{20})substituted heterocycle; or when taken together, R^2 and R^3 form a carbonyl (=O), or spiro carbocyclic ring of 3 to 7 carbon atoms; and R^5 and R^6 are independently selected from H, -(C_1-C_8)alkyl, -(C_1-C_8)substituted alkyl, -(C_2-C_8)alkenyl, -(C_2-C_8)substituted alkenyl, -(C_2-C_8)alkynyl, -(C_2-C_8)substituted alkynyl, -(C_6-C_{20})aryl, -(C_6-C_{20})substituted aryl, -(C_3-C_{20})heterocycle, and -(C_3-C_{20})substituted heterocycle; wherein -(C_1-C_8)substituted alkyl, -(C_2-C_8) substituted alkenyl, -(C_2-C_8)substituted alkynyl, -(C_6-C_{20})substituted aryl, and -(C_3-C_{20})substituted heterocycle are independently substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, OH, -N(R^5)$_2$, -N(R^5)$_3^+$, -(C_1-C_8)alkylhalide, carboxylate, sulfate, sulfamate, sulfonate, -(C_1-C_8)alkylsulfonate, -(C_1-C_8)alkylamino, 4-dialkylaminopyridinium, -(C_1-C_8)alkylhydroxyl, -(C_1-C_8)alkylthiol, -SO_2R^5, -S(=O)R^5, -SR^5, -SO_2N(R^5)$_2$, -C(=O)R^5, -CO_2R^5, -C(=O)N(R^5)$_2$, -CN, -N_3, -NO_2, -(C_1-C_8)alkoxy, -(C_1-C_8)trifluoroalkyl, -(C_1-C_8)alkyl, -(C_3-C_{12})carbocycle, -(C_6-C_{20})aryl, -(C_3-C_{20})heterocycle, polyethyleneoxy, phosphonate, and phosphate.

[00291] The conjugate comprising a heterocyclic self-immolative is stable extracellularly, or in the absence of an enzyme capable of cleaving the amide bond of the self-immolative moiety. However, upon entry into a cell, or exposure to a suitable enzyme, an amide bond is cleaved initiating a spontaneous self-immolative reaction resulting in the cleavage of the bond covalently linking the self-immolative moiety to the camptothecin moiety, to thereby effect release of the drug in its underivatized or pharmacologically active form.

[00292] The self-immolative moiety in conjugates either incorporates one or more heteroatoms and thereby may provide improved solubility, may improve the rate of cleavage and/or may decrease propensity for aggregation of the conjugate. Thus, the heterocyclic self-immolative linker constructs in some instances may result in increased efficacy, decreased toxicity, and/or desirable pharmacokinetic and/or pharmacodynamic properties.

[00293] It is understood that T in formulae **I-III** is O, as it is derived from the tertiary hydroxyl (-OH) on the lactone ring portion of a camptothecin moiety.

[00294] Not to be limited by theory or any particular mechanism, the presence of electron-withdrawing groups on the heterocyclic ring of formula **I**, **II** or **III** may moderate the rate of cleavage.

[00295] In one embodiment, the self-immolative moiety is the group of formula **I** in which Q is N, and U is O or S. Such a group has a non-linearity structural feature which improves solubility of the conjugates. In this context R is sometimes H, methyl, nitro, or CF_3. In one embodiment, Q is N and U is O thereby forming an oxazole ring and R is H. In another embodiment, Q is N and U is S thereby forming a thiazole ring optionally substituted at R with an Me or CF_3 group.

[00296] In another exemplary embodiment, the self-immolative moiety is the group of formula **II** in which Q is N and V^1 and V^2 are independently N or CH. In another embodiment, Q, V^1 and V^2 are each N. In another embodiment, Q and V^1 are N while V^2 is CH. In another embodiment, Q and V^2 are N while V^1 is CH. In another embodiment, Q and V^1 are both CH and V^2 is N. In another embodiment, Q is N while V^1 and V^2 are both CH.

[00297] In another embodiment, the self-immolative moiety is the group of formula **III** in which Q, V^1, V^2 and V^3 are each independently N or CH. In another embodiment Q is N while V^1, V^2 and V^3 are each N. In another embodiment, Q, V^1, and V^2 are each CH while V^3 is N. In another embodiment Q, V^2 and V^3 are each CH while V^1 is N. In another embodiment, Q, V^1 and V^3 are each CH while V^2 is N. In another embodiment, Q and V^2 are both N while V^1 and V^3 are both CH. In another embodiment Q and V^2 are both CH while V^1 and V^3 are both N. In another embodiment, Q and V^3 are both N while V^1 and V^2 are both CH.

[00298] Preferably, the linker (L) has the formula: $*–A_a–W_w–B_b–^{\#\#}$, wherein the integer a is 1, the integer b is 1, and the integer w is 2, 3 or 4, more preferably the integer w is 2 or 3; in very preferred embodiments the integer w is 2; and -A-, each -W- and -B- are as defined herein; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (C).

[00299] Preferably, the linker (L) has the following structure:

$*–A_a–W_w–B_b–^{\#\#}$,

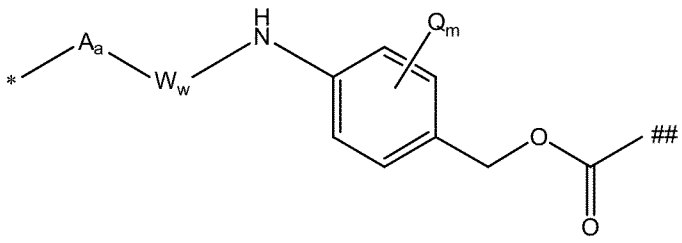
wherein -A- is a second spacer unit as described herein; a is an integer as described herein; preferably a is 1;

-B- is a first spacer unit as described herein; b is an integer as described herein; preferably b is 1;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C);

$-W_w-$ is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). Preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit $-W_w-$ may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00300] Preferably, the linker L has the following structure:



,

wherein -A- is a second spacer unit as described herein; a is an integer as described herein; preferably a is 1;

$-W_w-$ is an amino acid unit as described herein; w is an integer as described herein; preferably w is 2, 3 or 4 (i.e. preferably $-W_w-$ is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably $-W_w-$ is a dipeptide or a tripeptide), e.g. w

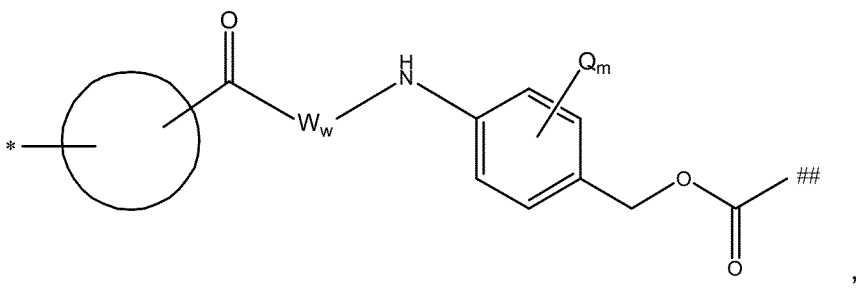
may be 1 or 2; in very preferred embodiments w is 2 (i.e. still more preferably -W_w- is a dipeptide);

Q is as defined herein;

m is an integer as defined herein, preferably m is 0;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00301] More preferably, the linker L has the following structure:



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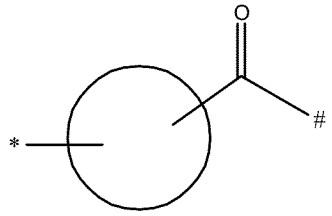
wherein:



is as defined herein; * denotes the attachment point to the Y; and # denotes the attachment point to the amino acid unit -W_w-, when present, or to the NH group;

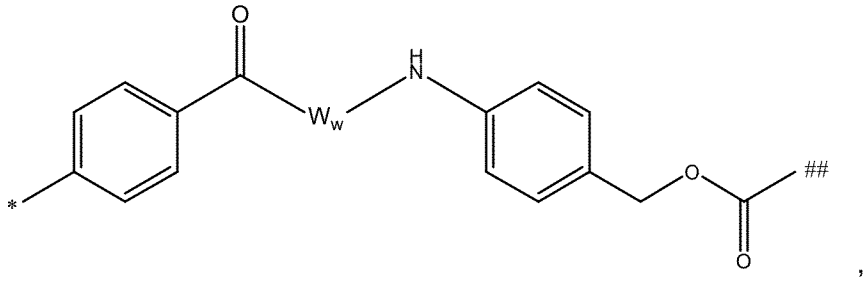
-W_w- is an amino acid unit as described herein; w is an integer as described herein, preferably w is 2, 3 or 4 (i.e. preferably -W_w- is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably -W_w- is a dipeptide or a tripeptide), in very preferred embodiments w is 2 (i.e. still more preferably -W_w- is a dipeptide);

Q is as defined herein;

m is an integer as defined herein, preferably m is 0;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

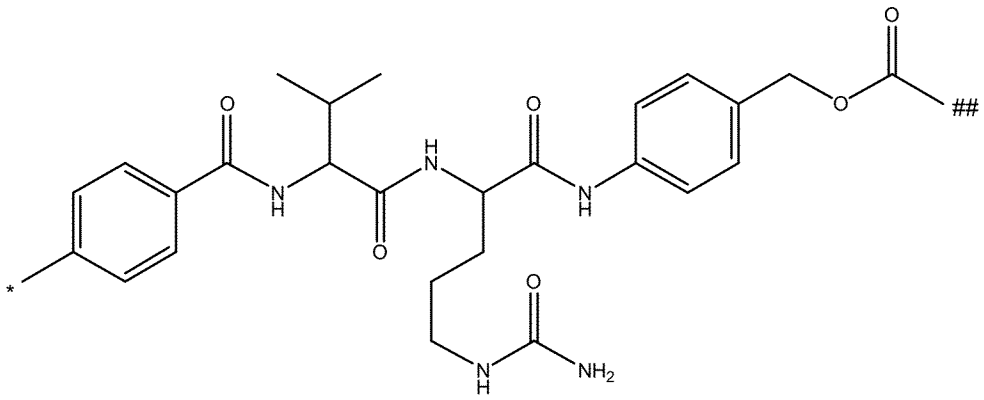
[00302] Still more preferably, the linker L has the following structure:



wherein:

-W_w- is an amino acid unit as described herein; w is an integer as described herein, preferably w is 2, 3 or 4 (i.e. preferably -W_w- is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably -W_w- is a dipeptide or a tripeptide), in very preferred embodiments w is 2 (i.e. still more preferably -W_w- is a dipeptide);
* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

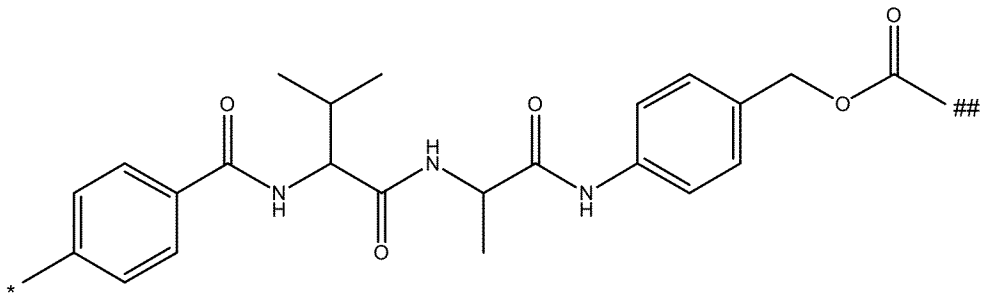
[00303] In a preferred embodiment, the linker L has the following structure:



,

which comprises the dipeptide valine-citrullin as the amino acid unit -W_w-; and
wherein * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Such linker is an illustrative example for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

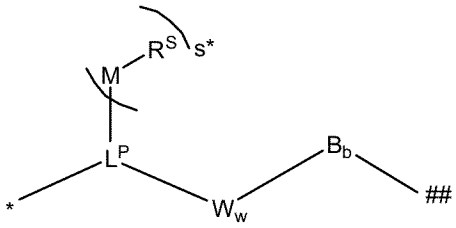
[00304] In another preferred embodiment, the linker L has the following structure:



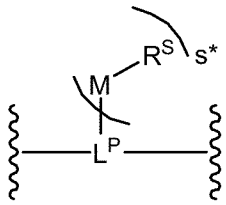
,

which comprises the dipeptide valine-alanine as the amino acid unit -W_w-; and
wherein * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Such linker is an illustrative example for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00305] Preferably, the linker (L) has the formula:

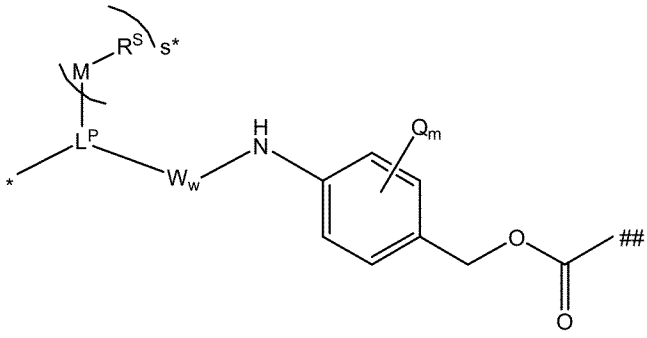


, wherein the integer b is 1, and the integer w is 2, 3 or 4, more preferably the integer w is 2 or 3, in very preferred embodiments the integer w is 2; and

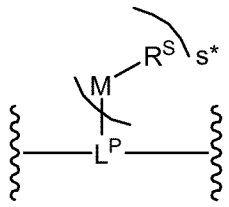


is as described herein; R^S is, each independently, a second polyalkylene glycol unit as described herein; preferably each R^S is, independently, a second polyethylene glycol unit as described herein; M is, each independently, as described herein, preferably each M is -O-; s* is an integer as described herein; preferably, s* is 1; each -W-, and -B- are as defined herein; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit $-W_w-$ is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). Preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). More preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit $-W_w-$ may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00306] Preferably, the linker L has the following structure:



,



wherein is as described herein; R^S is, each independently, a second polyalkylene glycol unit as described herein; preferably, each R^S is, independently, a second polyethylene glycol unit as described herein; M is, each independently, as described herein, preferably each M is -O-; s* is an integer as described herein; preferably, s* is 1;

$-W_w-$ is an amino acid unit as described herein; w is an integer as described herein; preferably w is 2, 3 or 4 (i.e. preferably $-W_w-$ is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably $-W_w-$ is a dipeptide or a tripeptide), in very preferred embodiments w is 2 (i.e. still more preferably $-W_w-$ is a dipeptide);

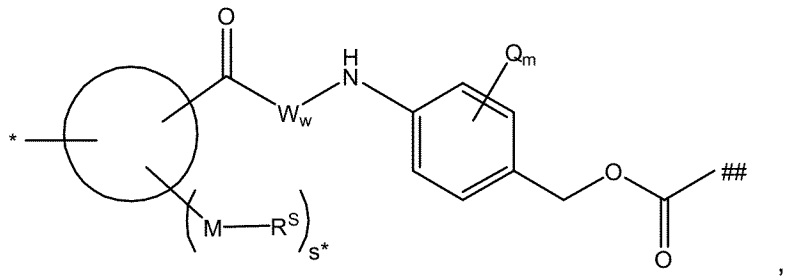
Q is as described herein;

m is an integer as described herein, preferably m is 0;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit $-W_w-$ is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit $-W_w-$ may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these

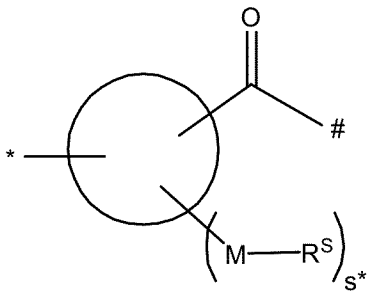
embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00307] More preferably, the linker L has the following structure:



,

wherein:



is as defined herein; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; s* is an integer as defined herein; preferably s* is 1; * denotes the attachment point to the Y; and # denotes the attachment point to the amino acid unit $-W_w-$, when present, or to the NH group;

$-W_w-$ is an amino acid unit as described herein; w is an integer as described herein, preferably w is 2, 3 or 4 (i.e. preferably $-W_w-$ is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably $-W_w-$ is a dipeptide or a tripeptide), in very preferred embodiments w is 2 (i.e. still more preferably $-W_w-$ is a dipeptide);

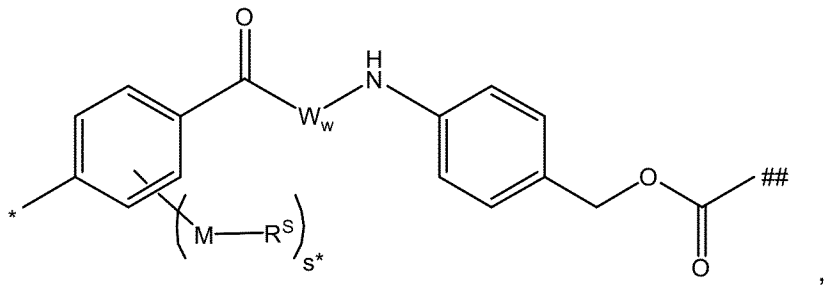
Q is as defined herein;

m is an integer as defined herein, preferably m is 0;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit $-W_w-$ is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e.

Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00308] Still more preferably, the linker L has the following structure:



,

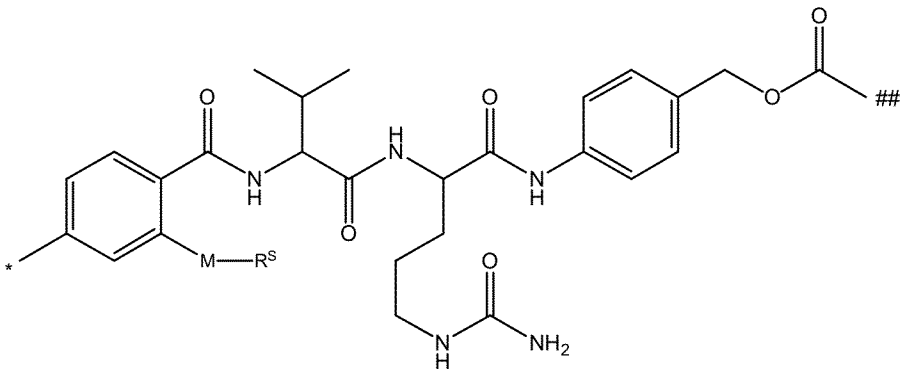
wherein:

R^S is, each independently a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein, preferably each M is -O-; s* is an integer as defined herein; preferably s* is 1;

-W_w- is an amino acid unit as described herein; w is an integer as described herein, preferably w is 2, 3 or 4 (i.e. preferably -W_w- is a dipeptide, a tripeptide or a tetrapeptide), more preferably w is 2 or 3 (i.e. more preferably -W_w- is a dipeptide or a tripeptide), still more preferably w is 2 (i.e. still more preferably -W_w- is a dipeptide);

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

[00309] In a preferred embodiment, the linker L has the following structure:

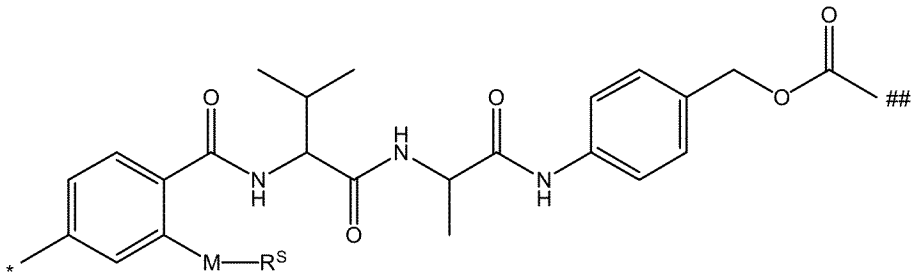


...,

which comprises the dipeptide valine-citrullin as the amino acid unit -W_w-;
wherein R^S is a second polyalkylene glycol unit as defined herein; preferably R^S is a second polyethylene glycol unit as defined herein; M is as defined herein; preferably M is -O-; and
* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Linkers according to these embodiments can be illustrative

examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

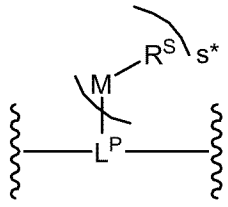
[00310] In another preferred embodiment, the linker L has the following structure:

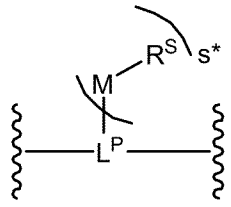


,

which comprises the dipeptide valine-alanine as the amino acid unit -W_w-; and
wherein R^S is a second polyalkylene glycol unit as defined herein; preferably R^S is a second polyethylene glycol unit as defined herein; M is as defined herein; preferably M is -O-; and
* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Linkers according to these embodiments can be illustrative examples for a linker which is cleavable, in particular by a protease, such as e.g. a cathepsin (e.g., cathepsin B).

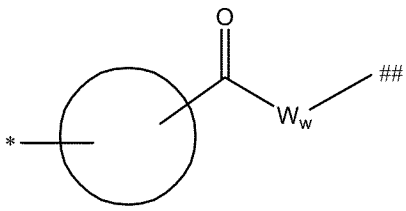
[00311] In some embodiments, the linker L has the formula: *–A_a–W_w–$^{##}$, wherein -A- is a second spacer unit as defined herein; the integer a associated with the second spacer unit is as defined herein; -W_w- is an amino acid unit as defined herein; the integer w associated with the amino acid unit W is a defined herein; the first spacer unit (-B_b-) is absent; * denotes the attachment point to the Y; and # denotes the attachment point to the camptothecin moiety (-C). Preferably, the integer a is 1. Preferably, the integer w is 2, 3 or 4, more preferably the integer w is 2 or 3, still more preferably the integer w is 2. Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected

from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ). In any one of these embodiments, the second spacer unit -A- may be a group Z having the structure 

, wherein 

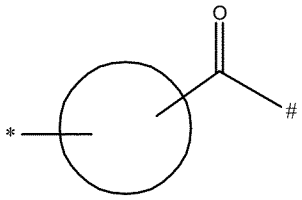
is as defined herein.

[00312] The linker L may have the following structure:



,

wherein:



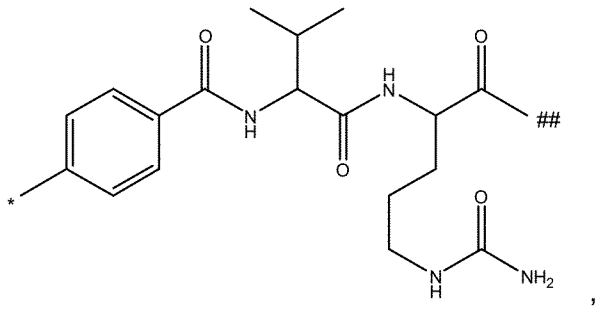
is as defined herein; * denotes the attachment point to the Y; and # denotes the attachment point to the amino acid unit -W_w-;

-W_w- is an amino acid unit as described herein; w is an integer as described herein, preferably w is 2, 3 or 4 (i.e. preferably -W_w- is a dipeptide, a tripeptide or a tetrapeptide), more preferably the integer w is 2 or 3 (i.e. more preferably -W_w- is a dipeptide or a tripeptide), still more preferably w is 2 (i.e. still more preferably -W_w- is a dipeptide);

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Preferably, in these embodiments, the amino acid unit -W_w- is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e. Val-Ala or VA), alanine-alanine (i.e. Ala-Ala or AA) and phenylalanine-lysine (i.e. Phe-Lys or FK). More preferably, in these embodiments the amino acid unit is a dipeptide selected from the group consisting of valine-citrulline (i.e. Val-Cit or VC), valine-alanine (i.e.

Val-Ala or VA), and phenylalanine-lysine (i.e. Phe-Lys or FK). Still more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC) or valine-alanine (i.e. Val-Ala or VA). Even more preferably, in these embodiments the amino acid unit is valine-citrulline (i.e. Val-Cit or VC). Alternatively, in these embodiments, the amino acid unit -W_w- may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), phenylalanine-glutamin (i.e. Phe-Gln or FQ) and threonine-threonine (i.e. Thr-Thr or TT). In these embodiments, the amino acid unit may be a dipeptide selected from the group consisting of valine-glutamine (i.e. Val-Gln or VQ), leucine-glutamine (i.e. Leu-Gln or LQ), and phenylalanine-glutamin (i.e. Phe-Gln or FQ). In these embodiments, the amino acid unit may be valine-glutamine (i.e. Val-Gln or VQ) or leucine-glutamine (i.e. Leu-Gln or LQ).

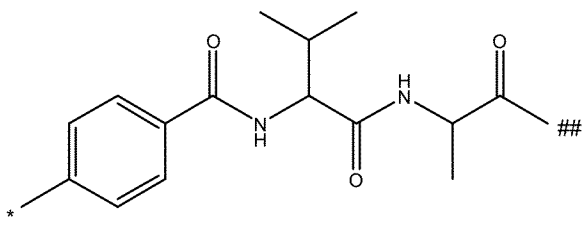
[00313] In some embodiments, the linker L may have the following structure:



,

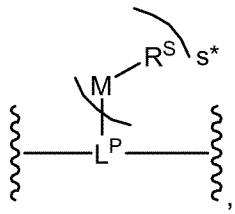
which comprises the dipeptide valine-citrulline as the amino acid unit -W_w-; and
wherein * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C).

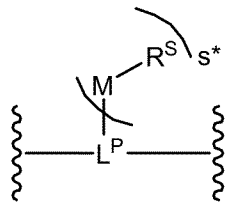
[00314] In some embodiments, the linker L may have the following structure:



,

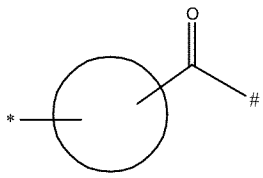
which comprises the dipeptide valine-alanine as the amino acid unit -W_w-; and
wherein * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C).

[00315] In some embodiments, the linker (-L-) has the formula: *–A_a–$^{##}$, wherein -A- is a second spacer unit as defined herein; the integer a associated with the second spacer unit is 1; the amino acid unit -W_w- is absent; the first spacer unit (-B-) is absent; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In any one of these embodiments, the second spacer unit -A- may be a group Z having the structure 

, wherein 

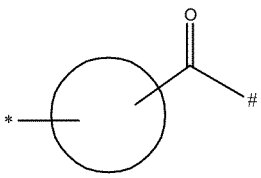
is as defined herein.

[00316] The linker (-L-) may have the following structure:



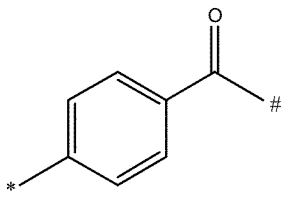
,

wherein:



is as defined herein; * denotes the attachment point to the Y; and # denotes the attachment point to the camptothecin moiety (-C).

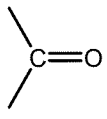
[00317] In some embodiments, the linker L may have the following structure:

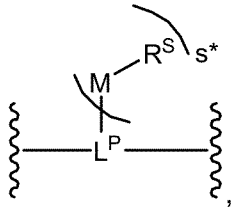


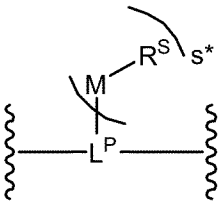
wherein * denotes the attachment point to the Y; and # denotes the attachment point to the camptothecin moiety (-C).

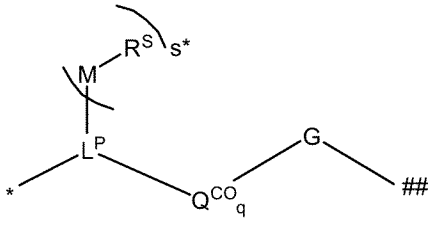
*Linker *–A_a–$Q^{CO}{}_q$–G–$^{##}$*

[00318] In some embodiments, the linker L has the following structure: *–A_a–$Q^{CO}{}_q$–G–##, wherein: –A– is a second spacer unit, as described herein; a is 0 or 1, as described herein; each –Q^{CO}– is independently a connector unit; q is 0 or 1; and –G– is a first spacer unit comprising a sugar moiety; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). Linkers comprising a sugar moiety, such as e.g. a glucuronic acid moiety, are described, e.g., in Jeffrey et al., *"Development and Properties of beta-Glucuronide Linkers for Monoclonal Antibody-Drug Conjugates"*, Bioconjugate Chem. 2006, 17, 831-840, doi: 10.1021/bc0600214; WO 2019/236954; and WO 2015/057699.

[00319] In the linker *–A_a–$Q^{CO}{}_q$–G–##, the second spacer unit -A-, when present, may be any second spacer unit as described herein. In the linker having the structure *–A_a–$Q^{CO}{}_q$–G–##, the second spacer unit A serves to connect the Y with the connector unit Q^{CO}, when present, or with the first spacer unit comprising a sugar moiety. The second spacer unit (-A-), when present, may be any chemical group or moiety which is capable to link a Y to the connector unit (Q^{CO}). Alternatively, the second spacer unit (-A-) may link the Y to the first spacer unit comprising a sugar moiety (-G-), in case no connector unit Q^{CO} is present. In this regard, the Y, as described herein, is bonded to the second spacer unit (–A–). The second spacer unit (–A–) comprises or is a functional group that is capable to form a bond to a connector unit (–Q^{CO}–), or to a first spacer unit having a sugar moiety (–G–), depending on whether a connector unit (–Q^{CO}–) is present or not. Preferably, the functional group, which is capable to form a bond to a connector unit (–Q^{CO}–), or to a first spacer unit comprising a sugar moiety (–G–), is a carbonyl group which is depicted as, e.g.,  or –C(O)–. The integer a associated with the second spacer unit may be 0 or 1. Preferably, the integer a is 1. Alternatively, in other embodiments the second spacer unit is absent (a = 0).

[00320] In the linker *–A_a–$Q^{CO}{}_q$–G–##, the second spacer unit -A-, when present, may be any second spacer unit as described herein. In some embodiments, the second spacer unit -A-, when present, may be a group Z having the structure  , wherein



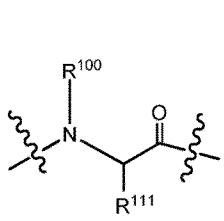
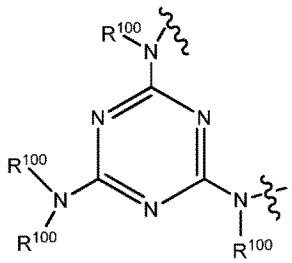
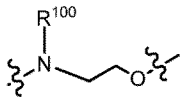
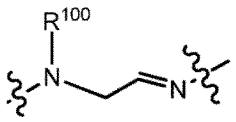
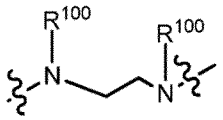
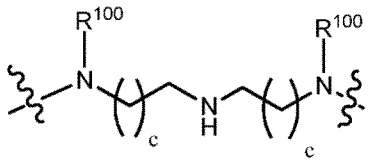
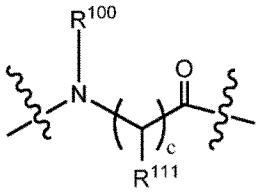
is as defined herein. Accordingly, in some embodiments, the linker (L) may have the structure 

, wherein L^P, R^S, s*, M, Q^{CO}, q, and G are as defined herein; * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

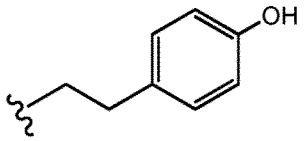
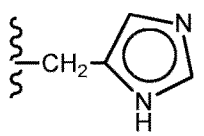
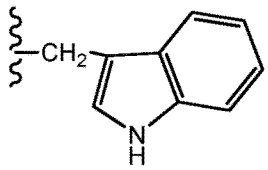
[00321] The connector unit (-Q^{CO}-) may be included in instances where it is desirable to add additional distance between the -Y- or, when present, between the second spacer unit (-A-) and the first spacer unit comprising a sugar moiety (-G-). In some embodiments, the extra distance may aid with activation within the first spacer unit comprising a sugar moiety (-G-). Accordingly, the connector unit (-Q^{CO}-), when present, extends the framework of the linker (-L-). In this regard, a connector unit (-Q^{CO}-) is covalently bonded with the -Y- or, when a second spacer unit -A- is present, with the second spacer unit (-A-) at one terminus, and the connector unit (-Q^{CO}-) is covalently bonded to the first spacer unit comprising a sugar moiety (-G-) at its other terminus. The integer q associated with the connector unit Q^{CO} may be 0 or 1. Preferably, the integer q is 1. Alternatively, in other embodiments, the connector unit Q^{CO} is absent (q = 0).

[00322] The connector unit (-Q^{CO}-), when present, serves to link the first spacer unit comprising a sugar moiety (-G-) to the second spacer unit (-A-), when present, or to the -Y-. The connector unit Q^{CO} may be any chemical group or moiety that serves to provide for attachment of the first spacer unit comprising a sugar moiety (-G-) to the second spacer unit (-A-), when present, or to the -Y-. The connector unit may be, for example, comprised of one or more (e.g., 1-10, preferably, 1, 2, 3, or 4) natural or non-natural amino acid, amino alcohol, amino aldehyde, and diamino residues. In some embodiments, the connector unit (-Q^{CO}-) is a single natural or non-natural amino acid, amino alcohol, amino aldehyde, or diamino residue. In some embodiments, the amino acid capable of acting as connector unit is beta-alanine. In particular, the connector unit may be a single beta-alanine.

[00323] In some embodiments, the connector unit (-Q^{CO}-) has the formula denoted below:

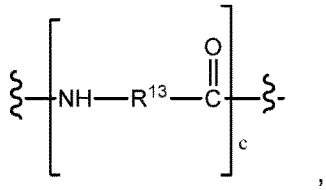






or


wherein the wavy lines indicate attachment of the connector unit within the linker (-L-) or to -Y-, when the second spacer unit (-A-) is absent; and wherein R^{111} is independently selected from the group consisting of hydrogen, hydroxybenzyl, methyl, isopropyl, isobutyl, sec-butyl, -CH_2OH, -$CH(OH)CH_3$, -$CH_2CH_2SCH_3$, -CH_2CONH_2, -CH_2COOH, -$CH_2CH_2CONH_2$, -CH_2CH_2COOH, -$(CH_2)_3NHC(=NH)NH_2$, -$(CH_2)_3NH_2$, -$(CH_2)_3NHCOCH_3$, -$(CH_2)_3NHCHO$, -$(CH_2)_4NHC(=NH)NH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_4NHCOCH_3$, -$(CH_2)_4NHCHO$, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NHCONH_2$, -$CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-,



, and


and each R^{100} is independently selected from hydrogen or -(C_1-C_3)alkyl, preferably hydrogen or CH_3; and subscript c is an independently selected integer from 1 to 10, preferably 1 to 3.

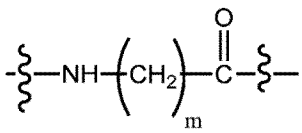
[00324] In preferred embodiments, a connector unit has the following structure (-Q^{CO}-) having a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-), and an NH group for attachment to the second spacer unit (-A-), when present, as follows:



,

wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1.

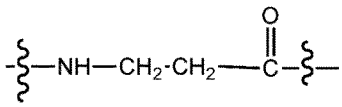
[00325] More preferably, the connector unit (-Q^{CO}-) has the following structure of:



,

wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), when present, and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4.

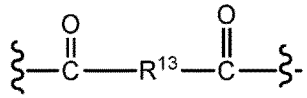
[00326] Still more preferably, the connector unit (-Q^{CO}-) has the following structure of:



,

wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), when present, and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

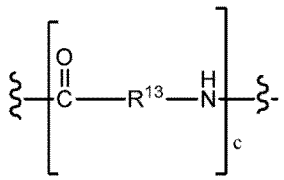
[00327] Another representative connector unit (-Q^{CO}-) having a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) is as follows:



,

wherein R^{13} is -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, or -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-. In some embodiments R^{13} is –(C_1-C_6)alkylene.

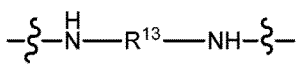
[00328] Another representative connector unit having an NH moiety that attaches to the first spacer unit comprising a sugar moiety (-G-) is as follows:



,

wherein in each instance, R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and subscript c is from 1 to 14. In some embodiments R^{13} is -(C_1-C_6)alkylene and subscript c is 1.

[00329] Another representative connector unit (-Q^{CO}-) having a NH moiety that attaches to the first spacer unit comprising a sugar moiety (-G-) is as follows:



,

wherein R^{13} is –(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, -C(=O)(C_1-C_{10})alkylene- or -(C_1-C_6)alkylene-C(=O)-(C_1-C_6)alkylene.

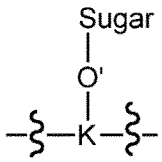
[00330] The first spacer unit having a sugar moiety (-G-) is the only component of the linker having the structure *–A_a–$Q^{CO}{}_q$–G–$^{\#\#}$ that must be present. In some embodiments, the first spacer unit comprising a sugar moiety (-G-) forms a cleavable bond with the camptothecin moiety (-C). In some embodiments, the first spacer unit comprising a sugar moiety (-G-) forms a cleavable bond with the connector unit (-Q^{CO}-), when present. In some embodiments, the cleavable bond is within the first spacer unit comprising a sugar moiety (-G-) but allows for release of free drug (e.g., by a 1,6-elimination reaction following cleavage). Functional groups for forming cleavable bonds can include, for example, sugars to form glycosidic bonds.

[00331] The structure and sequence of the first spacer unit comprising a sugar moiety (-G-) may be such that the unit is cleaved by the action of enzymes present at the target site. In other embodiments, the first spacer unit comprising a sugar moiety (-G-) may be cleavable by other mechanisms. The first spacer unit comprising a sugar moiety (-G-) may comprise one or multiple cleavage sites.

[00332] Preferably, the first spacer unit comprising a sugar moiety (-G-) comprises a sugar cleavage site. In some such embodiments, the first spacer unit comprising a sugar moiety (-G-) comprises a sugar moiety (Su) linked via an oxygen glycosidic bond to a self-immolative group. In such aspects, the self-immolative group is considered to be part of the first spacer unit comprising a sugar moiety (-G-). In this regard, the "self-immolative group" may be a tri-functional chemical moiety that is capable of covalently linking together three spaced chemical moieties (i.e., the sugar moiety (via a glycosidic bond), a camptothecin moiety (-C), and a connector unit -Q^{CO}-, a second spacer unit -A-, or -Y-, depending on whether a -Q^{CO}- unit and/or an -A- unit are present or not. The glycosidic bond may be one that can be cleaved at the target site to initiate a self-immolative reaction sequence that leads to a release of the drug. Particular sugar moieties may be selected, e.g., from the group consisting of glucuronic acid, galactose, glucose, arabinose, mannose-6-phosphate,

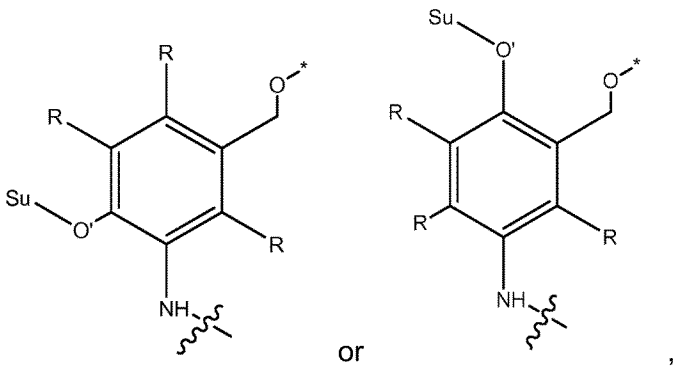
fucose, rhamnose, gulose, allose, 6-deoxy-glucose, lactose, maltose, cellobiose, gentiobiose, maltotriose, GlcNAc, GalNAc and maltohexaose.

[00333] Accordingly, the first spacer unit comprising a sugar moiety (-G-) may comprise a sugar moiety (Su) linked via a glycoside bond (-O'-) to a self-immolative group (K) of the formula:



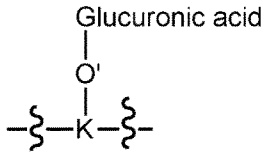
wherein the self-immolative group K forms a covalent bond with the camptothecin moiety and a covalent bond with -Q^{CO}-, -A-, or -Y-, as the case may be.

[00334] The first spacer unit comprising a sugar moiety (-G-) may be, for example, represented by the formula:



wherein Su is a Sugar moiety, -O'- represents an oxygen glycosidic bond; each R is independently hydrogen, a halogen, -CN, or -NO_2; and wherein the wavy line indicates attachment to -Q^{CO}-, -A-, or -Y-, as the case may be, and the asterisk indicates attachment to the camptothecin moiety (either directly or indirectly via a spacer unit; the spacer unit, when present, may be, for example -(C=O)-).

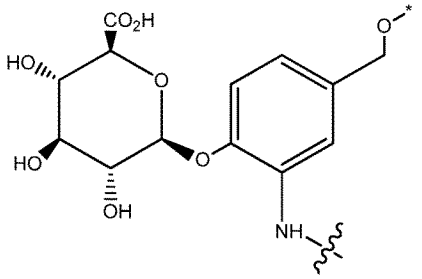
[00335] In some such embodiments, the sugar cleavage site is recognized by a beta-glucuronidase and the first spacer unit comprising a sugar moiety (-G-) comprises a glucuronide unit. The glucuronide unit may comprise glucuronic acid linked via a glycoside bond (-O'-) to a self-immolative group (K) of the formula:



,

wherein the self-immolative group K forms a covalent bond with the camptothecin moiety (either directly or indirectly via a spacer unit; the spacer unit, when present, may be, for example -(C=O)-) and a covalent bond with -Q^{CO}-, -A-, or -Y-, as the case may be.

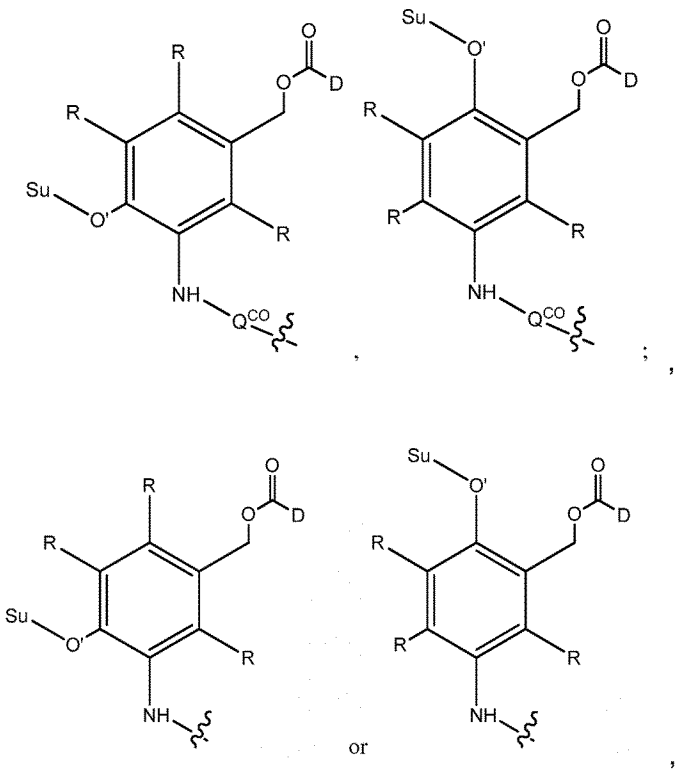
[00336] The glucuronide unit may be, for example, represented by the formula:



,

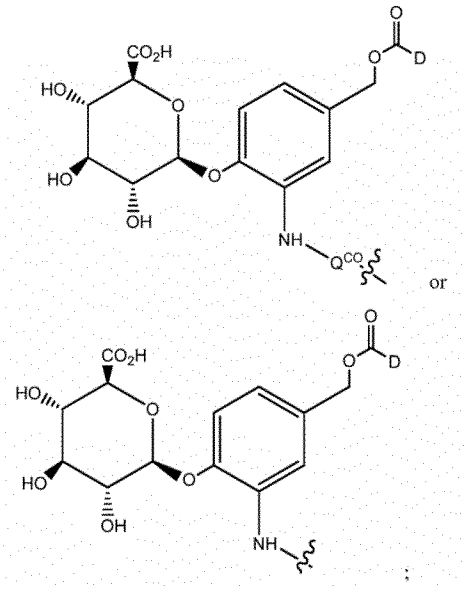
wherein the wavy line indicates covalent attachment to the -Q^{CO}-, -A- or -Y-, as the case may be, and the asterisk indicates covalent attachment to the camptothecin moiety -C (either directly or indirectly via a spacer unit; the spacer unit, when present, may be, for example -(C=O)-).

[00337] In some embodiments the first spacer unit comprising a sugar moiety (-G-) comprises a sugar cleavage site, and -S-C, i.e. the combination of the first spacer unit comprising a sugar moiety (-G-) and the camptothecin moiety (in the following formulae, the camptothecin moiety is exceptionally denoted as “D”), is represented by the following formulae:



wherein Su is a sugar moiety, D is a camptothecin moiety, -O'- represents an oxygen glycosidic bond; each R is each independently hydrogen or halogen, -CN, $-NO_2$ or other electron withdrawing group, $-Q^{CO}-$ is a connector unit, as described herein; wherein the wavy bond indicates covalent attachment to the -A- or -Y-, as the case may be.

[00338] When the first spacer unit comprising a sugar moiety (-G-) comprises a glucuronide unit, then -S-C, i.e. the combination of the first spacer unit comprising a sugar moiety (-G-) and the camptothecin moiety (in the following formulae, the camptothecin moiety is exceptionally denoted as “D”) may be, for example, represented by the following formulae:



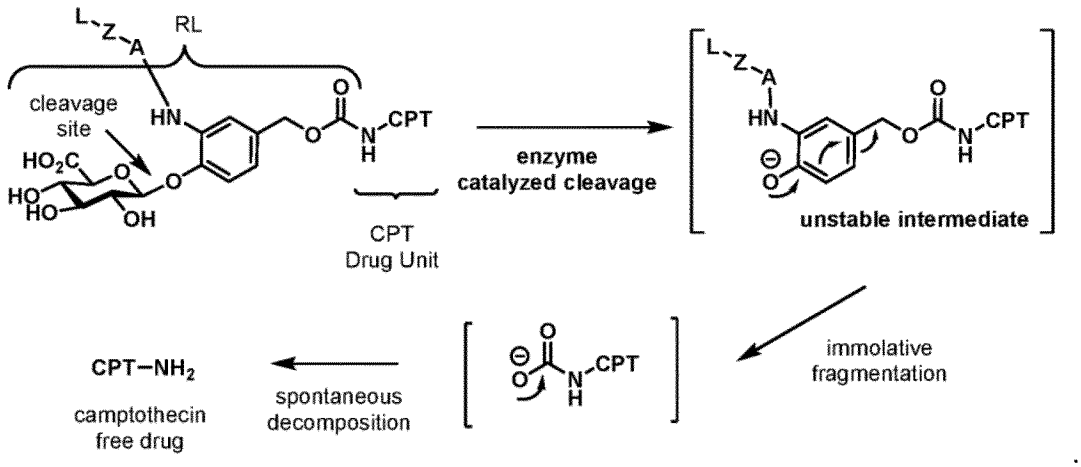
or

;

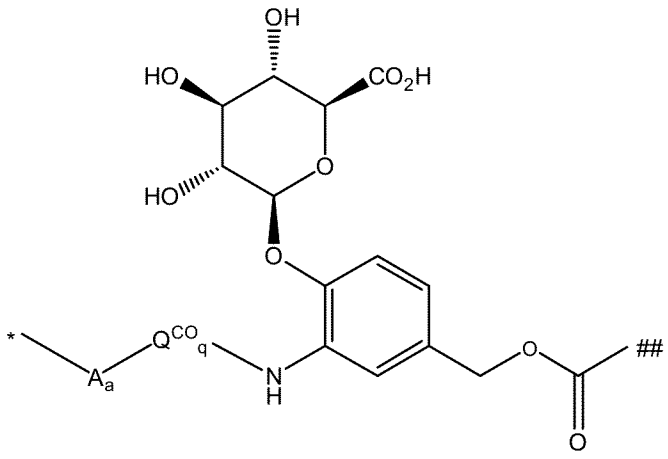
wherein the wavy bond indicates covalent attachment to the -A- or -Y-, as the case may be; D is a camptothecin moiety; and -Q^{CO}- is a connector unit, as described herein.

[00339] Without being bound by theory, Scheme 1a depicts a mechanism of free drug release of a camptothecin drug unit attached through a nitrogen atom of an amine substituent from the free drug to a releasable linker that comprises a glucuronide unit.

Scheme 1a



[00340] In preferred embodiments, the linker (L) has the following structure:

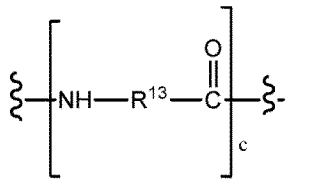


wherein:

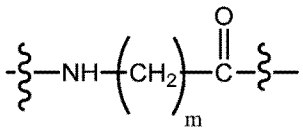
-A- is a second spacer unit as described herein; a is an integer as described herein, preferably a is 1;

-Q^{CO}- is a connector unit as described herein; q is an integer as defined herein, preferably q is 1;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), when present, and may be as follows:

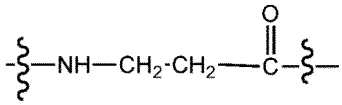


wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1; Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

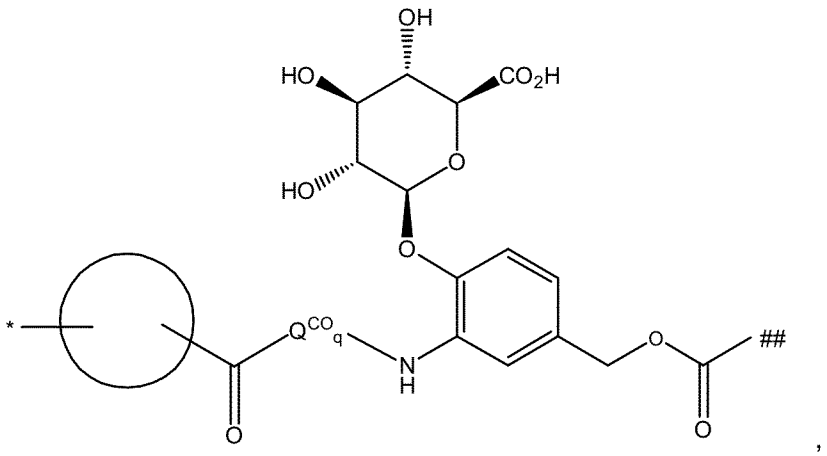
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), when present, and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4; More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

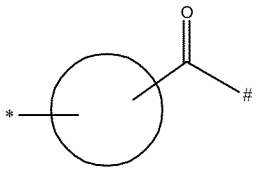
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), when present, and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

[00341] More preferably, the linker (L) has the following structure:



,

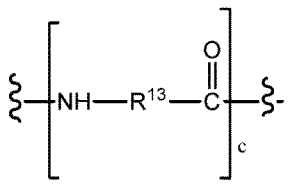
wherein:



is as defined herein; * denotes the attachment point to the -Y-; and # denotes the attachment point to the connector unit (-Q^{CO}-), when present, or to the NH group;

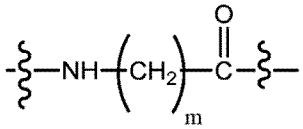
-Q^{CO}- is a connector unit as defined herein; q is an integer as defined herein, preferably q is 1;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), and may be as follows:



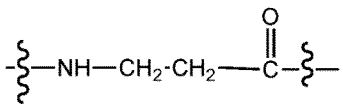
,

wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1. Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

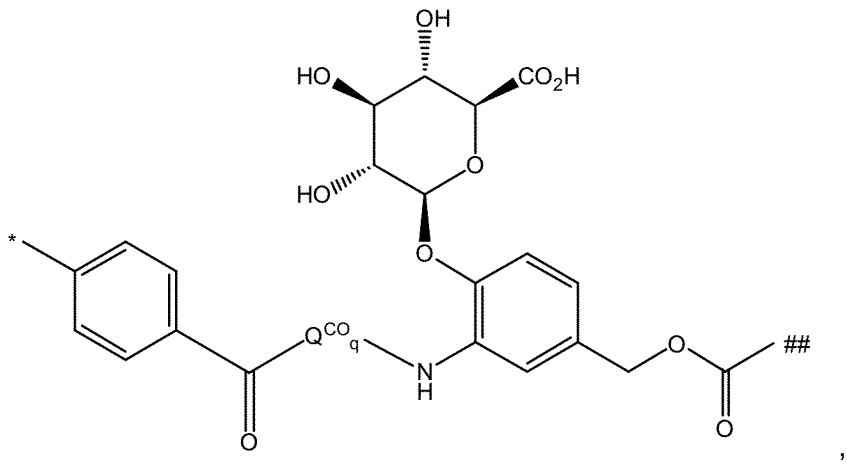
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4. More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

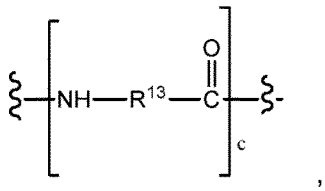
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), when present, and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

[00342] Still more preferably, the linker (L) has the following structure:

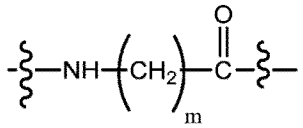


wherein:

Q^{CO} is a connector unit as defined herein; q is an integer as defined herein, preferably q is 1; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), and may be as follows:

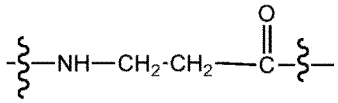


wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1. Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

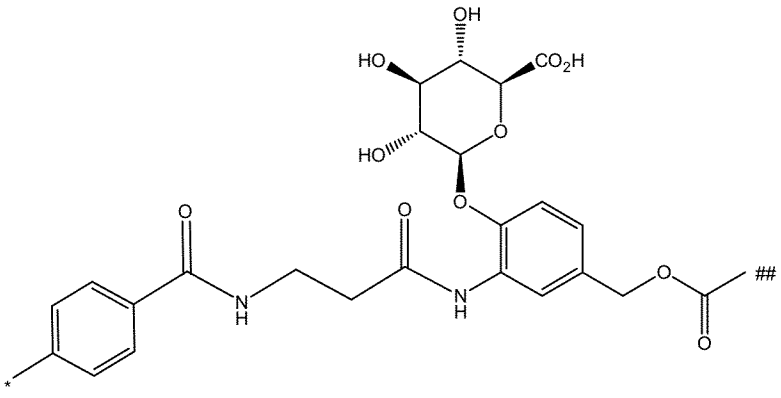
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4; More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

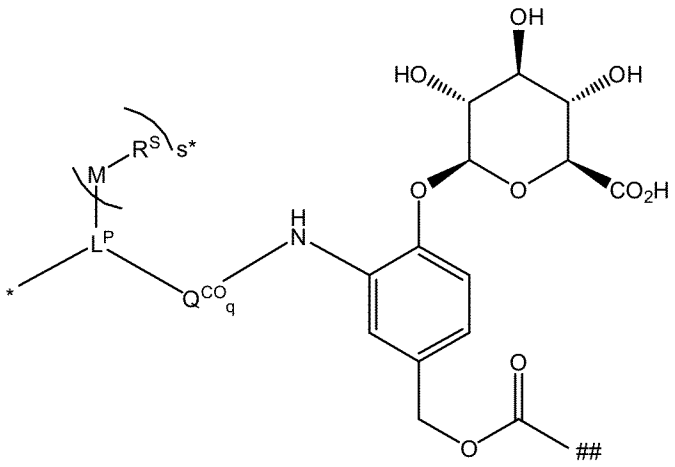
[00343] Still more preferably, the linker L may have the following structure:



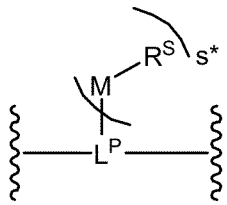
,

wherein * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00344] In preferred embodiments, the linker L has the following structure:



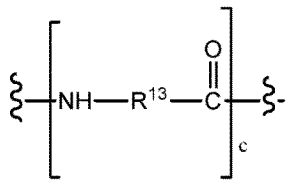
wherein:



is as described herein; R^S is, each independently, a second polyalkylene glycol unit as described herein; preferably each R^S is, independently, a second polyethylene glycol unit as described herein; M is, each independently, as described herein; preferably each M is -O-; s* is an integer as described herein; preferably, s* is 1;

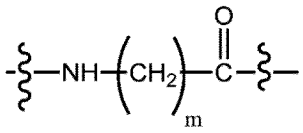
$-Q^{CO}-$ is a connector unit as described herein; q is an integer as defined herein, preferably q is 1;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), and may be as follows:



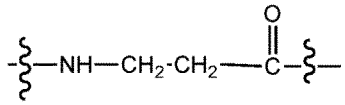
wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-

C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1. Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

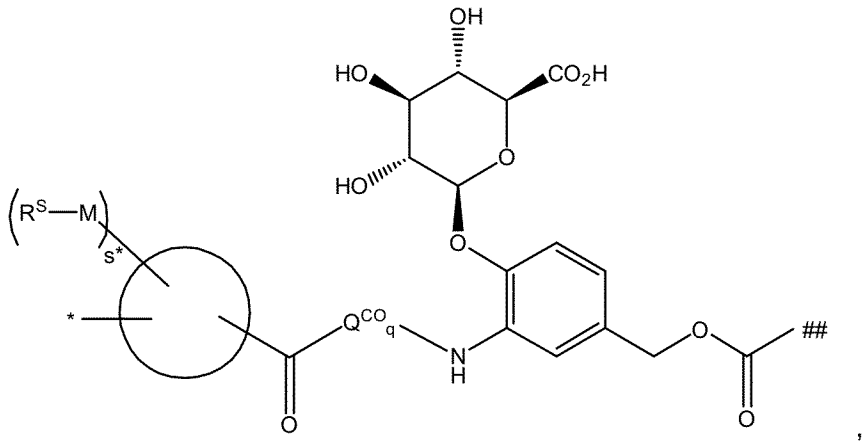
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4. More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

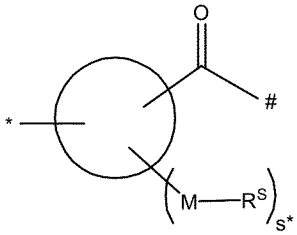
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

[00345] More preferably, the linker (L) has the following structure:



,

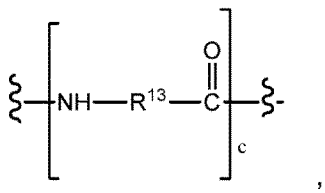
wherein:



is as defined herein; R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably, each R^S is, independently, a second polyethylene glycol unit as defined herein; M is, each independently, as defined herein; preferably each M is -O-; s* is an integer as defined herein; preferably s* is 1; * denotes the attachment point to the Y; * denotes the attachment point to the -Y-; and # denotes the attachment point to the connector unit (-Q^{CO}-), when present, or to the NH group;

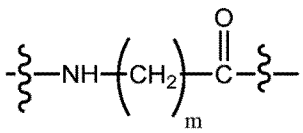
-Q^{CO}- is a connector unit as defined herein; q is an integer as defined herein, preferably q is 1;

* denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), and may be as follows:



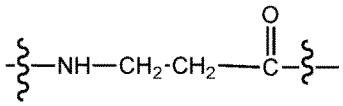
,

wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1. Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

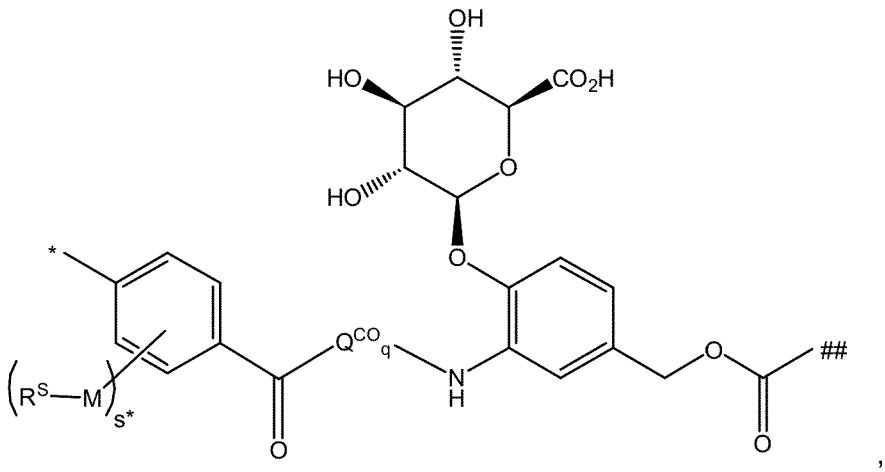
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4. More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

[00346] More preferably, the linker (L) has the following structure:

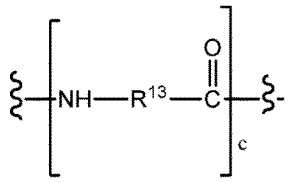


,

wherein:

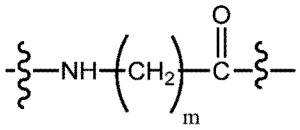
R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably each R^S is, independently, a second polyethylene glycol unit as described herein; M is, each independently, as defined herein; preferably each M is -O-; s* is an integer as defined herein; preferably s* is 1.

Q^{CO} is a connector unit as defined herein; q is an integer as defined herein, preferably q is 1; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). In these embodiments, the connector unit (Q^{CO}), when present, may have a carbonyl group for attachment to the first spacer unit comprising a sugar moiety (-G-) and an NH group for attachment to the second spacer unit (-A-), and may be as follows:



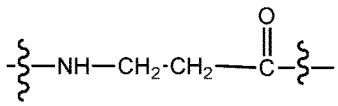
,

wherein in each instance R^{13} is independently selected from the group consisting of -(C_1-C_6)alkylene-, -(C_3-C_8)carbocyclo-, -arylene-, -(C_1-C_{10})heteroalkylene-, -(C_3-C_8)heterocyclo-, -(C_1-C_{10})alkylene-arylene-, -arylene-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)carbocyclo)-, -(C_3-C_8)carbocyclo-(C_1-C_{10})alkylene-, -(C_1-C_{10})alkylene-(C_3-C_8)heterocyclo-, and -(C_3-C_8)heterocyclo-(C_1-C_{10})alkylene-, and the subscript c is an integer ranging from 1 to 4. In some embodiments R^{13} is -(C_1-C_6)alkylene and c is an integer ranging from 1 to 4. In preferred embodiments, R^{13} is -(C_1-C_6)alkylene and c is 1. Preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, may have the following structure of:



,

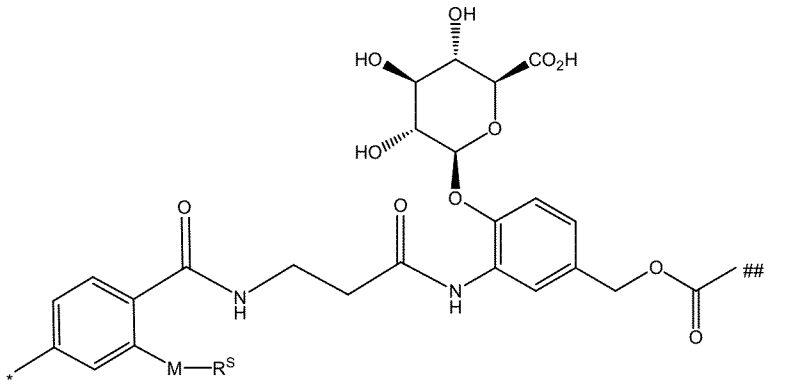
wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-); and m is an integer ranging from 1 to 6, preferably 2 to 6, more preferably 2 to 4. More preferably, in these embodiments, the connector unit (-Q^{CO}-), when present, has the following structure of:



,

wherein the wavy line adjacent to the nitrogen indicates covalent attachment to a second spacer unit (-A-), and the wavy line adjacent to the carbonyl indicates covalent attachment to the first spacer group comprising a sugar moiety (-G-).

[00347] Still more preferably, the linker L has the following structure:

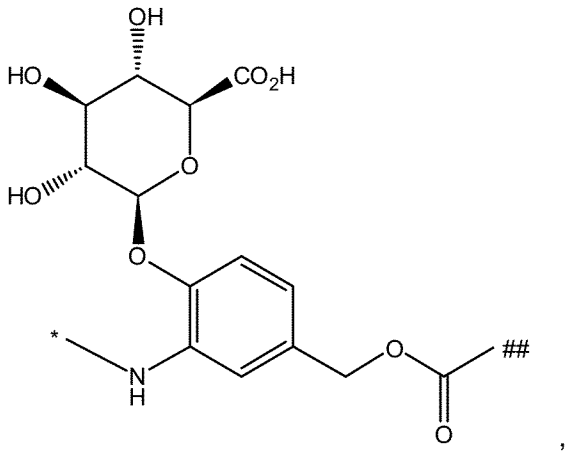


wherein:

R^S is, each independently, a second polyalkylene glycol unit as defined herein; preferably each R^S is, independently, a second polyethylene glycol unit as described herein; M is, each independently, as defined herein; preferably each M is -O-; s* is an integer as defined herein; preferably s* is 1.

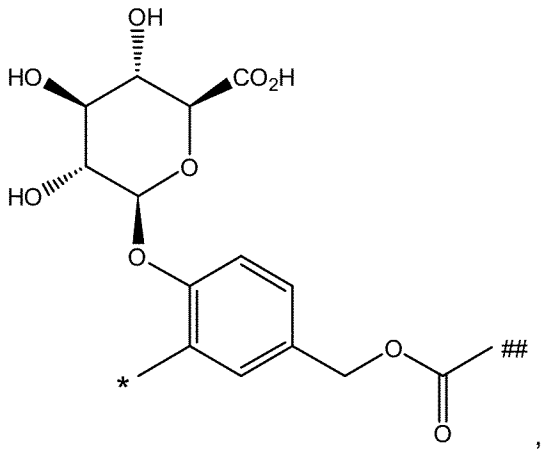
* denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00348] Also preferably, the linker L has the following structure:



wherein * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00349] In one embodiment, the linker L has the following structure:



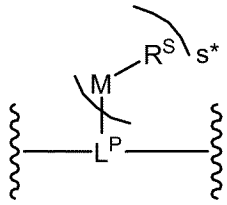
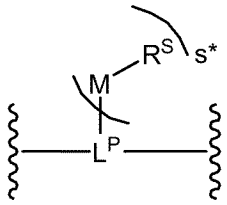
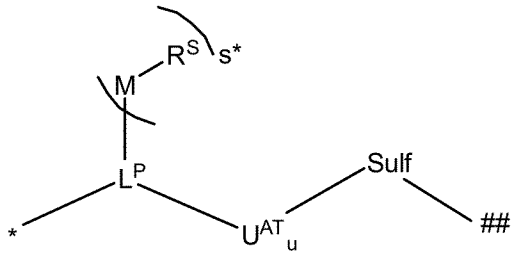
wherein * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C). In this embodiment, Y may be as defined herein; preferably, Y may be NH.

*Linker *–A_a–U^{AT}_u–Sulf–$^{##}$*

[00350] In some embodiments, the linker L has the following structure: *–A_a–U^{AT}_u–Sulf–$^{##}$, wherein: –A– is a second spacer unit; a is 0 or 1; each –U^{AT}_u– is independently an attachment unit; u is 0 or 1; and –Sulf– is a first spacer unit comprising a sulfatase-cleavable moiety; * denotes the attachment point to the Y; and ## denotes the attachment point to the camptothecin moiety (-C). For sulfatase-cleavable linkers, see e.g. Bargh et al., *"Sulfatase-cleavable linkers for antibody-drug conjugates"*, Chemical Science, 2020, 11, 2375-2380, doi: 10.1039/c9sc06410a.

[00351] In the linker having the structure *–A_a–U^{AT}_u–Sulf–$^{##}$, the second spacer unit (-A-), when present, serves to connect a Y to the attachment unit (U^{AT}), when present, or to the first spacer unit comprising a sulfatase-cleavable moiety. The second spacer unit (-A-) may be any chemical group or moiety which is capable to link a Y to the attachment unit (U^{AT}). Alternatively, the second spacer unit (-A-) may link the Y to the first spacer unit comprising a sulfatase-cleavable moiety (-Sulf-), in case no attachment unit (U^{AT}) is present. In this regard, the Y, as described herein, is bonded to the second spacer unit (–A–). The second spacer unit (–A–) comprises or is a functional group that is capable to form a bond to an attachment unit (–U^{AT}–), or to a first spacer unit having a sulfatase-cleavable moiety (–Sulf–), depending on whether an attachment unit (–U^{AT}–) is present or not. Preferably, the functional group, which is capable to form a bond to an attachment unit (–U^{AT}–), or to a first spacer unit comprising a sulfatase-cleavable moiety (–Sulf–), is a carbonyl group which is depicted as,

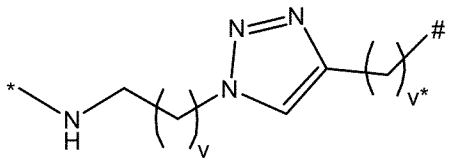
e.g.,  or –C(O)–. The integer a associated with the second spacer unit may be 0 or 1. Preferably, the integer a is 1. Alternatively, in other embodiments the second spacer unit is absent (a = 0).

[00352] In the linker *-A_a-$U^{AT}{}_u$-Sulf-$^{\#\#}$, the second spacer unit -A- may be any second spacer unit as described herein. In some embodiments, the second spacer unit -A-, when present, may be a group Z having the structure  , wherein  is as defined herein. Accordingly, in some embodiments, the linker (L) may have the structure,  , wherein L^P, R^S, s*, M, U^{AT}, u, and Sulf are as defined herein; * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00353] The attachment unit (-U^{AT}-) may be included in instances where it is desirable to add additional distance between the -Y- or, when present, the second spacer unit (-A-) and the first spacer unit comprising a sulfatase-cleavable moiety (-Sulf-). Accordingly, the attachment unit (-U^{AT}-), when present, extends the framework of the linker (-L-). In this regard, an attachment unit (-U^{AT}-) may be covalently bonded with the -Y- or, when a second spacer unit -A- is present, with the second spacer unit (-A-) at one terminus, and the attachment unit (-U^{AT}-) is covalently bonded to the first spacer unit comprising a sulfatase-cleavable group (-Sulf-) at its other terminus.

[00354] The attachment unit (-U^{AT}-) may be any chemical group or moiety that serves to provide for attachment of the first spacer unit comprising a sulfatase-cleavable moiety (-Sulf-) to the second spacer unit (-A-), when present, or to the -Y-.

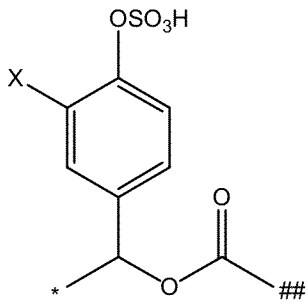
[00355] In some embodiments, the attachment unit (U^{AT}) has the formula denoted below:



...;

wherein v is an integer ranging from 1 to 6; preferably, v is 1 or 2; more preferably v is 2; and v* is an integer ranging from 1 to 6; preferably, v* is 1 or 2; more preferably v* is 1;
wherein * denotes the attachment point to the second spacer unit (-A-), when present, and # denotes the attachment point to the sulfatase-cleavable moiety (Sulf).

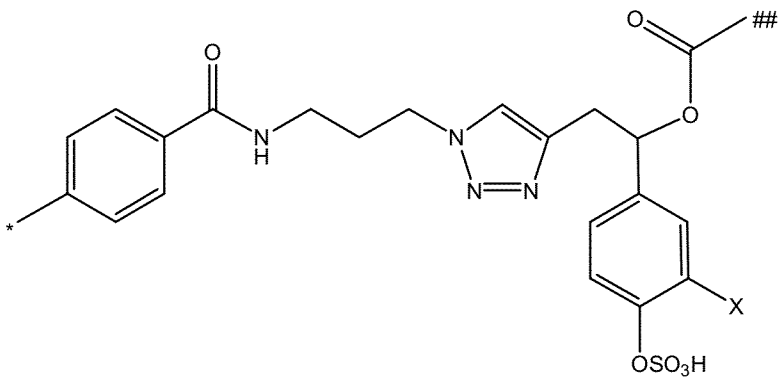
[00356] Preferably, the first spacer unit comprising a sulfatase-cleavable moiety (Sulf) has the formulae denoted below:



,

wherein X is hydrogen (H) or an electron withdrawing group, such as e.g. NO_2; * denotes the attachment point to the attachment unit (U^{AT}), when present, or (-A-), when present; and # denotes the attachment point to the camptothecin moiety (-C).

[00357] In some embodiments, the linker L may have the following structure:.

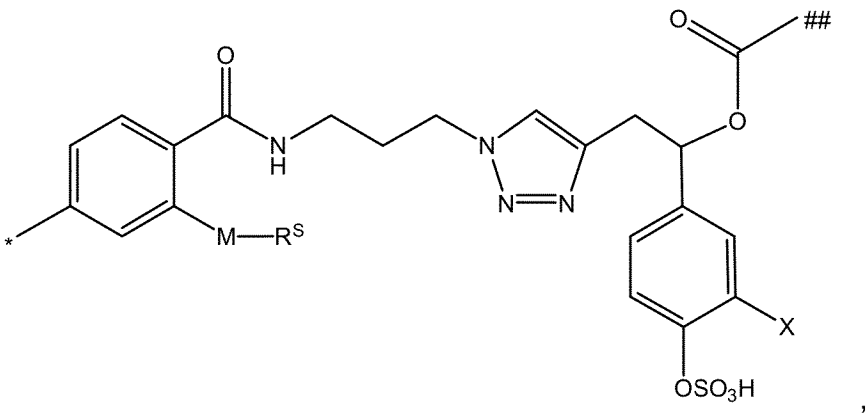


,

wherein X is H or NO_2;

* denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00358] In some embodiments, the linker L may have the following structure:



,

wherein:

X is H or NO_2;

R^S is a second polyalkylene glycol unit as defined herein; preferably R^S is a second polyethylene glycol unit as defined herein;

M is as defined herein; preferably M is -O-;

* denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

Third Spacer Unit

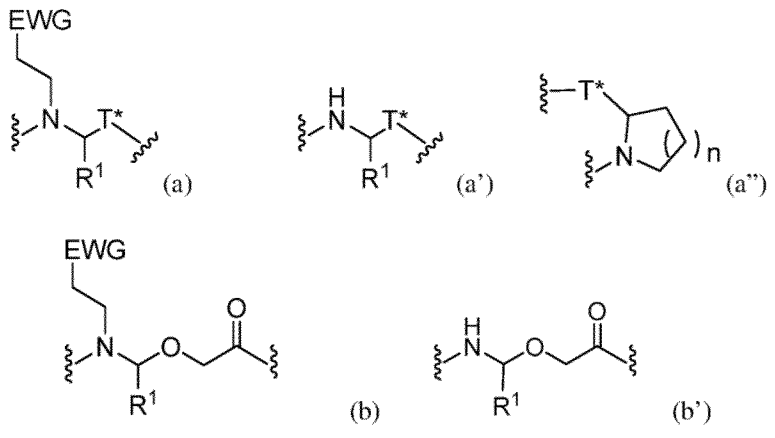
[00359] In some embodiments, when a first spacer unit (-B-), or a first spacer unit comprising a sugar moiety (-G-), or a first spacer unit comprising a sulfatase-cleavable moiety (Sulf) is present, the linker (L) may comprise an optional third spacer unit (-E-), which is arranged between the first spacer unit (-B-), or the first spacer unit comprising a sugar moiety (-G-), or the first spacer unit comprising a sulfatase-cleavable moiety (Sulf) and the camptothecin moiety (-C). The third spacer unit can be a functional group which may facilitate attachment of the first spacer unit (-B-), or the first spacer unit comprising a sugar moiety (-G-), or the first spacer unit comprising a sulfatase-cleavable moiety (Sulf) to the camptothecin moiety (-C), or it can provide additional structural components which may facilitate release of the camptothecin moiety (-C) from the remainder of the conjugate. Suitable third spacer units are described, e.g., in WO 2019/236954.

[00360] In some embodiments, the third spacer unit (-E-) is bound to the first spacer unit (-B-) and to the camptothecin moiety (-C). Accordingly, the linker (-L-) may have the structure *–A_a–W_w–B_b–E–$^{\#\#}$; wherein -E- is a third spacer unit as described herein; wherein -A-, a, -W-, w, and -B- are as described herein, in particular as with regard to the linker (L) having the structure *–A_a–W_w–B_b–$^{\#\#}$; b is 1; wherein, in each instance, * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00361] In other embodiments, the third spacer unit (-E-) is bound to the first spacer unit comprising a sugar moiety (-G-) and to the camptothecin moiety (-C-). Accordingly, the linker (-L-) may have the structure *–A_a–$Q^{CO}{}_q$–G–E–$^{\#\#}$; wherein -E- is a third spacer unit as described herein; wherein -A-, a, -Q^{CO}-, q, and G are as described herein, in particular as with regard to the linker (-L-) having the structure *–A_a–$Q^{CO}{}_q$–G–$^{\#\#}$; wherein, in each instance, * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

[00362] In other embodiments, the third spacer unit (-E-) is bound to the first spacer unit comprising a sulfatase-cleavable moiety (-Sulf-) and to the camptothecin moiety (-C-). Accordingly, the linker (-L-) may have the structure *–A_a–$U^{AT}{}_u$–Sulf–E–$^{\#\#}$; wherein -E- is a third spacer unit as described herein; wherein -A-, a, -U^{AT}-, u, and Sulf are as described herein, in particular as with regard to the linker (-L-) having the structure *–A_a–$U^{AT}{}_u$–Sulf–$^{\#\#}$; wherein, in each instance, * denotes the attachment point to the -Y-; and ## denotes the attachment point to the camptothecin moiety (-C).

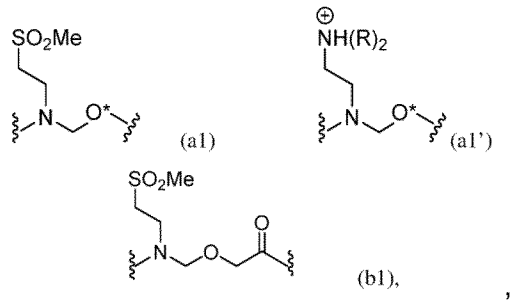
[00363] In some embodiments, exemplary third spacer units -E- are represented by the formulae:



,

wherein EWG represents an electron-withdrawing group, R^1 is -H or $(C_1$-$C_4)$alkyl and subscript n is 1 or 2. In some embodiments, EWG is selected from the group consisting of -CN, $-NO_2$, $-CX_3$, -X, C(=O)OR', $-C(=O)N(R')_2$, -C(=O)R', -C(=O)X, $-S(=O)_2R'$, $-S(=O)_2OR'$, $-S(=O)_2NHR'$, $-S(=O)_2N(R')_2$, $-P(=O)(OR')_2$, $-P(=O)(CH_3)NHR'$, -NO, $-N(R')_3^+$, wherein X is -F, -Br, -Cl, or -I, and R' is independently selected from the group consisting of hydrogen and $(C_1$-$C_6)$alkyl, and wherein the wavy line adjacent to the nitrogen atom in each of formula (a), (a'), (a"), (b) and (b') is the point of covalent attachment to the first spacer unit (-B-) and the wavy line adjacent to the carbonyl carbon atom of formula (b) and formula (b') is the point of covalent attachment to a heteroatom of a hydroxyl or primary or secondary amine of a camptothecin moiety (-C); and wherein formula (a), formula (a') and formula (a") represents exemplary units in which T* is the heteroatom from a hydroxyl or primary or secondary amine functional group of a camptothecin moiety (-C); and wherein the wavy line adjacent to T* is the point of covalent attachment to the remainder of the camptothecin moiety. In these embodiments, the third spacer unit -E- may facilitate release of the camptothecin moiety as free drug.

[00364] In still other embodiments, third spacer units are represented by the formulae:

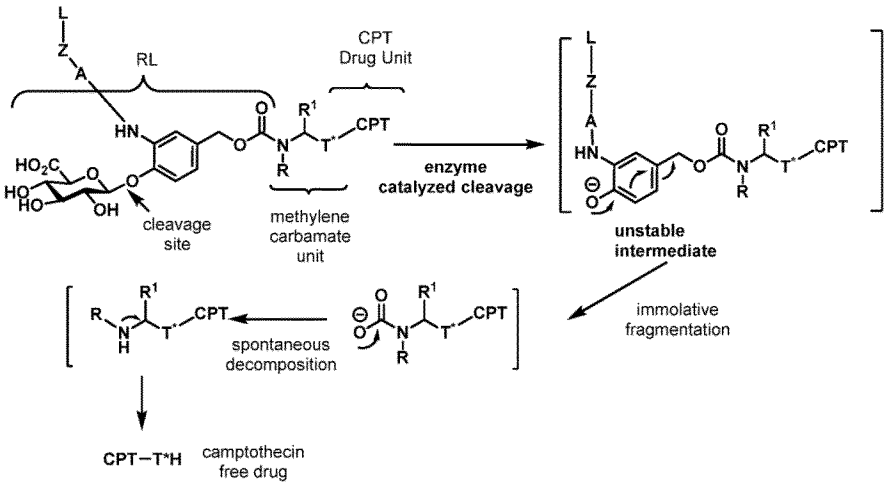


wherein formula (a1) and formula (a1') in which each R is independently -H or $(C_1$-$C_4)$alkyl represents units in which O* is the oxygen atom from a hydroxyl substituent of a camptothecin moiety (-C); and the wavy lines of formula (a1), formula (a1') and formula (b1) retain their previous meanings from formulae (a), (a') and (b), respectively. In formula (a1') the $-CH_2CH_2N^+(R)_2$ moiety represents exemplary basic units in protonated form.

[00365] Without being bound by theory, Scheme 1b depicts a mechanism of free drug release from a camptothecin moiety attached to a methylene carbamate unit in a conjugate having a self-immolative moiety. In that scheme, T* is a heteroatom from the hydroxyl or

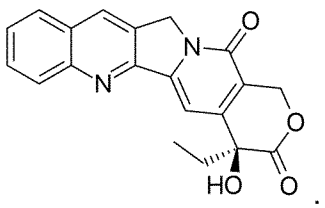
primary or secondary amine of a camptothecin moiety that is incorporated into the methylene carbamate unit.

Scheme 1b

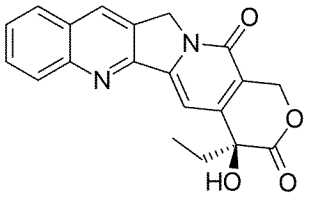


Camptothecin Moiety (-C)

[00366] The term “camptothecin moiety” includes camptothecin itself and analogues of camptothecin. Camptothecin is a topoisomerase poison, which was discovered in 1966 by M. E. Wall and M. C. Wani in systematic screening of natural products for anticancer drugs. Camptothecin was isolated from the bark and stem of *Camptotheca acuminata* (Camptotheca, Happy tree), a tree native to China used as a cancer treatment in Traditional Chinese Medicine. Camptothecin has the following structure:



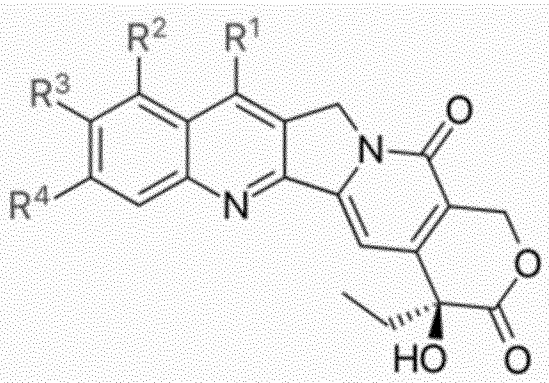
The term „campthothecin moiety“ also comprises camptothecin analogoues. In this regard, the term “camptothecin moiety” denotes any moiety which comprises the structure of camptothecin:

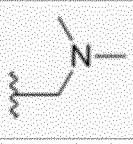
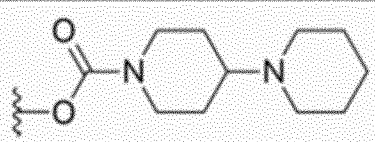
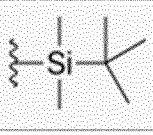


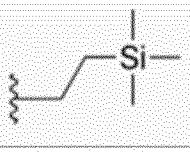
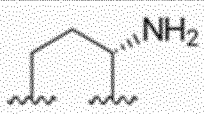
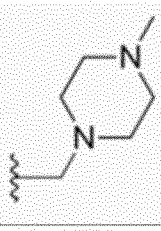
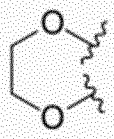
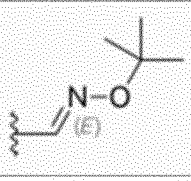
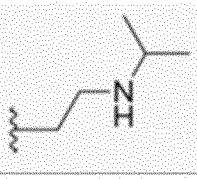
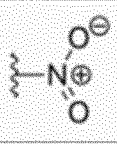
,

and which may be optionally substituted. The optional substituents may include, as illustrative non-limiting examples, (C_1-C_{10})alkyl, (C_3-C_8)carbocyclo, (C_3-C_8)heterocyclo, aryl, an amino group, a hydroxy group, a carbonyl group, an amide group, an ester group, a carbamate group, a carbonate group and/or a silyl group. The camptothecin moiety may have one or more functional group(s) which are capable to form a bond to the linker L. A person skilled in the will readily select a suitable camptothecin moiety having a desired biological activity. Camptothecin analogues have been approved and are used in cancer chemotherapy today, such as e.g. topotecan, irinotecan, or belotecan.

[00367] The following camptothecin analogues are also envisioned by the term camptothecin moiety:

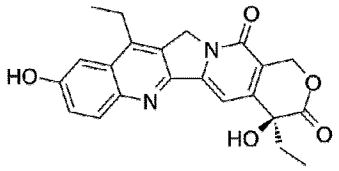


Analogue	R^1	R^2	R^3	R^4
Topotecan	—H		—OH	—H
Irinotecan (CPT-11)		—H		—H
Silatecan (DB-67, AR-67)		—H	—OH	—H

Cositecan (BNP-1350)		—H	—H	—H
Exatecan			—CH_3	—F
Lurtotecan		—H		
Gimatecan (ST1481)		—H	—H	—H
Belotecan (CKD-602)		—H	—H	—H
Rubitecan	—H		—H	—H

Further camptothecin analogues, which may be used as camptothecin moiety, are described in WO 2019/236954 and EP 0 495 432.

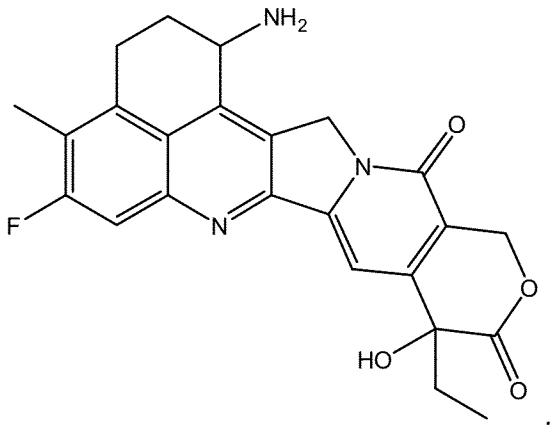
[00368] In some embodiments, the camptothecin moiety (C) is selected from the group consisting of exatecan, SN38, camptothecin, topotecan, irinotecan, belotecan, lurtotecan, rubitecan, silatecan, cositecan and gimatecan. Preferably, the camptothecin moiety is selected from the group consisting of exatecan, SN38, camptothecin, topotecan, irinotecan and belotecan. SN38 has the following structure:



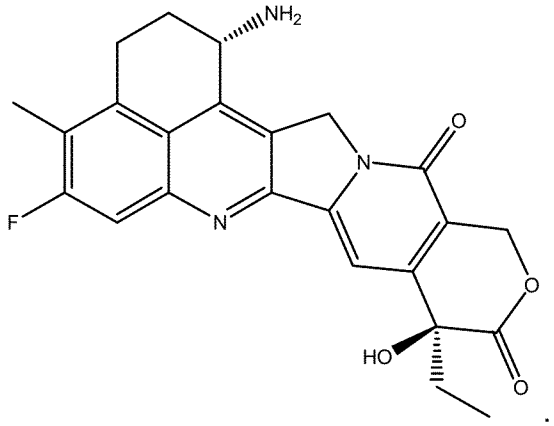
;

and the structures of exatecan, camptothecin, topotecan, irinotecan and belotecan are as described herein.

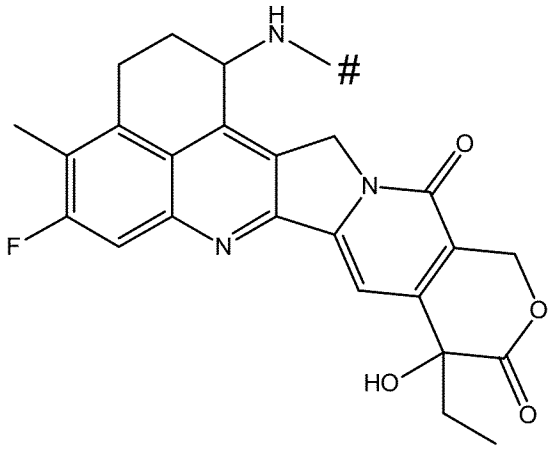
[00369] More preferably, in any one of the embodiments described herein, the camptothecin moiety C is exatecan having the following structure:



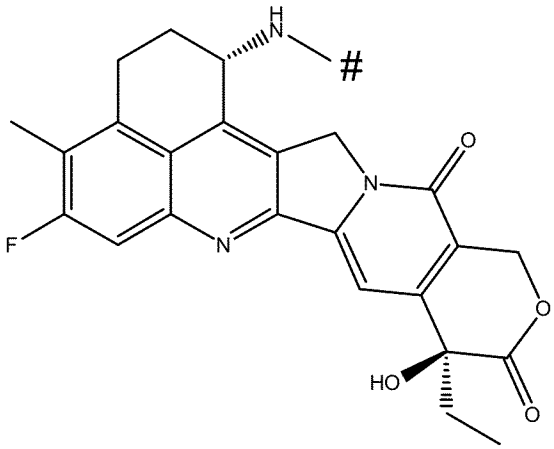
Still more preferably, the camptothecin moiety is exatecan having the following structure:



Preferably, in any one of these embodiments the exatecan is bound to the linker L via the amino group (i.e., via the NH_2 group of exatecan). Exatecan bound to the linker L via the amino group can be depicted, e.g., as follows:



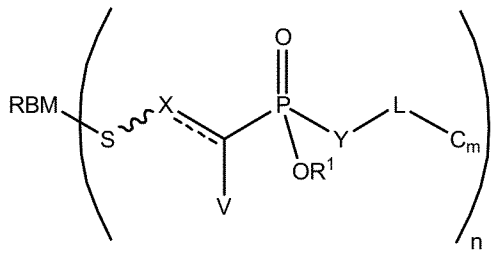
or



,

wherein # indicates the attachment point to the linker L.

[00370] The present invention also relates to a conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

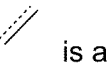
RBM is an antibody;

is a double bond; or

is a bond;

V is absent when  is a double bond; or

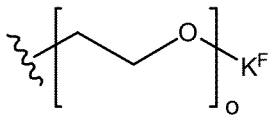
V is H when  is a bond;

X is  when is a double bond; or

X is  when  is a bond;

Y is NH;

R^1 is a polyethylene glycol unit having the structure:



wherein:

 indicates the position of the O;

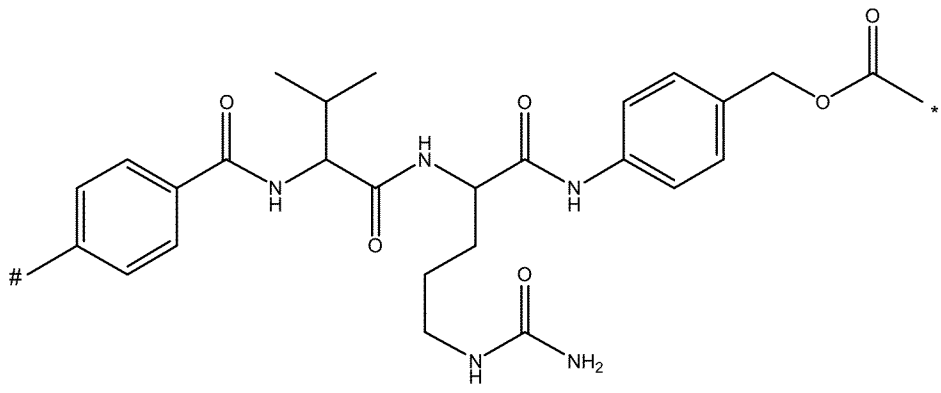
K^F is as defined herein; preferably K^F is H; and

o is an integer as defined herein; preferably o is an integer ranging from 8 to 30; more preferably from 16 to 30; still more preferably from 20 to 28; still more preferably, o is 22, 23, 24, 25 or 26; still more preferably, o is 23, 24 or 25; even more preferably o is 24;

R^3 is H;

R^4 is H;

L is a linker having the following structure:



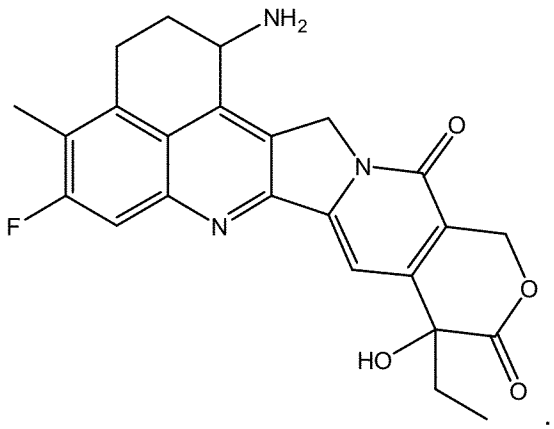
wherein # indicates the attachment point to the Y and * indicates the attachment point to the camptothecin moiety (C);

C is a camptothecin moiety;

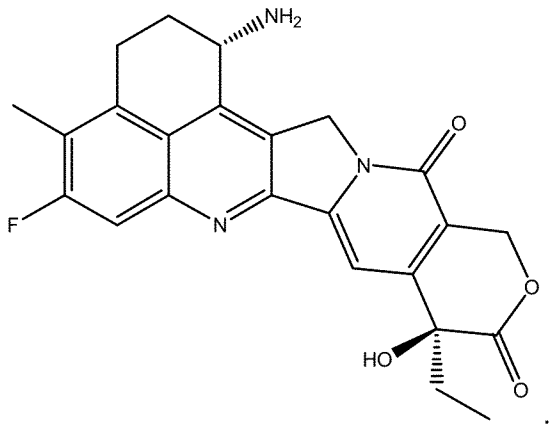
m is 1; and

n is an integer as defined herein;
preferably n is an integer ranging from 1 to 10; more preferably from 2 to 10; still more preferably from 4 to 10; still more preferably from 6 to 10, still more preferably from 7 to 10, even more preferably n is 8; or
preferably n is an integer ranging from 1 to 10, more preferably from 2 to 8, still more preferably from 3 to 6, still more preferably n is 4 or 5, even more preferably n is 4.

Preferably, the camptothecin moiety C is exatecan having the following structure:

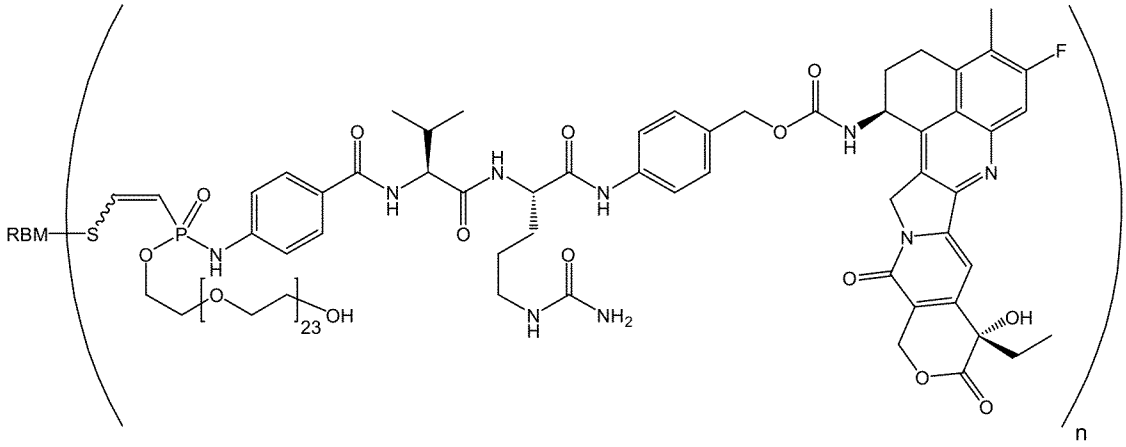


More preferably, the camptothecin moiety is exatecan having the following structure:



Preferably, in any one of these embodiments the exatecan is bound to the linker L via the amino group.

[00371] The present invention also relates to a conjugate having the following formula (Ia):



(Ia)

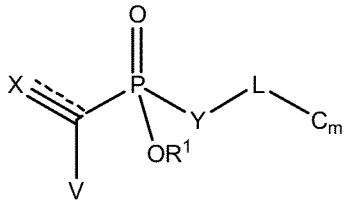
wherein:

RBM is an antibody; and

n is an integer a defined herein;
preferably n is an integer ranging from 1 to 10; more preferably from 2 to 10; still more preferably from 4 to 10; still more preferably from 6 to 10, still more preferably from 7 to 10, even more preferably n is 8; or
preferably n is an integer ranging from 1 to 10, more preferably from 2 to 8, still more preferably from 3 to 6, still more preferably n is 4 or 5, even more preferably n is 4.

Compound of Formula (II)

[00372] The present invention also relates to a compound having the formula (II):



(II),

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is $R_3-C\equiv$ when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10; and

[00373] Preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H. Preferably R^4, when present, is H or (C_1-C_8)alkyl; more preferably R^4, when present, is H. Preferably R^5, when present is H or (C_1-C_8)alkyl; more preferably R^5, when present, is H. Preferably R^6, when present is H or (C_1-C_8)alkyl; more preferably R^6, when present, is H. Preferably R^7, when present is H or (C_1-C_8)alkyl; more preferably R^7, when present, is H.

[00374] Preferably,  is a triple bond; V is absent; X is $^{R_3-C}$; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H.

[00375] More preferably,  represents a triple bond; V is absent; X represents $^{R_3-C}$, and R_3 represents H or (C_1-C_8)alkyl. Preferably, R_3 represents H or (C_1-C_6)alkyl, more preferably H or (C_1-C_4)alkyl, still more preferably H or (C_1-C_2)alkyl. Even more preferably, R_3 is H.

[00376] In some embodiments,  may be a double bond; V is H or (C_1-C_8)alkyl, preferably V is H; X is $^{R_3-\overset{R_4}{\overset{|}{C}}}$; R_3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; more preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H; R^4 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably, R^4 is H or (C_1-C_8)alkyl, preferably R^4 is H.

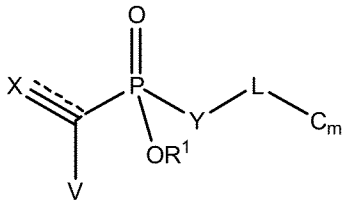
[00377] In some embodiments,  may represent a double bond; V may be H or (C_1-C_8)alkyl; X may represent $^{R_3-\overset{R_4}{\overset{|}{C}}}$; and R_3 and R_4 may independently represent H or (C_1-C_8)alkyl. Preferably, R_3 and R_4 independently represent H or (C_1-C_6)alkyl, more preferably H or (C_1-C_4)alkyl, still more preferably H or (C_1-C_2)alkyl. Preferably, R_3 and R_4 are the same; even more preferably, R_3, R_4 and V are the same. More preferably, R_3 and R_4 are both H. Preferably, V is H or (C_1-C_6)alkyl, more preferably H or (C_1-C_4)alkyl, still more preferably H or (C_1-C_2)alkyl. Even more preferably, V is H. In preferred embodiments, R_3, R_4 and V are each H.

[00378] In any one of the compounds of formula (II), any variable may be defined as described herein, in particular as with regard to the conjugates of formula (I) and/or the thiol-containing molecule of formula (III). Accordingly, RBM,  , V, X, Y, R^1, R^3, R^4, R^5, R^6, R^7, L, C, m and n may be as defined herein. Preferably, Y is NH.

Method of Preparing a Conjugate of Formula (I)

[00379] The present invention also relates to a method of preparing a conjugate of formula (I), said method comprising:

reacting a compound of formula (II)



(II),

or a pharmaceutically acceptable salt or solvate thereof,

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is  when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

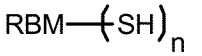
R^7 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10;

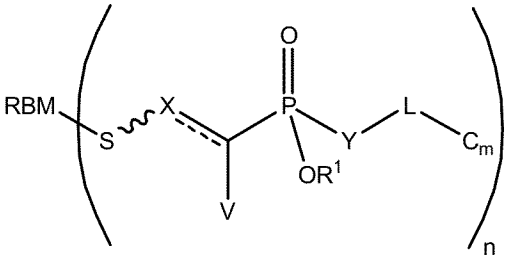
with a thiol-containing molecule of formula (III)



(III),

wherein RBM is a receptor binding molecule; and
n is an integer ranging from 1 to 20;

resulting in a compound of formula (I)



(I)

wherein:

 is a double bond when  in a compound of formula (II) is a triple bond; or

 is a bond when  in a compound of formula (II) is a double bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NH, S, O, or CH_2;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic or optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

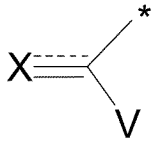
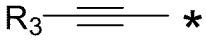
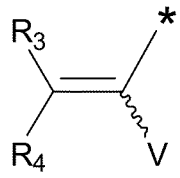
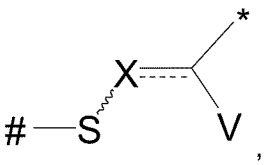
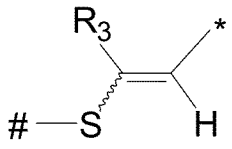
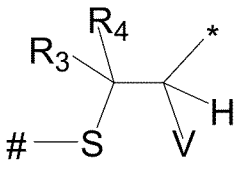
[00380] Preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H. Preferably R^4, when present, is H or (C_1-C_8)alkyl; more preferably R^4, when present, is H. Preferably R^5, when present is H or (C_1-C_8)alkyl; more preferably R^5, when present, is H. Preferably R^6, when present is H or (C_1-C_8)alkyl; more preferably R^6, when present, is H. Preferably R^7, when present is H or (C_1-C_8)alkyl; more preferably R^7, when present, is H.

[00381] Preferably,  is a triple bond; V is absent; X is  ; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H; and  represents a double bond.

[00382] More preferably,  represents a triple bond; V is absent; X represents , R_3 represents H or (C_1-C_8)alkyl; and  represents a double bond. Preferably, R_3 represents H or (C_1-C_6)alkyl, more preferably H or (C_1-C_4)alkyl, still more preferably H or (C_1-C_2)alkyl. Even more preferably, R_3 is H.

[00383] In some embodiments,  may be a double bond; V is H or (C_1-C_8)alkyl, preferably V is H; X is  ; R_3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; and  may represent a bond; more preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H; R^4 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably, R^4 is H or (C_1-C_8)alkyl, preferably R^4 is H.

[00384] In some embodiments,  may represent a double bond; V may be H or (C_1-C_8)alkyl; X may represent  ; R_3 and R_4 may independently represent H or (C_1-C_8)alkyl; and  may represents a bond. Preferably, R_3 and R_4 independently represent H or C_1-C_6-alkyl, more preferably H or C_1-C_4-alkyl, still more preferably H or C_1-C_2-alkyl. Preferably, R_3 and R_4 are the same; even more preferably, R_3, R_4 and V are the same. More preferably, R_3 and R_4 are both H. Preferably, V is H or C_1-C_6-alkyl, more preferably H or C_1-C_4-alkyl, still more preferably H or C_1-C_2-alkyl. Even more preferably, V is H. In preferred embodiments, R_3, R_4 and V are each H.

[00385] With regard to the representations  and  used herein, it is noted that, as commonly known to a person skilled in the art, each carbon atom is tetravalent. Accordingly, a structure  , wherein X and V are as defined herein and the asterisk (*) indicates attachment to the phosphorus, includes the structures  and  , wherein R_3, R_4 and V are as defined herein. A structure  , wherein X and V are as defined herein, the asterisk (*) indicates attachment to the phosphorus and # indicates attachment to the receptor binding molecule (RBM), includes the structures  and  , wherein R_3, R_4 and V are as defined herein, and H is hydrogen. A wavy bond indicates that the configuration of the double bond may be E or Z. It is also possible that the compound is present as a mixture of the E and Z isomers.

[00386] When the receptor binding molecule comprises one or more disulfide bridges, such as e.g. an antibody, the method may further comprise reducing at least one disulfide

bridge of the receptor binding molecule in the presence of a reducing agent to form a thiol group (SH). The resulting compound of formula (III) may then be reacted with a compound of formula (II) to yield a conjugate of formula (I). The reducing agent may be selected from the group consisting of tris(2-carboxyethyl)phosphine (TCEP), dithiothreitol (DTT), sodium dithionite, sodium thiosulfate, and sodium sulfite. Accordingly, the reducing agent may be dithiothreitol (DTT). The reducing agent may be sodium dithionite. The reducing agent may be sodium sulfite. Preferably, the reducing agent is tris(2-carboxyethyl)phosphine (TCEP).

[00387] Preferably, the reducing of at least one disulfide bridge comprises using about 1 to about 3 equivalents, preferably about 1 to about 2 equivalents, more preferably about 1 equivalent of the reducing agent per 1 disulfide bridge to be reduced. In this context, it is noted that in theory 1 eq. of the reducing agent, in particular of a reducing agent as described herein, is necessary to reduce 1 disulfide bridge to give 2 thiol groups (SH).

[00388] Preferably, the thiol-containing molecule of formula (III) is reacted with about 1 to about 4 equivalents, preferably about 1 to about 3 equivalents, more preferably about 1 to about 2 equivalents, still more preferably about 1.5 equivalents of the compound of formula (II) per thiol group (SH).

[00389] Preferably, the reaction of a compound of formula (II) with a thiol-containing molecule of formula (III) is carried out in an aqueous medium.

[00390] Preferably, the reaction of the compound of formula (II) with the thiol-containing molecule of formula (III) is performed under neutral pH or slightly basic conditions. Still more preferably the reaction is performed at a pH of from 6 to 10. Even more preferably, the reaction is performed at a pH of from 7 to 9.

[00391] In any one of the methods, any variable may be defined as described herein, in particular as with regard to the conjugates of formula (I) and/or the compound of formula (II). Accordingly, RBM,  ,  , V, X, Y, R^1, R^3, R^4, R^5, R^6, R^7, L, C, m and n may be as defined herein. Preferably, Y is NH.

[00392] Methods of preparing compounds of formula (II) are known in the art. As illustrative examples, compounds of formula (II), wherein the group Y is NH, may be prepared by using techniques and conditions, e.g. a Staudinger phosphonite reaction, as e.g.

described in WO 2018/041985 A1, which is hereby incorporated by reference. Compounds of formula (II), wherein Y is S or O, may be prepared by using techniques and conditions as e.g. described in WO 2019/170710, which is hereby incorporated by reference. In an analogous manner to the compounds of formula (I), wherein Y is S or O, as described in WO 2019/170710, compounds of formula (II), wherein Y is CR^6R^7, may be prepared, as illustrative examples, by substitution at the phosphorus atom using, e.g., a suitable organometallic compound, such as e.g. a Grignard compound or an organolithium compound. A person skilled in the art readily selects suitable methods and conditions to prepare compounds of formula (II). The Examples section of the present disclosure also comprises guidance on how to prepare or obtain compounds of formula (II) and/or conjugates of formula (I).

[00393] The present invention also relates to a conjugate of formula (I) obtainable or being obtained by any method of preparing a conjugate of formula (I) as described herein.

Pharmaceutical Composition

[00394] The present invention further relates to a pharmaceutical composition comprising a conjugate of formula (I).

[00395] The pharmaceutical composition may comprise a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from more than 0 to about 14, preferably from about 1 to about 14, more preferably from about 2 to about 14, still more preferably from about 4 to about 14, still more preferably from about 5 to about 12, still more preferably from about 6 to about 12, still more preferably from about 6 to about 10, even more preferably about 8. Accordingly, the pharmaceutical composition may comprise a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from more than 0 to about 14. Preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 1 to about 14. More preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 2 to about 14. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 4 to about 14. Still more preferably, the

pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 5 to about 12. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 6 to about 12. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from about 6 to about 10. Even more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties per receptor binding molecule in the composition is about 8. When the receptor binding molecule is, in some preferred embodiments, an antibody or an antibody fragment, such average number is also denoted as “average drug to antibody ratio (DARav)”. In this context, a person skilled in the art understands that a composition may comprise a population of conjugates, which may differ in the number of camptothecin moieties per receptor binding molecule, and which may optionally also comprise unconjugated receptor binding molecule, so that the result is an average number of camptothecin moieties per receptor binding molecule.

[00396] The pharmaceutical composition may comprise a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from more than 0 to about 14, preferably from about 1 to about 14, more preferably from about 1 to about 12, still more preferably, from about 2 to about 10, still more preferably from about 2 to about 8, still more preferably from about 2 to about 6, still more preferably from about 3 to about 5, even more preferably about 4. Accordingly, the pharmaceutical composition may comprise a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from more than 0 to about 14. Preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 1 to about 14. More preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 1 to about 12. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 2 to about 10. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 2 to

about 8. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 2 to about 6. Still more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is from about 3 to about 5. Even more preferably, the pharmaceutical composition comprises a population of a conjugate of formula (I), wherein the average number of camptothecin moieties C per receptor binding molecule is about 4. When the receptor binding molecule is, in some preferred embodiments, an antibody or an antibody fragment, such average number is also denoted as “average drug to antibody ratio (DARav)”.

[00397] The pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water, 5% dextrose, or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters that are suitable for administration to a human or non-human subject. Particular exemplary pharmaceutically acceptable carriers include (biodegradable) liposomes; microspheres made of the biodegradable polymer poly(D,L-lactic-coglycolic acid (PLGA), albumin microspheres; synthetic polymers (soluble); nanofibers, protein-DNA complexes; protein conjugates; erythrocytes; or virosomes. Various carrier based dosage forms comprise solid lipid nanoparticles (SLNs), polymeric nanoparticles, ceramic nanoparticles, hydrogel nanoparticles, copolymerized peptide nanoparticles, nanocrystals and nanosuspensions, nanocrystals, nanotubes and nanowires, functionalized nanocarriers, nanospheres, nanocapsules, liposomes, lipid emulsions, lipid microtubules/microcylinders, lipid microbubbles, lipospheres, lipopolyplexes, inverse lipid micelles, dendrimers, ethosomes, multicomposite ultrathin capsules, aquasomes, pharmacosomes, colloidosomes, niosomes, discomes, proniosomes, microspheres, microemulsions and polymeric micelles. Other suitable pharmaceutically acceptable carriers and excipients are *inter alia* described in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1991) and Bauer et al., Pharmazeutische Technologie, 5th Ed., Govi-Verlag Frankfurt (1997). See, e.g., Remington: The Science and Practice of Pharmacy, 21st edition; Lippincott Williams & Wilkins, 2005.

[00398] In some embodiments, a pharmaceutically acceptable carrier or composition is sterile. A pharmaceutical composition can comprise, in addition to the active agent, physiologically acceptable compounds that act, for example, as bulking agents, fillers, solubilizers, stabilizers, osmotic agents, uptake enhancers, etc. Physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose, lactose; dextrans; polyols such as mannitol; antioxidants, such as ascorbic acid or glutathione; preservatives; chelating agents; buffers; or other stabilizers or excipients.

[00399] The choice of a pharmaceutically acceptable carrier(s) and/or physiologically acceptable compound(s) can depend for example, on the nature of the active agent, e.g., solubility, compatibility (meaning that the substances can be present together in the composition without interacting in a manner that would substantially reduce the pharmaceutical efficacy of the pharmaceutical composition under ordinary use situations) and/or route of administration of the composition.

[00400] Pharmaceutical compositions of the invention may comprise a therapeutically effective amount of the conjugate of formula (I) described herein and can be structured in various forms, e.g. in solid, liquid, gaseous or lyophilized form and may be, inter alia, in the form of an ointment, a cream, transdermal patches, a gel, powder, a tablet, solution, an aerosol, granules, pills, suspensions, emulsions, capsules, syrups, liquids, elixirs, extracts, tincture or fluid extracts or in a form which is particularly suitable for topical or oral administration. A variety of routes are applicable for administration of the conjugate of formula (I), including, but not limited to, orally, topically, transdermally, subcutaneously, intravenously, intraperitoneally, intramuscularly or intraocularly. However, any other route may readily be chosen by the person skilled in the art if desired.

Use in Methods of Treatment

[00401] As shown in the Examples, conjugates of formula (I) of the present invention can be used for the treatment, in particular the treatment of cancer. Accordingly, the present invention further relates to a conjugate of formula (I) of the invention for use in a method of treating a disease, optionally comprising the administration of an effective amount of the conjugate of the invention or the pharmaceutical composition of the invention to a subject or patient in need thereof. Also, the present invention relates to a pharmaceutical composition of the invention for use in a method of treating a disease, optionally comprising the administration of an effective amount of the conjugate of the invention or the pharmaceutical composition of the invention to a subject or patient in need thereof. The disease may be

associated with overexpression of CD30. The disease may by associated with overexpression of Her2. The disease may be cancer. The cancer may be a solid tumor. The disease may be a cancer associated with overexpression of CD30. The disease may be a cancer associated with overexpression of Her2.

[00402] The present invention also relates to the use of a conjugate of formula (I) of the invention for the manufacture of a medicament for treating a disease. The present invention also relates to the use of a pharmaceutical composition of the invention for the manufacture of a medicament for treating a disease. The disease may be associated with overexpression of CD30. The disease may by associated with overexpression of Her2. The disease may be cancer. The cancer may be a solid tumor. The disease may be a cancer associated with overexpression of CD30. The disease may be a cancer associated with overexpression of Her2.

[00403] The present invention also relates to a method of treating a disease, comprising the administration of an effective amount of a conjugate of formula (I) of the invention to a subject or patient in need thereof. The present invention also relates to a method of treating a disease, comprising the administration of an effective amount of a pharmaceutical composition of the invention to a subject or patient in need thereof. The disease may be associated with overexpression of CD30. The disease may by associated with overexpression of Her2. The disease may be cancer. The cancer may be a solid tumor. The disease may be a cancer associated with overexpression of CD30. The disease may be a cancer associated with overexpression of Her2.

[00404] The phrase " effective amount" in general refers to an amount of a therapeutic agent (e.g., the conjugate of the invention) that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in in vitro assays. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[00405] Further, the present invention relates to a conjugate of formula (I) as described herein for use in a method of treating cancer in a patient. The present invention also relates to a pharmaceutical composition as described herein for use in a method of treating cancer in a patient. The term "patient" means according to the invention a human being, a non-human primate or another animal, in particular a mammal such as a cow, horse, pig, sheep, goat, dog, cat or a rodent such as a mouse and rat. In a particularly preferred embodiment, the patient is a human being. Except when noted, the terms “patient” or “subject” are used herein interchangeably. The term “treatment” in all its grammatical forms includes therapeutic or prophylactic treatment. A “therapeutic or prophylactic treatment” comprises prophylactic treatments aimed at the complete prevention of clinical and/or pathological manifestations or therapeutic treatment aimed at amelioration or remission of clinical and/or pathological manifestations. The term “treatment” thus also includes the amelioration or prevention of diseases.

[00406] A conjugate of formula (I) of the present invention may be administered at any dose that is therapeutically effective. The upper limit is usually a dose that is still safe to administer in terms of side effects. As illustrative examples a conjugate of the invention may be administered at a(n effective) dose of 20 mg/kg, 18 mg/kg, 16 mg/kg, 14 mg/kg, 12 mg/kg, 10 mg/kg, 9 mg/kg, 8 mg/kg, 7 mg/kg, 6 mg/kg, 5 mg/kg, 4 mg/kg, 3 mg/kg, 2 mg/kg, 1 mg/kg, 0.5 mg/kg or 0.25 mg/kg. This dose may be administred over a given amount of time, for example over three to four week (21 day to 28 days), a period that is also called “treatment cycle”. Such a treatment cycle may be repeated, depending on the disease progression or regression, i.e treatment outcome. As one illustratrative example, a conjugate of formula (I) may be administered in the amounts that, for example, are recommended for Enhertu for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer. In this indication, the recommended dosage of Enhertu is 5.4 mg/kg given as an intravenous infusion once every 3 weeks (21-day cycle) until disease progression or unacceptable toxicity. In such a treatment cycle, the conjugate may be administered in a similar or same way as Enhertu, i.e. in first infusion in which the conjugate is infused over 90 minutes and in subsequent infusion, where the conjugate is administered over 30 minutes if prior infusions were well tolerated. In line with this, a conjugate of the invention may be administered to a patient by any suitable way, for example by injection or infusion such as intravenous infusion. In particular, a conjugate of the invention may be administered at a(n effective) dose of 5 mg/kg given as an intravenous infusion, e.g., once every three weeks.

[00407] The term “cancer”, as used herein, can denote any cancer, e.g., preferably said cancer is selected from the group consisting of: Breast cancer, Head and neck cancer, Ovary cancer, Endometrium cancer, Uterine cervix cancer, Rectum cancer, Colon cancer, Esophagus cancer, Stomach cancer, Lung cancer, Kidney cancer, Adrenal gland cancer, Bladder cancer, Liver cancer, Sarcoma, Brain cancer, Nevi and Melanomas, Urogenital cancer, Prostate cancer, Vulva Squamous cell carcinoma, Oropharyngeal cancer, Endocrine gland cancer, Thoracic Cancer, Mesothelioma, Pancreas cancer, Cholangiocarcinoma, Blood cancers, Retinoblastom, Thyroid cancer, Fallopian tube cancer; further preferably said cancer is a solid cancer, e.g., selected from the group consisting of: Breast cancer, Head and neck cancer, Ovarian cancer, Endometrial cancer, Uterus cancer (e.g., including cancers of the muscle sheets), Cervical cancer, Rectum cancer, Colon cancer, Anal cancer, Esophagus cancer, Stomach cancer, Lung cancer, Kidney cancer, Adrenal gland cancer, Bladder cancer, Liver cancer, Sarcoma (e.g., including osteosarcoma and Kaposi sarcoma), Brain cancer (e.g., including pituitary tumor/s), Nevi and Melanoma cancers, Skin cancers (e.g., including squamous cell carcinoma and melanoma), Urogenital cancer (e.g., ureter and bladder cancer, testicular cancer, prostate cancer, penile cancer), Prostate cancer, Vulva Squamous cell carcinoma, Oropharyngeal cancer, Endocrine gland cancer, Thoracic Cancer, Mesothelioma, Pancreas cancer, Cholangiocarcinoma, Blood cancers (e.g., including lymphoma, leukemia, myeloma, Myelodysplastic syndromes, myelofibrosis), Eye cancers (e.g., including Retinoblastoma), Neuroendocrine tumors, Cancer of unknown primary (CUP)). Preferably said cancer is a solid and/or metastatic cancer, further preferably said cancer is selected from the group consisting of: lung cancer, ovarian cancer, thyroid cancer, nonsquamous non-small cell lung carcinoma, nonmucinous ovarian carcinoma, papillary thyroid carcinoma, renal cancer, endometrial cancer, uterus cancer, ureter cancer, bladder cancer and fallopian tube cancer. Said cancer may be also selected from the group consisting of Cancer in Adolescents, Adrenocortical Carcinoma, Anal Cancer, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor, Basal Cell Carcinoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Tumors, Breast Cancer, Bronchial Tumors, Cervical Cancer, Chordoma, Chronic Myeloproliferative Neoplasms, Colorectal Cancer, Craniopharyngioma, Embryonal Tumors, Medulloblastoma and Other Central Nervous System, Childhood (Brain Cancer), Endometrial Cancer (Uterine Cancer), Ependymoma, Childhood (Brain Cancer), Esophageal Cancer, Esthesioneuroblastoma (Head and Neck Cancer), Ewing Sarcoma (Bone Cancer), Extracranial Germ Cell Tumor, Childhood, Extragonadal Germ Cell Tumor, Fallopian Tube Cancer, Gallbladder Cancer, Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumors (GIST), Gestational Trophoblastic Disease, Head and Neck Cancer, Heart Tumors, Hepatocellular (Liver) Cancer, Histiocytosis, Langerhans Cell,

Hypopharyngeal Cancer (Head and Neck Cancer), Intraocular Melanoma, Islet Cell Tumors, Pancreatic Neuroendocrine Tumors, Kaposi Sarcoma (Soft Tissue Sarcoma), Kidney (Renal Cell) Cancer, Langerhans Cell Histiocytosis, Laryngeal Cancer (Head and Neck Cancer), Lip and Oral Cavity Cancer (Head and Neck Cancer), Liver Cancer, Lung Cancer, Male Breast Cancer, Melanoma, Melanoma, Intraocular (Eye), Merkel Cell Carcinoma (Skin Cancer), Mesothelioma, Malignant, Mouth Cancer (Head and Neck Cancer), Multiple Endocrine Neoplasia Syndromes, Nasal Cavity and Paranasal Sinus Cancer (Head and Neck Cancer), Nasopharyngeal Cancer (Head and Neck Cancer), Neuroblastoma, Non-Small Cell Lung Cancer, Oral Cancer, Lip and Oral Cavity Cancer and Oropharyngeal Cancer (Head and Neck Cancer), Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Papillomatosis (Childhood Laryngeal), Paraganglioma, Paranasal Sinus and Nasal Cavity Cancer (Head and Neck Cancer), Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer (Head and Neck Cancer), Pheochromocytoma, Pituitary Tumor, Pleuropulmonary Blastoma (Lung Cancer), Primary Central Nervous System (CNS) Lymphoma, Primary Peritoneal Cancer, Prostate Cancer, Pulmonary Inflammatory Myofibroblastic Tumor (Lung Cancer), Rectal Cancer, Renal Cell (Kidney) Cancer, Retinoblastoma, Rhabdomyosarcoma, Childhood (Soft Tissue Sarcoma), Salivary Gland Cancer (Head and Neck Cancer), Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma of the Skin – see Skin Cancer, Squamous Neck Cancer with Occult Primary, Metastatic (Head and Neck Cancer), Stomach (Gastric) Cancer, Testicular Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Tracheobronchial Tumors (Lung Cancer), Transitional Cell Cancer of the Renal Pelvis and Ureter (Kidney (Renal Cell) Cancer), Ureter and Renal Pelvis, Transitional Cell Cancer (Kidney (Renal Cell) Cancer, Urethral Cancer, Uterine Cancer, Endometrial, Uterine Sarcoma, Vaginal Cancer, Vascular Tumors (Soft Tissue Sarcoma), Vulvar Cancer, and Wilms Tumor and Other Childhood Kidney Tumors.

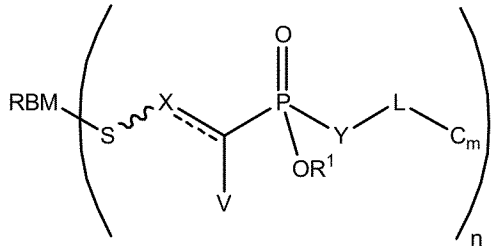
[00408] By "tumor" is meant a group of cells or tissue that is formed by misregulated cellular proliferation, in particular cancer. Tumors may show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be either benign or malignant. In particular, the term "tumor" refers to a malignant tumor. The term “tumor” may refer to a solid tumor. According to one embodiment, the term "tumor" or "tumor cell" also refers to non-solid cancers and cells of non-solid cancers such as leukemia cells. According to another embodiment, respective non-solid cancers or cells thereof are not encompassed by the terms "tumor" and "tumor cell".

[00409] By "metastasis" is meant the spread of cancer cells from its original site to another part of the body. The formation of metastasis is a very complex process and normally involves detachment of cancer cells from a primary tumor, entering the body circulation and settling down to grow within normal tissues elsewhere in the body. When tumor cells metastasize, the new tumor is called a secondary or metastatic tumor, and its cells normally resemble those in the original tumor. This means, for example, that, if breast cancer metastasizes to the lungs, the secondary tumor is made up of abnormal breast cells, not of abnormal lung cells. The tumor in the lung is then called metastatic breast cancer, not lung cancer.

Items of the Invention

The invention further relates to the following items:

1. A conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

RBM is a receptor binding molecule;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is R_3-C when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

2. The conjugate of item 1, wherein:

R^3 is H or $(C_1$-$C_8)$alkyl; preferably R^3 is H;

R^4 when present is H or $(C_1$-$C_8)$alkyl; preferably R^4, when present, is H;

R^5 when present is H or $(C_1$-$C_8)$alkyl; preferably R^5, when present, is H;

R^6 when present is H or $(C_1$-$C_8)$alkyl; preferably R^6, when present, is H; and

R^7 when present is H or $(C_1$-$C_8)$alkyl; preferably R^7, when present, is H.

3. The conjugate of item 1 or 2, wherein  is a double bond; V is absent; X is $R_3{-}C$; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or $(C_1$-$C_8)$alkyl; more preferably R^3 is H.

4. The conjugate of item 1 or 2, wherein  is a bond; V is H or $(C_1$-$C_8)$alkyl, preferably V is H; X is  ; R_3 is H or an optionally substituted aliphatic residue or

an optionally substituted aromatic residue; more preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H; R^4 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably, R^4 is H or (C_1-C_8)alkyl, preferably R^4 is H.

5. The conjugate of any one of the preceding items, wherein the receptor binding molecule is selected from the group consisting of an antibody, an antibody fragment, and a proteinaceous binding molecule with antibody-like binding properties.

6. The conjugate of item 5, wherein the receptor binding molecule is an antibody, preferably wherein the antibody is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, and a single domain antibody, such as a camelid or shark single domain antibody.

7. The conjugate of item 5, wherein the receptor binding molecule is an antibody fragment,

preferably wherein the antibody fragment is a divalent antibody fragment, more preferably wherein the divalent antibody fragment is selected from the group consisting of a $(Fab)_2$'-fragment, a divalent single-chain Fv fragment, a dual affinity re-targeting (DART) antibody, and a diabody; or

preferably wherein the antibody fragment is a monovalent antibody fragment, more preferably wherein the monovalent antibody fragment is selected from the group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv).

8. The conjugate of item 5, wherein the receptor binding molecule is a proteinaceous binding molecule with antibody-like binding properties,
preferably wherein the proteinaceous binding molecule with antibody-like binding properties is selected from the group consisting of a mutein based on a polypeptide of the lipocalin family, a glubody, a protein based on the ankyrin scaffold, a protein based on the crystalline scaffold, an adnectin, an avimer, a DARPin, and an affibody.

9. The conjugate of any one of the preceding items, wherein Y is NH.

9a. The conjugate of item 9, wherein the receptor binding molecule is an antibody.

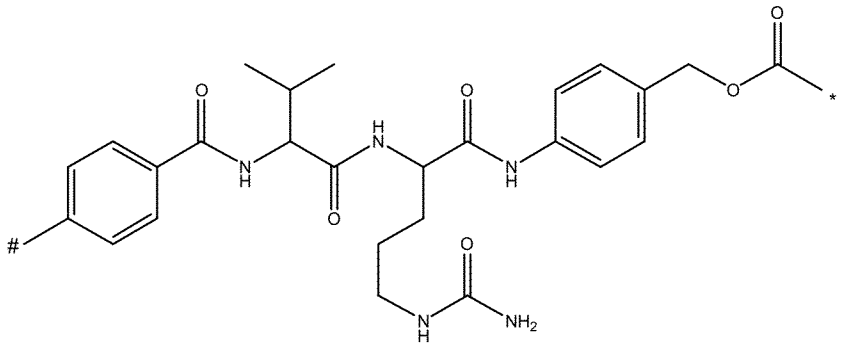
10. The conjugate of any one of the preceding items, wherein the linker L is cleavable.

11. The conjugate of item 10, wherein the linker L is cleavable by a protease, a glucuronidase, a sulfatase, a phosphatase, an esterase, or by disulfide reduction.

12. The conjugate of item 11, wherein the linker L is cleavable by a protease, preferably by a cathepsin such as cathepsin B.

13. The conjugate of any one of the preceding items, wherein the linker L comprises a valine-citrulline moiety.

14. The conjugate of item 13, wherein the linker L is:

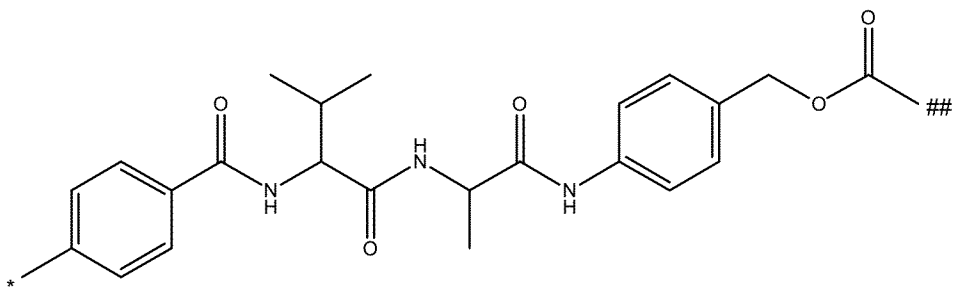


,

wherein # indicates the attachment point to the Y and * indicates the attachment point to the camptothecin moiety.

15. The conjugate of any one of items 1 to 12, wherein the linker L comprises a valine-alanine moiety.

16. The conjugate of item 15, wherein the linker L is:



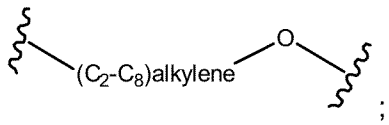
,

wherein * indicates the attachment point to the Y and ## indicates the attachment point to the camptothecin moiety.

17. The conjugate of any one of items 1 to 9, wherein the linker is non-cleavable.

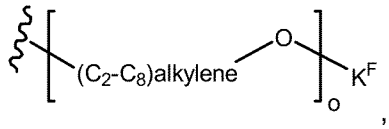
18. The conjugate of any one of the preceding items, wherein R^1 is a first polyalkylene glycol unit R^F.

19. The conjugate of items 18, wherein the first polyalkylene glycol unit R^F comprises 1 to 100 subunits having the structure:



;

preferably wherein R^F is



,

wherein:

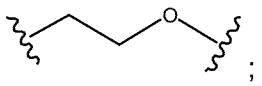
 indicates the position of the O;

K^F is selected from the group consisting of -H, $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$; preferably K^F is H; and

o is an integer ranging from 1 to 100.

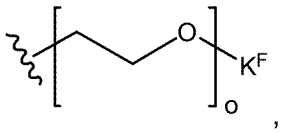
20. The conjugate of item 18 or 19, wherein R^1 is a first polyethylene glycol unit.

21. The conjugate of item 17, wherein the first polyethylene glycol unit R^F comprises 1 to 100 subunits having the structure:



;

preferably wherein R^F is:



,

wherein

indicates the position of the O;

K^F is selected from the group consisting of -H, $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$; preferably K^F is H; and

o is an integer ranging from 1 to 100.

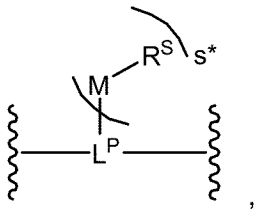
21a. The conjugate of item 21, wherein K^F is H.

21b. The conjugate of item 21 or 21a, wherein o ranges from 8 to 30.

21c. The conjugate of item 21b, wherein o ranges from 20 to 28.

21d. The conjugate of item 21c, wherein o is 22, 23, 24, 25 or 26.

22. The conjugate of any one of items 1 to 13, 15 and 17 to 21d, in particular of any one of items 18 to 21d, wherein the linker comprises a second spacer unit A, said second spacer unit A being a group Z, said group Z having the following structure:



,

wherein:

L^P is a parallel connector unit;

R^S is, each independently, a second polyalkylene glycol unit;

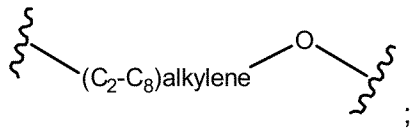
M is, each independently, a bond or a moiety that binds R^S with L^P;

s* is an integer ranging from 1 to 4; preferably, s* is 1; and

the wavy lines indicate the attachment point to the -Y- and to another part of the linker, when present, or to a camptothecin moiety (-C).

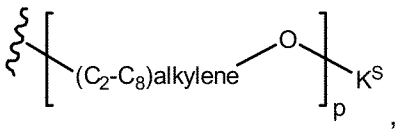
23. The conjugate of item 22, wherein M is each independently selected from the group consisting of -NH-, -O-, -S-, -C(O)-O-, -C(O)-NH- and -(C_1-C_{10})alkylene-; preferably each M is -O-.

24. The conjugate of any one of items 22 to 23, wherein the second polyalkylene glycol unit R^S, each independently, comprises 1 to 100 subunits having the structure:



;

preferably wherein R^S is, each independently,



,

wherein:

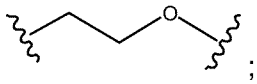

indicates the position of the M in group Z;

K^S is selected from the group consisting of -H, -PO_3H, -(C_1-C_{10})alkyl, -(C_1-C_{10})alkyl-SO_3H, -(C_2-C_{10})alkyl-CO_2H, -(C_2-C_{10})alkyl-OH, -(C_2-C_{10})alkyl-NH_2, -(C_2-C_{10})alkyl-NH(C_1-C_3)alkyl and -(C_2-C_{10})alkyl-N((C_1-C_3)alkyl)$_2$; preferably K^S is H; and

p is an integer ranging from 1 to 100.

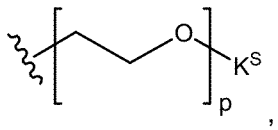
25. The conjugate of any one of items 22 to 24, wherein R^S is, each independently, a second polyethylene glycol unit.

26. The conjugate of item 25, wherein the second polyethylene glycol unit R^S, each independently, comprises 1 to 100 subunits having the structure:



;

preferably wherein R^S is, each independently:



,

wherein

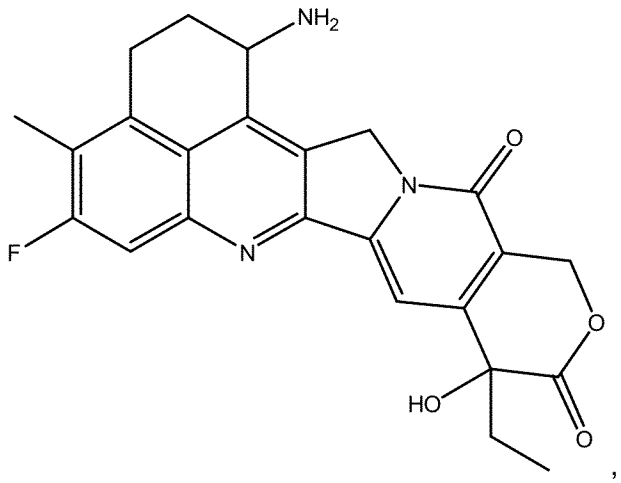
indicates the position of the M in group Z;

K^S is selected from the group consisting of -H, $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$; preferably K^S is H; and

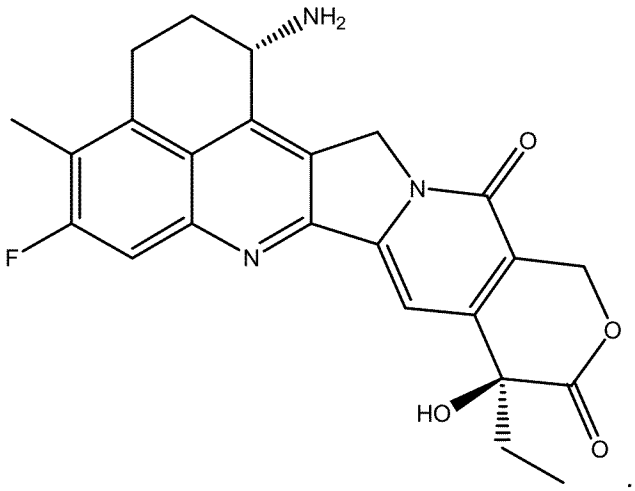
p is an integer ranging from 1 to 100.

27. The conjugate of any one of the preceding items, wherein the camptothecin moiety C is selected from the group consisting of exatecan, SN38, camptothecin, topotecan, irinotecan, belotecan, lurtotecan, rubitecan, silatecan, cositecan, and gimatecan.

28. The conjugate of item 27, wherein the campthothecin moiety C is exatecan having the formula:



preferably having the formula:



29. The conjugate of item 28, wherein the exatecan is bound to the linker L via the amino group.

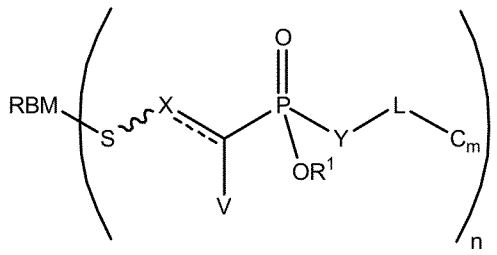
30. The conjugate of any one of the preceding items, wherein the number of camptothecin moieties C per receptor binding molecule is from 1 to 14, preferably from 2 to 14, more preferably from 4 to 14, still more preferably from 5 to 12, still more preferably from 6 to 12, still more preferably from 7 to 10, even more preferably 8.

31. The conjugate of any one of items 1 to 29, wherein the number of camptothecin moieties C per receptor binding molecule is from 1 to 14, preferably from 1 to 12, more preferably from 2 to 10, still more preferably from 2 to 8, still more preferably from 2 to 6, still more preferably from 3 to 5, even more preferably 4.

32. The conjugate of any one of items 1 to 29, wherein m is an integer ranging from 1 to 4, preferably 1 or 2, more preferably 1; and
n is an integer ranging from 1 to 20, preferably from 1 to 10, more preferably from 2 to 10, still more preferably from 4 to 10, still more preferably from 6 to 10, still more preferably from 7 to 10, even more preferably 8.

33. The conjugate of any one of items 1 to 29, wherein m is an integer ranging from 1 to 4, preferably 1 or 2, more preferably 1; and
n is an integer ranging from 1 to 20, preferably from 1 to 10, more preferably from 2 to 8, still more preferably from 3 to 6, still more preferably 4 or 5, even more preferably 4.

33a. A conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

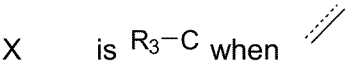
RBM is an antibody;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

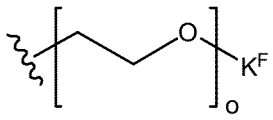
V is H when  is a bond;

X is  when is a double bond; or

X is  when  is a bond;

Y is NH;

R^1 is a polyethylene glycol unit having the structure:



wherein:

 indicates the position of the O;

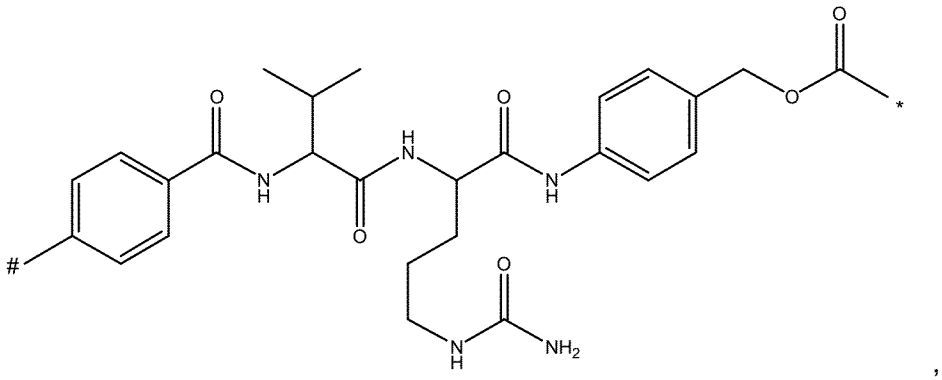
K^F is H; and

o is an integer ranging from 8 to 30;

R^3 is H;

R^4 is H;

L is a linker having the following structure:



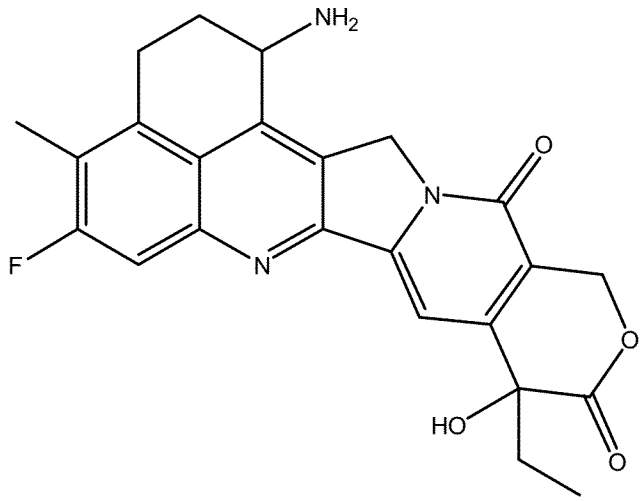
wherein # indicates the attachment point to the Y and * indicates the attachment point to the camptothecin moiety (C);

C is a camptothecin moiety;

m is 1; and

n is an integer ranging from 1 to 10.

33b. The conjugate of item 33a, wherein the camptothecin moiety C is exatecan having the formula:



.

33c. The conjugate of item 33a or 33b, wherein the exatecan is bound to the linker L via the amino group.

33d. The conjugate of any one of items 33a to 33c, wherein o ranges from 20 to 28.

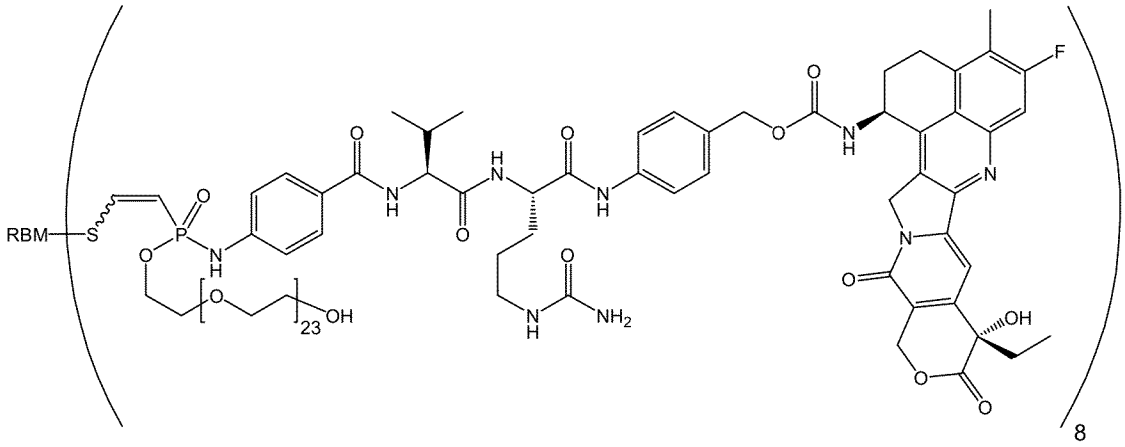
33e. The conjugate of item 33d, wherein o is 22, 23, 24, 25 or 26.

33f. The conjugate of any one of items 33a to 33e, wherein n ranges from 2 to 10.

33g. The conjugate of item 33f, wherein n is 4.

33h. The conjugate of item 33f, wherein n is 8.

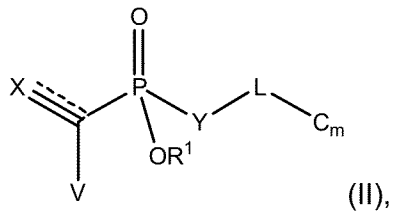
33i. A conjugate having the following formula (Ia):



(Ia),

wherein RBM is an antibody.

34. A compound having the formula (II):



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is  when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10.

35. The compound of item 34, wherein:

R^3 is H or (C_1-C_8)alkyl; preferably R^3 is H;

R^4 when present is H or (C_1-C_8)alkyl; preferably R^4, when present, is H;

R^5 when present is H or (C_1-C_8)alkyl; preferably R^5, when present, is H;

R^6 when present is H or (C_1-C_8)alkyl; preferably R^6, when present, is H; and

R^7 when present is H or (C_1-C_8)alkyl; preferably R^7, when present, is H.

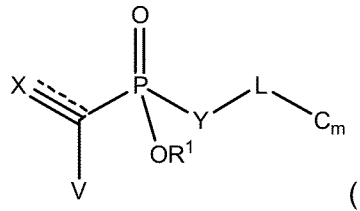
36. The compound of item 34 or 35, wherein  is a triple bond; V is absent; X is R_3-C; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H.

37. The compound of item 34 or 35, wherein  is a double bond; V is H or (C_1-C_8)alkyl, preferably V is H; X is  ; R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H; R^4 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^4 is H or (C_1-C_8)alkyl, more preferably R^4 is H.

38. The compound of any one of items 34 to 37, wherein RBM, V, X, Y, R^1, R^3, R^4, R^5, R^6, R^7, L, C, m and n are as defined in any one of items 1 to 33i; preferably, Y is NH.

39. A method of preparing a conjugate of formula (I), said method comprising:

reacting a compound of formula (II)



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is R_3-C when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

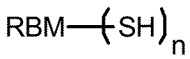
R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10; and

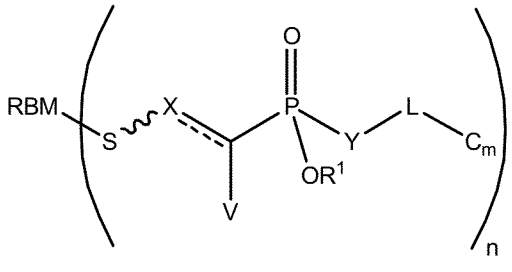
with a thiol-containing molecule of formula (III)



(III),

wherein RBM is a receptor binding molecule; and
n is an integer ranging from 1 to 20;

resulting in a compound of formula (I)



(I)

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a double bond when  in a compound of formula (II) is a triple bond; or

 is a bond when  in a compound of formula (II) is a double bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

40. The method of item 39, wherein:

R^3 is H or $(C_1\text{-}C_8)$alkyl; preferably R^3 is H;

R^4 when present is H or $(C_1\text{-}C_8)$alkyl; preferably R^4, when present, is H;

R^5 when present is H or $(C_1\text{-}C_8)$alkyl; preferably R^5, when present, is H;

R^6 when present is H or $(C_1\text{-}C_8)$alkyl; preferably R^6, when present, is H; and

R^7 when present is H or $(C_1\text{-}C_8)$alkyl; preferably R^7, when present, is H.

41. The method of item 39 or 40, wherein  is a triple bond; V is absent; X is ; R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue, preferably R3 is H orr $(C_1\text{-}C_8)$alkyl, more preferably R^3 is H, and is a double bond.

42. The method of item 39 or 40, wherein  is a double bond; V is H or (C_1-C_8)alkyl, preferably V is H; X is  , R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue, preferably R^3 is H or (C_1-C_8)alkyl, more preferably R^3 is H; R^4 is H or (C_1-C_8)alkyl, preferably R^4 is H; and  is a bond.

43. The method of any one of items 39 to 42, wherein the reaction is performed under neutral pH or slightly basic conditions, preferably at a pH of from 6 to 10.

44. The method of any one of items 39 to 43, further comprising reducing at least one disulfide bridge of the receptor binding molecule in the presence of a reducing agent to form a thiol group (SH).

45. The method of item 44, wherein the reducing agent is selected from the group consisting of tris(2-carboxyethyl)phosphine (TCEP), dithiothreitol (DTT), sodium dithionite, sodium thiosulfate, and sodium sulfite;
preferably wherein the reducing agent is tris(2-carboxyethyl)phosphine (TCEP).

46. The method of item 44 or 45, wherein the reducing of at least one disulfide bridge comprises using about 1 to about 3 equivalents, preferably about 1 to about 2 equivalents, more preferably about 1 equivalent of the reducing agent per disulfide bridge to be reduced.

47. The method of any one of items 39 to 46, wherein the thiol-containing molecule of formula (III) is reacted with about 1 to about 4 equivalents, preferably about 1 to about 3 equivalents, more preferably about 1 to about 2 equivalents, still more preferably about 1.5 equivalents of the compound of formula (II) per thiol group (SH).

48. The method of any one of items 39 to 47, wherein the reacting a compound of formula (II) with a thiol-containing molecule of formula (III) is carried out in an aqueous medium.

49. The method of any one of items 39 to 48, wherein RBM, V, X, Y, R^1, R^3, R^4, R^5, R^6, R^7, L, C, m and n are as defined in any one of items 1 to 33i; preferably Y is NH_2.

50. A conjugate of formula (I) obtainable or being obtained by a method of any one of items 39 to 49.

51. A pharmaceutical composition comprising a conjugate of any one of items 1 to 33i or 50.

52. The pharmaceutical composition of item 51, wherein the pharmaceutical composition comprises a population of a conjugate of any one of items 1 to 33, and wherein the average number of camptothecin moieties per receptor binding molecule in the composition is from more than 0 to about 14, preferably from about 1 to about 14, more preferably from about 2 to about 14, still more preferably from about 4 to about 14, still more preferably from about 5 to about 12, still more preferably from about 6 to about 12, still more preferably from about 6 to about 10, even more preferably about 8.

53. The pharmaceutical composition of item 51, wherein the pharmaceutical composition comprises a population of a conjugate of any one of items 1 to 33, and wherein the average number of camptothecin moieties C per receptor binding molecule is from more than 0 to about 14, preferably from about 1 to about 14, more preferably from about 1 to about 12, still more preferably, from about 2 to about 10, still more preferably from about 2 to about 8, still more preferably from about 2 to about 6, still more preferably from about 3 to about 5, even more preferably about 4.

54. A conjugate of any one of items 1 to 33i or 50 for use in a method of treating a disease.

55. The conjugate for use of item 54, wherein the disease is cancer.

55a. The conjugate for use of item 55, wherein the cancer is a solid tumor.

56. A pharmaceutical composition of any one of items 51 to 53 for use in a method of treating a disease.

57. The pharmaceutical composition for use of item 56, wherein the disease is cancer.

58. The pharmaceutical composition for use of item 57, wherein the cancer is a solid tumor.

59. Use of a conjugate of any one of items 1 to 33i or 50 for the manufacture of a medicament for treating a disease.

60. The use of item 59, wherein the disease is cancer.

61. The use of item 60, wherein the cancer is a solid tumor.

62. Use of a pharmaceutical composition of any one of items 51 to 53 for the manufacture of a medicament for treating a disease.

63. The use of item 62, wherein the disease is cancer.

64. The use of item 63, wherein the cancer is a solid tumor.

65. A method of treating a disease, comprising the administration of an effective amount of a conjugate of any one of items 1 to 33f or 50 to a subject or patient in need thereof.

66. The method of claim 65, wherein the disease is cancer.

67. The method of claim 66, wherein the cancer is a solid tumor.

68. A method of treating a disease, comprising the administration of an effective amount of a pharmaceutical composition of any one of items 51 to 53 to a subject or patient in need thereof.

69. The method of item 68, wherein the disease is cancer.

70. The method of item 69, wherein the cancer is a solid tumor.

*****

[00410] It is noted that as used herein, the singular forms “a”, “an”, and “the”, include plural references unless the context clearly indicates otherwise. Thus, for example, reference to “a reagent” includes one or more of such different reagents and reference to “the method” includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[00411] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

[00412] The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[00413] The term “less than” or in turn “more than” does not include the concrete number. For example, less than 20 means less than the number indicated. Similarly, more than or greater than means more than or greater than the indicated number, e.g. more than 80 % means more than or greater than the indicated number of 80 %.

[00414] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term “comprising” can be substituted with the term “containing” or “including” or sometimes when used herein with the term “having”. When used herein “consisting of" excludes any element, step, or ingredient not specified.

[00415] The term “including” means “including but not limited to”. “Including” and “including but not limited to” are used interchangeably.

[00416] As used herein the terms "about", "approximately" or “essentially” mean within 20%, preferably within 15%, preferably within 10%, and more preferably within 5% of a given value or range. It also includes the concrete number, i.e. “about 20” includes the number of 20.

[00417] It should be understood that this invention is not limited to the particular methodology, protocols, material, reagents, and substances, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00418] All publications cited throughout the text of this specification (including all patents, patent application, scientific publications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

[00419] The content of all documents and patent documents cited herein is incorporated by reference in their entirety.

EXAMPLES

[00420] An even better understanding of the present invention and of its advantages will be evident from the following examples, offered for illustrative purposes only. The examples are not intended to limit the scope of the present invention in any way.

General Information, Materials and Methods

Chemicals, Solvents and Antibodies

[00421] Chemicals and solvents were purchased from Merck (Merck group, Germany), TCI (Tokyo chemical industry CO., LTD., Japan), Iris Biotech (Iris Biotech GmbH, Germany), MCE (MedChemExpress, USA) and Carl Roth (Carl Roth GmbH + Co. KG, Germany) and used without further purification. Amino acids all had their naturally occurring configuration (i.e., the L configuration), unless indicated to the contrary. Dry solvents were purchased from Merck (Merck group, Germany). Trastuzumab was purchased from Roche (Hoffmann-La Roche AG, Switzerland). Enhertu was purchased from Daichi-Sankyo (Daiichi Sankyō K.K , Japan) and Brentuximab was produced by Evitria (evitria AG, Switzterland). PEG24 was purchased from BiochemPEG (Pure Chemistry Scientific Inc., United States).

Preparative HPLC

[00422] Preperative HPLC was performed on a BÜCHI Pure C-850 Flash-Prep system (BÜCHI Labortechnik AG, Switzerland) using a VP 250/10 Macherey-Nagel Nucleodur C18 HTec Spum column (Macherey-Nagel GmbH & Co. Kg, Germany) for smaller scales. The following gradients were used: Method C: (A = H2O + 0.1% TFA (trifluoroacetic acid), B = MeCN (acetonitrile) + 0.1% TFA, flow rate 6 ml/min, 30% B 0-5 min, 30-70% B 5-35 min, 99% B 35-45 min. For bigger scales, a VP 250/21 Macherey-Nagel Nucleodur C18 HTec Spum column (Macherey-Nagel GmbH & Co. Kg, Germany) was used with the following gradients: Method D: (A = H2O + 0.1% TFA (trifluoroacetic acid), B = MeCN (acetonitrile) + 0.1% TFA, flow rate 14 ml/min, 30% B 0-5 min, 30-70% B 5-35 min, 99% B 35-45 min.

LC/MS

[00423] Small molecules, linker-payloads, antibodies and ADCs were analyzed using a Waters H-class instrument equipped with a quaternary solvent manager, a Waters sample manager-FTN, a Waters PDA detector and a Waters column manager with an Acquity UPLC protein BEH C4 column (300 Å, 1.7 µm, 2.1 mm x 50 mm) for antibodies and ADCs. Here, samples were eluted at a column temperature of 80°C. The following gradient was used: A: 0.1% formic acid in H_2O; B: 0.1% formic acid in MeCN. 25% B 0-1 min, 0.4 mL/min, 25-95% B 1-3.5 min 0.2 mL/min, 95% B 3.5-4.5 min 0.2 mL/min, 95-25% B 4.5-5 min 0.4 mL/min, 25-95% B 5-5.5 min 0.4 mL/min, 95-25%B 5.5-7.5 min 0.4 mL/min. Mass analysis was conducted with a Waters XEVO G2-XS QTof analyzer. Proteins were ionized in positive ion mode applying a cone voltage of 40 kV. Raw data was analyzed with MaxEnt 1. Small molecules and linker-payloads were analyzed with an Acquity UPLC-BEH C18 column (300 Å, 1.7 µm,2.1 mm x 50 mm). Here, samples were eluted at a column temperature of 45°C with a flow rate of 0.4 mL/min. The following gradient was used: A: 0.1% formic acid in H_2O; B: 0.1% formic acid in MeCN. 2% B 0-1 min, 2-98% B 1-5 min, 98%B 5-5.5 min, 98-2% B 5.5-6 min, 2% B 6-7min.

Preparative Size-Exclusion-Chromatography (SEC)

[00424] Protein purification by size-exclusion chromatography was conducted with an ÄKTA Pure FPLC system (GE Healthcare, United States) equipped with a F9-C-fraction collector.

ADC Concentration Determination

[00425] The ADC concentrations were determined in a 96-well plate with a Pierce™ Rapid Gold BCA Protein Assay Kit (Thermo Fisher Scientific, USA) and a Bradford reagent

B6916 (Merck, Germany) with pre-diluted protein assay standards of bovine gamma globulin (Thermo Fisher Scientific, USA). Results of both Assays were arithmetically averaged.

Sample Preparation of ADCs and Antibodies for MS

[00426] 0.5 µl PNGase-F solution (Pomega, Germany, Recombinant, cloned from Elizabethkingia miricola 10 u/µl) and 5 µL of a 100 mM solution of DTT in water were added to 50 µl of 0.2 mg/mL antibody or ADC in PBS and the solution was incubated at 37 °C for at least 2 hours. Samples were subjected to LC/MS, injecting 2 µl for each sample.

Analytical Size-Exclusion Chromatography (SEC)

[00427] Analytical size-exclusion chromatography (A-SEC) of the ADCs was conducted on a Vanquish Flex UHPLC System with a DAD detector, Split Sampler FT (4°C), Column Compartment H (25°C) and binary pump F (Thermo Fisher Scientific, USA) using a MAbPac SEC-1 300 Å, 4 x 300 mm column (Thermo Fisher Scientific, USA) with a flow rate of 0.15 mL/min. Separation of different ADC/mAb populations have been achieved during a 30 minute isocratic gradient using a phosphate buffer at pH 7 (20 mM Na_2HPO_4/NaH_2PO_4, 300 mM NaCl, 5% v/v isopropyl alcohol as a mobile phase. 8 µg ADC/mAb where loaded onto the column for A-SEC analysis. UV chromatograms were recorded at 220 and 280 nm.

Analytical Hydrophobic Interaction Chromatography (HIC)

[00428] The measurements were conducted on a Vanquish Flex UHPLC System (2.9) with a MabPac HIC Butyl 4.6 x 100 mm column (Thermo Fischer Scientific, USA). Separation of different ADCs/antibodies have been achieved with the following gradient: A: 1 M (NH4)2SO4, 500 mM NaCl, 100 mM NaH2PO4 pH 7.4 B: 20 mM NaH_2PO_4, 20% (v/v) Isopropyl alcohol, pH 7.4. 0% B: 0-1 min, 0-95% B: 1-15 min, 95% B: 15-20 min, 95-0% B: 20-23 min, 0% B: 23-25 min, with a flow of 700 uL/min. 15 µg sample were loaded onto the column for each analysis. UV chromatograms were recorded at 220 and 280 nm.

General Method for the Conjugation of P5-based Exatecan Linker-Payload Constructs to Antibodies to Achieve DAR8

[00429] 50 µl of the antibody solution of 10.0 mg/ml in conjugation buffer (freshly prepared 100 mM NH_4HCO_3-buffer, pH 8.0) were mixed with 3.33 µl of a 10 mM TCEP solution in P5-conjugation buffer. Directly afterwards, 1.67 µl of a 40 mM solution of the P5-Exatecan construct dissolved in DMSO were added. The mixture was shaken at 350 rpm and 25°C for 16 hours. The reaction mixtures were purified by preparative size-exclusion chromatography with a 25 ml Superdex™ 200 Increase 10/300GL (Cytiva, Sweden) and a

flow of 0.8 ml/min eluting with sterile PBS (Merck, Germany). The antibody containing fractions were pooled and concentrated by spin-filtration (Amicon® Ultra- 2mL MWCO: 30 kDa, Merck, Germany).

In vitro Cytotoxicity

[00430] To investigate direct cytotoxicity of ADCs, respective cells were incubated for 4 days for brentuximab ADCs and 7 days for trastuzumab ADCs with increasing concentrations of ADCs (0-3 µg/ml) to generate a dose-response curve. Killing was analysed using resazurin cell viability dye at a final concentration of 55 µM (Sigma-Aldrich) by dividing the fluorescence from control cells in medium by the flourescence of ADC-treated cells. Fluorescence emission at 590 nM was measured on a Microplate reader Infinite M1000 Pro (Tecan).

In vitro Bystander Capacity

[00431] To analyze bystander activity of ADCs on target-negative cells, 20.000 target-positive cells (SKBR-3 for trastuzumab ADCs) were incubated with increasing concentrations of ADCs (0-3 µg/ml). After 5 days, half of the cell culture supernatant volumes was transferred to 5.000 target-negative cells (MDA-MB-468 for trastuzumab ADCs) and incubated for another 5 days. Killing was analyzed by a resazurin-based viability measurement as described above.

DNA-Damage

[00432] The upregulation of DNA damage markers as response to ADCs or small molecules (Exatecan and Camptothecin) were analyzed by a flow cytometry-based readout. For this, 50.000 Her2-positive SKBR-3 cells were incubated for 24 hours to 72 hours with 5 µg/ml of ADCs or 5 nM of the small molecules. After the end of the incubation time, cells were stained with LIVE/DEAD™ Fixable Aqua (Thermo Fisher Scientific) followed by fixation and permeabilization using the BD Cytofix/Cytoperm Kit (BD Biosciences) according to the manufacturer's instructions. Intracellular staining of DNA damage markers was performed using anti-cleaved PARP (Asp214) PE, anti-H2AX (pSer139) AF647 and anti-active caspase 3 FITC (all BD Biosciences). Cells were acquired on a Cytoflex LX flow cytometer (Beckmann Coulter).

Serum-Stability of the Constructs

[00433] 40 µl of normal rat serum (, containing the corresponding ADCs in a concentration of 0.4 mg/ml in at least 80% rat serum (Thermo Fisher Scientific, USA) were

sterile filtered with UFC30GV0S centrifugal filter units (Merck, Germany) and incubated at 37°C for 1, 3 and 7 days. Samples for day 0 were directly processed further.

[00434] The supernatant of 50 µl anti human igG (Fc-Specific) agarose slurry (Sigma Aldrich, United States) was removed by centrifugation and the remaining resin washed three times with 300 µL PBS. The resin was incubated with 40 µl of the serum-ADC mix for 1h at room temperature. Afterwards, the supernatant was removed and the resin washed 3 times with 300 µL PBS. Following by incubation for 5 minutes with 60 µl 100 mM Glycin buffer pH 2.3 at room temperature. This solution was rebuffered to PBS by using 0.5 mL Zeba™ Spin Desalting Columns with 7K MWCO (Thermo Fisher Scientific, USA). The samples were processed further for MS-measurements, as described above. The 0 days sample has been analysed in the same way, directly after serum and ADC have been mixed.

Serum-stability followed by in vitro toxicity measurements

[00435] 50 µl of normal rat serum (Thermo Fisher Scientific, USA) or human serum, containing the corresponding ADCs (Trastuzumab-P5(PEG24)-VC-PAB-Exatecan or Enhertu in a concentration of 0.4 mg/ml in at least 80% rat serum (Thermo Fisher Scientific, USA) was sterile filtered with UFC30GV0S centrifugal filter units (Merck, Germany) and incubated at 37°C for 0, 1, 3 and 7 days. Samples for day 0 were directly processed further. After the respective incubation time, samples were deep-frozen in liquid Nitrogen and stored at -80°C until the cytotoxicity was measured. The serum samples containing the ADCs in the assumed starting concentration of 0.4 mg/mL were diluted without any further processing to achieve a concentration gradient with increasing concentrations of ADCs (0-3 µg/ml) to generate a dose-response curve. The in vitro cytotoxicity with SKBR3 and MDA-MB-468 cells was measured afterwards, exactly as described before.

In vivo Pharmacokinetics (PK) Study

[00436] Female Sprague-Dawley rats were treated intravenously via the tail-vein with 5 mg ADC per Kilogram bodyweight (bolus) with the respective ADC. Approximately 1 mL of blood was collected after 0.5h, 1h, 4h, 24h. 48h. 96h. 168h, 336h, and 504h. Blood samples were allowed to stand at room temperature to clot for 30 minutes. Blood serum was isolated from the samples after centrifugation and collection of the supernatant. Serum samples were analyzed via ELISA as follows.

Analysis of the in vivo Samples by ELISA

[00437] **Method 1:** To evaluate the pharmacokinetics (PK) of the ADCs in vivo, the total antibody concentration was measured at different time points in serum of ADC-treated SD rats. Total humanized anti-CD30 antibody was analyzed in rat serum over the range 2000 – 15,6 ng/ml. Nunc 96-well plate with (100 µl/well) were coated with Recombinant Human CD30/TNFRSF8 diluted in PBS (required concentration: 0,25 µg/ml) and sealed with PCR Foil. Plates were incubated in a fridge to maintain a temperature between 2-8°C overnight. The coated plates were washed 3x with 300 µl PBST. 200 µl/well of blocking solution (2 % Albumin in PBST) was added, the plate was sealed and an incubated at room temperature for 1 hour. The coated plates were washed 3x with 300 µl PBST. 100 µl/well of prepared standards (2000 – 15,6 ng/ml of the respective ADCs, QCs and test samples were added, the plates were sealed and incubated at room temperature for 1 hour. The plates were washed 3x with 300 µl PBST. 100 µl/well Anti-Human IgG (γ-chain specific)-Peroxidase antibody (dilution 1:60000 in PBS) was added and incubated for 1h at rt. The plates were washed 3x with 300 µl PBST. 50 µl/well TMB was added, the plates were sealed and incubated at room temperature for 15. 50 µl/well of 1 M Sulfuric Acid was added. Using a Tecan Plate Reader, the absorbance at a wavelength of 450 nm was measured.

[00438] **Method 2:** To evaluate the pharmacokinetics (PK) of the ADCs in vivo, the total antibody concentration was measured at different time points in serum of ADC-treated SD rats or SCID mice. Total antibody was analyzed in serum over the range 2000 – 15,6 ng/ml. Nunc 96-well plate with (100 µl/well) were coated with the target of the antibody in PBS (Her2 for Trastuzumab based ADCs; required concentration: 0,25 µg/ml) and sealed with PCR Foil. Plates were incubated in a fridge to maintain a temperature between 2-8°C overnight. The coated plates were washed 3x with 300 µl PBST. 200 µl/well of blocking solution (2 % Albumin in PBST) was added, the plate was sealed and an incubated at room temperature for 1 hour. The coated plates were washed 3x with 300 µl PBST. 100 µl/well of prepared standards (2000 – 15,6 ng/ml of the respective ADCs, QCs and test samples were added, the plates were sealed and incubated at room temperature for 1 hour. The plates were washed 3x with 300 µl PBST. 100 µl/well Anti-Human IgG (γ-chain specific)-Peroxidase antibody (dilution 1:60000 in PBS) was added and incubated for 1h at rt. The plates were washed 3x with 300 µl PBST. 50 µl/well TMB was added, the plates were sealed and incubated at room temperature for 15 min. 50 µl/well of 1 M Sulfuric Acid was added. Using a Tecan Plate Reader, the absorbance at a wavelength of 450 nm was measured.

To evaluate the stability of the ADCs in vivo, the intact ADC concentration was measured at different time points in serum of ADC-treated SD rats. Intact ADC was analyzed in rat serum

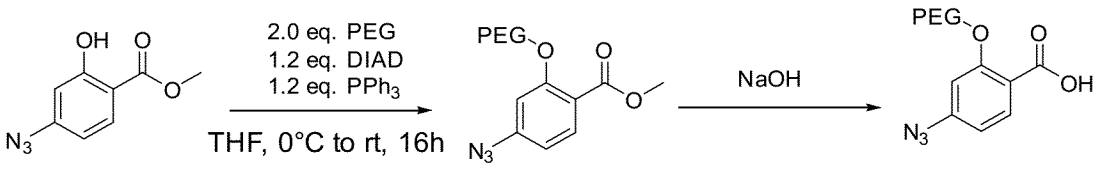
over the range 2000 – 15,6 ng/ml. Nunc 96-well plate with (100 µl/well) were coated with rabbit anti-Exatecan mAb diluted in PBS (required concentration: 1 µg/ml) and sealed with PCR Foil. Plates were incubated in a fridge to maintain a temperature between 2-8°C overnight. The coated plates were washed 3x with 300 µl PBST. 200 µl/well of blocking solution (2 % Albumin in PBST) was added, the plate was sealed and an incubated at room temperature for 1 hour. The coated plates were washed 3x with 300 µl PBST. 100 µl/well of prepared standards (2000 – 15,6 ng/ml of the respective ADCs, QCs and test samples were added, the plates were sealed and incubated at room temperature for 1 hour. The plates were washed 3x with 300 µl PBST. 100 µl/well Goat Anti-Human IgG (H+L) Preabsorbed (dilution 1:25000 in PBS was added and incubated for 1h at rt. The plates were washed 3x with 300 µl PBST. 100 µl/well TMB was added, the plates were sealed and incubated at room temperature for 10 min. 100 µl/well of 1 M Sulfuric Acid was added. Using a Tecan Plate Reader, the absorbance at a wavelength of 450 nm was measured.

Analysis of serum samples via MS

[00439] The supernatant of 50 µl anti human igG (Fc-Specific) agarose slurry (Sigma Aldrich, United States) was removed by centrifugation and the remaining resin washed three times with 300 µL PBS. The resin was incubated with 100 µl of serum, that was collected from the rhodents that were treated with the respective ADC after certain times of circulation, for 1h at room temperature. Afterwards, the supernatant was removed and the resin washed 3 times with 300 µL PBS. Following by incubation for 5 minutes with 60 µl 100 mM Glycin buffer pH 2.3 at room temperature. This solution was rebuffered to PBS by using 0.5 mL Zeba™ Spin Desalting Columns with 7K MWCO (Thermo Fisher Scientific, USA). The samples were processed further for MS-measurements, as described above.

Example 1: Linker-Payload Synthesis

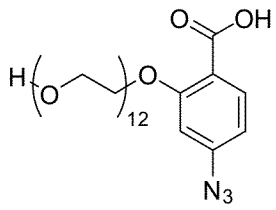
General method 1 for the synthesis of PEGylated phenyl azides



[00440] In a 25-mL round-botom flask, 50 mg Methyl-4-azido-2-hydroxybenzoate (0.259 mmol, 1.0 eq.), 0.518mmol of the desired PEG-alcohol (2.0 eq.) and 82 mg triphenylphosphine (0.311 mmol, 1.2 eq.) were dissolved in 5 mL of dry THF and the reaction

mixture was cooled to 0°C. 54 mg diisopropyl azodicarboxylate (0.311 mmol, 1.2 eq.) were added drop-wise and the solution was allowed warm to room temperature over night while stirring. All volatiles were removed in an N2-Stream and the solids were dissolved in 1 mL of 2N NaOH. The mixture was stirred for 30 min at room temperature, neutralized with 2N HCL and the crude product was purified by preparative HPLC.

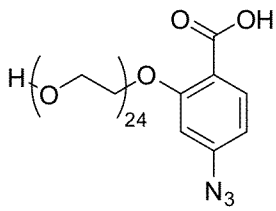
Methyl 4-azido-2-(dodecaethyleneglycol)benzoate



[00441] The title compound was synthesized in accordance to general Method 1 from 18 mg Methyl-4-azido-2-hydroxybenzoate (91 µmol, 1.00 eq.), 100 mg of dodecaethylene glycol (183 µmol, 2.0 eq), 29 mg triphenylphosphine (110 µmol, 1.2 eq.), 19 mg diisopropyl azodicarboxylate (110 µmol, 1.2 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (6.2 mg, 8.8 µmol, 10%). HR for $C_{31}H_{54}N_3O_{15}^+$ $[M+H]^+$ calcd.: 708.3550, found 708.74.

Figure 1 shows an analytical HPLC chromatogram of the compound methyl 4-azido-2-(dodecaethyleneglycol)benzoate. The horizontal axis depicts the retention time in minutes.

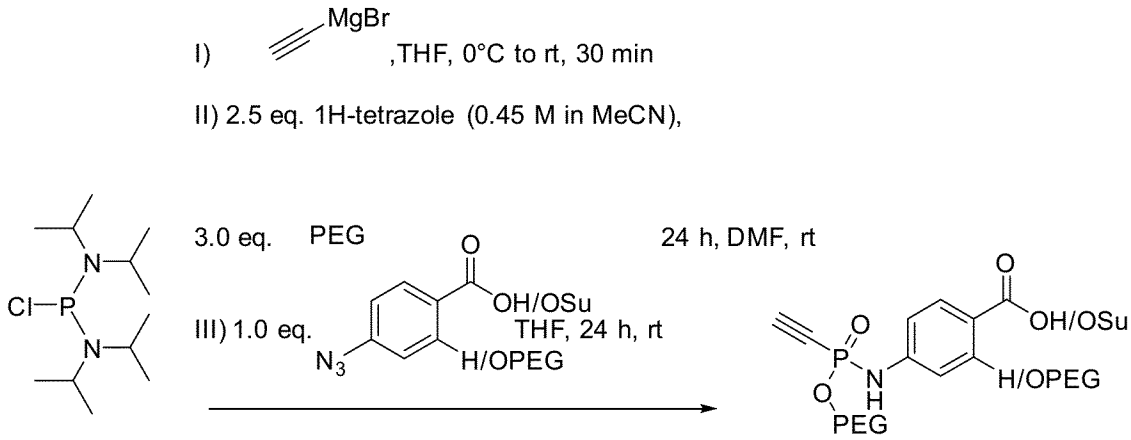
Methyl 4-azido-2-(tetracosaethyleneglycol)benzoate



The title compound was synthesized in accordance to general Method 1 from 36 mg Methyl-4-azido-2-hydroxybenzoate (186 µmol, 1.00 eq.), 400 mg of PEG24 (372 µmol, 2.0 eq), 59 mg triphenylphosphine (223 µmol, 1.2 eq.), 39 mg diisopropyl azodicarboxylate (223 µmol, 1.2 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (58 mg, 46.9 µmol, 10%). MS for $C_{55}H_{102}N_3O_{27}^+$ $[M+H]^+$ calcd.: 1236.6696, found 1237.05.

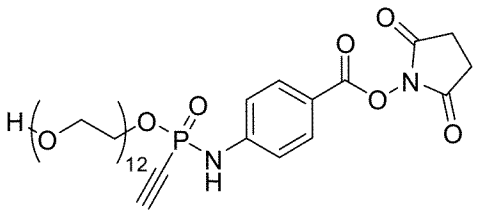
Figure 2 shows an analytical HPLC chromatogram of the compound methyl 4-azido-2-(tetracosaethyleneglycol)benzoate. The horizontal axis depicts the retention time in minutes.

General Method 2 for the Synthesis of PEGylated P5 Building Blocks via the Staudinger Phosphonite Reaction



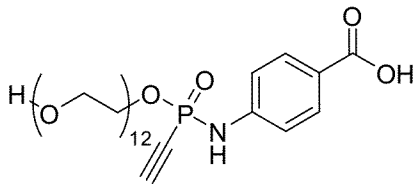
[00442] A 25-ml Schlenk flask was charged with 267 mg bis(diisopropylamino)chlorophosphine (1.00 mmol, 1.00 eq.) under an argon atmosphere, cooled to 0 °C and 2.20 mL ethynylmagnesium bromide solution (0.5 M in THF, 1.10 mmol, 1.10 eq.) was added drop wise. The yellowish solution was allowed to warm to room temperature and stirred for further 30 minutes. 3.00 mmol (3.0 eq.) of the desired PEG-alcohol, dissolved in 5.56 mL 1H tetrazole solution (0.45 M in MeCN, 2.50 mmol, 2.50 eq.) were added and the white suspension was stirred overnight at room temperature. The formation of the desired phosphonite was monitored by ^{31}P-NMR. 1.0 mmol (1.0 eq.) of the desired azide dissolved in 2 mL of DMF, THF or MeCN was added and the suspension further stirred for 24h at room temperature. The crude reaction mixture was purified using preparative HPLC.

P5(PEG12)-OSu



[00443] The title compound was synthesized in accordance to general Method 2 from 19.5 mg bis(diisopropylamino)chlorophosphine (73 µmol, 1.00 eq.), 146 µL ethynylmagnesium bromide solution (0.5 M in THF, 73 µmol, 1.00 eq.), 100 mg of dodecaethylene glycol (183 µmol, 2.50 eq), 400 µL 1H-tetrazole solution (0.45 M in MeCN, 183 µmol) and 19 mg 4-azidobenzoic-acid-*N*-hydroxysuccinimide ester (73 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (42.5 mg, 50 µmol, 68%). ^{1}H NMR (300 MHz, Acetonitrile-d3) δ 8.06 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 4.40 – 4.14 (m, 2H), 3.79 – 3.69 (m, 2H), 3.66 – 3.47 (m, 40H), 3.21 (d, J = 13.1 Hz, 1H), 2.86 (s, 4H), 1.30 (m, 2H), 1.13 – 0.79 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.77, 169.46, 161.66, 161.47, 152.75, 146.09, 132.90, 132.24, 117.82, 113.97, 113.29, 89.25, 88.92, 77.27, 77.06, 76.85, 74.69, 72.57, 71.19, 70.62, 70.54, 70.51, 70.47, 70.44, 70.36, 70.27, 70.20, 69.74, 69.70, 68.14, 65.77, 65.73, 61.63, 61.60, 40.72, 30.34, 25.68. ^{31}P NMR (122 MHz, Acetonitrile-d_3) δ -10.87. HRMS $C_{37}H_{60}N_2O_{19}P^+$ calc.: 851.3573 $[M+H]^+$, 851.3571.

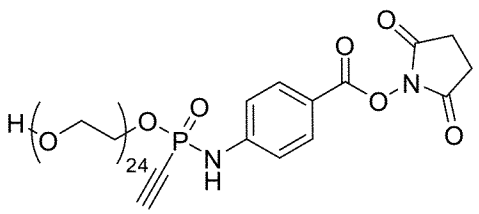
P5(PEG12)-COOH



[00444] The title compound was synthesized in accordance to general Method 2 from 40 mg bis(diisopropylamino)chlorophosphine (150 µmol, 1.00 eq.), 360 µL ethynylmagnesium bromide solution (0.5 M in THF, 180 µmol, 1.2 eq.), 245 mg of PEG12 (450 µmol, 3.0 eq), 0.83 mL 1H-tetrazole solution (0.45 M in MeCN, 450 µmol, 2.5 eq.) and 39 mg 4-azidobenzoic-acid (150 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (25 mg, 34 µmol, 23%). HR-MS for $C_{33}H_{57}NO_{16}P^+$ $[M+H]^+$ calcd.: 754.3410, found 754.3398

Figure 3 shows an analytical HPLC chromatogram of the compound P5(PEG12)-COOH.

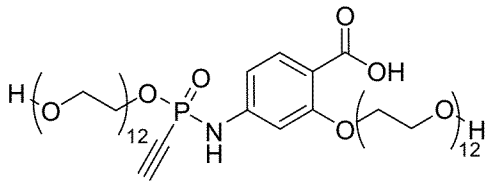
P5(PEG24)-OSu



[00445] The title compound was synthesized in accordance to general Method 2 from 41 mg bis(diisopropylamino)chlorophosphine (159 µmol, 1.00 eq.), 370 µL ethynylmagnesium bromide solution (0.5 M in THF, 185 µmol, 1.2 eq.), 450 mg of PEG24 (388 µmol, 2.50 eq), 1.02 mL 1H-tetrazole solution (0.45 M in MeCN, 466 µmol, 3.0 eq.) and 40 mg 4-azidobenzoic-acid-*N*-hydroxysuccinimide ester (155 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (79 mg, 57 µmol, 37%). MS for $C_{61}H_{109}N_2O_{30}P^{2+}$ $[M+2H]^{2+}$ calcd.: 690.3396, found 690.81.

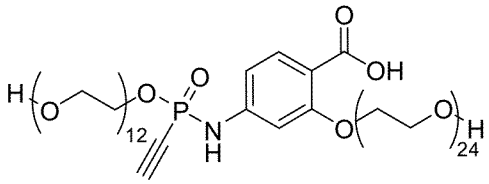
Figure 4 shows an analytical HPLC chromatogram of the compound P5(PEG24)-OSu. The horizontal axis depicts the retention time in minutes.

P5(PEG12, PEG12)-COOH



[00446] The title compound was synthesized in accordance to general Method 2 from 6.8 mg bis(diisopropylamino)chlorophosphine (25 µmol, 1.00 eq.), 61 µL ethynylmagnesium bromide solution (0.5 M in THF, 31 µmol, 1.2 eq.), 42 mg of Dodecaethyleneglycol (76 µmol, 3.0 eq), 164 µL 1H-tetrazole solution (0.45 M in MeCN, 64 µmol, 2.5 eq.) and 18.4 mg Methyl-4-azido-2-(dodecaethyleneglycol) benzoate (25 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (4.8 mg, 3.4 µmol, 13%). MS for $C_{57}H_{106}NO_{29}P^{2+}$ $[M+2H]^{2+}$ calcd.: 649.8289, found 650.22.

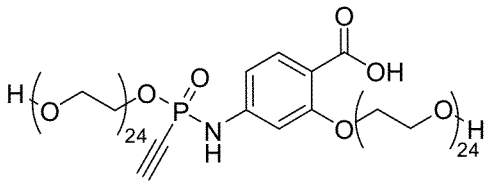
P5(PEG12,PEG24)-COOH



[00447] The title compound was synthesized in accordance to general Method 2 from 5.4 mg bis(diisopropylamino)chlorophosphine (20 µmol, 1.00 eq.), 50 µL ethynylmagnesium bromide solution (0.5 M in THF, 24 µmol, 1.2 eq.), 33 mg of Dodecaethyleneglycol (61 µmol, 3.0 eq), 115 µL 1H-tetrazole solution (0.45 M in MeCN, 51 µmol, 3.0 eq.) and 25 mg Methyl-4-azido-2-(tetracosaethyleneglycol) benzoate (20 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (6.6 mg, 3.6 µmol, 18%). MS for $C_{81}H_{154}NO_{41}P^{2+}$ $[M+2H]^{2+}$ calcd.: 913.9862, found 914.45.

Figure 5 shows an analytical HPLC chromatogram of the compound P5(PEG12,PEG24)-COOH. The horizontal axis depicts the retention time in minutes.

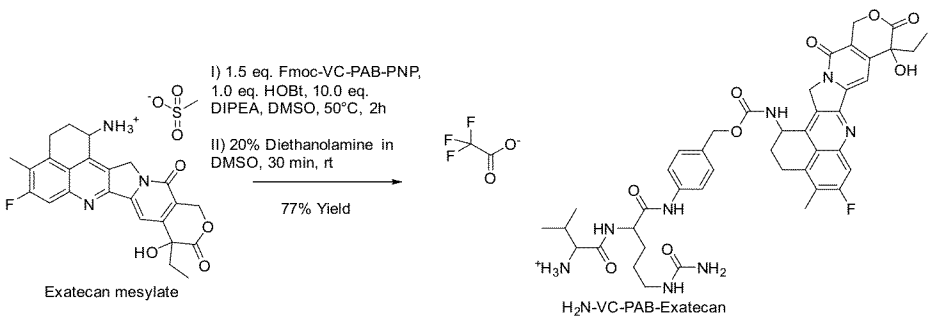
P5(PEG24,PEG24)-COOH



[00448] The title compound was synthesized in accordance to general Method 2 from 5.4 mg bis(diisopropylamino)chlorophosphine (20 µmol, 1.00 eq.), 50 µL ethynylmagnesium bromide solution (0.5 M in THF, 24 µmol, 1.2 eq.), 65 mg of PEG24 (61 µmol, 3.0 eq), 115 µL 1H-tetrazole solution (0.45 M in MeCN, 51 µmol, 3.0 eq.) and 25 mg Methyl-4-azido-2-(tetracosaethyleneglycol) benzoate (20 µmol, 1.00 eq.). The product was obtained as colourless oil after preparative HPLC (Method D) and lyophilization. (18.4 mg, 7.5 µmol, 37%). MS for $C_{105}H_{202}NO_{53}P^{2+}$ $[M+2H]^{2+}$ calcd.: 1178.6451, found 1178.69.

Figure 6 shows an analytical HPLC chromatogram of the compound P5(PEG24,PEG24)-COOH. The horizontal axis depicts the retention time in minutes.

NH_2-VC-PAB-Exatecan TFA salt

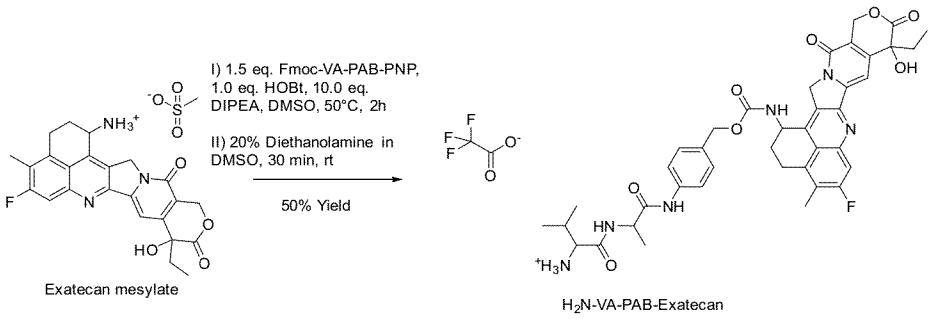


[00449] A screw-cap-vial was charged with 34.3 mg of Exatecan Mesylate (0.0645 mmol, 1.0 eq.) and suspended in 645 µL of dry DMSO. 241 µL of a solution of a 0.4 mol/L solution of Fmoc-VC-PAB-PNP in dry DMSO (0.0967 mmol, 1.5 eq.), 64,5 µL of a 1 mol/L solution of HOBt hydrate in dry DMSO (0.0645 mmol, 1.0 eq.) and 113 µL of DIPEA were added (0.645 mmol, 10.0 eq.). The yellow solution was stirred for 2 h at 50°C. Afterwards, 425 µL of a solution of 50% Diethanolamine in dry DMSO (w/w) was added and the reaction mixture was allowed to stir at room temperature for another 30 minutes. 1.5 ml MeCN and 2.5 mL H_2O added and the yellow solution was directly purified by preparative HPLC, using Method D. After lyophilization, 47.3 mg (76.7%, 0.0495mmol) of a yellowish solid were obtained as TFA-salt.

HR-MS for $C_{43}H_{50}FN_8O_9^+$ $[M+H]^+$ calcd.: 841.3680, found 841.3696

Figure 7 shows an analytical HPLC chromatogram of the compound NH_2-VC-PAB-Exatecan TFA salt.

NH_2-VA-PAB-Exatecan TFA salt



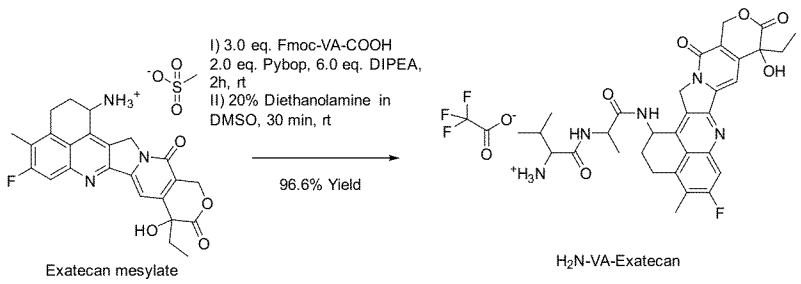
[00450] A screw-cap-vial was charged with 1.23 mg of Exatecan Mesylate (0.00232 mmol, 1.0 eq.) and suspended in 23 µL of dry DMSO. 8.7 µL of a solution of a 0.4 mol/L solution of Fmoc-VA-PAB-PNP in dry DMSO (0.00348 mmol, 1.5 eq.), 2.3 µL of a 1 mol/L solution of HOBt hydrate in dry DMSO (0.00232 mmol, 1.0 eq.) and 4 µL of DIPEA were

added (0.0232 mmol, 10.0 eq.). The yellow solution was stirred over night at room temperature. Afterwards, 15 µL of a solution of 50% Diethanolamine in dry DMSO (w/w) was added and the reaction mixture was allowed to stir at room temperature for another 30 minutes. 1.5 ml MeCN and 2.5 mL H_2O added and the yellow solution was directly purified by preparative HPLC, using Method C. After lyophilization, 1.01 mg (50.0 %, 0.00116 mmol) of a yellowish solid were obtained as TFA-salt.

HR-MS for $C_{40}H_{44}FN_6O_8^+$ $[M+H]^+$ calcd.: 755.3200, found 755.3201.

Figure 8 shows an analytical HPLC chromatogram of the compound NH_2-VA-PAB-Exatecan TFA salt.

NH_2-VA-Exatecan



[00451] A screw-cap-vial was charged with 5.12 mg of Exatecan Mesylate (0.00964 mmol, 1.0 eq.) and suspended in 96 µL of dry DMSO. 96 µL of a solution of a 300 mM of Fmoc-VA-COOH (0.02892, 3.0 eq.) in dry DMSO, 96 µL of a solution of a 200 mM Pybop (0.01928 mmol, 2.0 eq.) in dry DMSO and 33.4 µL DIPEA (0.0289 mmol, 6.0 eq.) were added and the solution was stirred for 2h at room temperature. 1.5 ml MeCN and 2.5 mL H_2O added and the yellow solution was directly purified by preparative HPLC, using Method C. Two Diastereoisomers were separated (Isomer A, firstly eluting and Isomer B, secondly eluting) After lyophilization, 5.04 mg of Isomer A (72.6 %, 0.0070 mmol), a yellowish solid and 1.68 mg of Isomer B (24.0 %, 0.00232 mmol), a yellowish solid were obtained as TFA-salts.

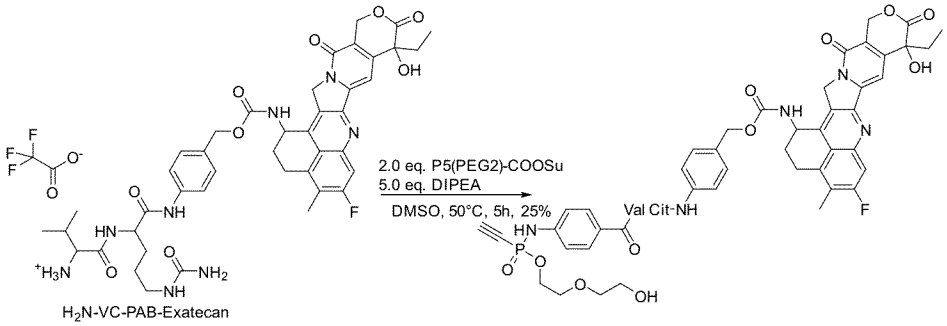
HR-MS for Isomer A $C_{32}H_{37}FN_5O_6^+$ $[M+H]^+$ calcd.: 606.2723, found 606.2744.

HR-MS for Isomer B $C_{32}H_{37}FN_5O_6^+$ $[M+H]^+$ calcd.: 606.2723, found 606.2744).

Figure 9 shows an analytical HPLC chromatogram of isomer A of the compound NH_2-VA-Exatecan.

Figure 10 shows an analytical HPLC chromatogram of isomer B of the compound NH_2-VA-Exatecan.

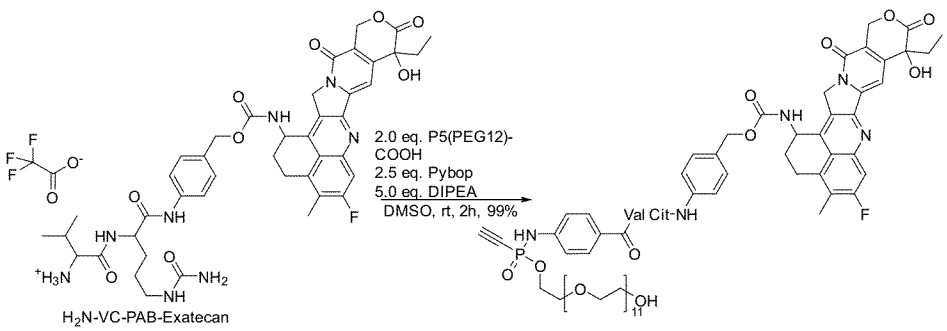
P5(PEG2)-VC-PAB-Exatecan



[00452] A screw-cap-vial was charged with 23,4 µL of a 200 mM solution of NH2-VC-PAB-Exatecan TFA salt in dry DMSO (0.00468 mmol, 1.0 eq.), 46,8 µL of a 200 mM solution of 2-(2-Hydroxyethoxy)ethyl-*N*-(4-benzoic-acid-*N*-hydroxysuccinimideester)-*P*-ethynyl phosphonamidate (P5(PEG2)-COOSu, 0.00936 mmol, 2.0 eq.) and 4.08 µL DIPEA (0.0234 mmol, 5.0 eq.). The solution was shaken for 5 hours at 50°C, cooled to room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method C. After lyophilization, 1.33 mg (25.0 %, 0.00117 mmol) of a yellowish solid were obtained.
HR-MS for $C_{56}H_{64}FN_9O_{14}P^+$ $[M+H]^+$ calcd.: 1136.4289, found 1136.4306.

Figure 11 shows an analytical HPLC chromatogram of the compound P5(PEG2)-VC-PAB-Exatecan.

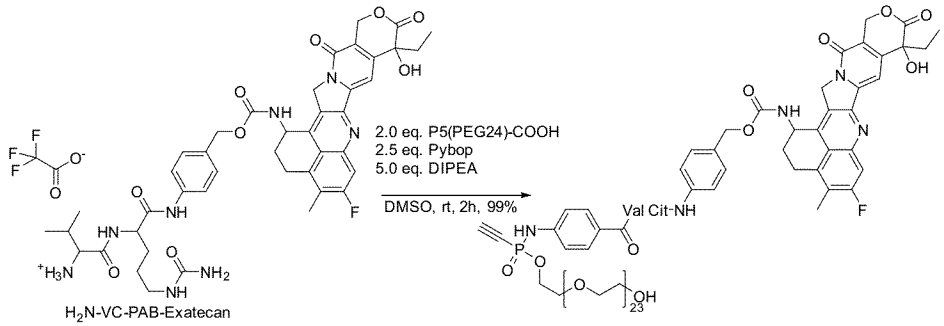
P5(PEG12)-VC-PAB-Exatecan



[00453] A screw-cap-vial was charged with 51 µL of a 200 mM solution of NH2-VC-PAB-Exatecan TFA salt in dry DMSO (0.0102 mmol, 1.0 eq.), 102 µL of a 200 mM solution of PEG12-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG12)-COOH, 0.0204 mmol, 2.0 eq.) in dry DMSO, 102 µL of a 250 mM solution of Pybop (0.0255 mmol, 2.5 eq.) in dry DMSO and 8.89 µL DIPEA (0.051 mmol, 5.0 eq.). The solution was shaken for 2 hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method D. After lyophilization, 15.91 mg (99.0 %, 0.0101 mmol) of a yellowish solid were obtained.
HR-MS for $C_{76}H_{105}FN_9O_{24}P^{2+}$ $[M+H]^{2+}$ calcd.: 788.8492, found 788.8485.

Figure 12 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VC-PAB-Exatecan.

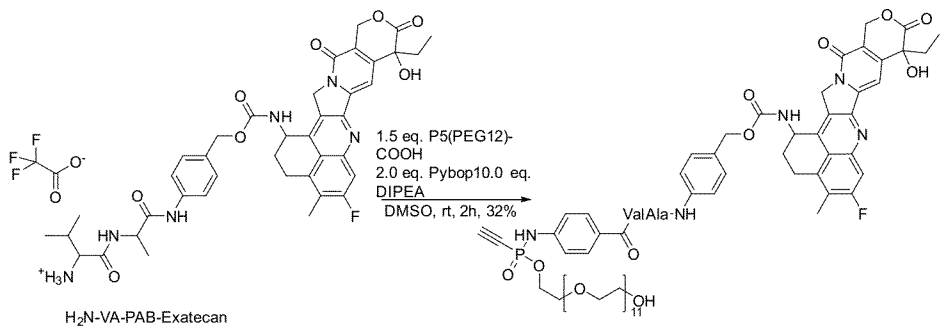
P5(PEG24)-VC-PAB-Exatecan



[00454] A screw-cap-vial was charged with 102 µL of a 200 mM solution of NH2-VC-PAB-Exatecan TFA salt in dry DMSO (0.0204 mmol, 1.0 eq.), 204 µL of a 200 mM solution of PEG24-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG24)-COOH, 0.0408 mmol, 2.0 eq.) in dry DMSO, 204 µL of a 250 mM solution of Pybop (0.051 mmol, 2.5 eq.) in dry DMSO and 17.78 µL DIPEA (0.102 mmol, 5.0 eq.). The solution was shaken for 2 hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method D. After lyophilization, 25,76 mg (60.0 %, 0.01224 mmol) of a yellowish solid were obtained.
HR-MS for $C_{100}H_{153}FN_9O_{36}P^{2+}$ $[M+H]^{2+}$ calcd.: 1053.5081, found 1053.50833.

Figure 13 shows an analytical HPLC chromatogram of the compound P5(PEG24)-VC-PAB-Exatecan.

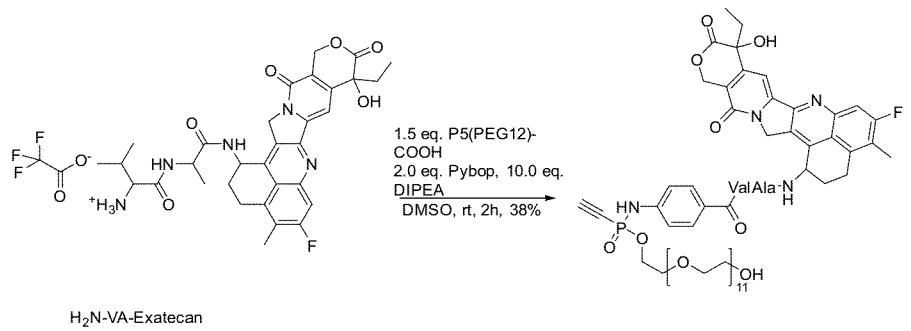
P5(PEG12)-VA-PAB-Exatecan



[00455] A screw-cap-vial was charged with 11.6 µL of a 100 mM solution of NH2-VA-PAB-Exatecan TFA salt in dry DMSO (0.00116 mmol, 1.0 eq.), 8.7 µL of a 200 mM solution of PEG12-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG12)-COOH, 0.00174 mmol, 1.5 eq.) in dry DMSO, 11.6 µL of a 200 mM solution of Pybop (0.00232 mmol, 2.0 eq.) in dry DMSO and 2.02 µL DIPEA (0.0116 mmol, 10.0 eq.). The solution was shaken for 2 hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method C. After lyophilization, 0.56 mg (32,2 %, 0.000375 mmol) of a yellowish solid were obtained.
HR-MS for $C_{73}H_{99}FN_7O_{23}P^{2+}$ $[M+H]^{2+}$ calcd.: 745.8252, found 745.8255.

Figure 14 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-PAB-Exatecan.

P5(PEG12)-VA-Exatecan from Isomer A

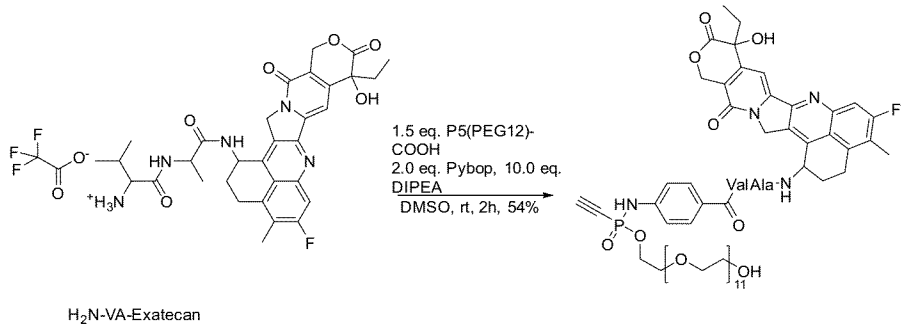


[00456] A screw-cap-vial was charged with 35 µL of a 200 mM solution of NH2-VA-Exatecan TFA salt (Isomer A) in dry DMSO (0.0070 mmol, 1.0 eq.), 52.5 µL of a 200 mM solution of PEG12-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG12)-COOH, 0.0104 mmol, 1.5 eq.) in dry DMSO, 70 µL of a 200 mM solution of Pybop (0.0140 mmol, 2.0 eq.) in dry DMSO and 12.2 µL DIPEA (0.07 mmol, 10.0 eq.). The solution was shaken for 2

hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method C. After lyophilization, 3.6 mg (38.4 %, 0.0026 mmol) of a yellowish solid were obtained.
HR-MS for $C_{65}H_{92}FN_6O_{21}P^{2+}$ $[M+H]^{2+}$ calcd.: 671.3013, found 671.3004.

Figure 15 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-Exatecan from Isomer A.

P5(PEG12)-VA-Exatecan from Isomer B

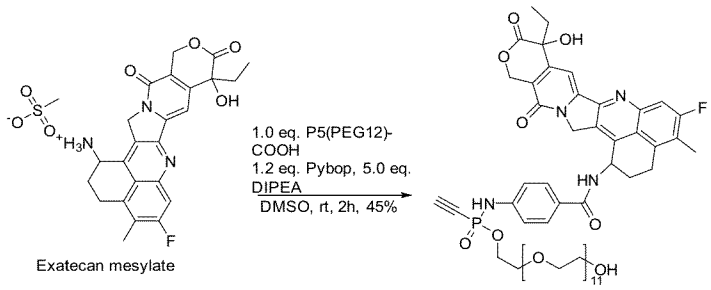


H_2N-VA-Exatecan

[00457] A screw-cap-vial was charged with 11.6 µL of a 200 mM solution of NH2-VA-Exatecan TFA salt (Isomer A) in dry DMSO (0.0023 mmol, 1.0 eq.), 17.4 µL of a 200 mM solution of PEG12-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG12)-COOH, 0.00345 mmol, 1.5 eq.) in dry DMSO, 23.3 µL of a 200 mM solution of Pybop (0.0046 mmol, 2.0 eq.) in dry DMSO and 4.04 µL DIPEA (0.023 mmol, 10.0 eq.). The solution was shaken for 2 hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method C. After lyophilization, 1.68 mg (54.0 %, 0.0012 mmol) of a yellowish solid were obtained.
HR-MS for $C_{65}H_{92}FN_6O_{21}P^{2+}$ $[M+H]^{2+}$ calcd.: 671.3013, found 671.3004.

Figure 16 shows an analytical HPLC chromatogram of the compound P5(PEG12)-VA-Exatecan from Isomer B.

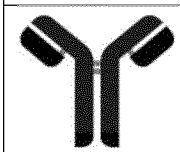
P5(PEG12)-Exatecan

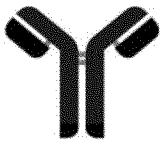
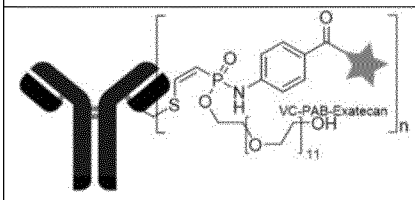


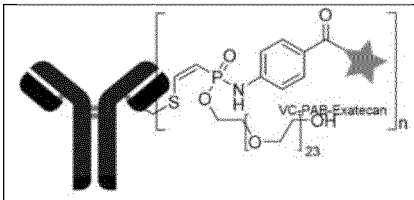
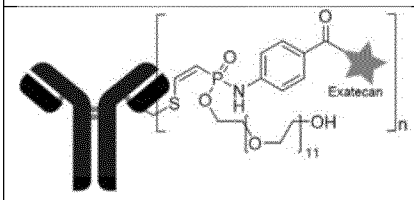
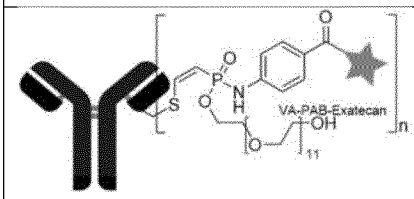
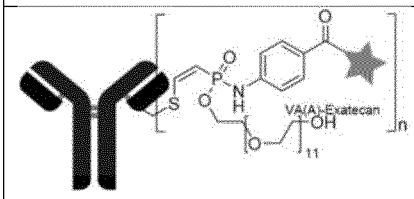
[00458] A screw-cap-vial was charged with 50 µL of a 100 mM suspension of Exatecan Mesylate in dry DMSO (0.005 mmol, 1.0 eq.), 20 µL of a 250 mM solution of PEG12-*N*-(4-benzoic-acid)-*P*-ethynyl phosphonamidate (P5(PEG12)-COOH, 0.005 mmol, 1.0 eq.) in dry DMSO, 20 µL of a 300mM solution of Pybop (0.006 mmol, 1.2 eq.) in dry DMSO and 4.33 µL DIPEA (0.025 mmol, 5.0 eq.). The solution was shaken for 2 hours at room temperature, 1.5 ml MeCN and 2.5 mL H_2O were added and the solution was directly purified by preparative HPLC, using Method C. After lyophilization, 2.63 mg (45.0 %, 0.0023 mmol) of a yellowish solid were obtained.
HR-MS for $C_{57}H_{77}FN_4O_{19}P^+$ $[M+H]^+$ calcd.: 1171.4899, found 1171.4852.

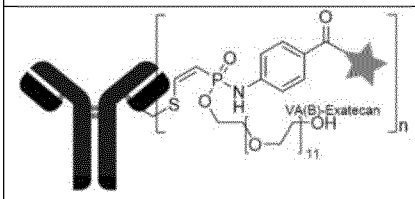
Figure 17 shows an analytical HPLC chromatogram of the compound P5(PEG12)-Exatecan.

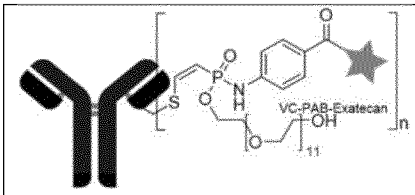
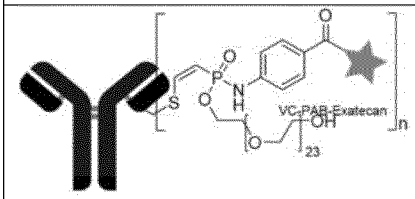
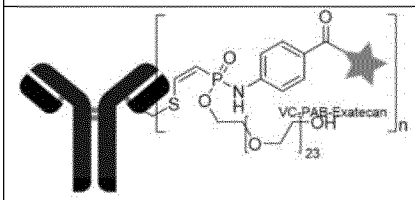
Example 2: Analytics of the Synthesized ADCs and Antibody Starting Materials

Antibody/ADC	**Analytical SEC**	**Analytical HIC**	**MS analysis of the fully conjugated DAR8 species, DAR**	**Yield ADC (protein concentration) after conjugation and purification**
 Trastuzumab	See **Figure 18**	See **Figure 19**	LC: calcd.: 23439 found: 23438 HC: calcd.: 49146 found: 49149	-/-

 Brentuximab	See **Figure 20**	See **Figure 21**	LC: calcd.: 23724 found: 23724 HC: calcd.: 48878 found: 48877	-/-
 Palivizumab	See **Figure 22**	See **Figure 23**	LC: calcd.: 23296 found: 23294 HC: calcd.: 49200 found: 49202	-/-
 Trastuzumab-P5(PEG12)-VC-PAB-Exatecan	See **Figure 24**	See **Figure 25**	DARav: 4,91 LC: calcd.: 25015 found: 25014 HC: calcd.: 53873 found: 53878	80%

 Trastuzumab-P5(PEG24)-VC-PAB-Exatecan	See **Figure 26**	See **Figure 27**	DARav: 7,91 LC: calcd.: 25544 found: 25543 HC: calcd.: 55461 found: 55464	99%
 Trastuzumab-P5(PEG12)-Exatecan	See **Figure 28**	See **Figure 29**	DARav: 7,19 LC: calcd.: 24609 found: 24609 HC: calcd.: 52658 found:52661	97%
 Trastuzumab-P5(PEG12)-VA-PAB-Exatecan	See **Figure 30**	See **Figure 31**	DARav: 4,39 LC: calcd.: 24929 found: 24928 HC: calcd.: 53615 found:53620	84%
 Trastuzumab-P5(PEG12)-VA- Exatecan (Isomer A)	See **Figure 32**	See **Figure 33**	DARav: 7,89 LC: calcd.: 24780 found: 24779 HC: calcd.: 53168	77%

			found:53173	
 Trastuzumab-P5(PEG12)-VA- Exatecan (Isomer B)	See **Figure 34**	See **Figure 35**	DARav: 7,71 LC: calcd.: 24780 found: 24779 HC: calcd.: 53168 found:53173	86%

 Brentuximab-P5(PEG12)-VC-PAB-Exatecan	See **Figure 36**	See **Figure 37**	DARav: 7,6 LC: calcd.: 25300 found: 25299 HC: calcd.: 53605 found: 53605	60%
 Brentuximab-P5(PEG24)-VC-PAB-Exatecan	See **Figure 38**	See **Figure 39**	DARav: 8,0 LC: calcd.: 25829 found: 25828 HC: calcd.: 55192 found: 55191	71%
 Palivizumab-P5(PEG24)-VC-PAB-Exatecan	See **Figure 40**	See **Figure 41**	DARav: 8,0 LC: calcd.: 25401 found: 25400 HC: calcd.: 55512 found: 55517	81%

DARav means the average drug-to-antibody ratio. LC: Mass of the light chain; HC: Mass of the heavy chain.

[00459] **Figure 18** shows an analytical SEC chromatogram of Trastuzumab. SEC means size-exclusion chromatography.

[00460] **Figure 19** shows an analytical HIC chromatogram of Trastuzumab. HIC means hydrophobic interaction chromatography.

[00461] **Figure 20** shows an analytical SEC chromatogram of Brentuximab.

[00462] **Figure 21** shows an analytical HIC chromatogram of Brentuximab.

[00463] **Figure 22** shows an analytical SEC chromatogram of Palivizumab.

[00464] **Figure 23** shows an analytical HIC chromatogram of Palivizumab.

[00465] **Figure 24** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VC-PAB-Exatecan.

[00466] **Figure 25** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VC-PAB-Exatecan.

[00467] **Figure 26** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan.

[00468] **Figure 27** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan.

[00469] **Figure 28** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[00470] **Figure 29** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[00471] **Figure 30** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[00472] **Figure 31** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-PAB-Exatecan.

[00473] **Figure 32** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer A).

[00474] **Figure 33** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer A).

[00475] **Figure 34** shows an analytical SEC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer B).

[00476] **Figure 35** shows an analytical HIC chromatogram of Trastuzumab-P5(PEG12)-VA-Exatecan (isomer B).

[00477] **Figure 36** shows an analytical SEC chromatogram of Brentuximab-P5(PEG12)-VC-PAB-Exatecan.

[00478] **Figure 37** shows an analytical HIC chromatogram of Brentuximab-P5(PEG12)-VC-PAB-Exatecan.

[00479] **Figure 38** shows an analytical SEC chromatogram of Brentuximab-P5(PEG24)-VC-PAB-Exatecan.

[00480] **Figure 39** shows an analytical HIC chromatogram of Brentuximab-P5(PEG24)-VC-PAB-Exatecan.

[00481] **Figure 40** shows an analytical SEC chromatogram of Palivizumab-P5(PEG24)-VC-PAB-Exatecan.

[00482] **Figure 41** shows an analytical HIC chromatogram of Palivizumab-P5(PEG24)-VC-PAB-Exatecan.

[00483] The SEC chromatograms (SEC = size-exclusion chromatography) show that the conjugates show low to substantially no aggregation in aqueous solution. Camptothecin based ADC conjugates have been shown to exhibit a strong tendency to aggregate already during the conjugation process to the antibody. This problem is in particular present when the camptothecin moiety is conjugated via a VC-PAB-Linker and a maleimide unit for antibody conjugation (Burke et al., *"Design, Synthesis, and Biological Evaluation of Antibody-Drug Conjugates Comprised of Potent Camptothecin Analogs"*, Bioconjugate Chem. 2009, 20, 1242-1250, https://doi.org/10.1021/bc9001097). In that case, up to 80% aggregated ADC has been reported.

[00484] In contrast, the SEC data described herein is showing highly monomeric camptothecin based-ADCs, including those carrying a VC-PAB-linker, with less than 5% aggregates after purification in all tested variants. In combination with the high conjugation yields described herein, typically in the range of 80-90% based on antibody concentration determination before and after the conjugation process, this clearly shows that only minor percentages of aggregates are being formed when the P5-conjugation technology described herein is used.

[00485] The HIC chromatograms (HIC = hydrophobic interaction chromatography) show that the conjugates exhibit a good hydrophilicity combined with excellent homogeneity of only one major DAR8 ADC species formed during the conjugation process.

Example 3: Study to investigate how to synthesize lower DAR ADCs

[00486] Lower DAR ADCs (DAR means "Drug to Antibody Ratio") can be synthesized from P5(PEG24)-VC by reducing the equivalents of TCEP in the antibody conjugation process. A dependency of the TCEP equivalents on the DAR is shown in **Figure 42**.

[00487] **Figure 42** shows MS spectra of glycosylated, reduced Trastuzumab after the reaction with different equivalents of TCEP (top) and 15 eq. of linker-payload (P5(PEG24)-VC-PAB-Exatecan). Calculation of the DAR from those spectra in dependency on the TCEP amounts is shown in the bottom graph.

Example 4: *In vitro* Cytotoxicity Evaluation of the Constructs

[00488] The ADCs described herein have been evaluated for in vitro potency on antigen positive (targeted) and antigen negative (non-targeted) cell lines. The results are depicted in **Figure 43**.

- Selectivity for the targeted (antigen positive-) cell line has been observed for all the tested constructs.

[00489] **Figure 43** shows the In vitro cytoxicity of Trastuzumab (anti-Her2) ADCs linked to different Exatecan-based linker-payload constructs on antigen positive cell lines (HCC-78, top and SKBR3, bottom left) and an antigen negative cell line (MDA-MB-468, bottom right).

- The VC-PAB cleavage side provides a high in vitro activity of the constructs. A non-cleavable control (P5(PEG12)-Exatecan) and the non-cleavable VA-Isomer P5(PEG12)-VA-Exatecan (Isomer B) leads to a decreased activity on the targeted cell line.

- Enhertu has been used as reference for the ADCs described herein and can be regarded as an important standard for camptothecin-based ADCs, since it is approved and marketed for the treatment of unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2-based regimens in the metastatic setting.

- The novel constructs described herein exhibit similar IC50-values in cell killing (**Figure 43**), and the P5-VC-PAB-Exatecan based constructs show better absolute cell killing on HCC-7, when compared to Enhertu (**Figure 44**).

[00490] **Figure 44** shows the in vitro cytoxicity of a Trastuzumab (anti-Her2) ADC (Tras-P5(PEG24)-VC-PAB-Exatecan) and a non-binding isotype control with Palivizumab (Pali-P5(PEG24)-VC-PAB-Exatecan) on an antigen positive cell line (HCC-78).

- The effect of the Exatecan based ADCs is not only selective for the targeted cell line, as shown in **Figure 43**, but also specific for a targeting antibody within a targeted cell line, as shown in **Figure 44**.

[00491] **Figure 45** shows the in vitro cytoxicity of a Brentuximab (anti-CD30) ADC (Bren-P5(PEG12)-VC-PAB-Exatecan) on two antigen positive cell lines (L-540, left and SU-DHL-1, right).

[00492] **Figure 46** shows the in vitro cytoxicity of a Brentuximab (anti-CD30) ADC (Bren-P5(PEG24)-VC-PAB-Exatecan) on a panel of antigen positive cell lines (SR-786, SU-DHL-1, HH, HBLM-2, L-540, MOTN-1) and a non-targeted control cell line (HL-60).

- Bren-P5(PEG12)-VC-PAB-Exatecan (**Figure 45**) and Bren-P5(PEG24)-VC-PAB-Exatecan (**Figure 46**) show selective effects on a whole panel of different CD30-postive cell line. HL-60 has been used to demonstrated target selectivity, since no effect was observed on this cell line.

Example 5: *In vitro* Bystander Effect

- A bystander effect is advantageous for the in vivo activity of ADCs especially in the context of solid tumors, since it enables killing of tumor cells with heterogenous target expression levels.

- Therefore, the inventors designed an experiment where targeted cells (SKBR3, Her2+) were incubated with Trastuzumab-based Tras-P5(PEG24)-VC-PAB-Exatecan constructs and Enhertu and treated non-targeted cells (MDA-MB-468) with the supernatant of the SKBR3 culture. MDA-MB-468 cells can only be killed by an effective bystander killing in this setting, since the ADCs do not induce killing alone on this cell line.

- The bystander effect was equally high for Enhertu as for P5(PEG24)-VC-PAB-Exatecan.

[00493] **Figure 47** shows the Evaluation of the bystander effect of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan in direct comparison to Enhertu. In vitro cytoxicity of the ADCs on an antigen positive cell line (SKBR3, top left) and an antigen negative cell line (MDA-MB-468, top right). Transfer of the supernatant after incubation of SKBR-3 with the ADCs to MDA-MB-468 has been performed in order to evaluate bystander killing (MDA-MB-468, bottom).

Example 6: DNA Damage

- The mechanism of action of Camtothecin-based ADCs is DNA-single strand breaks after inhibition of nuclear Topoisomerase-I, followed by double strand breaks after S Phase replication.

- A read-out for DNA-damage is Histone H2A.X phosphorylation at Ser139, activated Caspase 3 and activated PARP.

- The inventors investigated all three markers via FACS after treatment of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan and Enhertu

[00494] **Figure 48** shows the relative quantification of Histone H2A.X phosphorylation (top left), activated Caspase 3 (top right) and activated PARP (bottom left) and cell viability (bottom right) after treatment of SKBR-3 cells with Trastuzumab-P5(PEG24)-VC-PAB-

Exatecan, Enhertu, onconjugated Exatecan or unconjugated Camptothecin after 1, 2 or 3 days versus untreated.

- This dataset clearly demonstrates that cell killing, described in the previous examples is mediated by the mode of action of the payloads, which are delivered via the ADC.

- No significant difference has been observed when cells were treated either with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan or Enhertu.

Example 7: Serum Stability

- Stable conjugation is key factor for a steady efficacy during ADC circulation and to reduce off-target toxicity
- To assess the stability of the constructs described herein in direct comparison to Enhertu, the inventors incubated P5(PEG24)-VC-PAB-Exatecan and Enhertu with rat serum for different time points at 37°C, followed by measuring the Drug-to antibody ratio by LC/MS

[00495] **Figure 49** shows the drug to antibody ratio of Enhertu and P5(PEG24)-VC-PAB-Exatecan after incubation in rat serum at 37°C for 0, 1, 3 and 7 days. Drug to antibody ratio has been measured by MS after pulldown of the ADC from serum.

- Enhertu significantly loses linker-payload in serum (63% payload loss), leading to a drastically reduced DAR of 3 already after 3 days

- In contrast, P5 is stable with a DAR of 7.6 after 7 days (5% payload loss)

Example 8: Serum Stability Followed by In vitro Toxicity Measurements

[00496] The results of these experiments are given in **Figures 50** and **51**.

[00497] **Figure 50** shows the cytotoxicity of the ADCs Trastuzumab-P5(PEG12)-VC-PAB-Exatecan (top), Trastuzumab-P5(PEG24)-VC-PAB-Exatecan (mid) and Enhertu (bottom) measured after 0,1,3 and 7 days of incubation with rat serum at 37°C on a Her2-negative cell line MDA-MB-468 (left) and a Her2-positive cell line SKBR3 (right).

[00498] **Figure 51** shows the cytotoxicity of the ADCs Trastuzumab-P5(PEG12)-VC-PAB-Exatecan (top), Trastuzumab-P5(PEG24)-VC-PAB-Exatecan (mid) and Enhertu

(bottom) measured after 0,1,3 and 7 days of incubation with human serum at 37°C on a Her2-negative cell line MDA-MB-468 (left) and a Her2-positive cell line SKBR3 (right).

[00499] **Figures 50** and **51** show a decreasing in vitro efficacy on the targeted cell line SKBR3 with increasing incubation times for Enhertu in rat serum as well as in human serum. This effect is far less pronounced to absent for both Trastuzumab P5-VC-PAB-Exatecan constructs in human and rat serum. Thus, in contrast to Enhertu, the ADC in accordance with an embodiment of the invention maintains its efficacy over the incubation time.

[00500] Moreover, Enhertu shows an increasing unspecific effect on the targeted negative cell line MDA-MB-468 with increasing incubation times in rat- as well as human serum. This increasing unspecificity is absent for both Trastuzumab P5-VC-PAB-Exatecan constructs in human and rat serum. Accordingly, in contrast to Enhertu, the ADC in accordance with an embodiment of the invention maintains its selectivity for the targeted cell line over the incubation time.

Example 9: In vivo Evaluation of the Constructs

- In vivo Pharmacokinetics (PK)-experiments have been performed with Brentuximab-P5(PEG12)-VC-PAB-Exatecan

- Female Sprague Dawley rats have been treated with 5 mg/kg of Brentuximab-P5(PEG12)-VC-PAB-Exatecan

- Blood sampling has been performed after different time points and the ADC amount was quantified in a total Brentuximab antibody ELISA-assay according to Method 1 as described above under General Information, Materials and Methods.

[00501] **Figure 52** shows the quantification of the amount of total antibody in blood circulation after treatment of female Spraque-Dawley rats with Brentuximab-P5(PEG12)-VC-PAB-Exatecan-DAR8 via ELISA.

- The experiment clearly shows a good PK behaviour with a reasonable clearance rate.

Example10: Melting curves evaluated by NanoDSF

[00502] The thermal stability of proteins was determined using nano differential scanning fluorimetry (nanoDSF) that measures temperature-dependent changes in the intrinsic fluorescence of tryptophane and tyrosine residues (Tycho NT.6, NanoTemper Technologies). For this, 1 µM of antibody or ADC in PBS was absorbed by a capillary that was subsequently placed into the reader. Afterwards, the intrinsic protein fluorescence was measured at 330 nM and 350 nM while incubating at increasing temperatures. Changes in fluorescence signal indicated transitions in the folding state of the proteins and the temperatures at which a transition occurred are named as inflection temperatures (Ti) or also melting temperatures (Tm) (Haffke, M. et al., Label-free Thermal Unfolding Assay of G Protein-Coupled Receptors for Compound Screening and Buffer Composition Optimization. 2016.).

[00503] **Figure 53** shows the melting curves of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan and Enhertu determined using nano differential scanning fluorimetry (nanoDSF). The melting curves of Enhertu and Trastuzumab-P5(PEG24)-VC-PAB-Exatecan are overlapping, showing that the biophysical protein stability of the two ADCs is similar with almost identical melting temperatures.

Example 11: Binding to extracellular Her2 evaluated by Flow Cytometry

[00504] To determine equilibrium binding constants (K_D), SKBR3, Her2+-cells were incubated with antibodies and ADCs in concentrations ranging from 0.0001 to 200 nM and stained with an Alexa-dye-labeled anti-human IgG H+L secondary antibody (Thermo Fisher Scientific) and analyzed by flow cytometry. Mean fluorescence intensity (MFI) ratios were normalized to the non-specific binding control. The assay was performed in duplicates and data points were analyzed by a non-linear regression using a one-site specific binding model to derive K_D values using Prism 9 software. The graph in **Figure 54** shows means of n = 2 ± SEM.

[00505] **Figure 54** shows a graph for determining the equilibrium binding constants (K_D) for the binding of Enhertu and Trastuzumab-P5(PEG24)-VC-PAB-Exatecan to extracellular Her2, and the obtained values for the equilibrium binding constants (K_D). The results show that the binding of Enhertu and Trastuzumab-P5(PEG24)-VC-PAB-Exatecan to extracellular Her2 is not significantly different, indicating that the biophysical ability of the two ADCs to bind the targeted Her2 receptor is similar with almost identical K_D.

Example 12: Aggregation stress test

[00506] Trastuzumab-P5(PEG24)-VC-PAB-Exatecan having a drug to antibody ratio of 8 (denoted herein as "DAR8") and Enhertu have been formulated in a buffer containing 20 mM Phosphate, 20 mM Trehalose and 0.009% Polysorbate 20 at 1 mg/ml. The samples have been sterile filtered using UFC30GV0S centrifugal filter units (Merck, Germany) and incubated at either 4°C or at 37°C in the dark. 50µl samples were drawn after 1,2,3 and 4 weeks and analyzed via Size-Exclusion-Chromatography as described above.

[00507] ADC aggregation, occurring during circulation in the patient, is known to cause off target toxicities. **Figure 55** shows the percentage of aggregates formed when incubating Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu at 37°C and 4°C in the dark after 0, 1, 2 and 4 weeks. The results clearly shows reduced aggregation for the Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 ADC under the incubation conditions compared to Enhertu, as an advantage of the technology described herein.

Example 13: ADCC of conjugated antibodies

[00508] For the Calcein release-based antibody-dependent cellular cytotoxicity (ADCC) assay, frozen primary healthy donor-derived natural killer (NK) cells, isolated from peripheral blood mononuclear cells (PBMCS) by negative selection, were purchased as effector cells (Lonza). Her2-positive target cells were stained with 16µM Calcein AM (Thermo Fisher Scientific). NK and target cells were then incubated at an effector-to-target ratio of 3:1 for 4 h at 37°C in presence of 50 nM Trastuzumab, Trastuzumab-P5(PEG24)-VC-PAB-Exatecan, Enhertu and a commercial human IgG1 isotype (BioLegend). Cells permeabilized with 2.5% Triton X (Sigma-Aldrich) served as positive control. Supernatants were transferred to a flat black non-binding 96-well plate (Greiner Bio-One) and fluorescence was measured at 485/535 nM via the Infinite M1000 Pro reader (Tecan).

[00509] The percent specific killing was calculated by dividing the Calcein released by antibody-mediated killing minus background Calcein release (NKs + targets) from Calcein released by Triton X-permeabilized cells (maximum killing) minus background Calcein release (targets only). We determined either maximum ADCC/specific killing at 15 µg/ml or generated a concentration-dependent killing curve that was analyzed by a non-linear regression using a one-site specific binding model.

[00510] **Figure 56** shows the percent specific killing measured in a calcein release-based antibody-dependent cellular cyctotoxicity (ADCC) assay with Her2-positive target cells SKBR-3, SKOV-3 and N87 when using unconjugated Trastuzumab, the ADC Trastuzumab-

P5(PEG24)-VC-PAB-Exatecan having a drug to antibody ratio of 8 (DAR8), Enhertu and an isotype control. The results show better ADCC-effect for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu. Effector functions are less influenced when using Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 having a P5-linker, compared to Enhertu.

Example 14: Internalization of conjugated antibodies

[00511] For pHrodo-based investigation of internalization, antibodies and ADCs were labeled with pHrodo™ Deep Red Antibody Labeling Kit (Thermo Fisher Scientific) according to manufacturer's instructions. Her2-positive solid tumor cells and negative cells were incubated with 5 µg/ml of pHrodo-labeled antibodies or ADCs for 1 h, 5 h and 24 h at 37°C. An increase in MFI indicates the presence of antibodies in late endosomal and lysosomal compartments. The MFI ratio was determined by deviding the MFI of pHrodo-incubated cells by the MFI of unstained cells.

[00512] **Figure 57** shows the results of pHrodo-based investigation of internalization using unconjugated Trastuzumab, Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu with Her2-positive SKOV-3 cells and Her2-negative MDA-MB-468 cells. Internalization into targeted Her2 cells is similar for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu, while undesired internalization into targeted negative cells is lower. This observation ccould reduce off-target toxicities, caused by ADC internalization into non-targeted cells.

Example 15: Additional in vitro efficacy data in direct comparison to Enhertu

[00513] In vitro cytotoxicity has been measured as described above under General Information, Materials and Methods.

[00514] **Figure 58** shows the results of in vitro cytotoxicity measurements carried out using Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu with Her2-positive cells SKBR-3 (Her2++), N87 (Her2++), HCC-1569 (Her2++), HCC-78 (Her2+), OE-19 (Her2+), SK-GT-2 (Her2+) and SKOV-3 (Her2++). Better in vitro efficacy for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu has been observed in vitro in particular in cell lines which are not highly overexpressing Her2 (denoted in **Figure 58** as "Her2+").

Example 16: In vitro bystander capacity evaluated by supernatant transfer

[00515] In vitro bystander capacity has been measured as described above under General Information, Materials and Methods. In addition to the above-mentioned datasets, other cell lines (Karpas 299 (Her2-) and DU-145 (Her2-)) have been used for testing the transferred material.

[00516] **Figure 59** shows the results for the in vitro bystander capacity measured after incubation with Her2-positive SKBR3 cells with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu, and supernatant transfer to Her2-negative cells Karpas-299 and DU-145. Better bystander effect for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu has been observed.

Example 17: In vitro bystander capacity evaluated by FACS

[00517] For the co-culture-based bystander assay, Her2-positive SKBR-3 cells and Her2-negative MDA-MB-468 cells were incubated at a 5:1 ratio with increasing concentrations of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan and Enhertu up to a maximum concentration of 3 µg/ml. After 5 days incubation time, co-cultures were stained by αHer2-FITC (BioLegend) and Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific). As a readout for the bystander effect, the percentage of dead Her2-positive and -negative cells was determined by flow cytometry.

[00518] **Figure 60** shows the results of measurements of the in vitro bystander capacity of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu in a co-culture of Her2-positive SKBR-3 cells and Her2-negative MDA-MB-468 cells. Slightly better bystander effect for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu has been observed, also when cells were co-cultured.

Example 18: Cytotoxicity on human primary cells

[00519] In vitro cytotoxicity against human umbilical vein endothelial cells, human bronchial endothelial cells, liver sinusoidal endothelial cells, Schwann cells, human renal proximal tubular epithelial cells, normal human dermal fibroblasts, human corneal epithelial cells and THLE-3 (hepatocytes) has been measured, as described above under General Information, Materials and Methods.

[00520] **Figure 61** shows the results of cytotoxicity measurements carried out on human umbilical vein endothelial cells, human bronchial endothelial cells, liver sinusoidal endothelial cells, Schwann cells, human renal proximal tubular epithelial cells, normal human

dermal fibroblasts, human corneal epithelial cells and THLE-3 (hepatocytes) using Tras-P5(PEG24)-VC-PAB-Exatecan DAR8, Enhertu and Palivizumab-P5(PEG24)-VC-PAB-Exatecan DAR8. Shown is the cyctotoxicity of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Palivizumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu. The model uses cultured cells of healthy human tissue and is therefore an in vitro measure for undesired toxicity. Throughout all tested tissue types, the dataset shows that the undesired effect of in this case off-target toxicity is less pronounced for the Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 compared to Enhertu. The dataset indicates a broader therapeutic window, caused by the technology described in the current patent, since the panel of **Figure 61** shows less undesired effect on the non-targeted cell-lines, whereas the panel of **Figure 58** shows a better desired effect on the targeted cell lines.

Example 19: In vivo PK evaluation of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 versus Enhertu in SCID mice

[00521] In vivo pharmacokinetics experiments (PK-experiments) have been performed with an Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 in female SCID mice that have been treated with 20 mg/kg of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 or Enhertu. Blood sampling has been performed after different time points and the ADC amount was quantified in a total antibody ELISA-assay according to Method 2 as described above under General Information, Materials and Methods. The Drug-to-Antibody Ratio (DAR) of the ADC (linker integrity) was analyzed by intact MS, as described above under General Information, Materials and Methods.

[00522] **Figure 62** shows the results of the in vivo pharmacokinetics experiments carried out with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 in female SCID mice that have been treated with 20 mg/kg of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 or Enhertu as reference. The dataset obtained from the ELISA assay shows similar clearance from the organism during circulation for Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 and Enhertu. Moreover, the DAR analysis confirms the stability of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 in vivo with a drastic improvement over the stability of Enhertu.

Example 20: In vivo efficacy for tumor treatment

[00523] All animal experiments were conducted in accordance with German animal welfare law and approved by local authorities. In brief, $1x10^7$ MDA-MB-468 cells were subcutaneously injected to CB17-Scid mice. Treatment was initiated when tumours reached a mean tumour volume of 0.188 cm^3 28 days after implantation. 5 animals per group were

treated once with either 1 mg/kg, 3 mg/kg or 10 mg/kg of H8-P5(PEG24)-VC-PAB-Exatecan DAR 8 or vehicle, as intravenous injection after randomisation into treatment and control groups. Tumour volumes, body weights and general health conditions were recorded throughout the whole study.

[00524] The humanized H8 antibody sequence originated from international patent application WO 2006/031653, which is directed to 5T4 antibodies. Antibodies were transiently expressed in Expi-CHO-S cells (Thermo Fisher) by co-transfecting cells with pcDNA3.4 expression plasmids (Thermo Fisher), coding for the heavy and light chain of the respective sequences in a 1:1 ratio, using the Expi-CHO transfection system (Thermo Fisher). Cells were harvested by centrifugation at 300 g for 5 minutes at 4°C. To clear micro particles from supernatant, the supernatants were centrifuged at 4000–5000 g for 30 min at 4 °C. For further clarification the supernatants were passed through a 0.22 µm filter. Antibodies were purified from cleared and filtered supernatants via Protein A chromatography and analyzed by HPLC-SEC, HPLC-HIC, LC-MS and SDS-PAGE. Conjugation of P5(PEG24)-VC-PAB-Exatecan to generate a DAR8 ADC was performed afterwards, as described above under General Information, Materials and Methods. The 5T4 H8-antibody was chosen to generate an exemplary in vivo dataset in a solid tumor model, different from Her2.

[00525] **Figure 63** shows the mean tumor volume of CB17-Scid mice determined in a solid tumor model after treatment with H8-P5(PEG24)-VC-PAB-Exatecan DAR8. **Figure 64** shows the body weight of CB17-Scid mice after treatment with H8-P5(PEG24)-VC-PAB-Exatecan DAR8. No loss in body weight occurred in the solid tumor model. Accordingly, the measured in vitro effects also translate into high in vivo efficacy for the ADCs based on P5(PEG24)-VC-PAB-Exatecan DAR 8. A Dose dependent efficacy has been demonstrated in vivo when effective doses between 1 and 3 mg/kg were administered in a challenging solid tumor model. 5/5 complete responses at 1 and 3 mg/kg single dose.

[00526] Similar efficacy results were obtained in another solid tumor model, namely an OVCAR-3 based in vivo xenograft model, with an ADC derived from an anti-NaPi2B-antibody, the sequence of which originated from US patent application US 2017/0266311A1. The preparation of the ADC and the experiment was performed as described above for the H8 antibody.

[00527] Moreover, a study with a N87 based in vivo xenograft model, with Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 is currently ongoing and expected to deliver the same/comparable outcome in terms of in vivo efficacy.

Example 21: In vivo PK evaluation of anti-target-P5(PEG24)-VC-PAB-Exatecan DAR8 in SD-rats

[00528] In vivo pharmacokinetics experiments (PK-experiments) have been performed in female SD rats that have been treated with 10 mg/kg of H8-P5(PEG24)-VC-PAB-Exatecan DAR8 or the unmodified H8 antibody. The antibody and the ADC were obtained as described above.

[00529] Blood sampling has been performed after different time points and the ADC amount was quantified in a total antibody ELISA-assay according to Method 2 as described above under General Information, Materials and Methods. ADC integrity has been confirmed via intact ADC ELISA. The Drug-to-Antibody Ratio (DAR) of the ADC (linker integrity) was analyzed by intact MS.

[00530] **Figure 65** shows the results of the in vivo pharmacokinetics experiments (PK-experiments) obtained in female SD rats that have been treated with 10 mg/kg of H8-P5(PEG24)-VC-PAB-Exatecan DAR8 or the unmodified H8 antibody. The good overlap between unmodified mab and ADC in total mAb ELISA shows that the conjugation of eight molecules of the linker-payload is not negatively influencing the clearance of the whole ADC from circulation. The DAR8 ADC clears with mAb-like kinetics. High overlap between total mab and intact ADC confirms good in vivo stability, which is underlined by the DAR that has been estimated via MS. The MS measurements confirm that DAR 8, i.e. full conjugation of the linker-payload to the antibody, is maintained after 3 weeks of circulation in vivo.

Example 22: Trastuzumab P5(PEG24)-VC-PAB-Exatecan DAR4 Synthesis and characterization

[00531] In order to achieve a statistic conjugation to the eight cysteine residues of P5(PEG24)-VC-PAB-Exatecan to the Trastuzumab IgG1, the above-mentioned conjugation protocol under General Information, Materials and Methods has been slightly adjusted, as follows: The concentration of the mAb has been reduced to 1 mg/ml, the equivalents of P5(PEG24)-VC-PAB-Exatecan for the conjugation reaction have been reduced to 10 and the TCEP equivalents have been reduced to 3. The ADC has been characterized by MS and an average Drug-to-Antibody ratio of 4 (DAR4) has been calculated, as described above.

Moreover, the product has been characterized by HIC, demonstrating a distribution of DAR0 to DAR8 species. The product has been also characterized by SEC, showing homogeneity.

[00532] **Figure 66** shows the HIC and SEC chromatograms of Trastuzumab P5(PEG24)-VC-PAB-Exatecan DAR4 having an average DAR of 4.

[00533] It is noted that the term "Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR4" or "Tras-P5(PEG24)-VC-PAB-Exatecan DAR4" refers to an ADC having an average drug to antibody ratio of 4 (DAR4), which is used in **Examples 22** and **23**. On the other hand, the term "Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8", "Tras-P5(PEG24)-VC-PAB-Exatecan DAR8", "Trastuzumab-P5(PEG24)-VC-PAB-Exatecan", "Tras-P5(PEG24)-VC-PAB-Exatecan" or the like, i.e. also without indication of the DAR, when used herein always refers to an ADC having a drug to antibody ratio of 8 (DAR8); see, e.g., also the Table under **Example 2** above for the structure and DAR of "Trastuzumab-P5(PEG24)-VC-PAB-Exatecan".

Example 23: Trastuzumab P5(PEG24)-VC-PAB-Exatecan DAR4 In vivo PK evaluation

[00534] In vivo pharmacokinetics experiments (PK-experiments) have been performed in female SD rats that have been treated with 10 mg/kg of Tras-P5(PEG24)-VC-PAB-Exatecan DAR4 having an average drug to antibody ratio of 4 (DAR4). Blood sampling has been performed after different time points, and the ADC amount was quantified in a total antibody ELISA-assay. ADC integrity has been confirmed via intact ADC ELISA.

[00535] **Figure 67** shows the results of the in vivo pharmacokinetics experiments (PK-experiments) obtained in female SD rats that have been treated with 10 mg/kg of Tras-P5(PEG24)-VC-PAB-Exatecan DAR4. The good PK profile, highly comparable to the unmodified H8 tested in **Example 21** above and depicted in **Figure 65**, upper panel on the left, shows that the statistic conjugation of four P5(PEG24)-VC-PAB-Exatecan molecules to the eight cysteine residues of the linker payload is not negatively influencing the clearance of the whole ADC from circulation. The ADC having an average DAR of 4 clears with mAb-like kinetics. High overlap between total mab and intact ADC confirms good in vivo stability.

Example 24: In vivo evaluation of Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 versus Enhertu

[00536] All animal experiments were conducted in accordance with German animal welfare law and approved by local authorities. In brief, $2x10^6$ NCI-N87 cells (a solid human

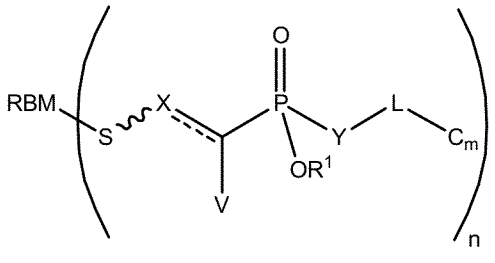
gastric cell cancer line) were subcutaneously injected to CB17-Scid mice. Treatment was initiated when tumours reached a mean tumour volume of 0.1-0.15cm^3. 10 animals per group were treated once with either 0.25, 0.5, 1 or 2 mg/kg, Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR 8 or Enhertu. 5 animals per group were treated with vehicle or with 2 mg/kg Palivizumab-P5(PEG24)-VC-PAB-Exatecan as Isotype control. All ADCs were administered as intravenous injection after randomisation of the animals into treatment and control groups. Tumour volumes, body weights and general health conditions were recorded throughout the whole study.

[00537] **Figure 68** shows results of an in vivo evaluation of trastuzumab-P5(PEG24)-VC-PAB-Exatecan having a drug to antibody ratio of 8 (DAR8). Reported are initial results after several days of observation of the tumor growth. Already after this initial period of 7 days, it is observed that the Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 construct leads to a greater tumor shrinkage compared to Enhertu in all treatment groups. Most pronounced is this effect in the 0.5 mg/kg, where Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8 leads to tumor regression, while Enhertu leads to progression. It is expected that this effect will be more pronounced, as the study proceeds further. The efficacy of all Trastuzumab based, targeted, ADCs is dose-dependent. It should be noted that a non-binding isotype control ADC Palivizumab-P5(PEG24)-VC-PAB-Exatecan at the highest dose did not lead to any effect, since the tumors behaved as in the vehicle control, showing the specific effect of drug delivery mediated by the antibody for the P5(PEG24)-VC-PAB-Exatecan linker system.

Claims

What is claimed is:

1. A conjugate having the formula (I):



(I),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

RBM is a receptor binding molecule;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

2. The conjugate of claim 1, wherein  is a double bond; V is absent; X is R_3-C; and R^3 is H or an optionally substituted aliphatic residue or an optionally substituted aromatic residue; preferably R^3 is H or (C_1-C_8)alkyl; more preferably R^3 is H.

3. The conjugate of claim 1 or 2, wherein the receptor binding molecule is selected from the group consisting of an antibody, an antibody fragment, and a proteinaceous binding molecule with antibody-like binding properties.

4. The conjugate of claim 3, wherein the receptor binding molecule is an antibody.

5. The conjugate of claim 4, wherein the antibody is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, and a single domain antibody, such as a camelid or shark single domain antibody.

6. The conjugate of any one of the preceding claims, wherein Y is NH.

7. The conjugate of claim 6, wherein the receptor binding molecule is an antibody.

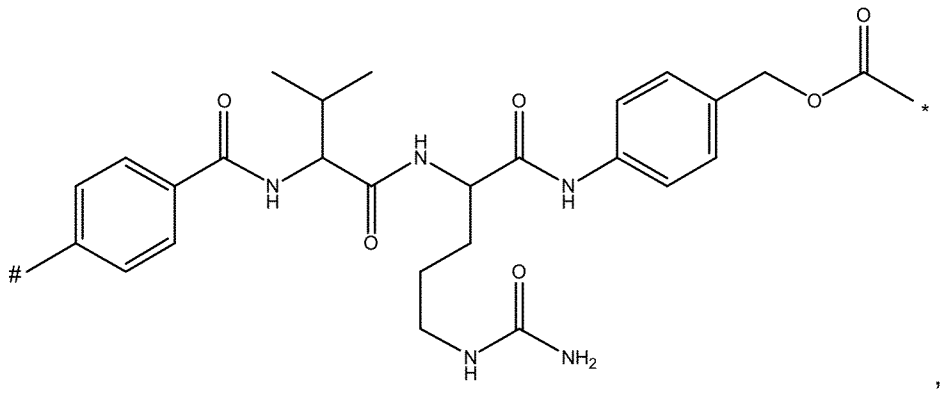
8. The conjugate of any one of the preceding claims, wherein the linker L is cleavable.

9. The conjugate of claim 8, wherein the linker L is cleavable by a protease, a glucuronidase, a sulfatase, a phosphatase, an esterase, or by disulfide reduction.

10. The conjugate of claim 9, wherein the linker L is cleavable by a protease, preferably by a cathepsin such as cathepsin B.

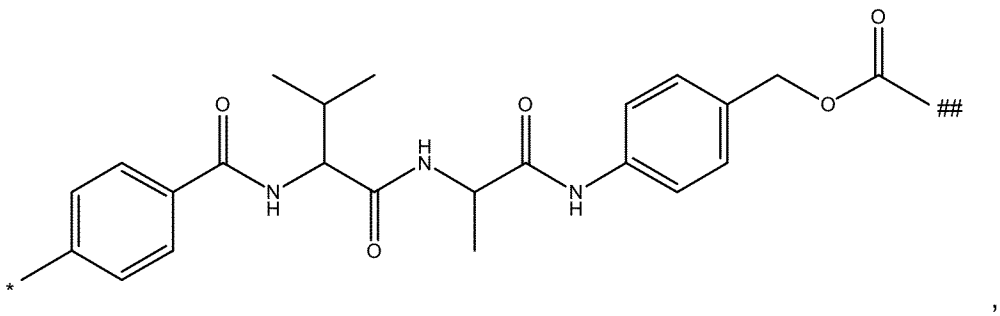
11. The conjugate of any one of the preceding claims, wherein the linker L comprises a valine-citrulline moiety or a valine-alanine moiety.

12. The conjugate of claim 11, wherein the linker L is:



wherein # indicates the attachment point to the Y and * indicates the attachment point to the camptothecin moiety.

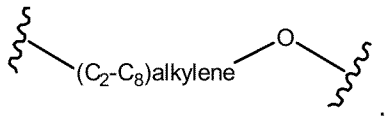
13. The conjugate of claim 11, wherein the linker L is:



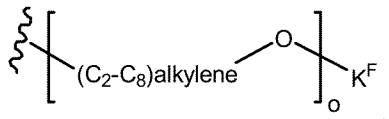
wherein * indicates the attachment point to the Y and ## indicates the attachment point to the camptothecin moiety.

14. The conjugate of any one of the preceding claims, wherein R^1 is a first polyalkylene glycol unit R^F.

15. The conjugate of claim 14, wherein the first polyalkylene glycol unit R^F comprises 1 to 100 subunits having the structure:



16. The conjugate of claim 15, wherein R^F is



wherein:

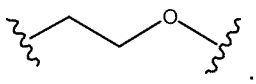
 indicates the position of the O;

K^F is selected from the group consisting of -H, $-PO_3H$, $-(C_1\text{-}C_{10})$alkyl, $-(C_1\text{-}C_{10})$alkyl-SO_3H, $-(C_2\text{-}C_{10})$alkyl-CO_2H, $-(C_2\text{-}C_{10})$alkyl-OH, $-(C_2\text{-}C_{10})$alkyl-NH_2, $-(C_2\text{-}C_{10})$alkyl-$NH(C_1\text{-}C_3)$alkyl and $-(C_2\text{-}C_{10})$alkyl-$N((C_1\text{-}C_3)\text{alkyl})_2$; and

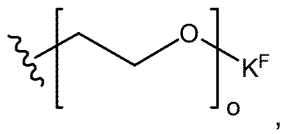
o is an integer ranging from 1 to 100.

17. The conjugate of any one of claims 14 to 16, wherein R^1 is a first polyethylene glycol unit.

18. The conjugate of claim 17, wherein the first polyethylene glycol unit R^F comprises 1 to 100 subunits having the structure:



19. The conjugate of claim 18, wherein R^F is:



wherein

 indicates the position of the O;

K^F is selected from the group consisting of -H, $-PO_3H$, $-(C_1-C_{10})$alkyl, $-(C_1-C_{10})$alkyl-SO_3H, $-(C_2-C_{10})$alkyl-CO_2H, $-(C_2-C_{10})$alkyl-OH, $-(C_2-C_{10})$alkyl-NH_2, $-(C_2-C_{10})$alkyl-NH(C_1-C_3)alkyl and $-(C_2-C_{10})$alkyl-N((C_1-C_3)alkyl)$_2$; and

o is an integer ranging from 1 to 100.

20. The conjugate of claim 19, wherein K^F is H.

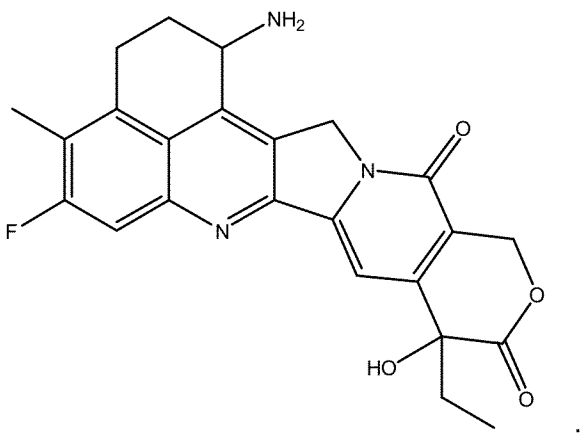
21. The conjugate of claim 20, wherein o ranges from 8 to 30.

22. The conjugate of claim 21, wherein o ranges from 20 to 28.

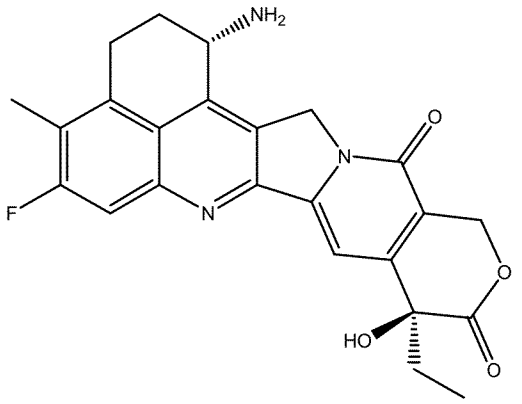
23. The conjugate of claim 22, wherein o is 22, 23, 24, 25 or 26.

24. The conjugate of any one of the preceding claims, wherein the camptothecin moiety C is selected from the group consisting of exatecan, SN38, camptothecin, topotecan, irinotecan, belotecan, lurtotecan, rubitecan, silatecan, cositecan, and gimatecan.

25. The conjugate of claim 24, wherein the camptothecin moiety C is exatecan having the formula:



26. The conjugate of claim 25, wherein the camptothecin moiety C is exatecan having the formula:



.

27. The conjugate of claim 25 or 26, wherein the exatecan is bound to the linker L via the amino group.

28. The conjugate of claim 1,

wherein:

RBM is an antibody;

 is a double bond; or

 is a bond;

V is absent when  is a double bond; or

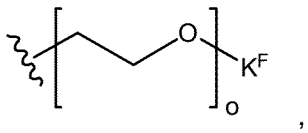
V is H when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NH;

R^1 is a polyethylene glycol unit having the structure:



,

wherein:

 indicates the position of the O;

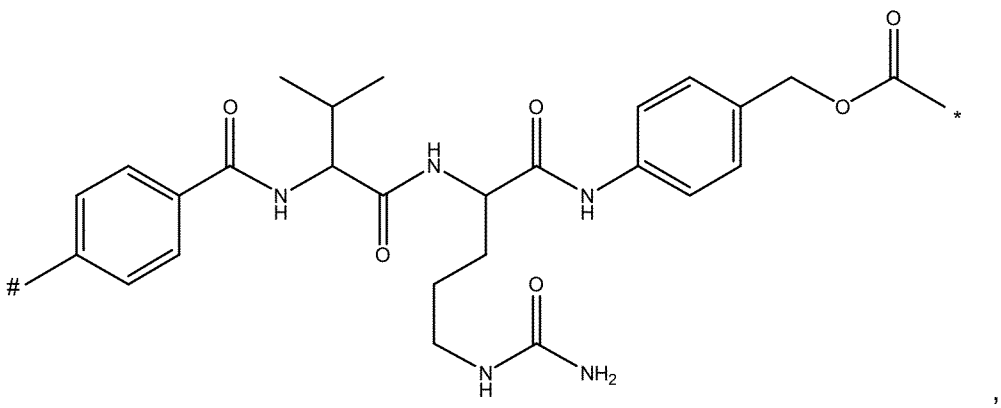
K^F is H; and

o is an integer ranging from 8 to 30;

R^3 is H;

R^4 is H;

L is a linker having the following structure:



,

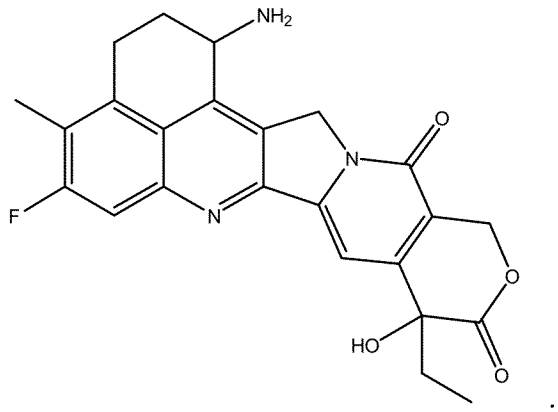
wherein # indicates the attachment point to the Y and * indicates the attachment point to the camptothecin moiety (C);

C is a camptothecin moiety;

m is 1; and

n is an integer ranging from 1 to 10.

29. The conjugate of claim 28, wherein the camptothecin moiety C is exatecan having the formula:



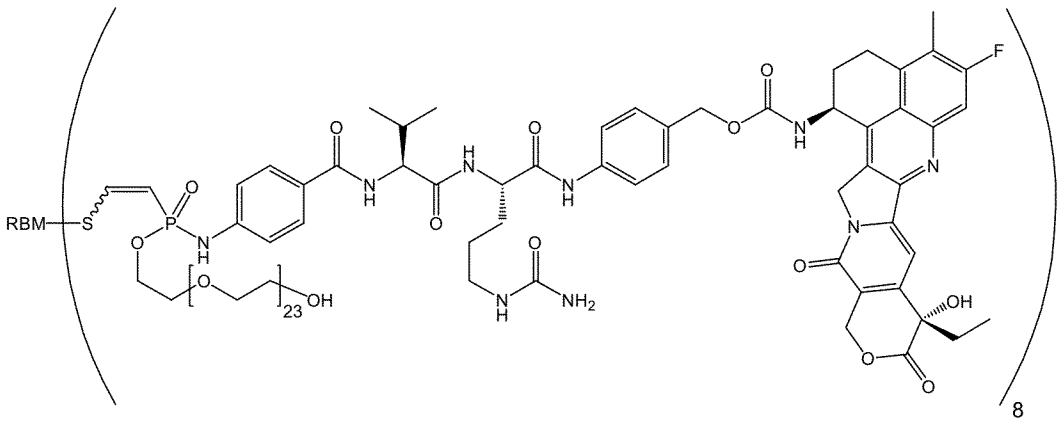
30. The conjugate of claim 29, wherein the exatecan is bound to the linker L via the amino group.

31. The conjugate of claim 30, wherein o ranges from 20 to 28.

32. The conjugate of claim 31, wherein o is 22, 23, 24, 25 or 26.

33. The conjugate of any one of claims 28 to 32, wherein n ranges from 2 to 10, preferably wherein n is 4 or 8.

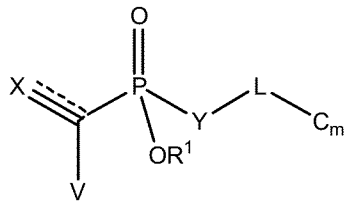
34. The conjugate of claim 33 having the following formula (Ia):



(Ia),

wherein RBM is an antibody.

35. A compound having the formula (II):



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is R_3-C when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

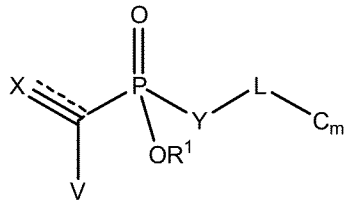
L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10.

36. A method of preparing a conjugate of formula (I), said method comprising:

reacting a compound of formula (II)



(II),

or a pharmaceutically acceptable salt or solvate thereof;

wherein:

 is a triple bond; or

 is a double bond;

V is absent when  is a triple bond; or

V is H or (C_1-C_8)alkyl when  is a double bond;

X is R_3-C when  is a triple bond; or

X is  when  is a double bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

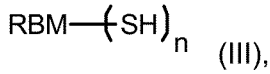
R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety; and

m is an integer ranging from 1 to 10; and

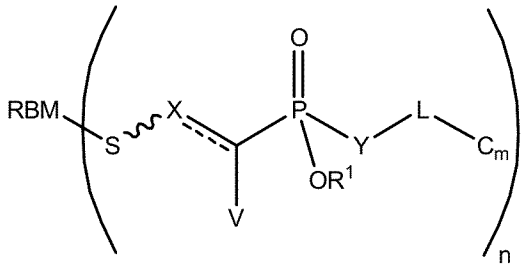
with a thiol-containing molecule of formula (III)



(III),

wherein RBM is a receptor binding molecule; and
n is an integer ranging from 1 to 20;

resulting in a compound of formula (I)



(I),

or a pharmaceutically acceptable salt or solvate thereof;

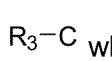
wherein:

 is a double bond when  in a compound of formula (II) is a triple bond; or

 is a bond when  in a compound of formula (II) is a double bond;

V is absent when  is a double bond; or

V is H or (C_1-C_8)alkyl when  is a bond;

X is  when  is a double bond; or

X is  when  is a bond;

Y is NR^5, S, O, or CR^6R^7;

R^1 is an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^3 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^4 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^5 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^6 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

R^7 is H; or an optionally substituted aliphatic residue or an optionally substituted aromatic residue;

L is a linker;

C is a camptothecin moiety;

m is an integer ranging from 1 to 10; and

n is an integer ranging from 1 to 20.

37. The method of claim 36, further comprising reducing at least one disulfide bridge of the receptor binding molecule in the presence of a reducing agent to form a thiol group (SH).

38. A pharmaceutical composition comprising a conjugate of any one of claims 1 to 34.

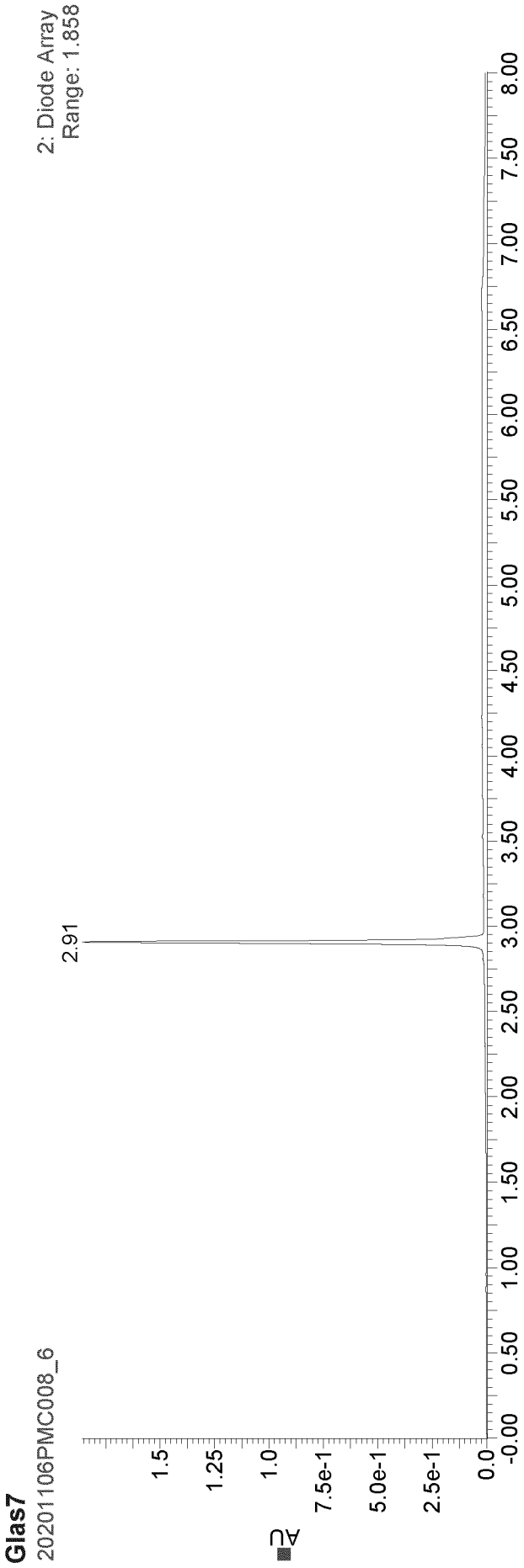
39. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition comprises a population of a conjugate of any one of claims 1 to 27, and wherein the average number of camptothecin moieties C per receptor binding molecule is from more than 0 to about 14.

40. A conjugate of any one of claims 1 to 34 or a pharmaceutical composition of claim 38 or 39 for use in a method of treating a disease.

41. The conjugate or the pharmaceutical composition for use of claim 40, wherein the disease is cancer.

42. The conjugate or the pharmaceutical composition for use of claim 41, wherein the cancer is a solid tumor.

Figure 1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1.25
1.0
7.5e-1
5.0e-1
2.5e-1
0.0
-0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
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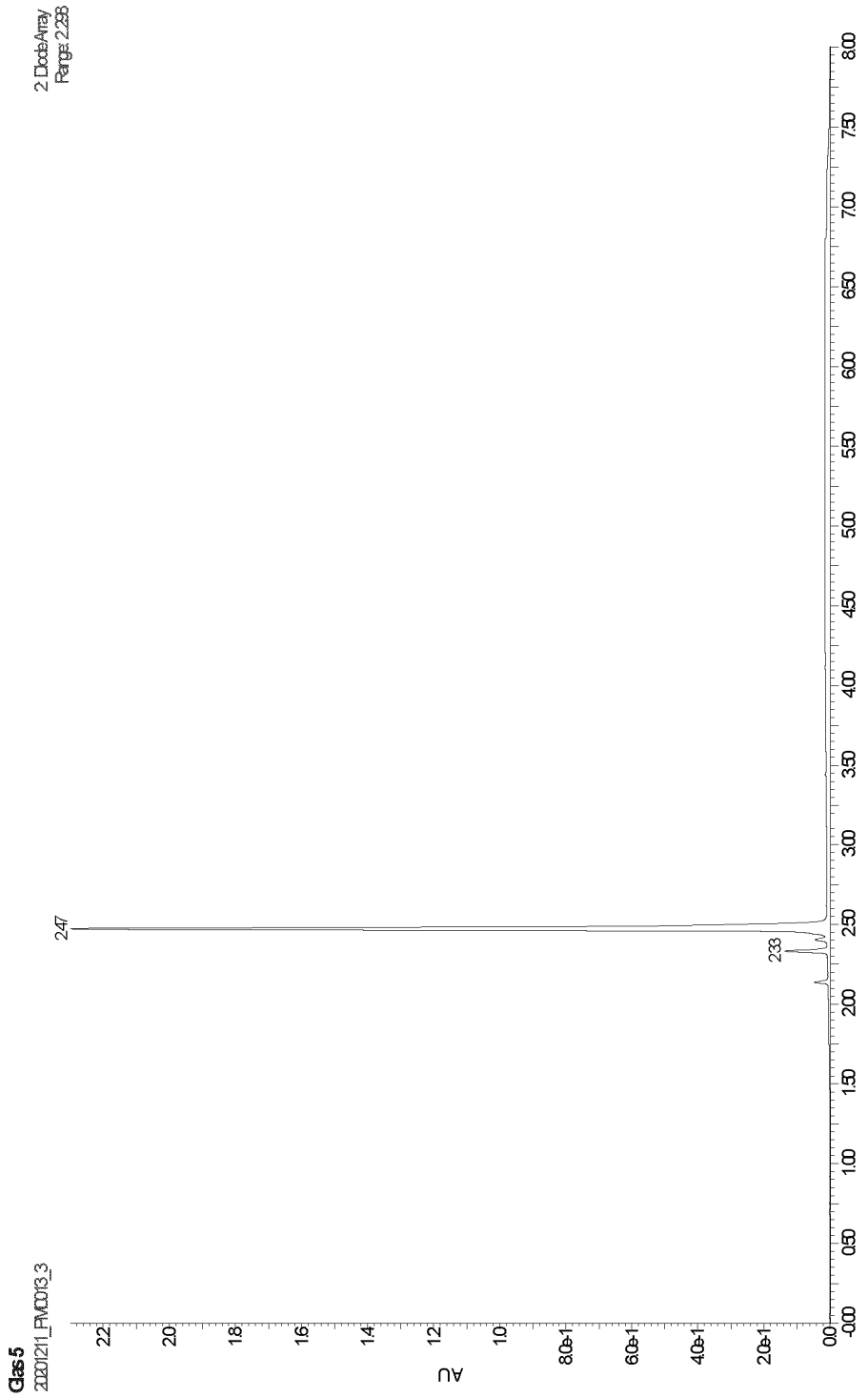
5.00
5.50
6.00
6.50
7.00
7.50
8.00

Figure 2


2 Diode Array
Range: 2.238
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AU
22
20
18
16
14
12
10
80e-1
60e-1
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20e-1
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0.50
1.00
1.50
2.00
2.50
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4.00
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6.00
6.50
7.00
7.50
8.00

Figure 3

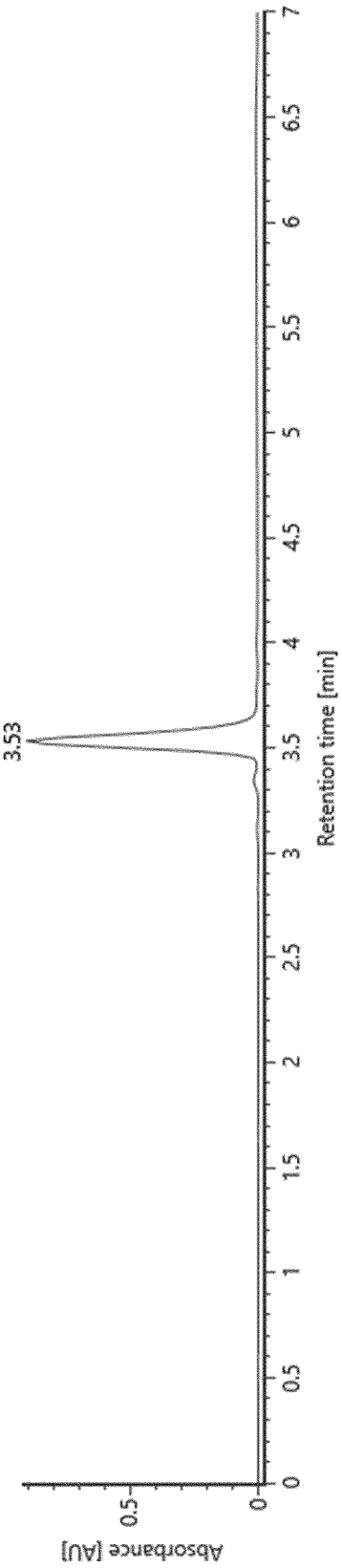


Figure 4

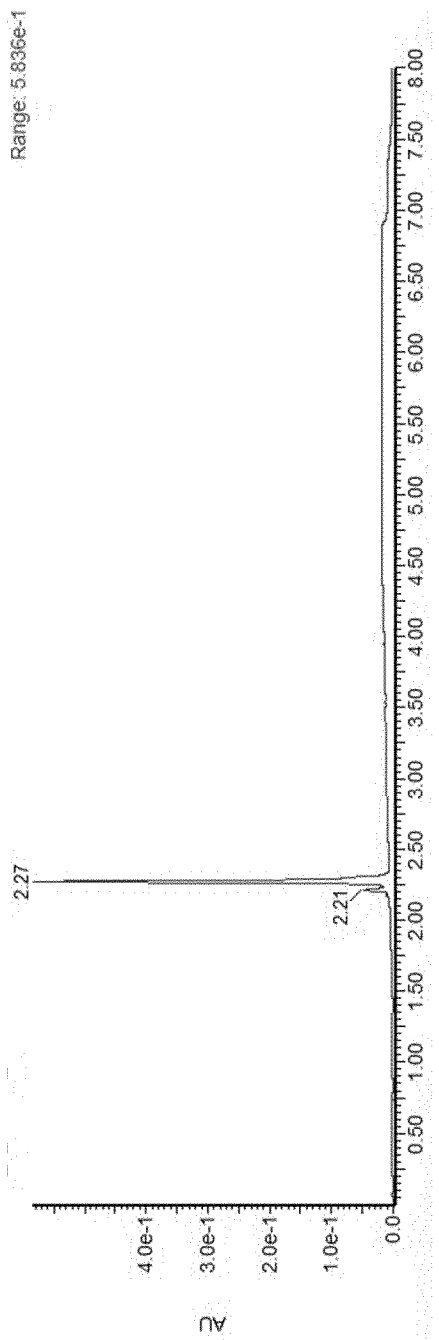
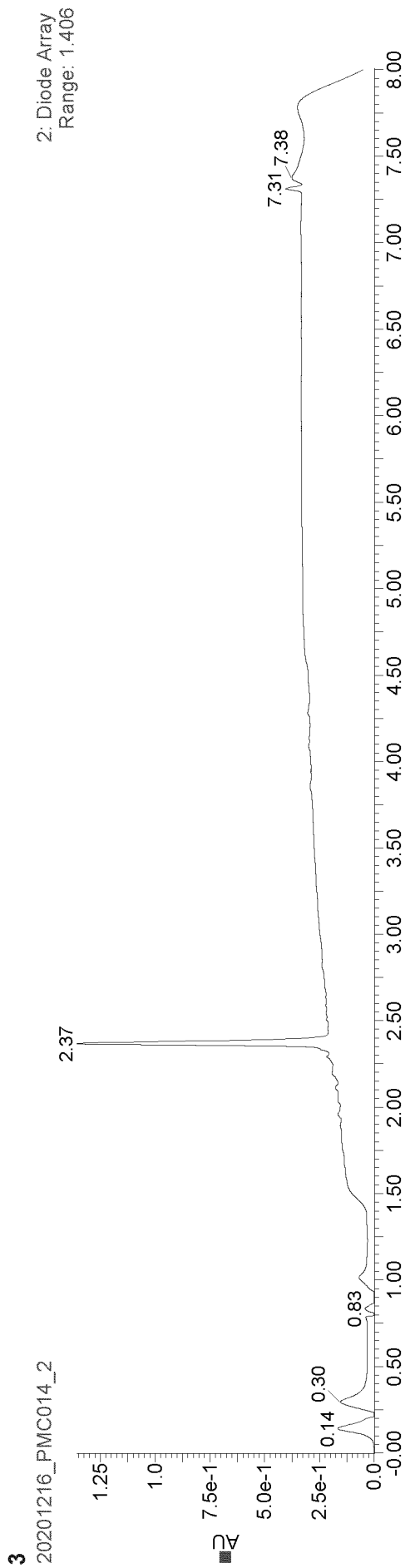


Figure 5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5.0e-1
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2.00
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3.00
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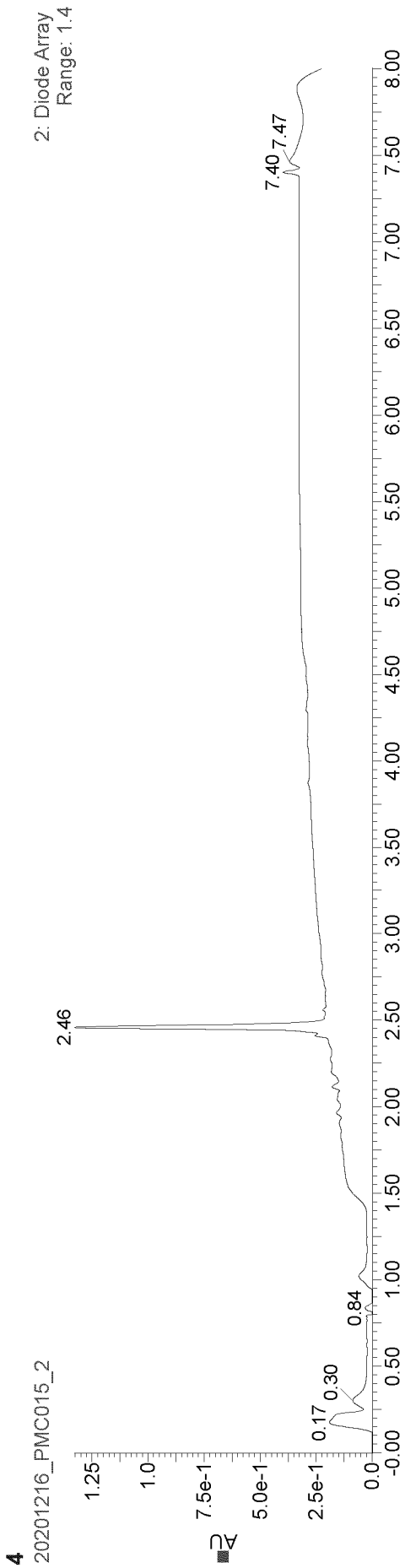
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
8.00
0.14
0.30
0.83
2.37
7.31
7.38

Figure 6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20201216_PMC015_2
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5.0e-1
2.5e-1
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1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
8.00
0.17
0.30
0.84
2.46
7.40
7.47

Figure 7

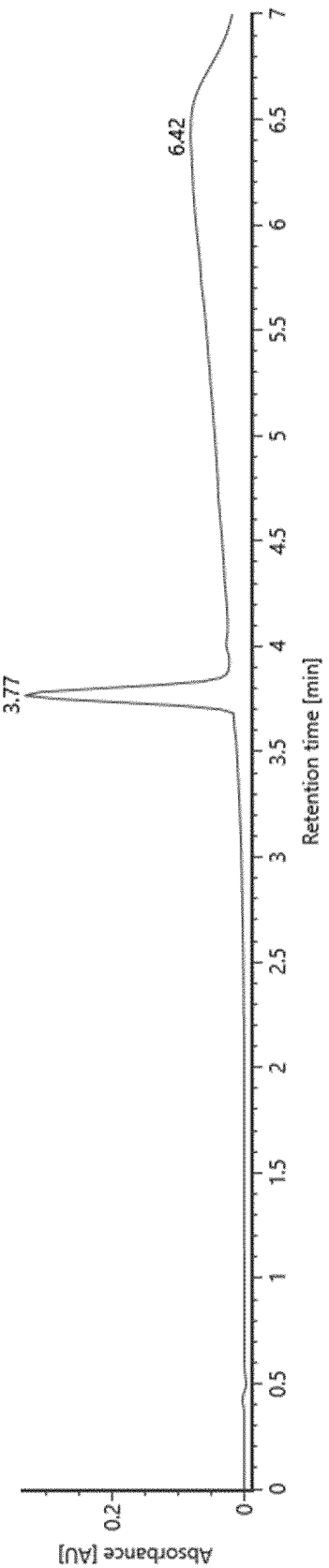


Figure 8

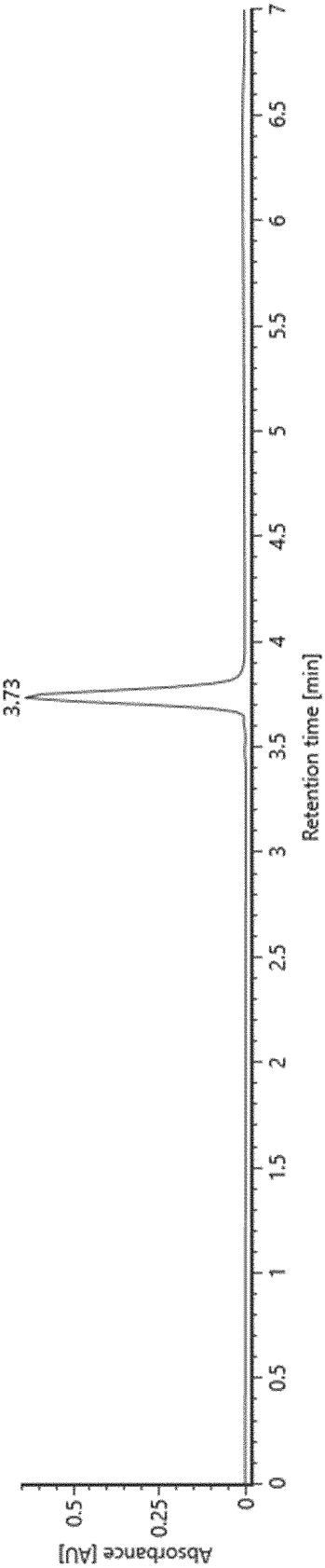


Figure 9

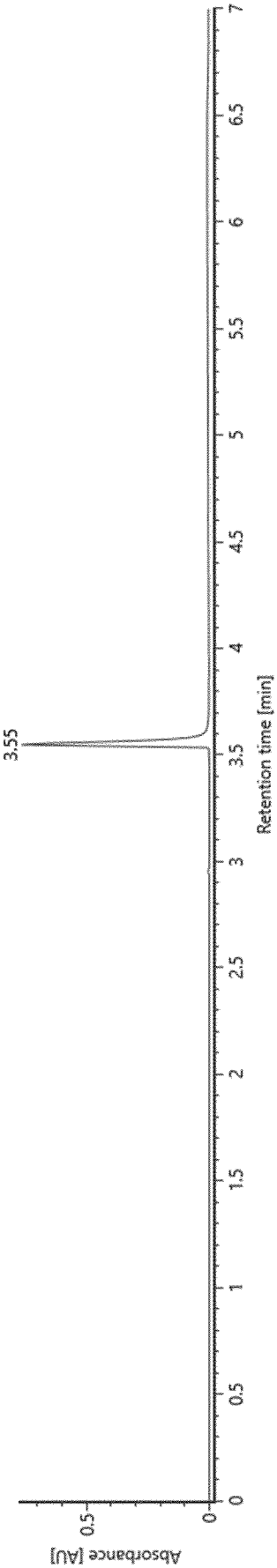


Figure 10

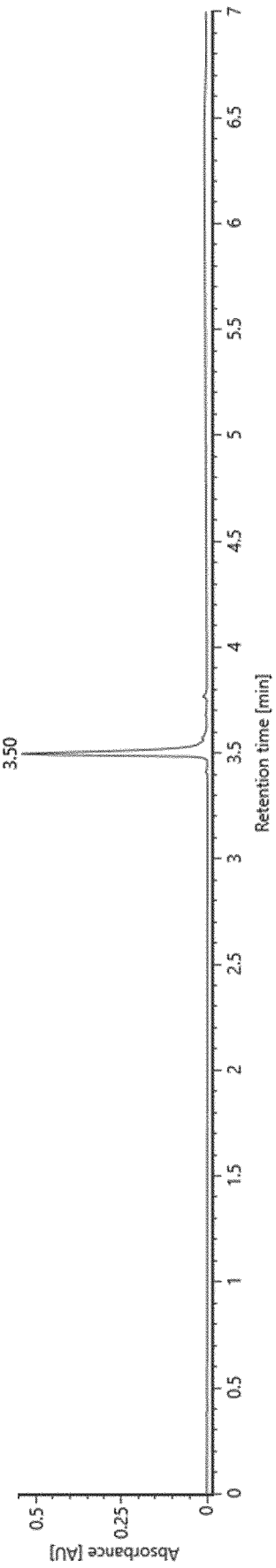


Figure 11

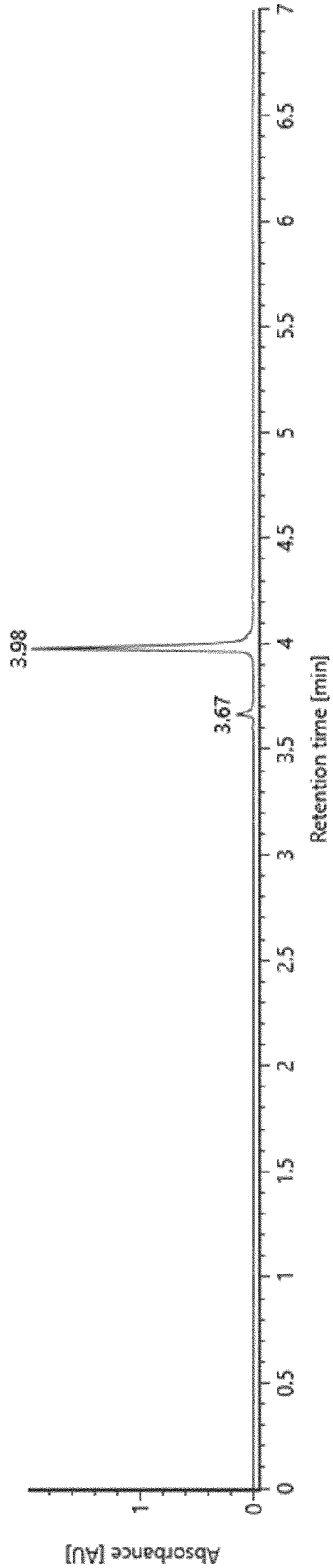


Figure 12

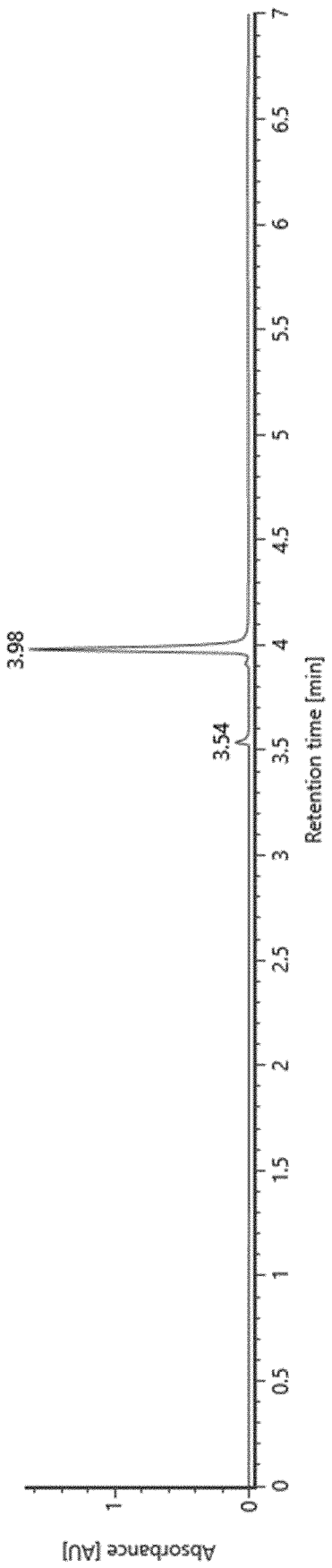


Figure 13

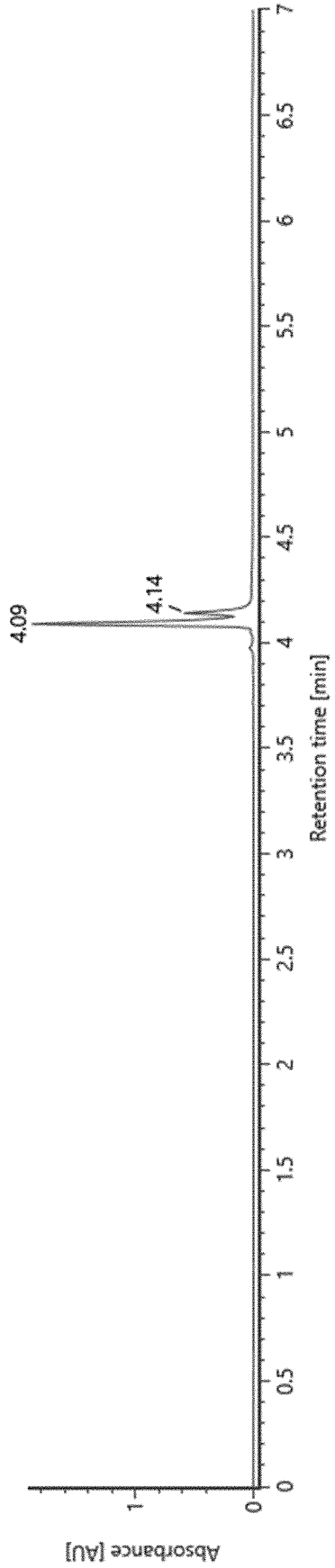


Figure 14

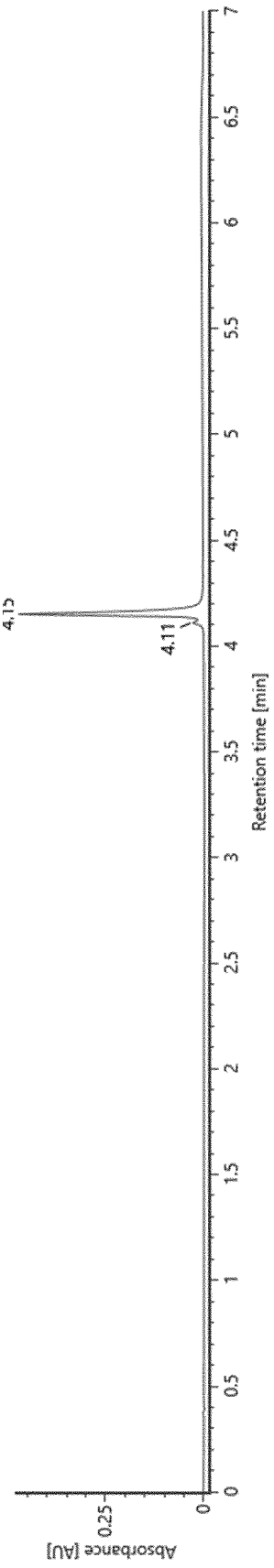


Figure 15

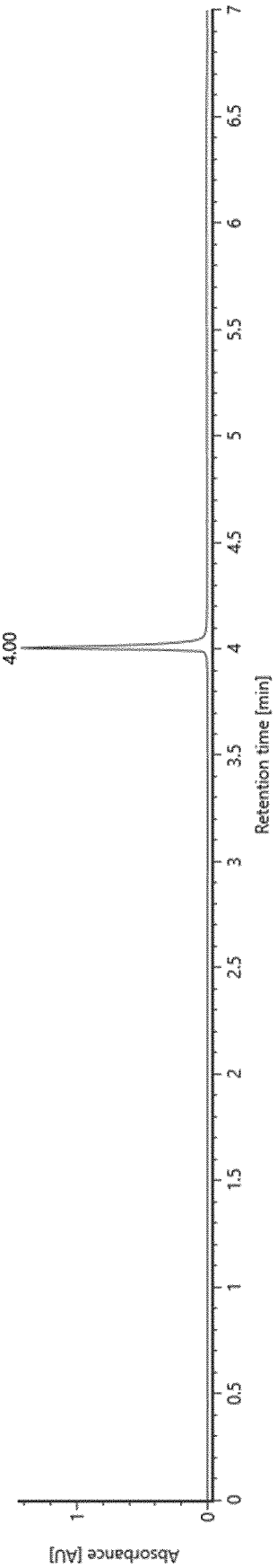


Figure 16

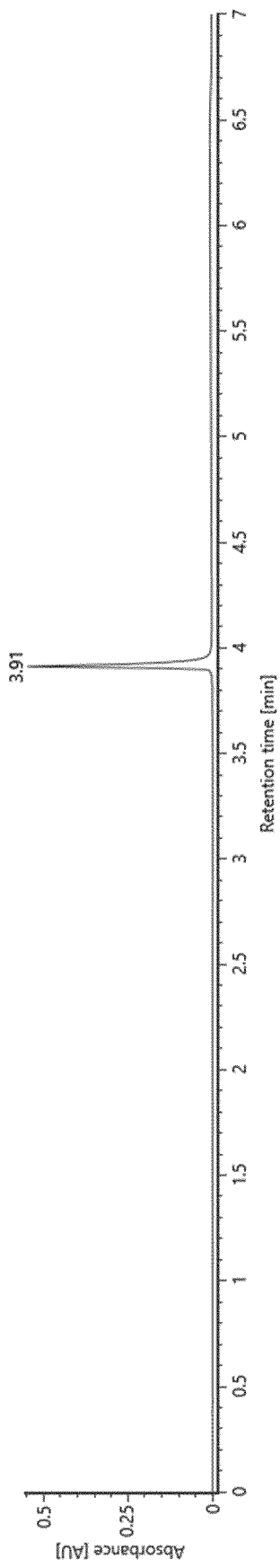


Figure 17

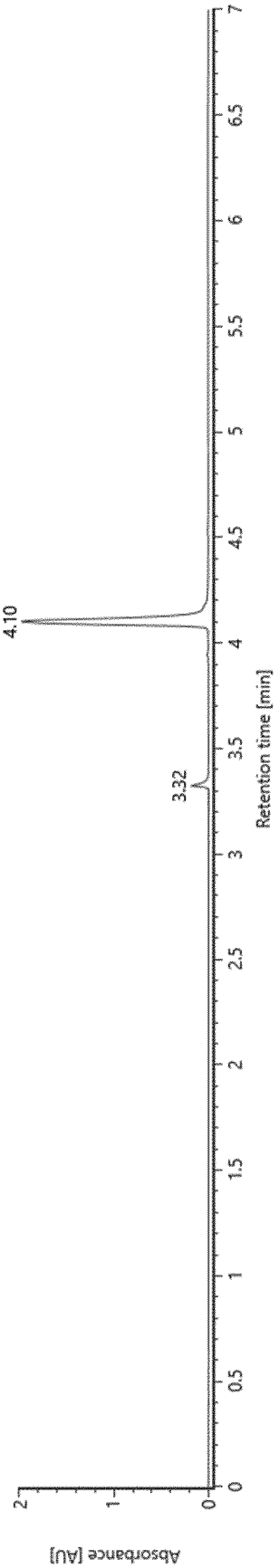

4.10
3.32
Retention time [min]
Absorbance [AU]
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
0
1
2

Figure 18

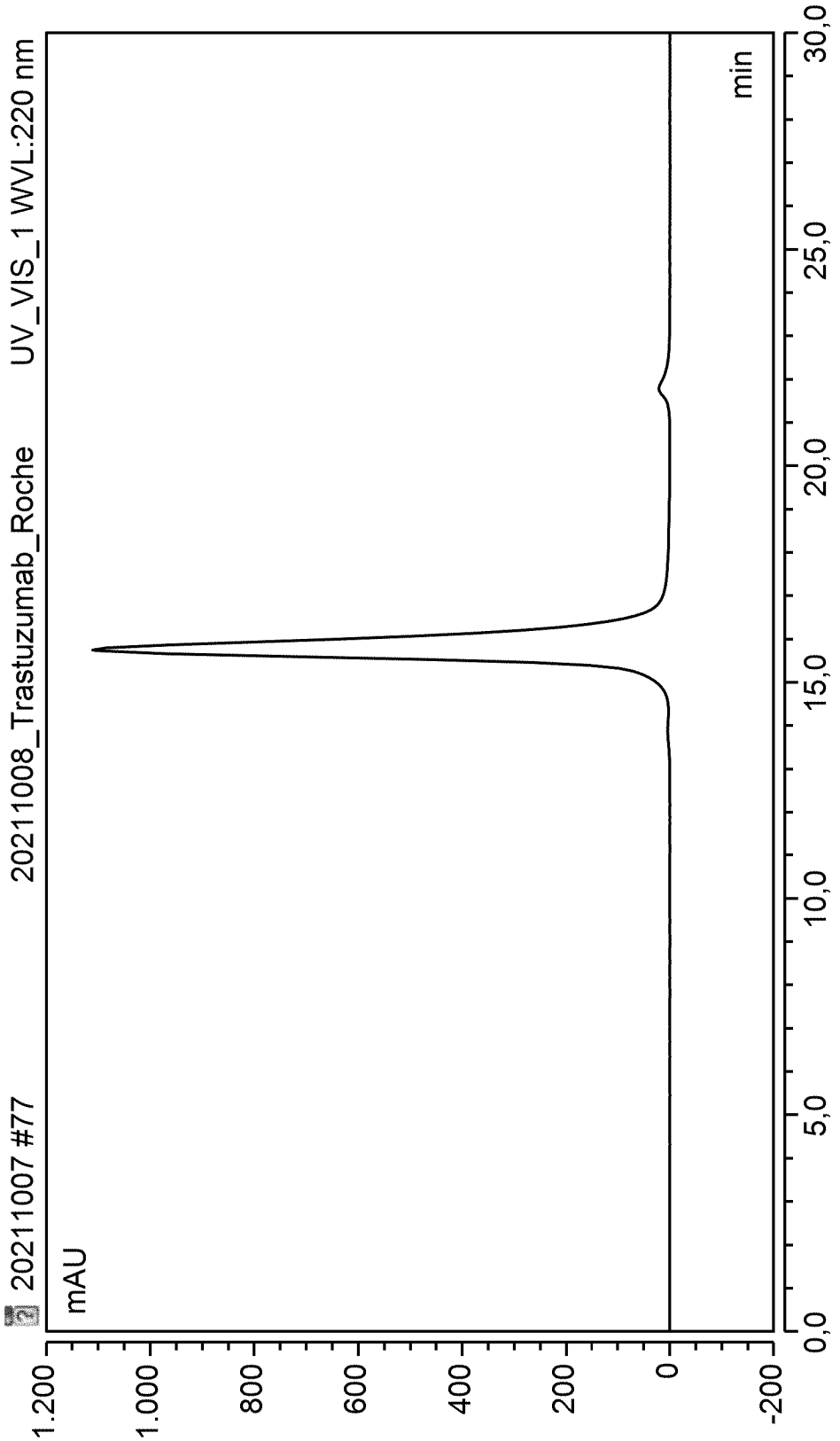

20211007 #77
20211008_Trastuzumab_Roche
UV_VIS_1 WVL:220 nm
mAU
min
1.200
1.000
800
600
400
200
0
-200
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 19

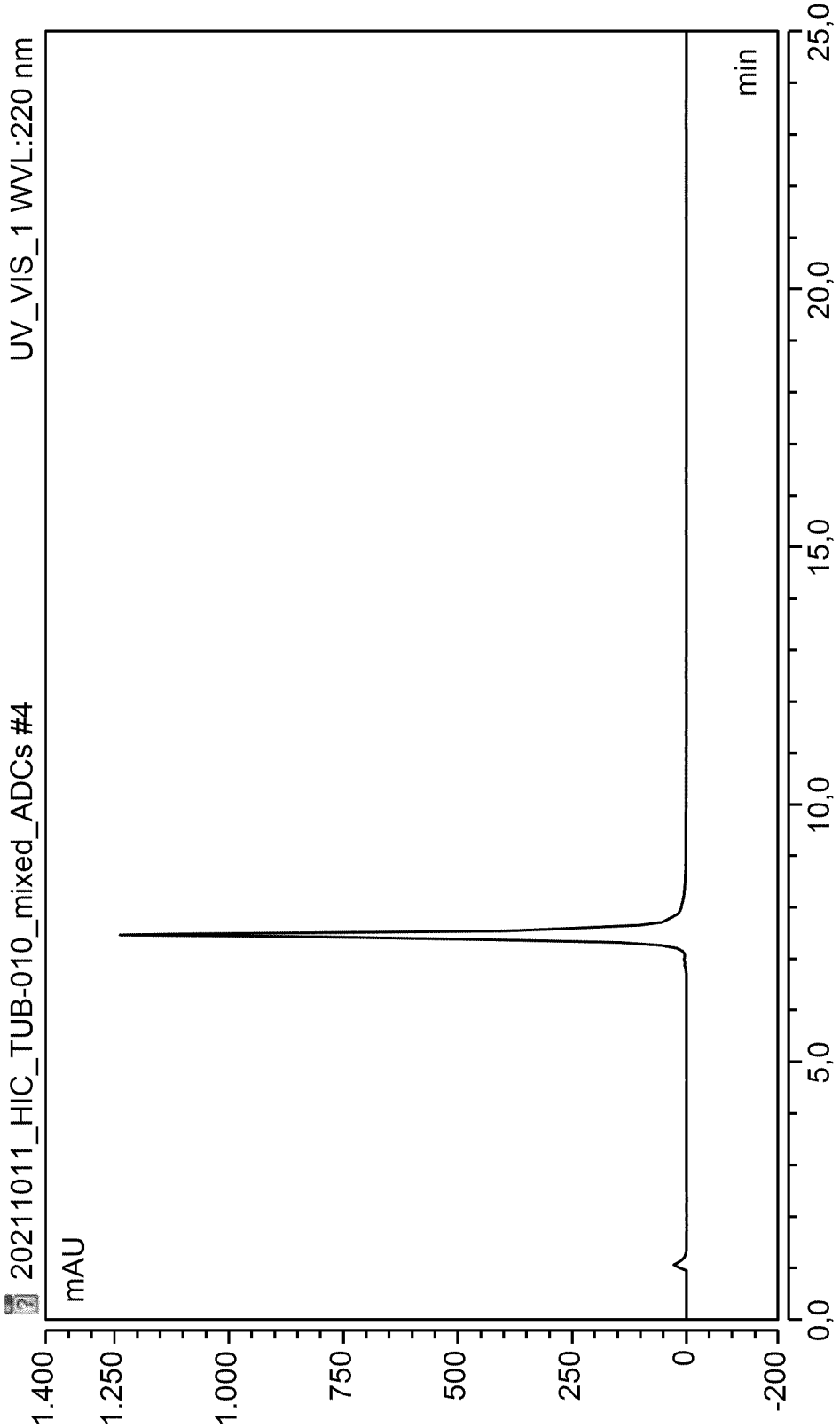

20211011_HIC_TUB-010_mixed_ADCs #4
UV_VIS_1 WVL:220 nm
mAU
min
1.400
1.250
1.000
750
500
250
0
-200
0,0
5,0
10,0
15,0
20,0
25,0

Figure 20

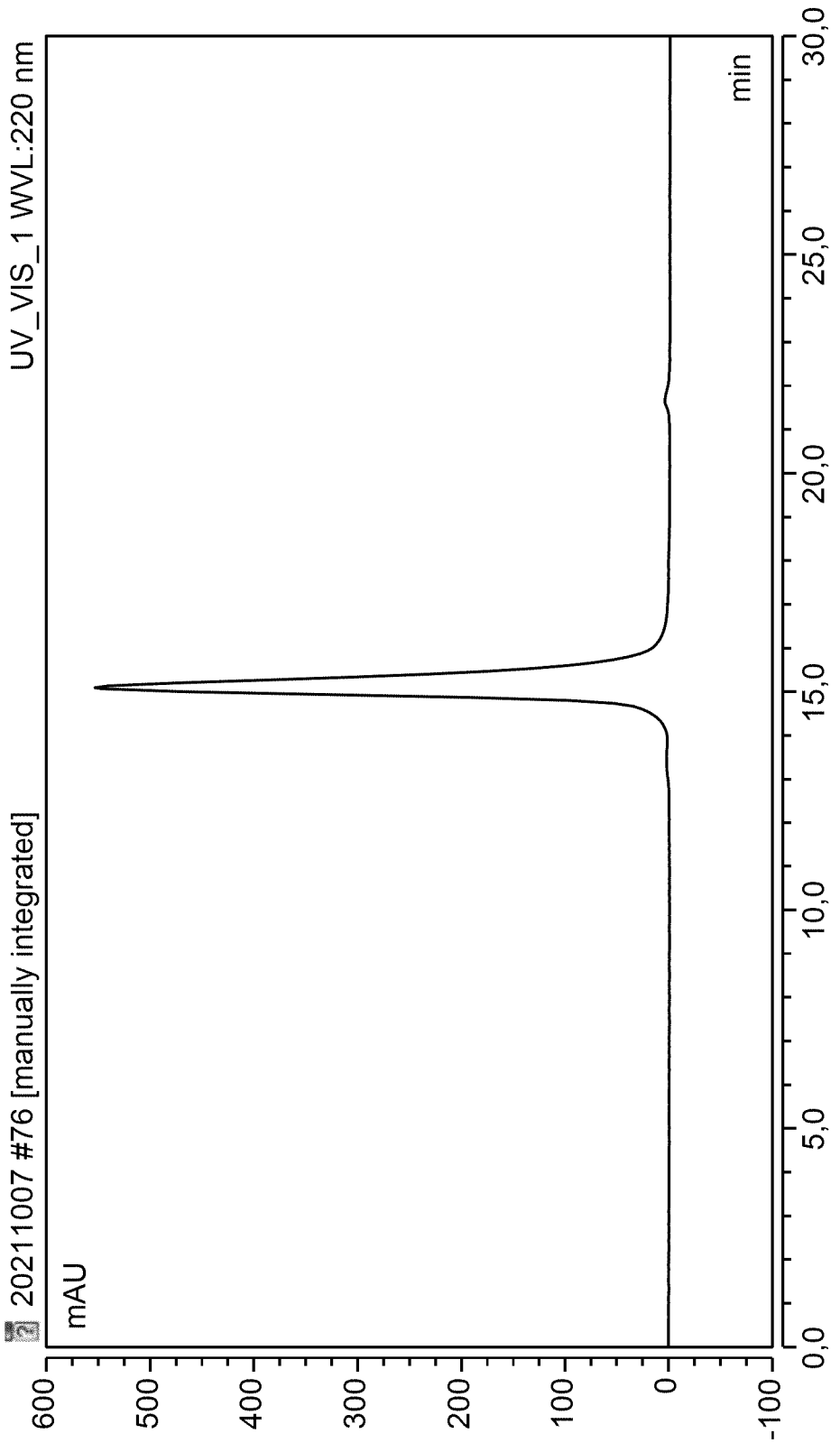

UV_VIS_1 WVL:220 nm
20211007 #76 [manually integrated]
mAU
min
600
500
400
300
200
100
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 21

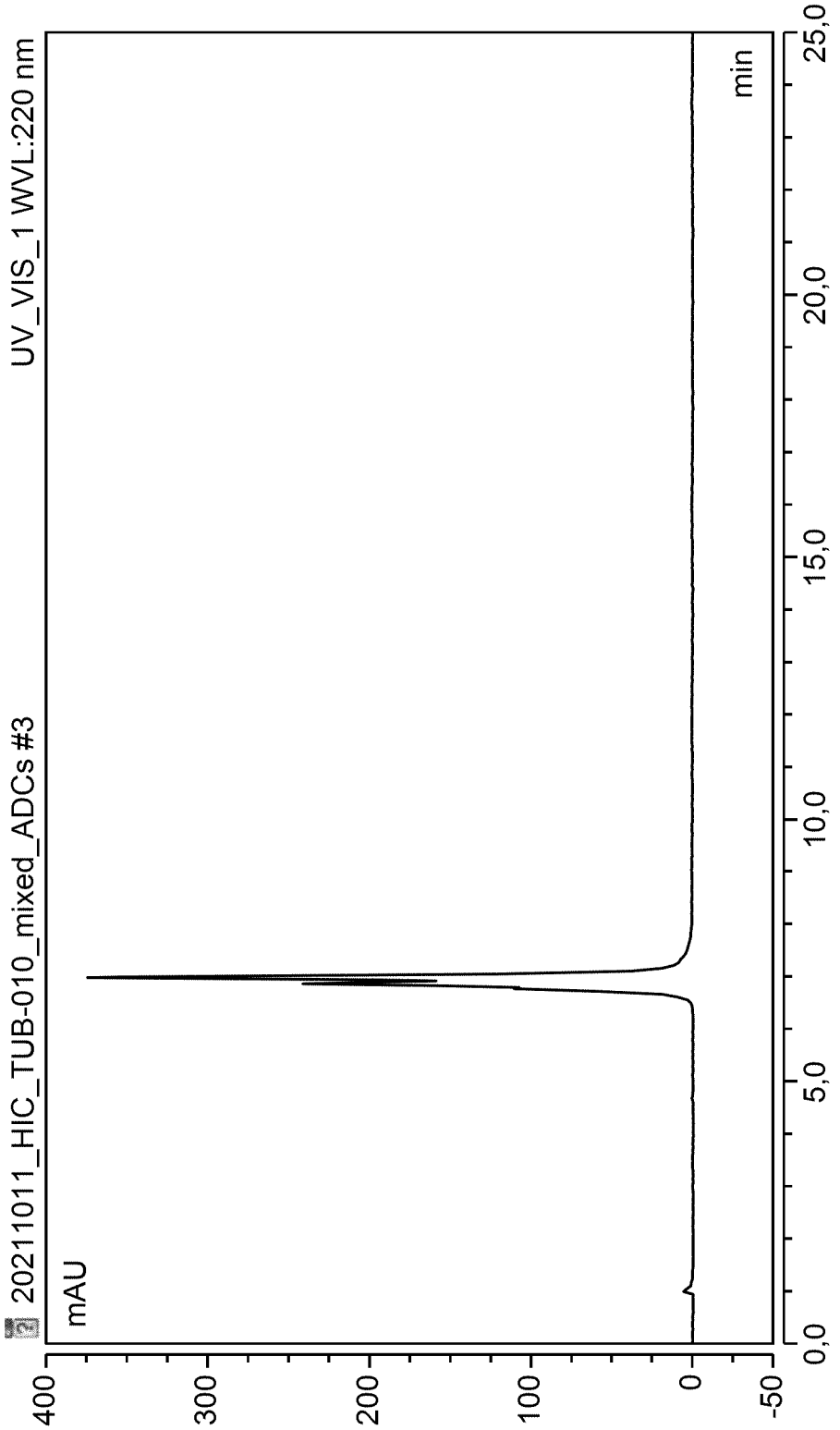

20211011_HIC_TUB-010_mixed_ADCs #3
UV_VIS_1 WVL:220 nm
mAU
min
400
300
200
100
0
-50
0,0
5,0
10,0
15,0
20,0
25,0

Figure 22

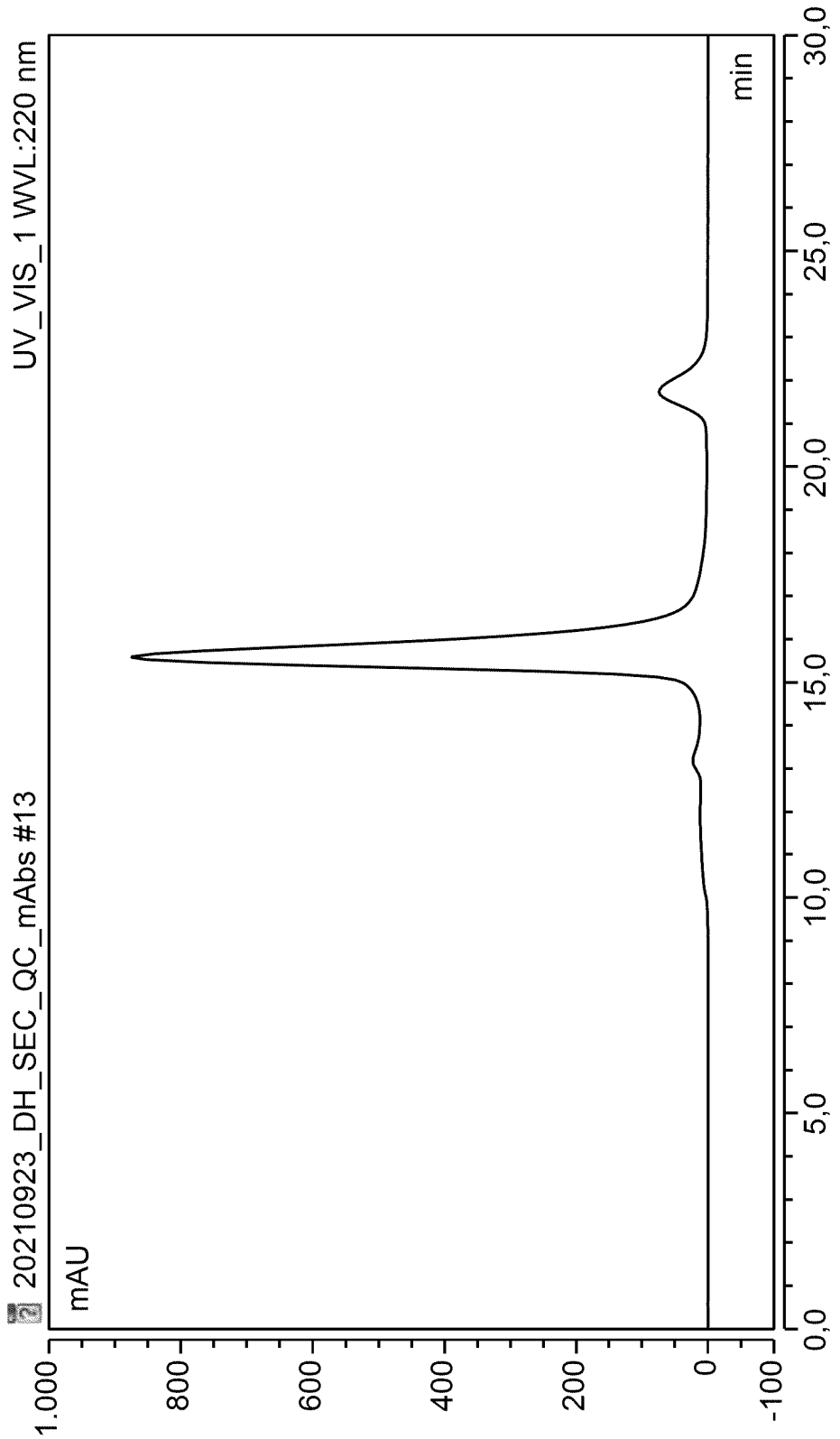

20210923_DH_SEC_QC_mAbs #13
UV_VIS_1 WVL:220 nm
mAU
min
1.000
800
600
400
200
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 23

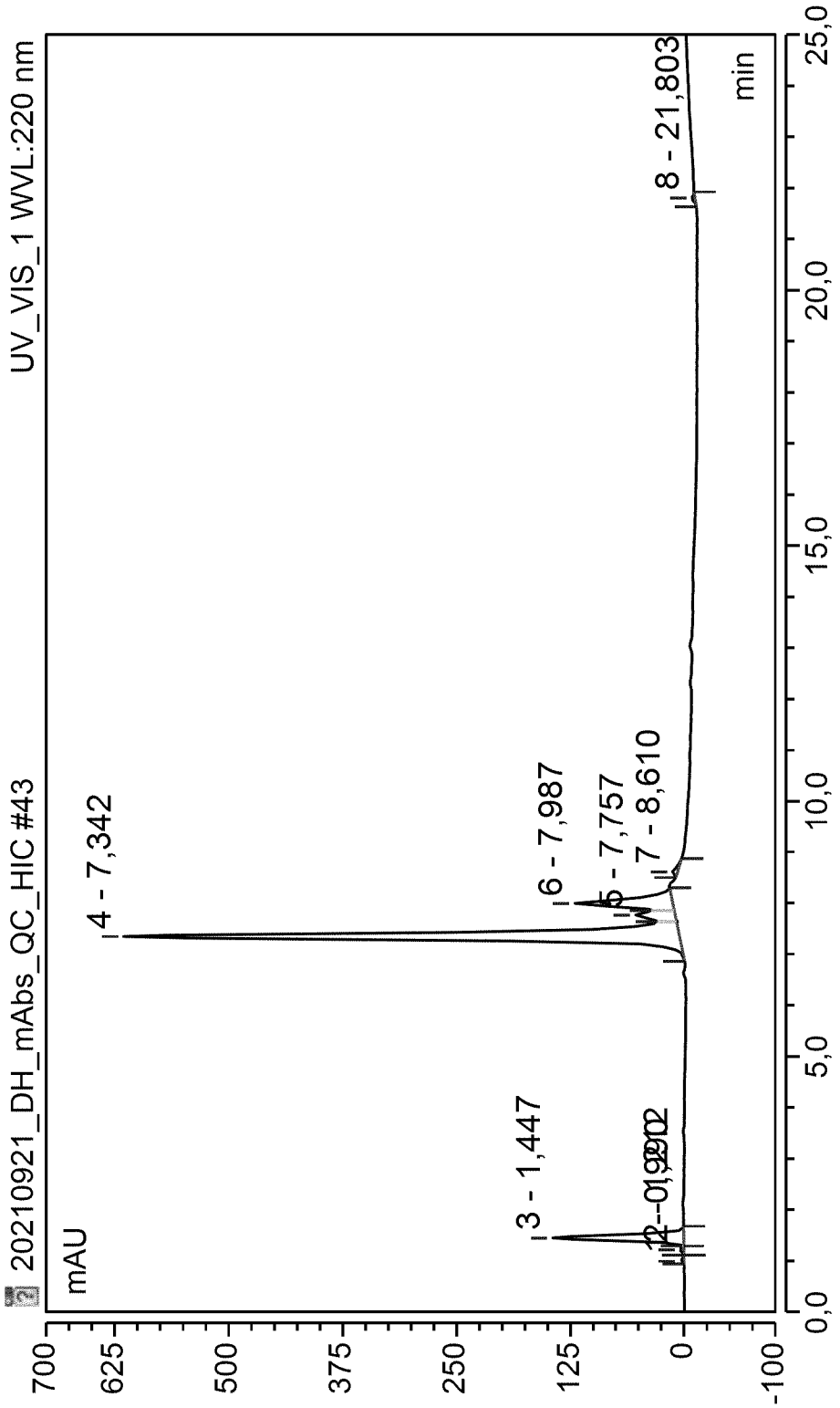

20210921_DH_mAbs_QC_HIC #43
UV_VIS_1 WVL:220 nm
mAU
min
700
625
500
375
250
125
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
3 - 1,447
4 - 7,342
6 - 7,987
7 - 8,610
8 - 21,803

Figure 24

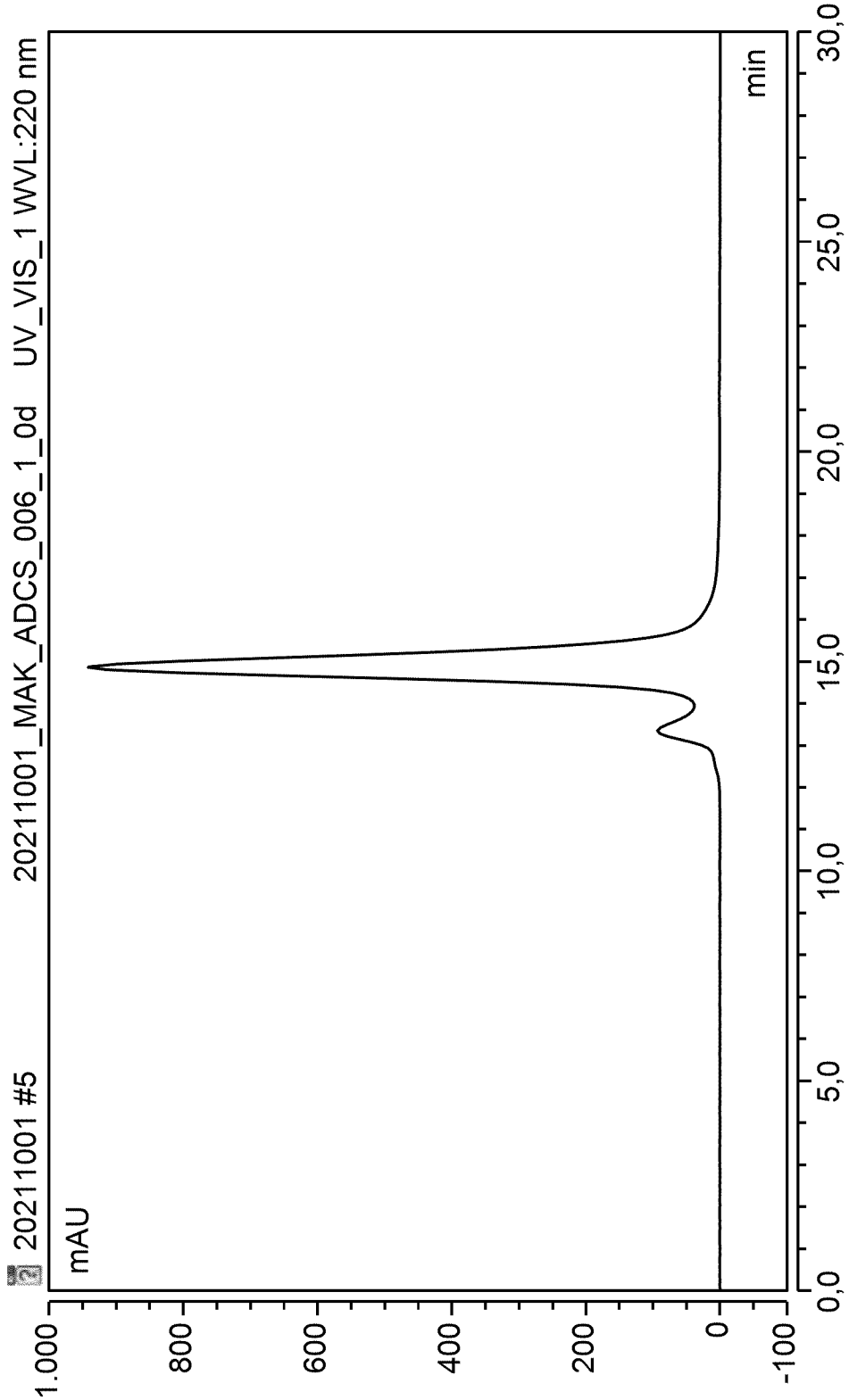

20211001 #5
20211001_MAK_ADCS_006_1_0d UV_VIS_1 WVL:220 nm
mAU
min
1.000
800
600
400
200
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 25

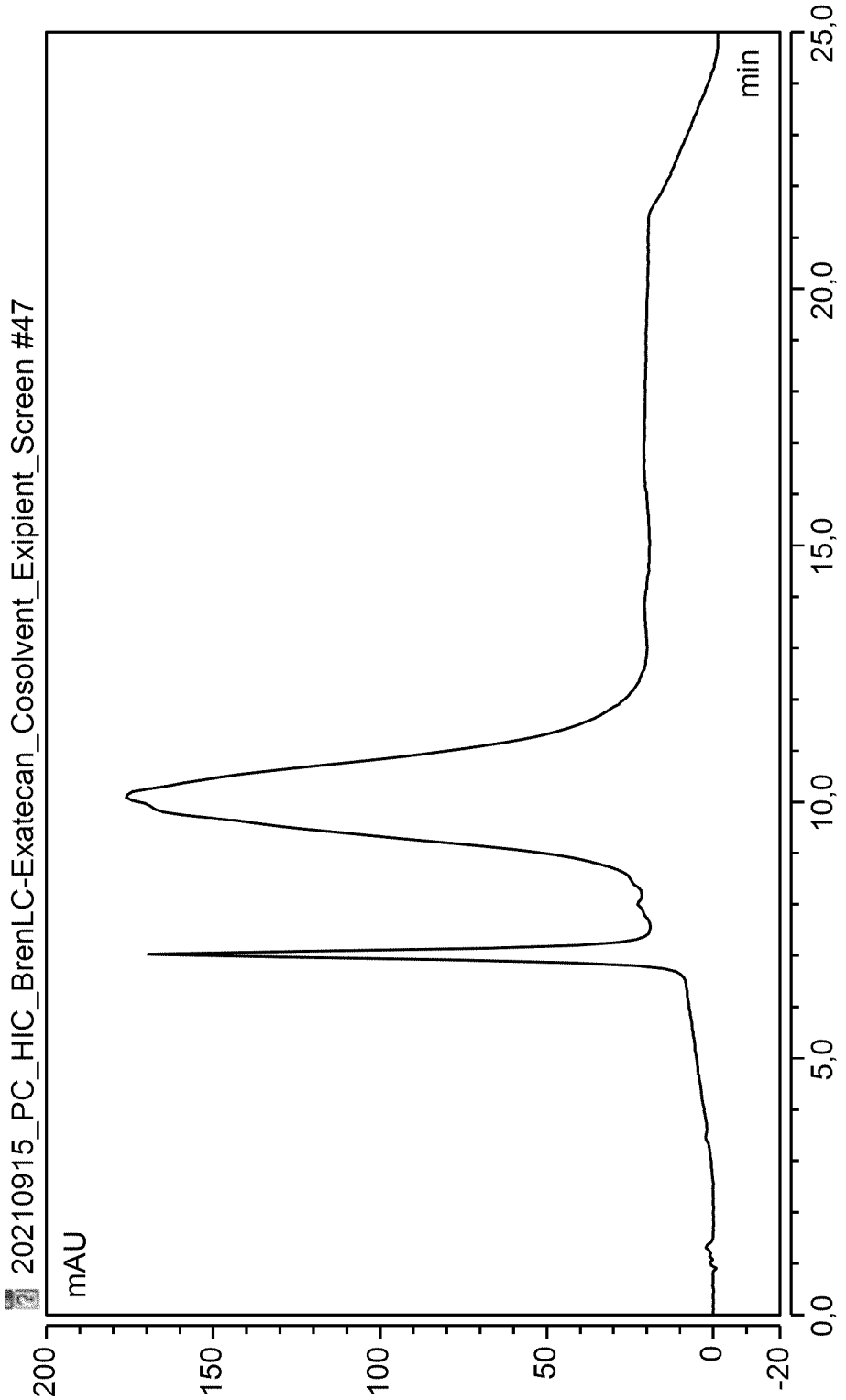

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #47
mAU
min
200
150
100
50
0
-20
0,0
5,0
10,0
15,0
20,0
25,0

Figure 26

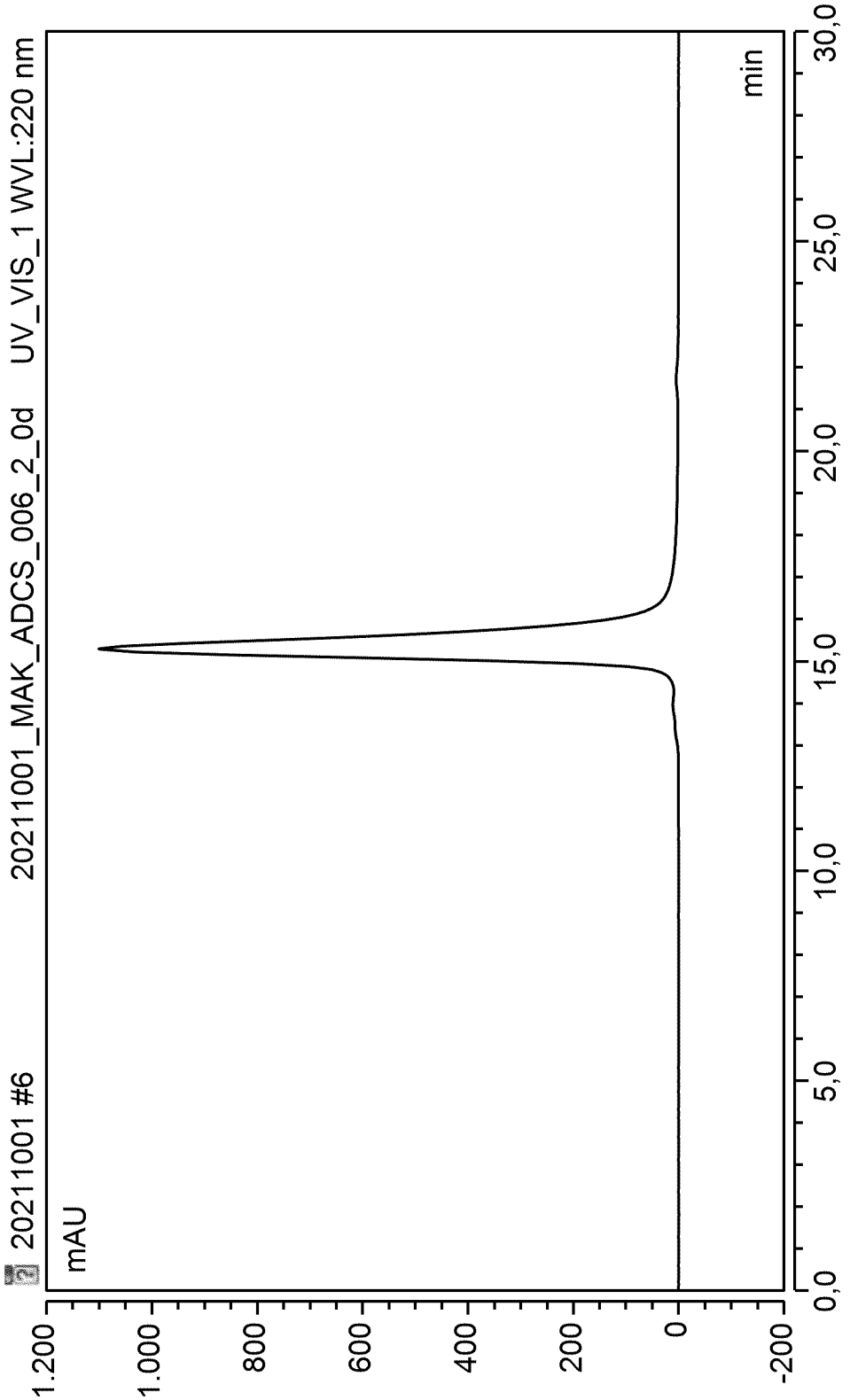

20211001 #6
20211001_MAK_ADCS_006_2_0d UV_VIS_1 WVL:220 nm
mAU
min
1.200
1.000
800
600
400
200
0
-200
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 27

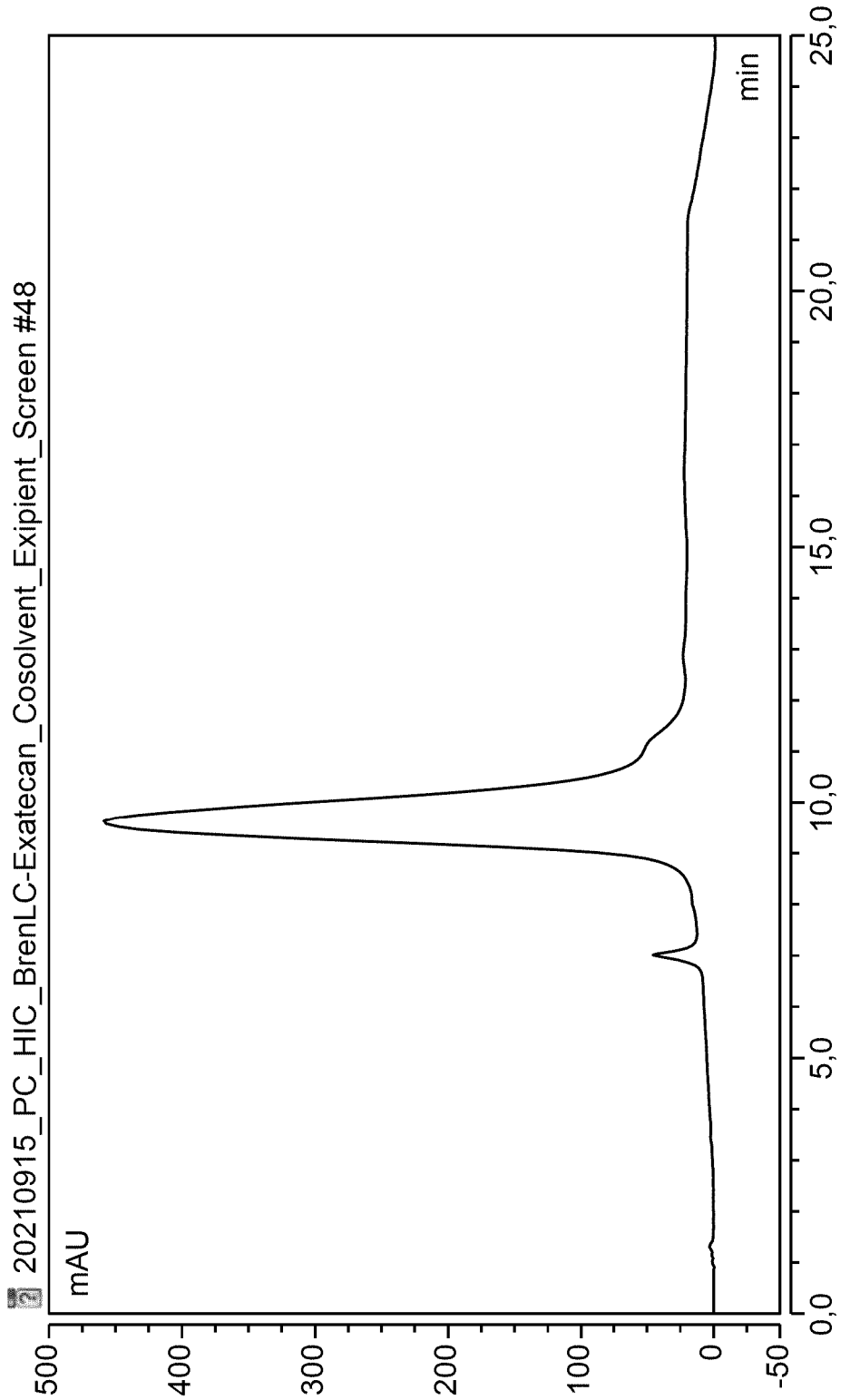

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #48
mAU
min
500
400
300
200
100
0
-50
0,0
5,0
10,0
15,0
20,0
25,0

Figure 28

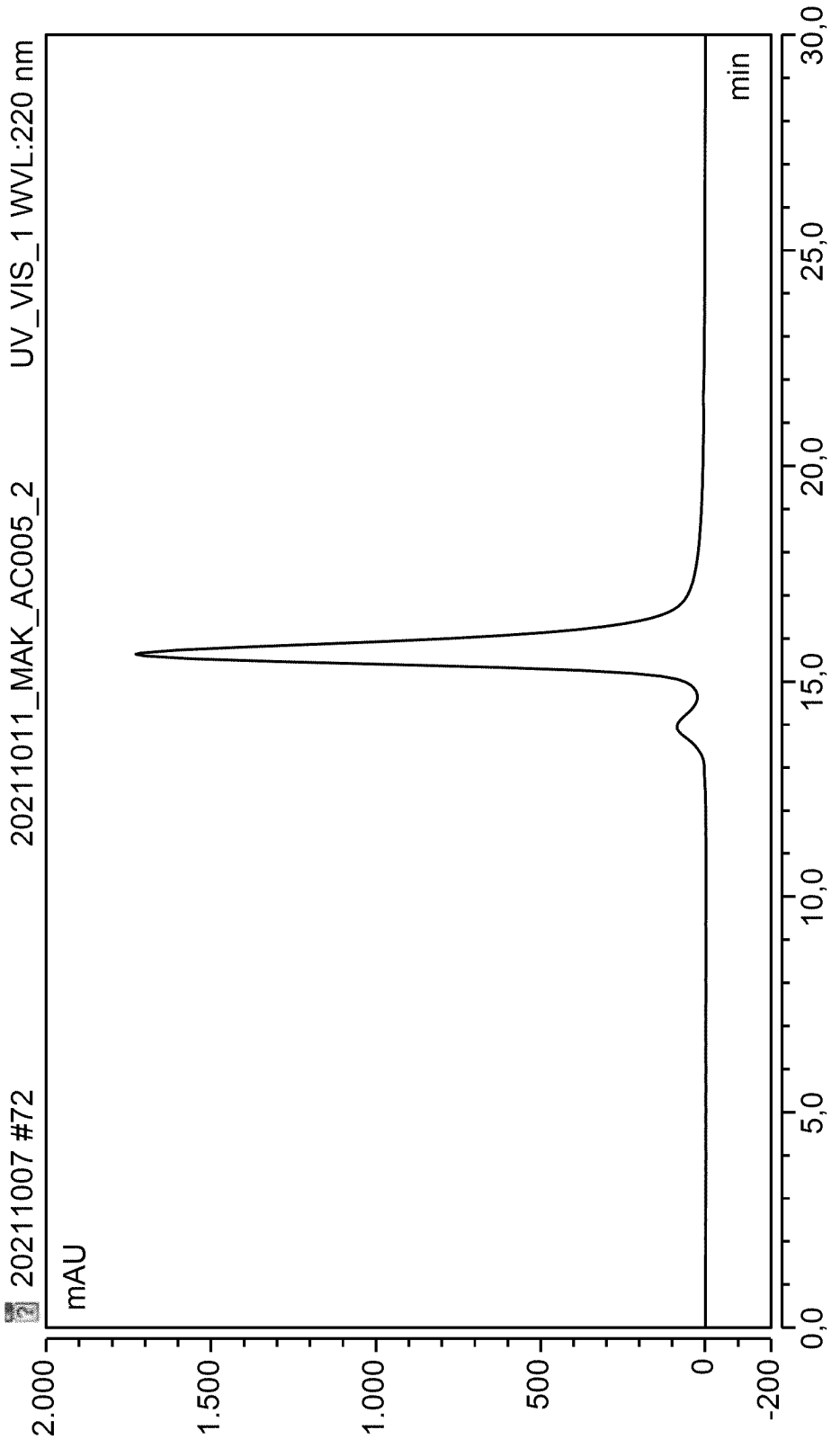

20211007 #72
20211011_MAK_AC005_2
UV_VIS_1 WVL:220 nm
mAU
min
2.000
1.500
1.000
500
0
-200
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 29

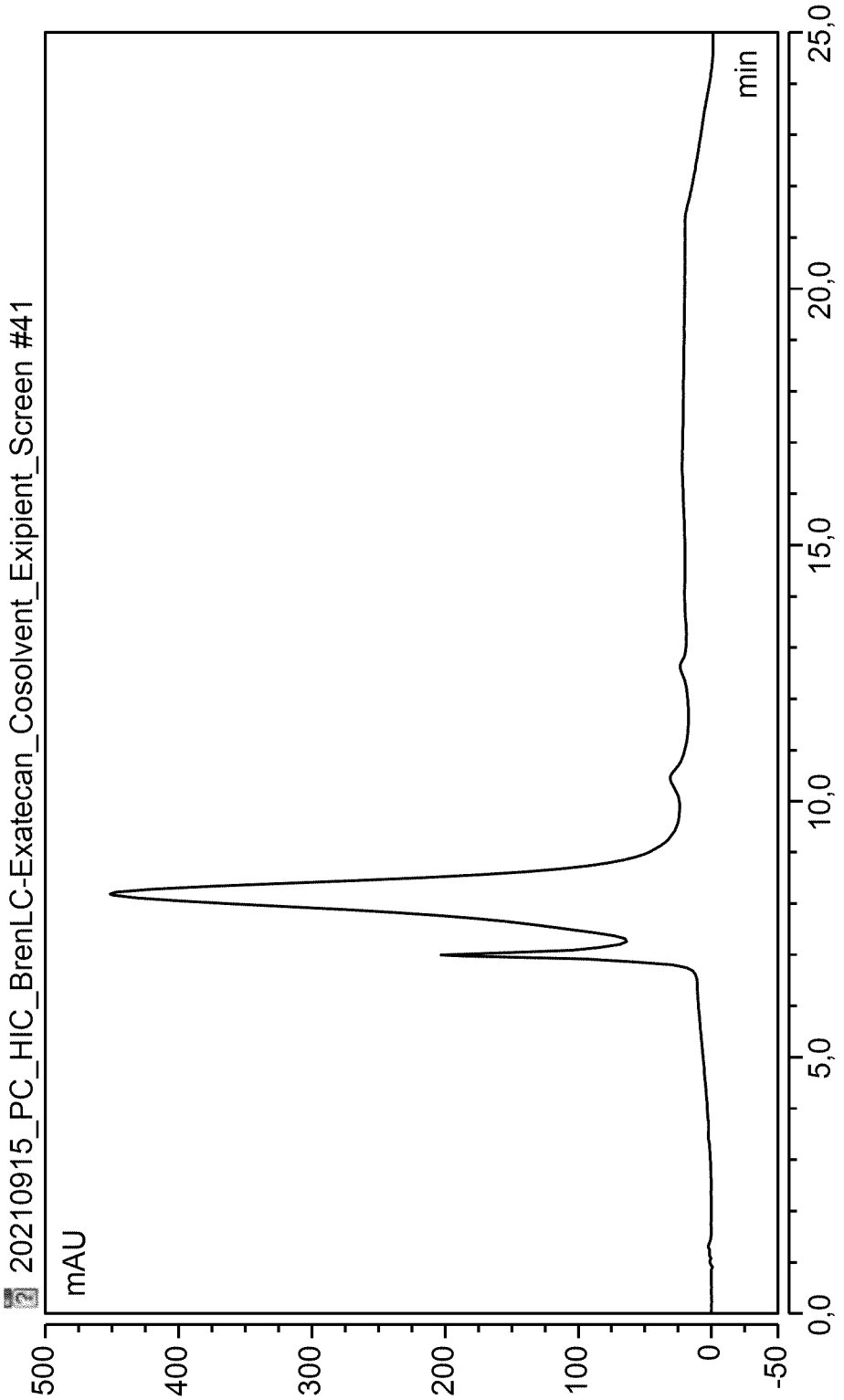

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #41
mAU
min
500
400
300
200
100
0
-50
0,0
5,0
10,0
15,0
20,0
25,0

Figure 30

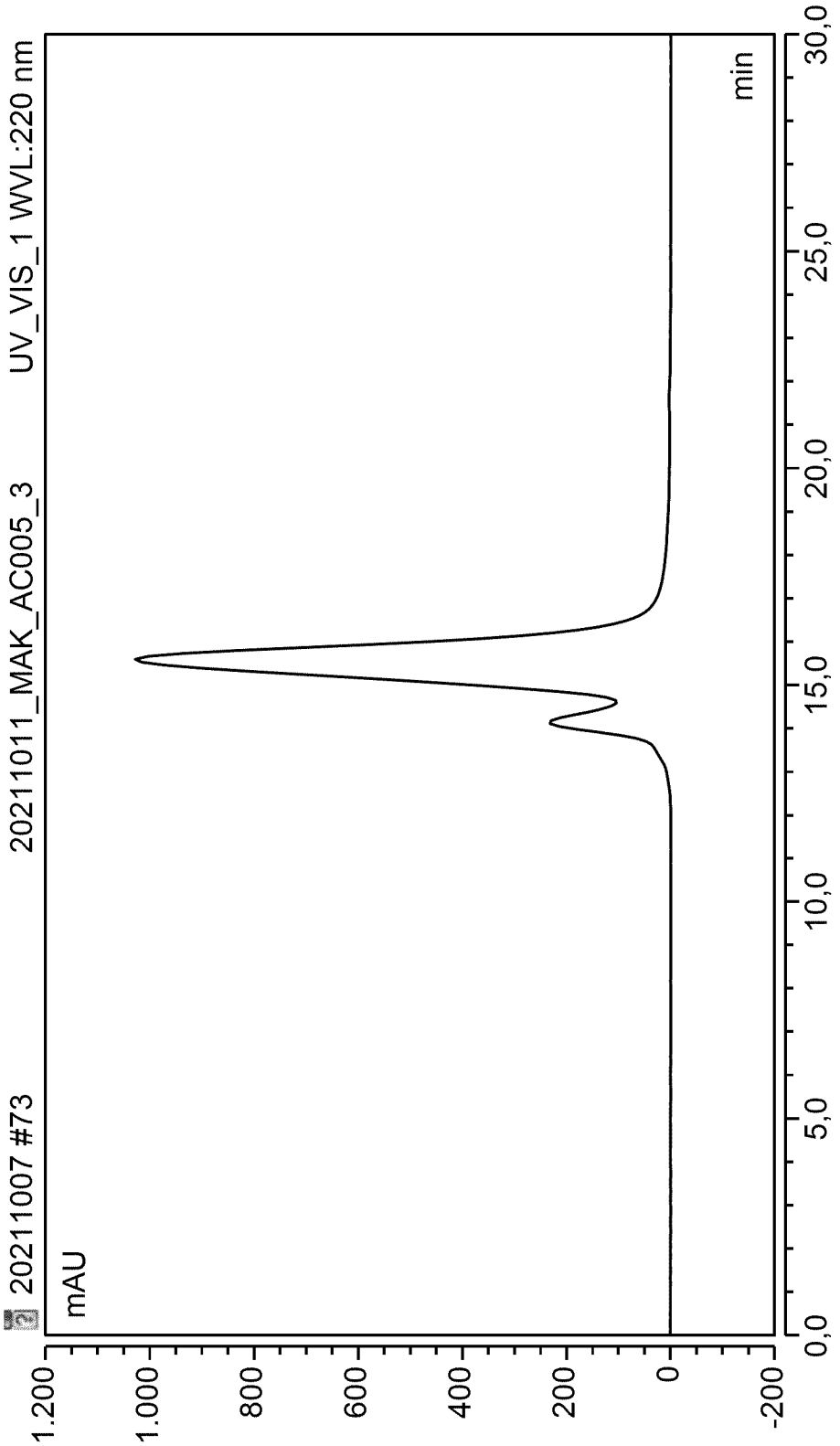

20211007 #73
20211011_MAK_AC005_3
UV_VIS_1 WVL:220 nm
mAU
min
1.200
1.000
800
600
400
200
0
-200
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 31

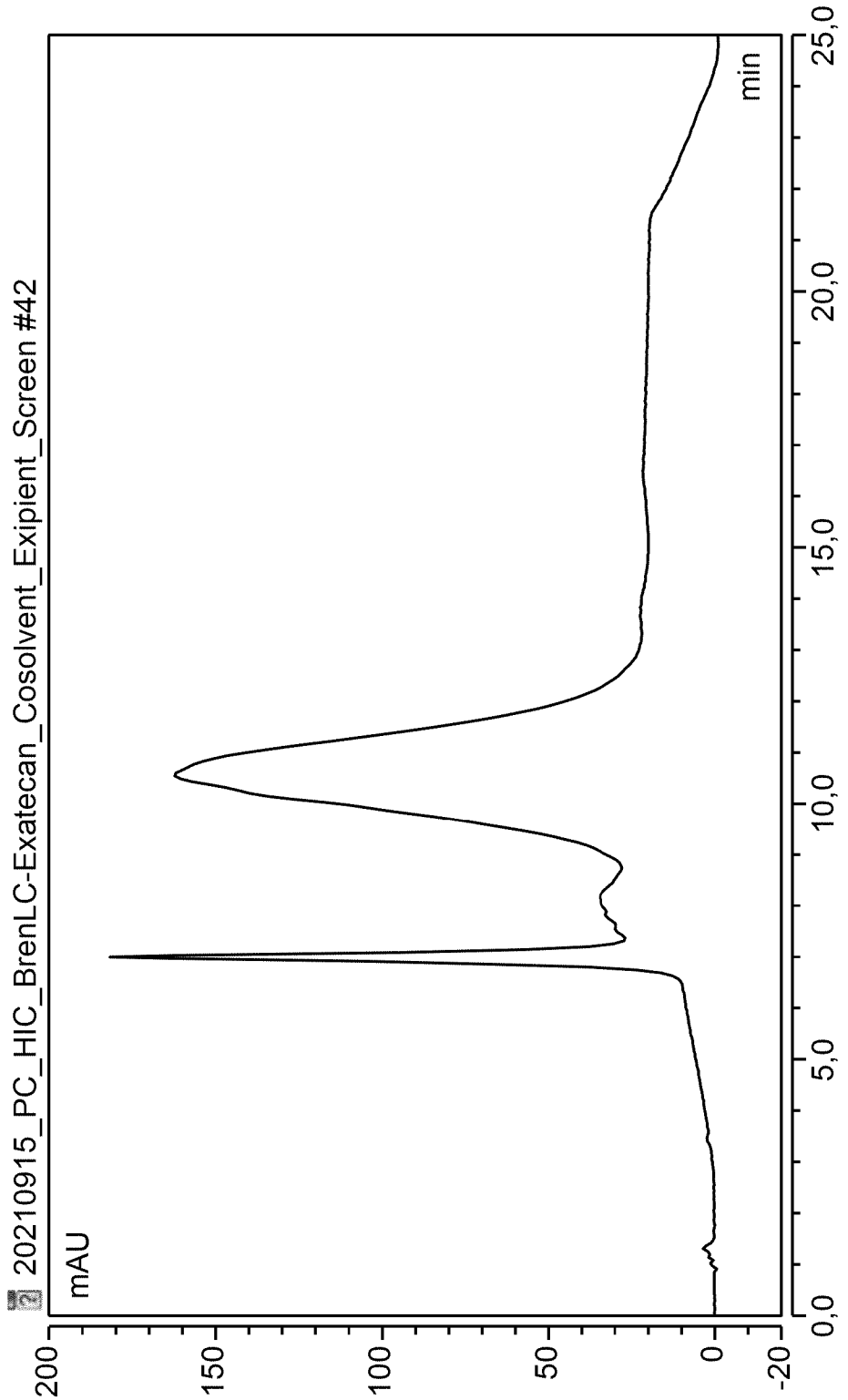

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #42
mAU
min
200
150
100
50
0
-20
0,0
5,0
10,0
15,0
20,0
25,0

Figure 32

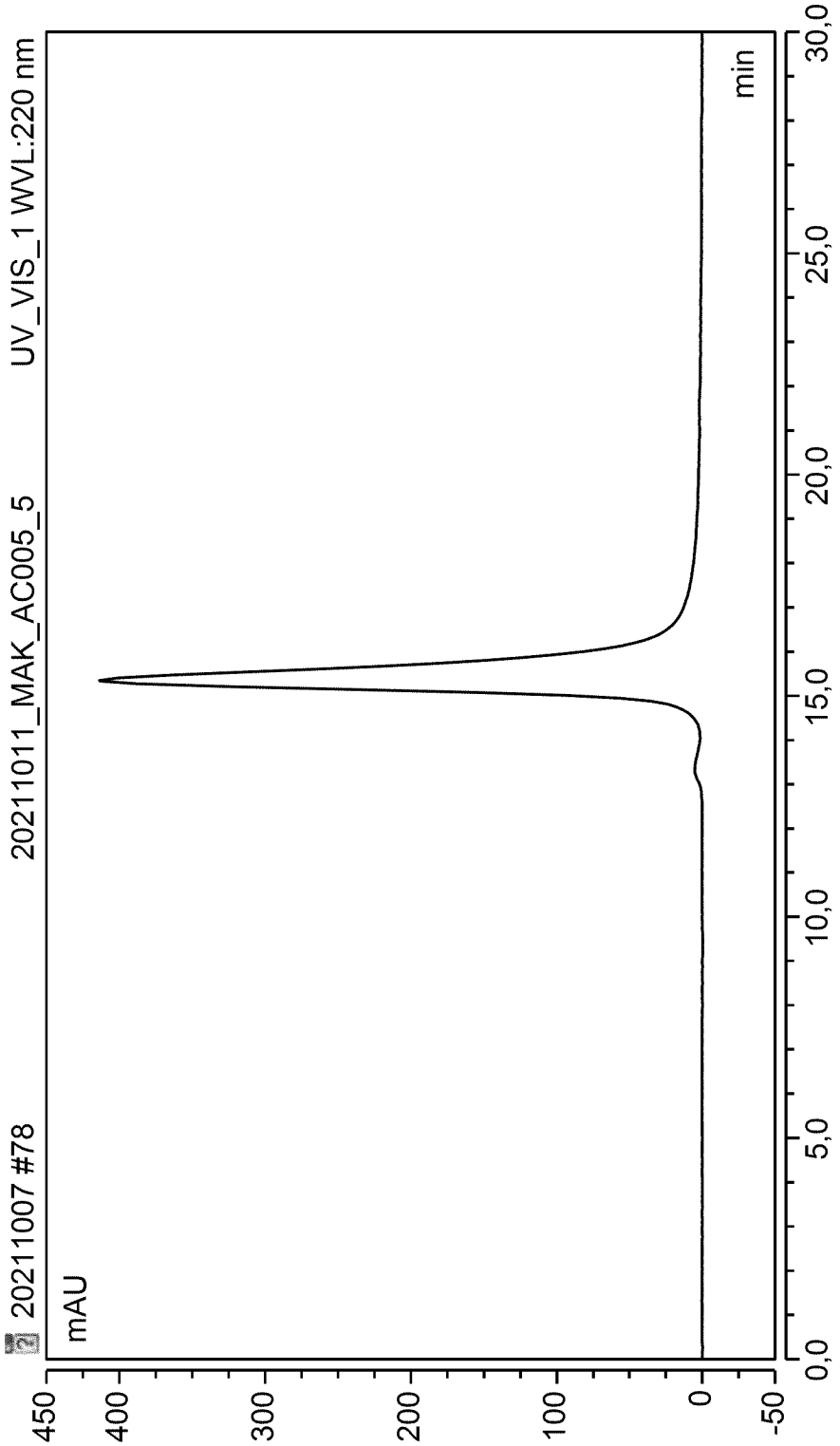

20211007 #78
20211011_MAK_AC005_5
UV_VIS_1 WVL:220 nm
mAU
min
450
400
300
200
100
0
-50
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 33

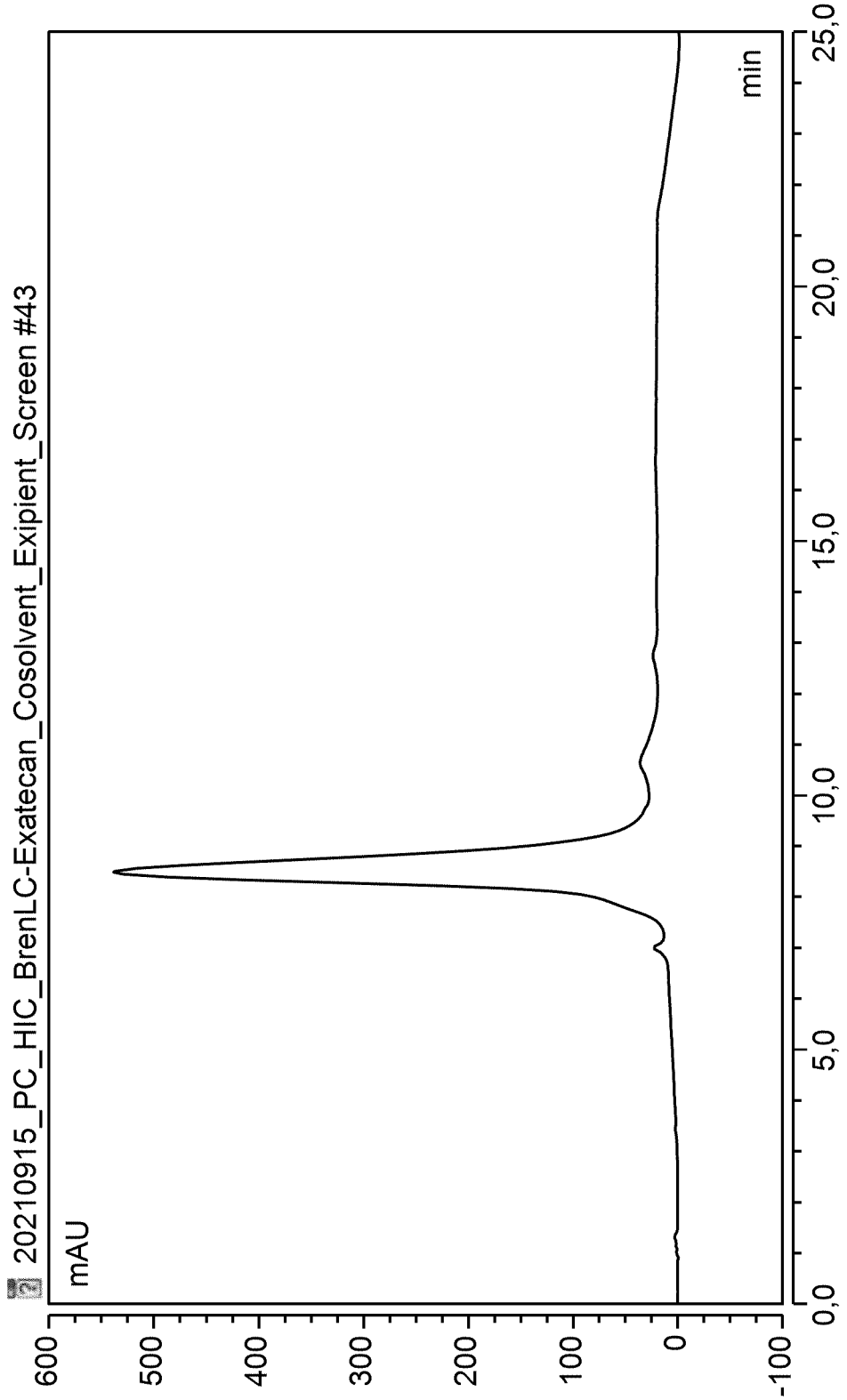

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #43
mAU
min
600
500
400
300
200
100
0
-100
0,0
5,0
10,0
15,0
20,0
25,0

Figure 34

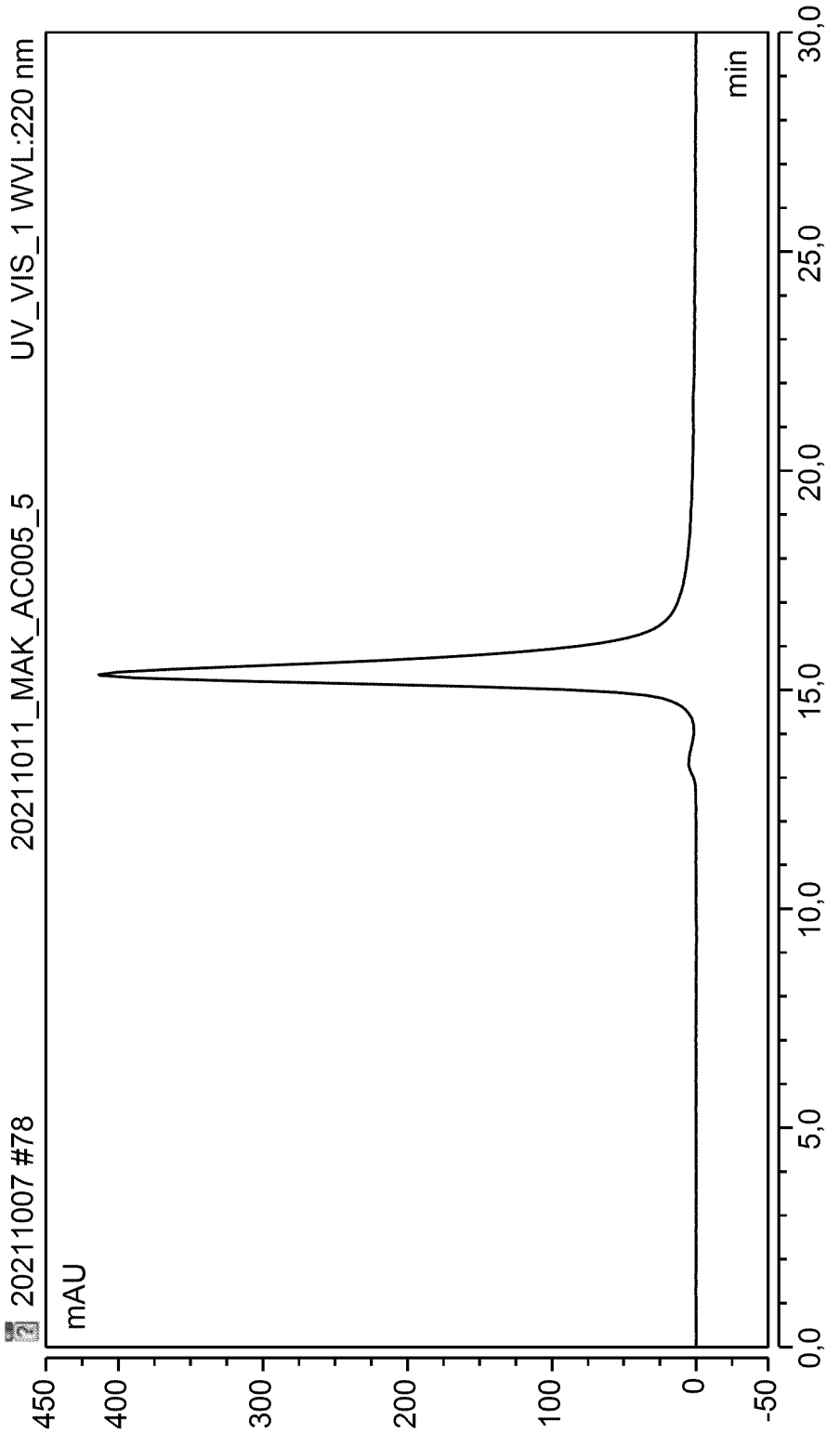

20211007 #78
20211011_MAK_AC005_5
UV_VIS_1 WVL:220 nm
mAU
min
450
400
300
200
100
0
-50
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 35

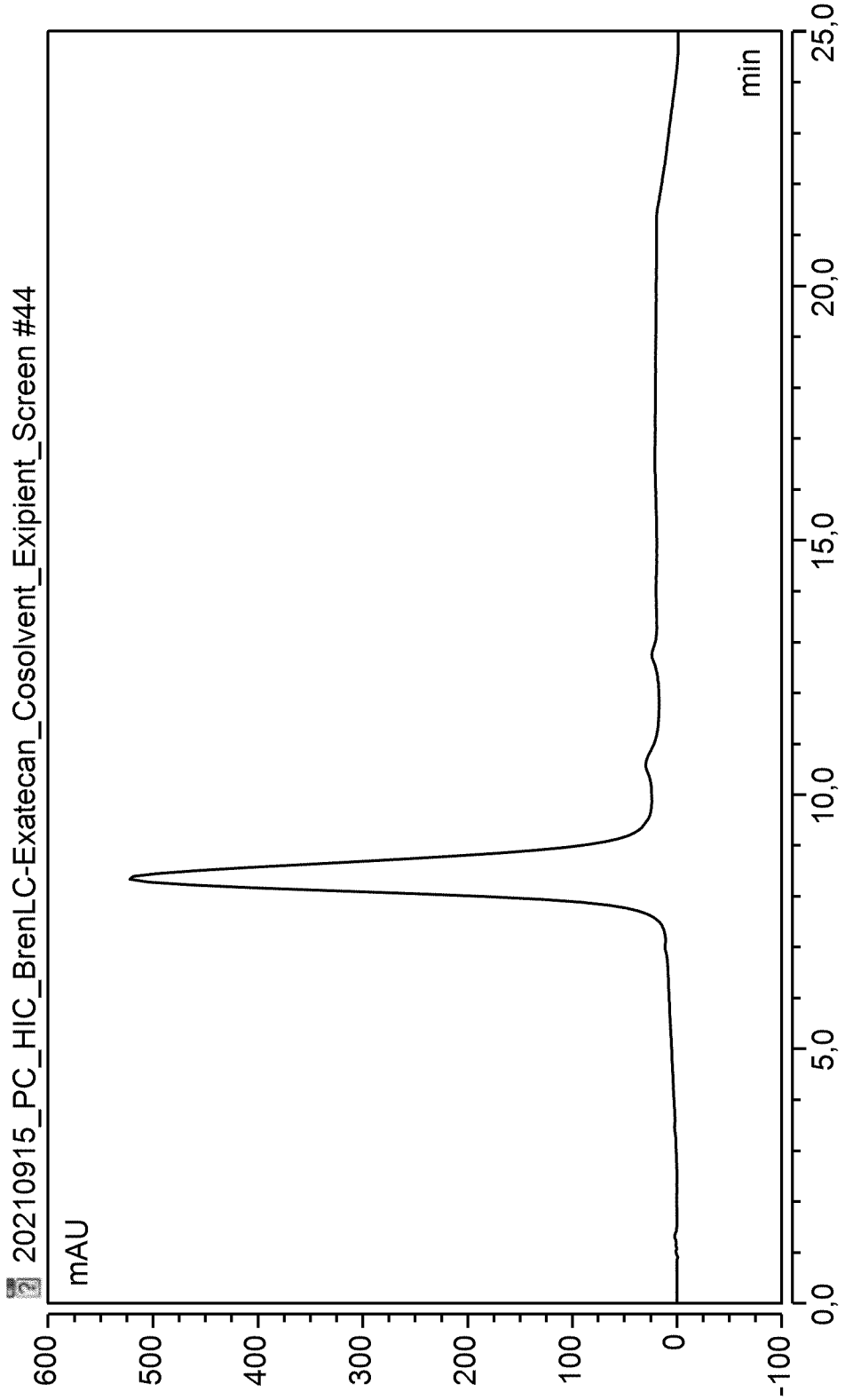

20210915_PC_HIC_BrenLC-Exatecan_Cosolvent_Exipient_Screen #44
mAU
min
600
500
400
300
200
100
0
-100
0,0
5,0
10,0
15,0
20,0
25,0

Figure 36

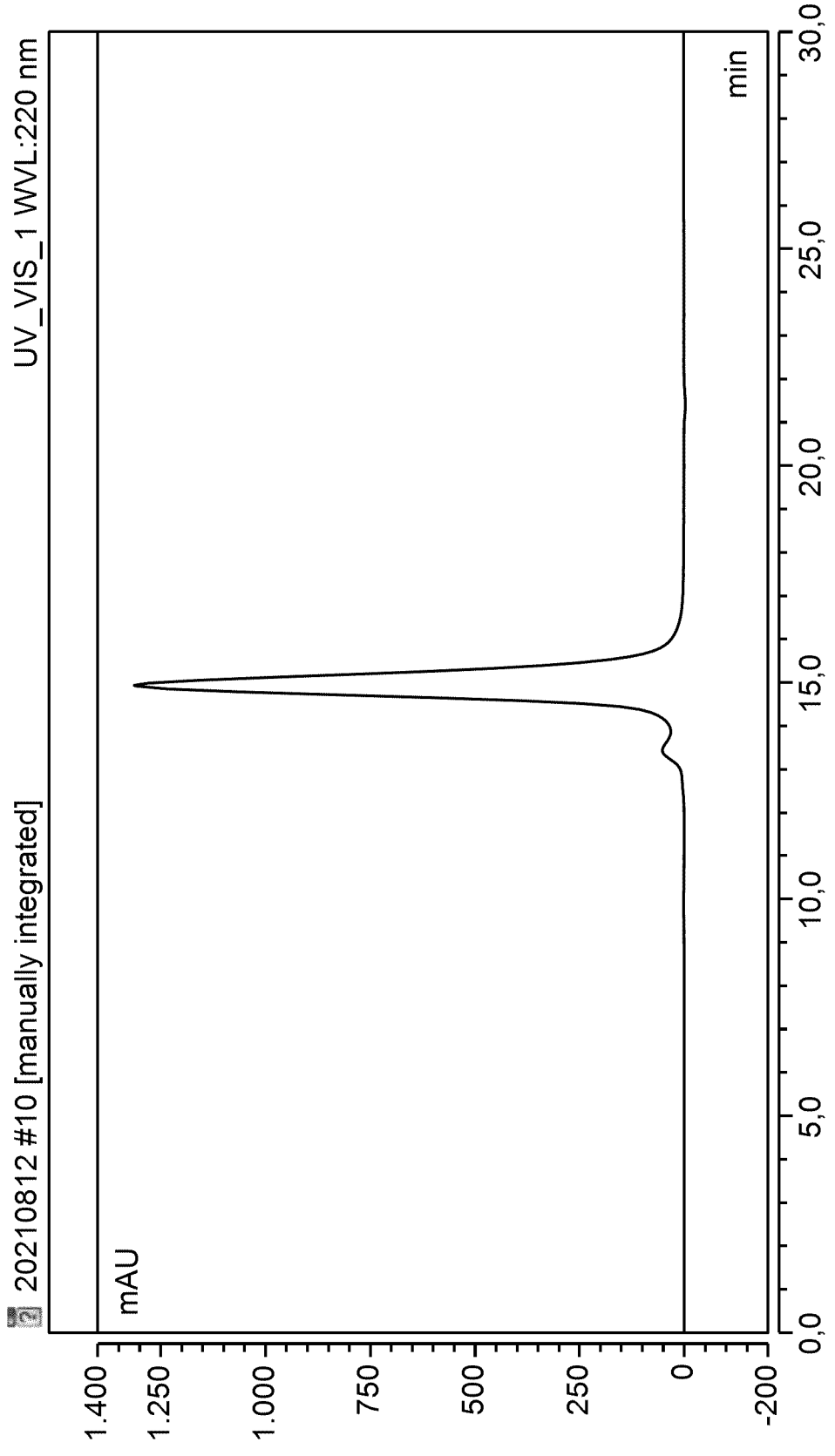

20210812 #10 [manually integrated]
UV_VIS_1 WVL:220 nm
mAU
min
1.400
1.250
1.000
750
500
250
0
-200
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 37

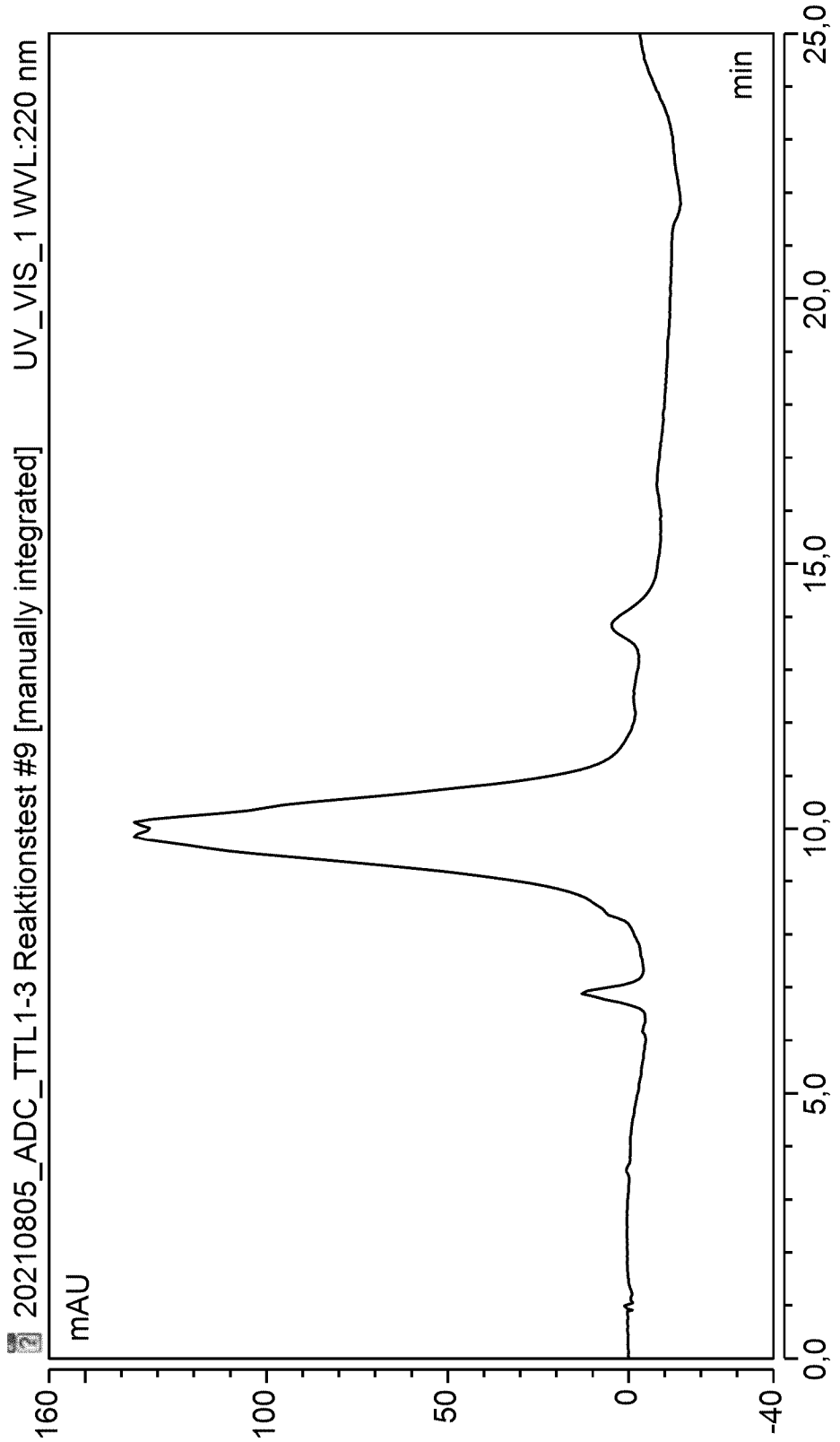

20210805_ADC_TTL1-3 Reaktionstest #9 [manually integrated] UV_VIS_1 WVL:220 nm
mAU
min
160
100
50
0
-40
0,0
5,0
10,0
15,0
20,0
25,0

Figure 38

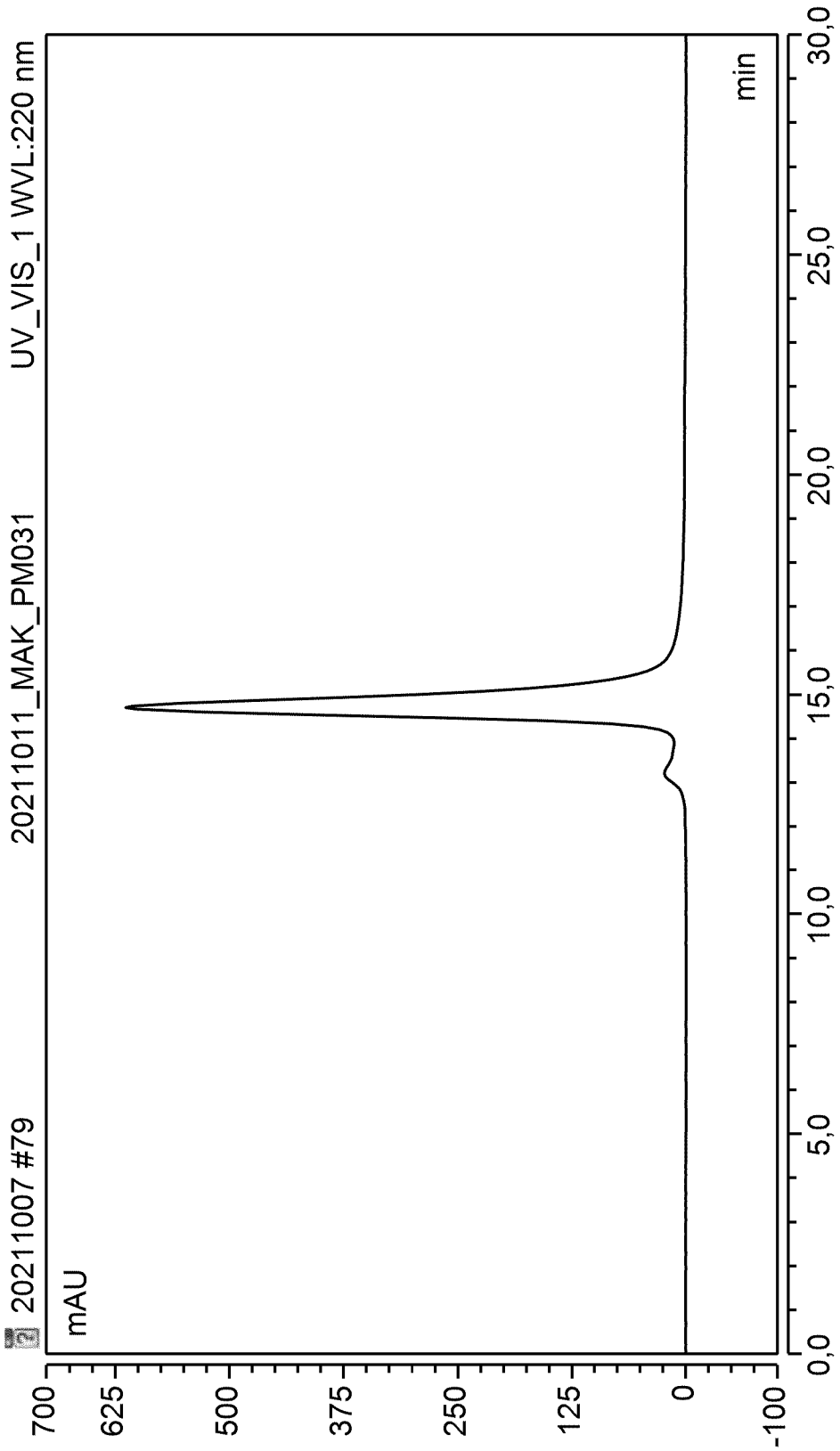

20211007 #79
mAU
20211011_MAK_PM031
UV_VIS_1 WVL:220 nm
min
700
625
500
375
250
125
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 39

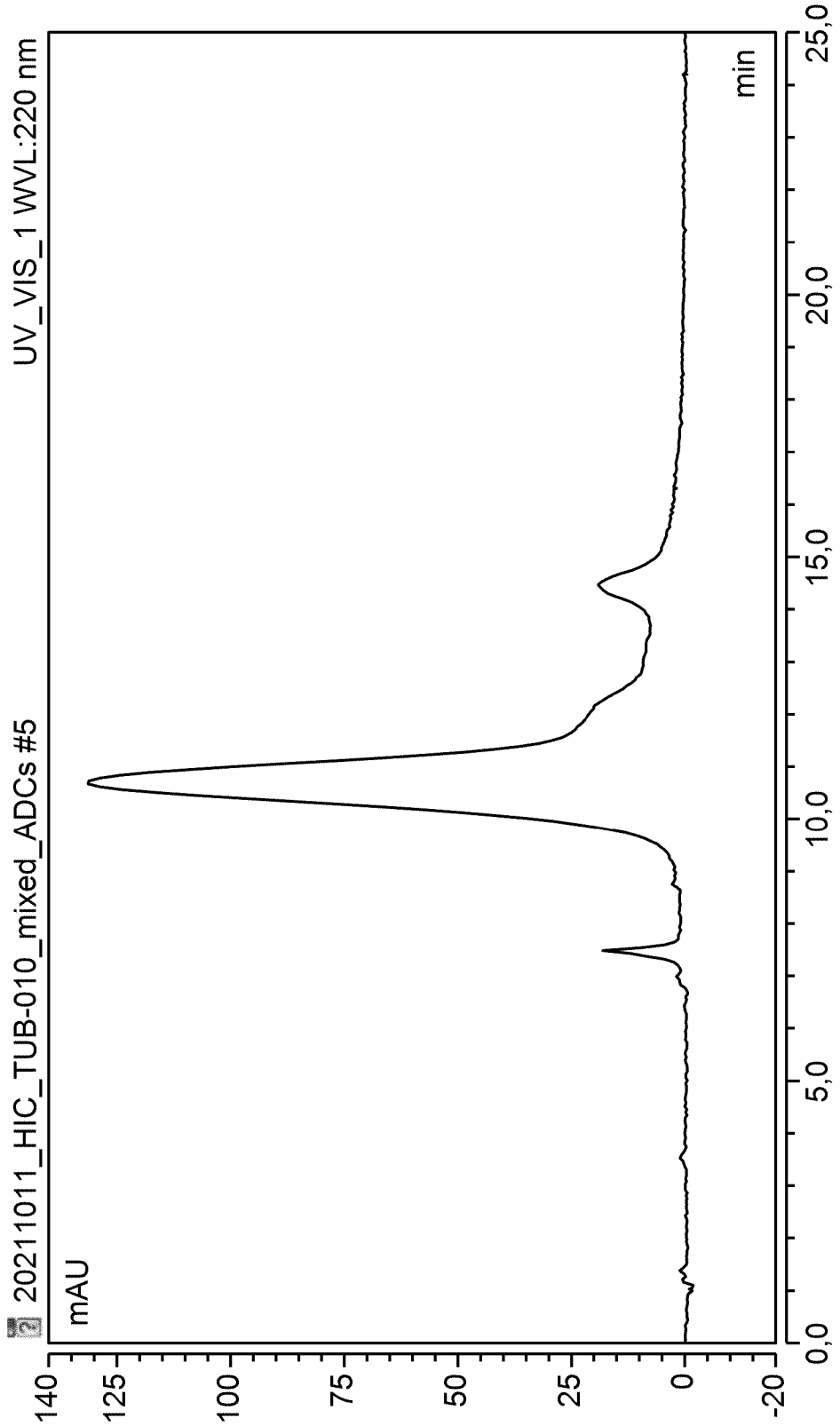

20211011_HIC_TUB-010_mixed_ADCs #5
UV_VIS_1 WVL:220 nm
mAU
min
140
125
100
75
50
25
0
-20
0,0
5,0
10,0
15,0
20,0
25,0

Figure 40

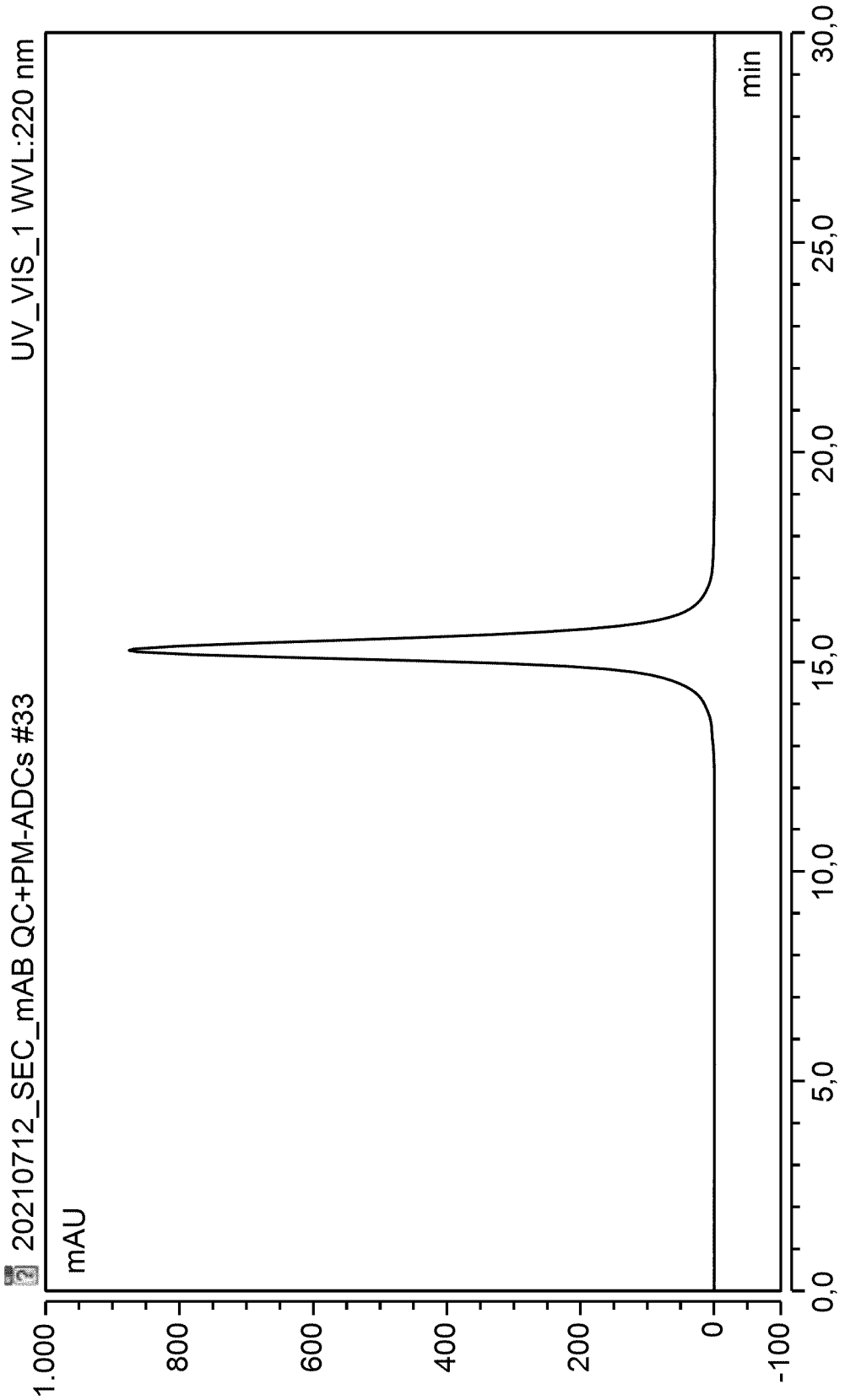

20210712_SEC_mAB QC+PM-ADCs #33
UV_VIS_1 WVL:220 nm
mAU
min
1.000
800
600
400
200
0
-100
0,0
5,0
10,0
15,0
20,0
25,0
30,0

Figure 41

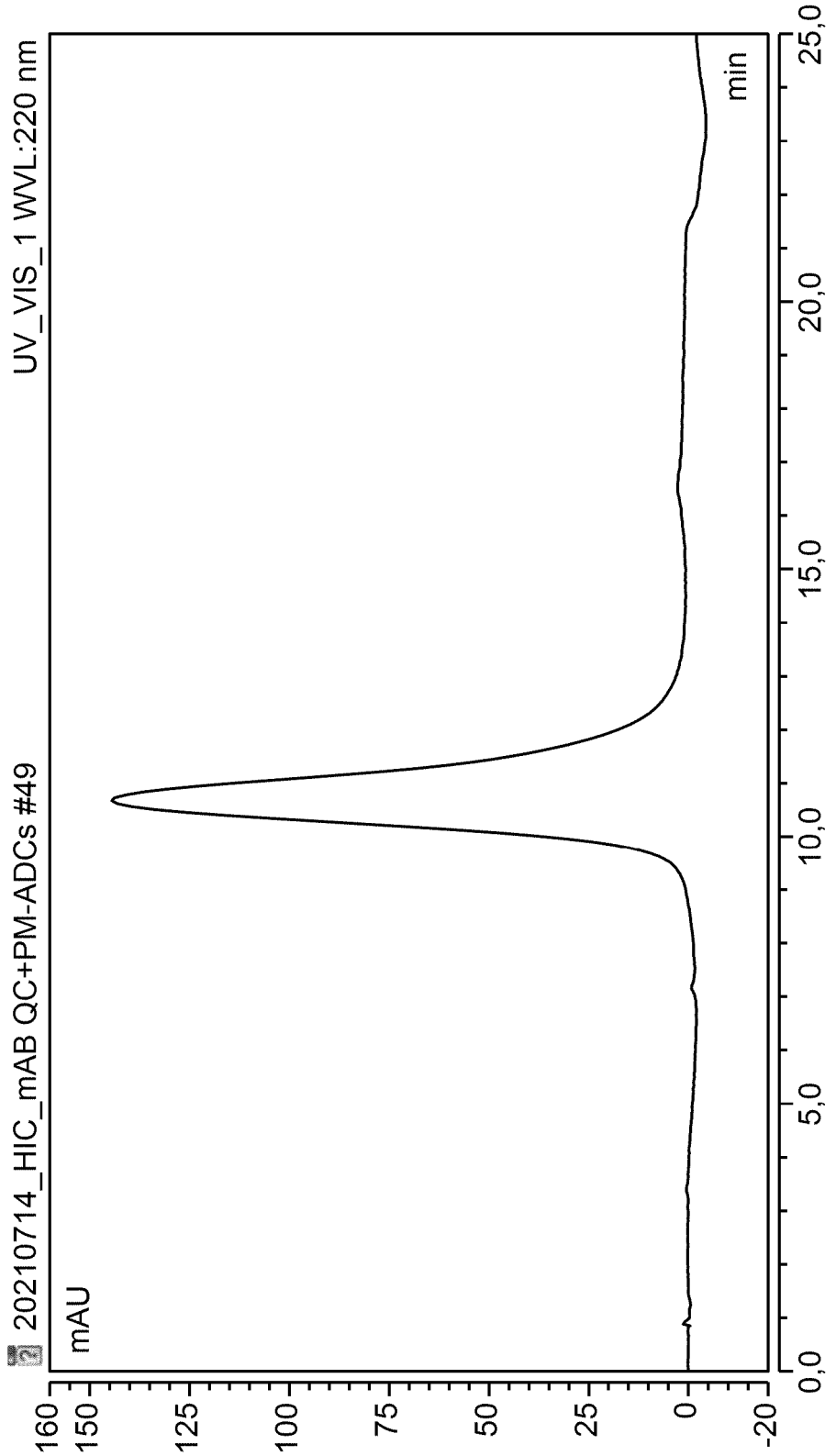

20210714_HIC_mAB QC+PM-ADCs #49
UV_VIS_1 WVL:220 nm
mAU
min
160
150
125
100
75
50
25
0
-20
0,0
5,0
10,0
15,0
20,0
25,0

Figure 42

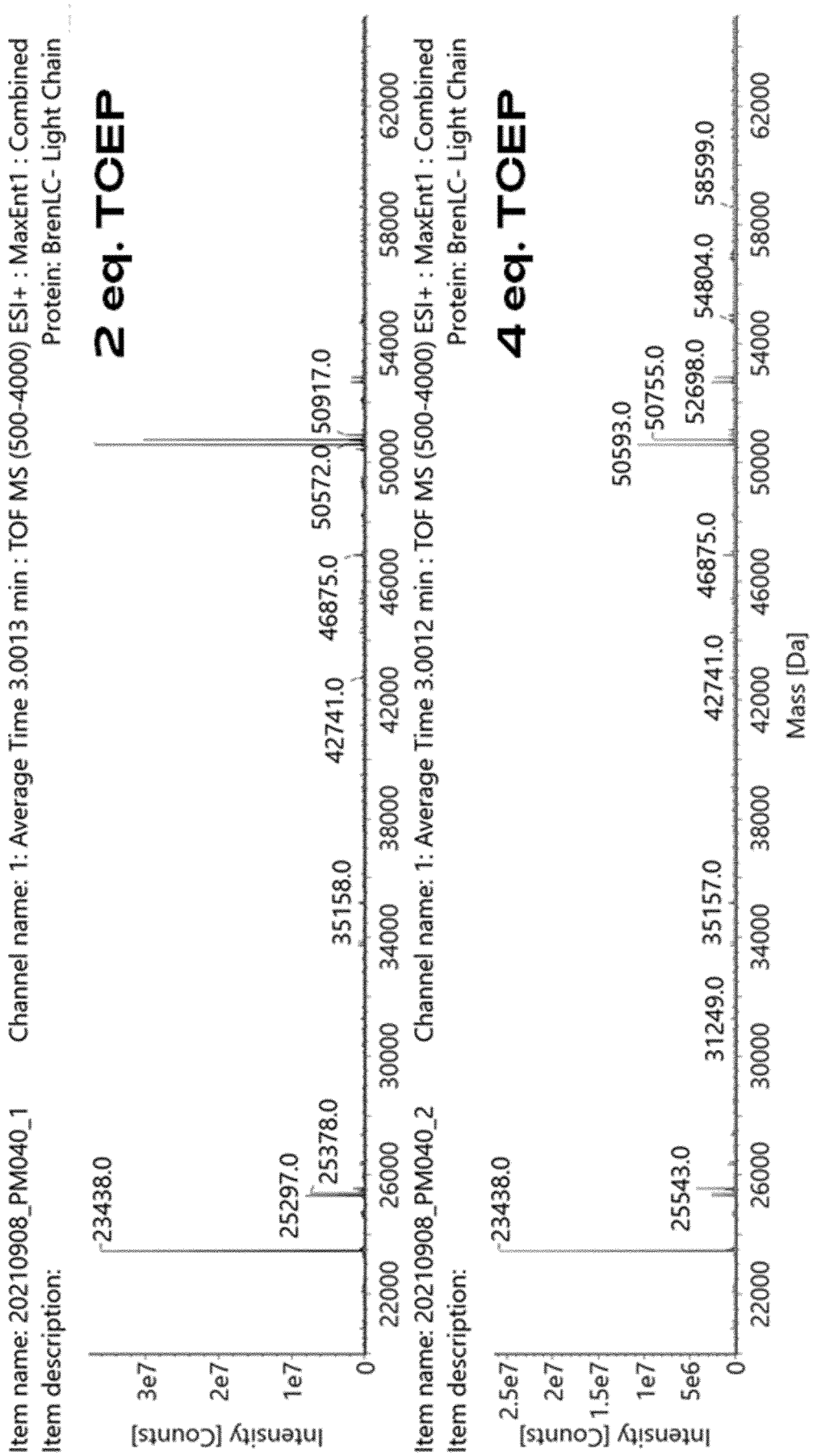

Item name: 20210908_PM040_1
Item description:
Channel name: 1: Average Time 3.0013 min : TOF MS (500-4000) ESI+ : MaxEnt1 : Combined
Protein: BrenLC- Light Chain
2 eq. TCEP
23438.0
25297.0
25378.0
35158.0
42741.0
46875.0
50572.0
50917.0
Intensity [Counts]
3e7
2e7
1e7
0
22000
26000
30000
34000
38000
42000
46000
50000
54000
58000
62000
Item name: 20210908_PM040_2
Item description:
Channel name: 1: Average Time 3.0012 min : TOF MS (500-4000) ESI+ : MaxEnt1 : Combined
Protein: BrenLC- Light Chain
4 eq. TCEP
23438.0
25543.0
31249.0
35157.0
42741.0
46875.0
50593.0
50755.0
52698.0
54804.0
58599.0
Intensity [Counts]
2.5e7
2e7
1.5e7
1e7
5e6
0
Mass [Da]

Figure 42 (continued)

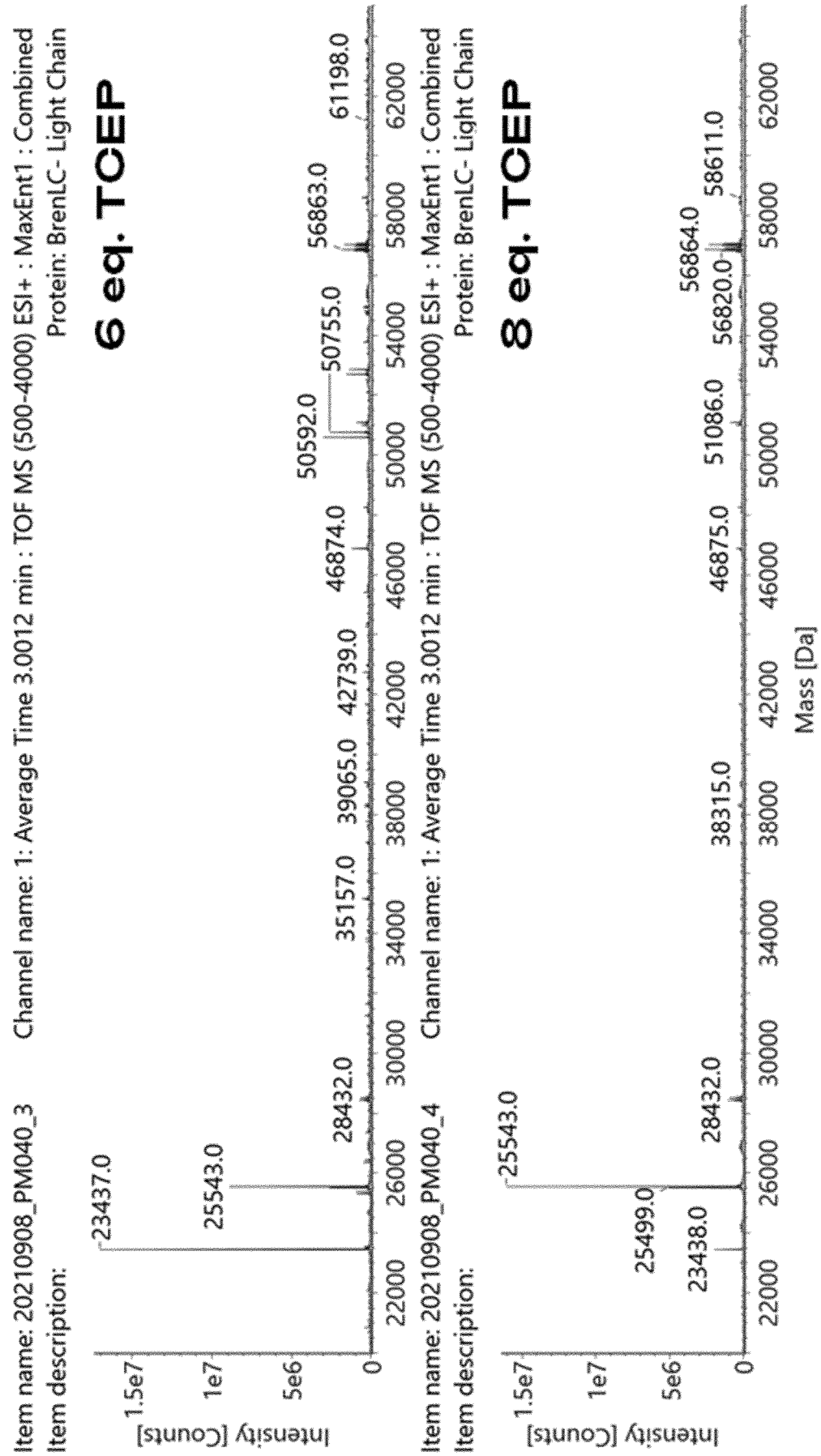


Figure 42 (continued)

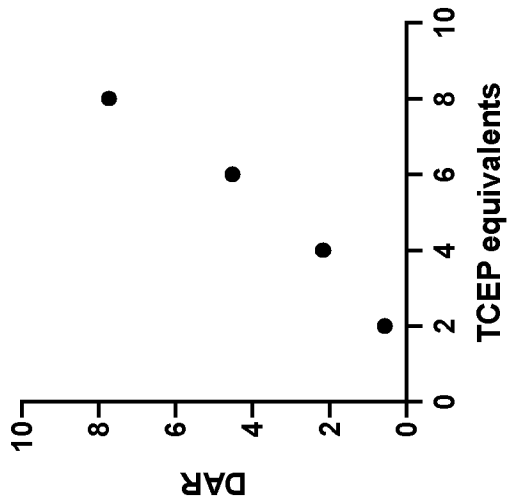


Figure 43

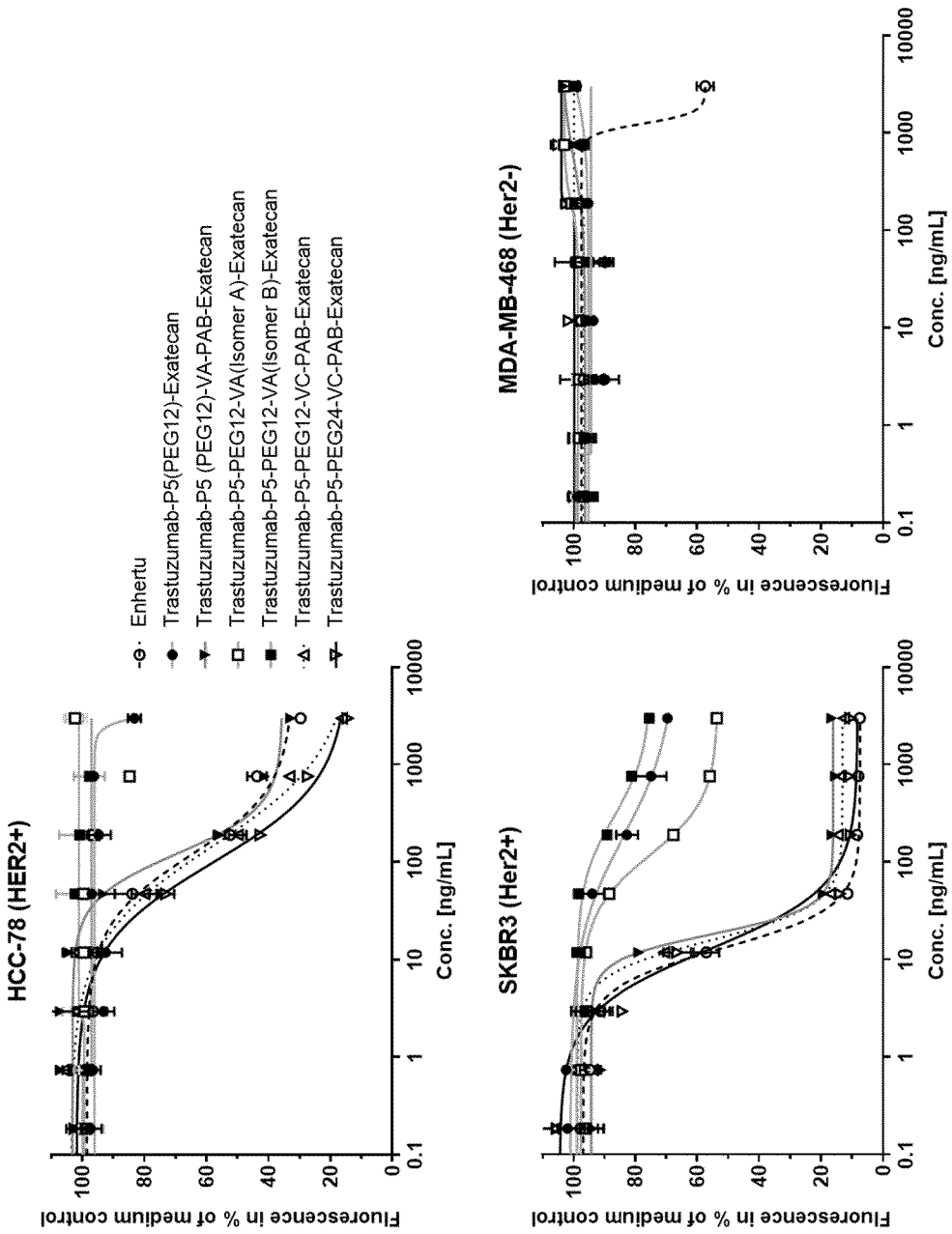

HCC-78 (HER2+)
SKBR3 (Her2+)
MDA-MB-468 (Her2-)
Enhertu
Trastuzumab-P5(PEG12)-Exatecan
Trastuzumab-P5 (PEG12)-VA-PAB-Exatecan
Trastuzumab-P5-PEG12-VA(Isomer A)-Exatecan
Trastuzumab-P5-PEG12-VA(Isomer B)-Exatecan
Trastuzumab-P5-PEG12-VC-PAB-Exatecan
Trastuzumab-P5-PEG24-VC-PAB-Exatecan
Fluorescence in % of medium control
Conc. [ng/mL]
0
20
40
60
80
100
0.1
1
10
100
1000
10000

Figure 44

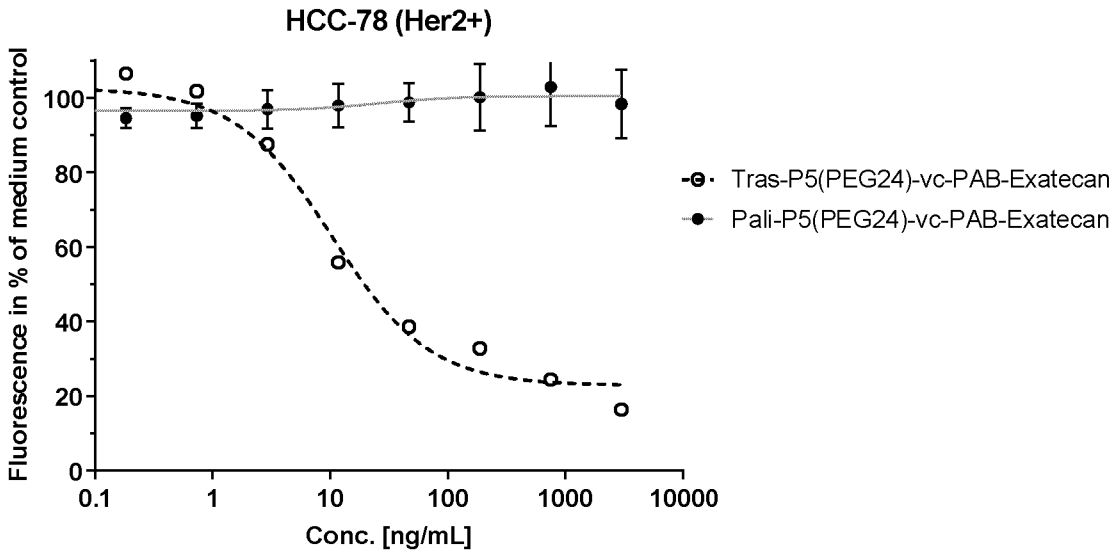


Figure 45

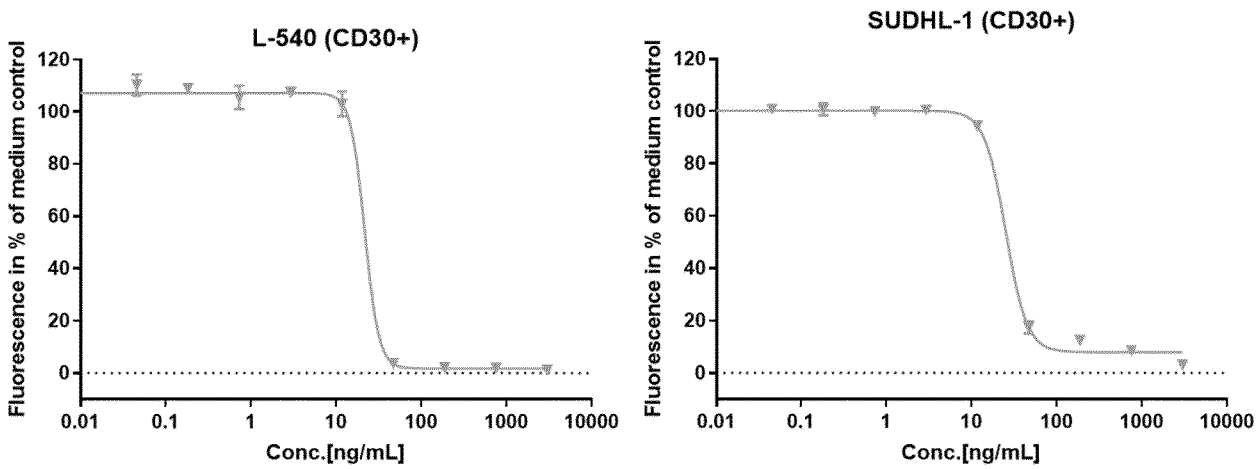


Figure 46

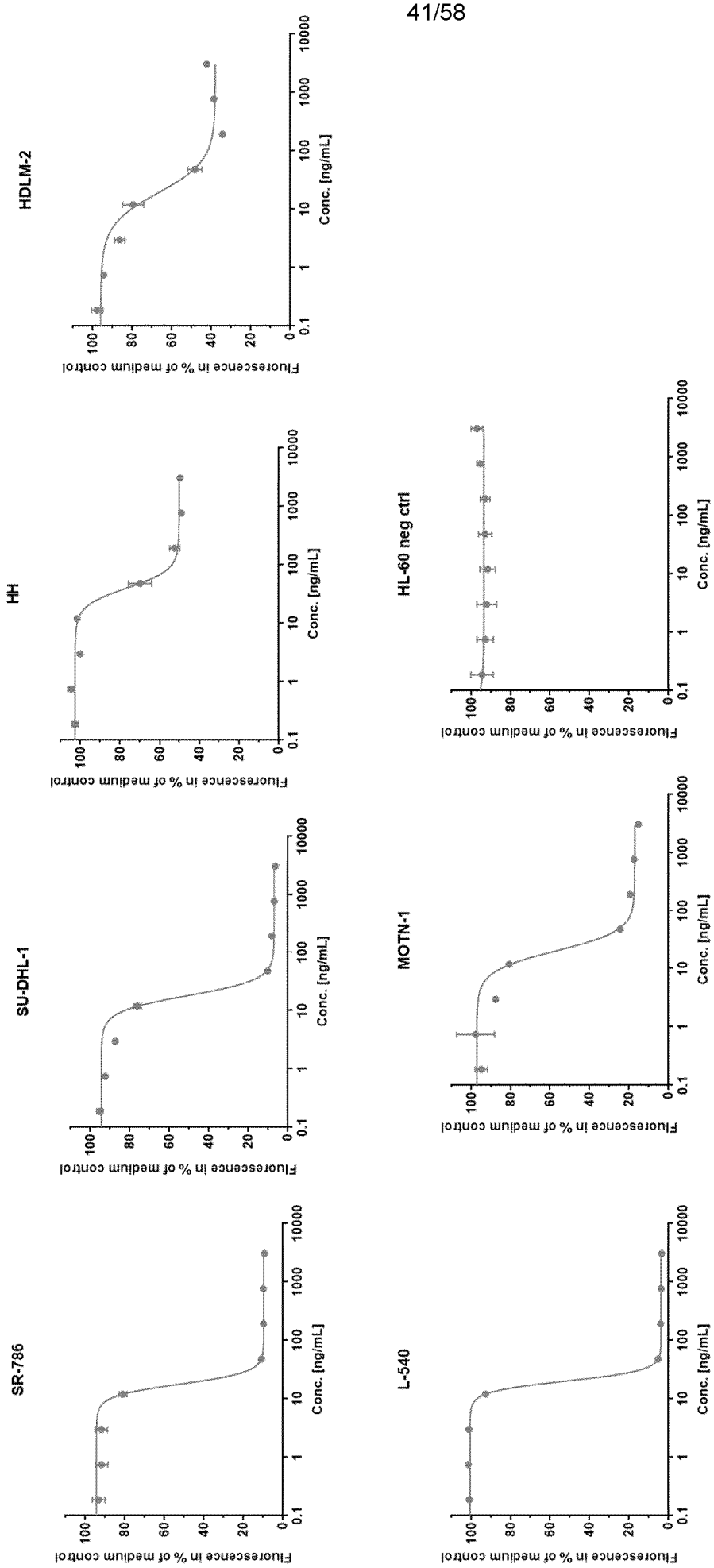

HDLM-2
HH
HL-60 neg ctrl
SU-DHL-1
MOTN-1
SR-786
L-540
Fluorescence in % of medium control
Conc. [ng/mL]
0
20
40
60
80
100
0.1
1
10
100
1000
10000

Figure 47

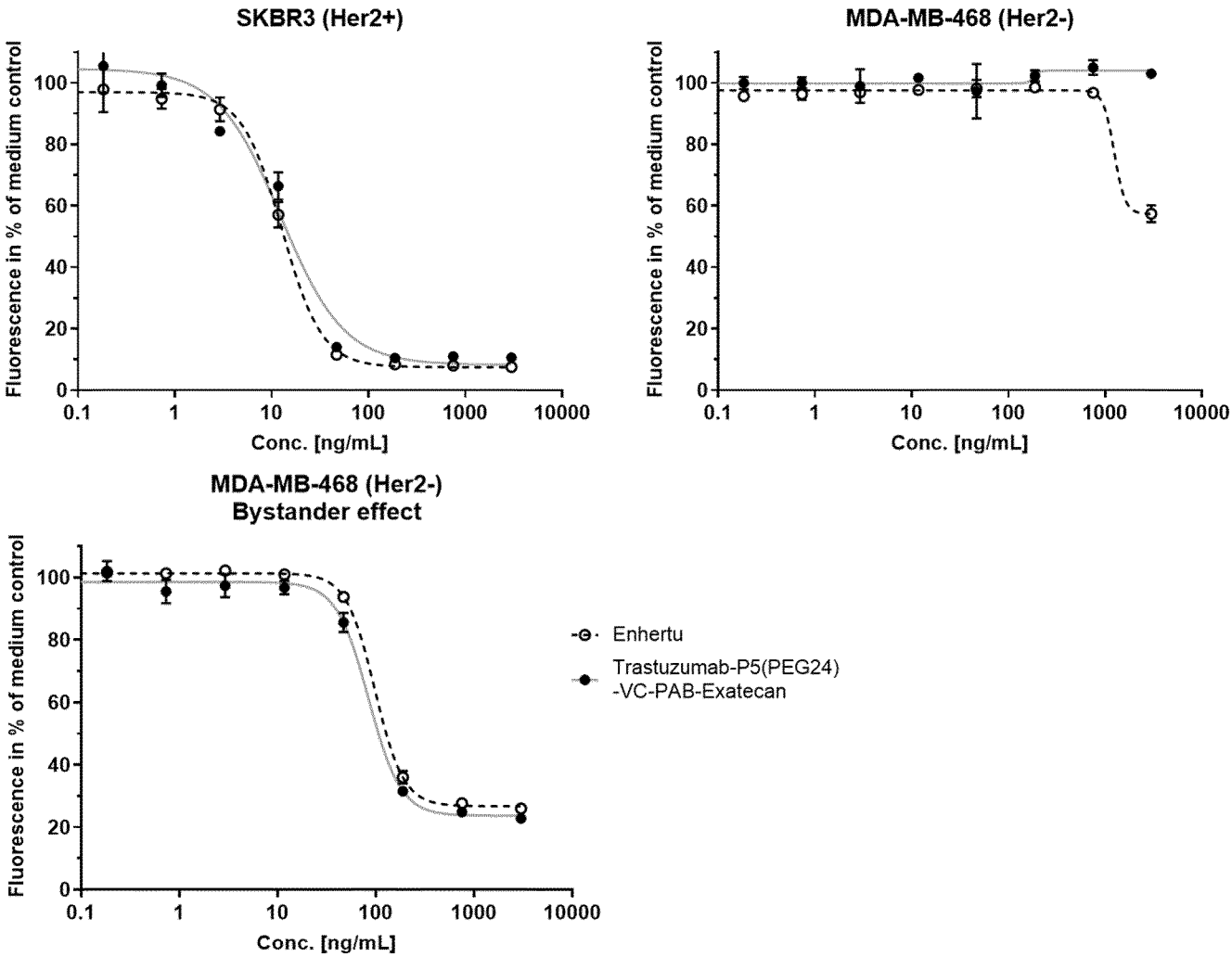

SKBR3 (Her2+)
MDA-MB-468 (Her2-)
MDA-MB-468 (Her2-)
Bystander effect
Fluorescence in % of medium control
Conc. [ng/mL]
0
20
40
60
80
100
0.1
1
10
100
1000
10000
Enhertu
Trastuzumab-P5(PEG24)
-VC-PAB-Exatecan

Figure 48

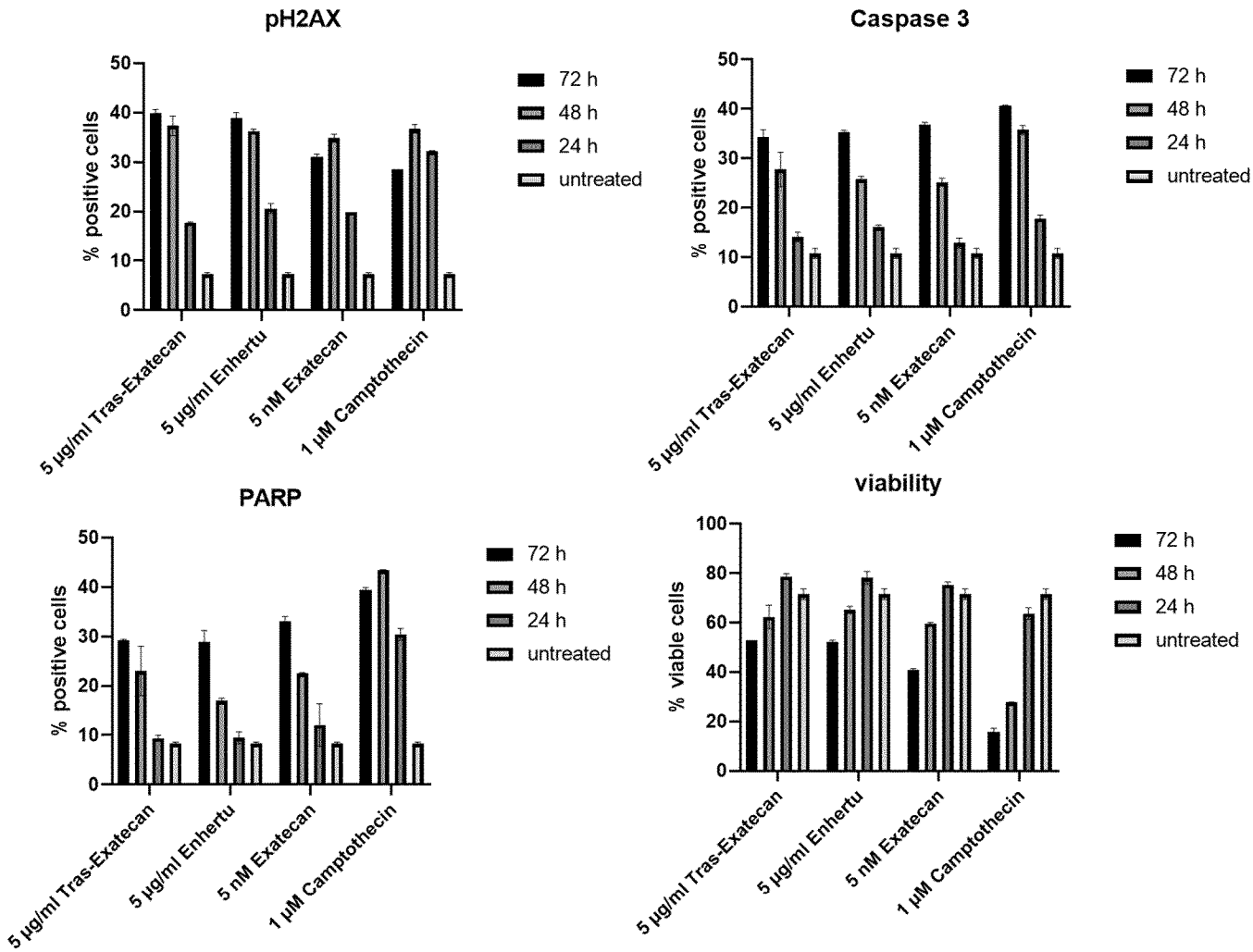

pH2AX
% positive cells
0
10
20
30
40
50
72 h
48 h
24 h
untreated
5 µg/ml Tras-Exatecan
5 µg/ml Enhertu
5 nM Exatecan
1 µM Camptothecin
Caspase 3
% positive cells
0
10
20
30
40
50
72 h
48 h
24 h
untreated
5 µg/ml Tras-Exatecan
5 µg/ml Enhertu
5 nM Exatecan
1 µM Camptothecin
PARP
% positive cells
0
10
20
30
40
50
72 h
48 h
24 h
untreated
5 µg/ml Tras-Exatecan
5 µg/ml Enhertu
5 nM Exatecan
1 µM Camptothecin
viability
% viable cells
0
20
40
60
80
100
72 h
48 h
24 h
untreated
5 µg/ml Tras-Exatecan
5 µg/ml Enhertu
5 nM Exatecan
1 µM Camptothecin

Figure 49

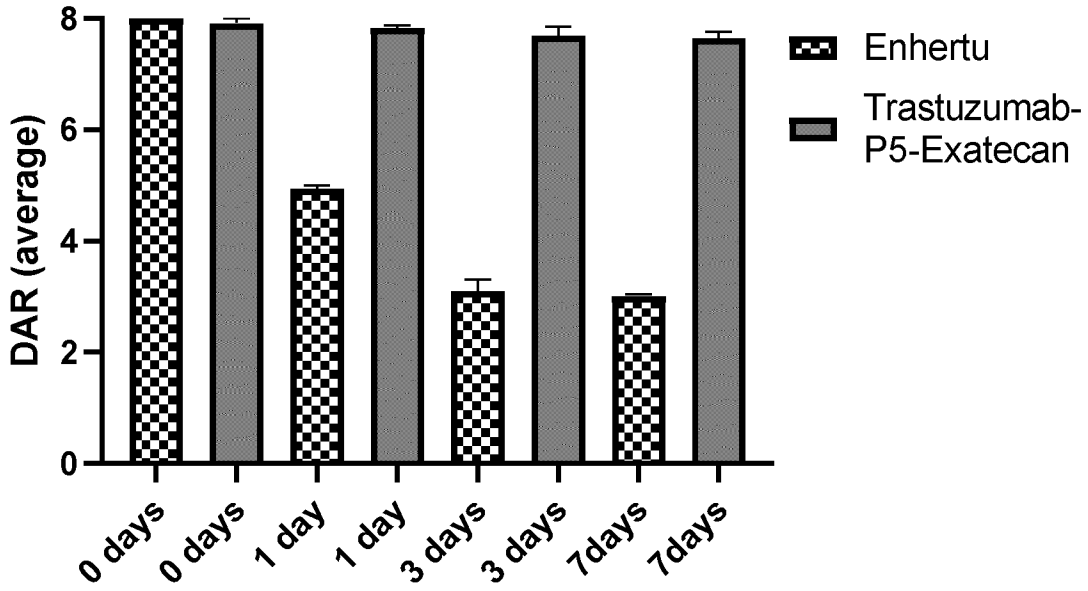

DAR (average)
8
6
4
2
0
0 days
0 days
1 day
1 day
3 days
3 days
7days
7days
Enhertu
Trastuzumab-P5-Exatecan

Figure 50

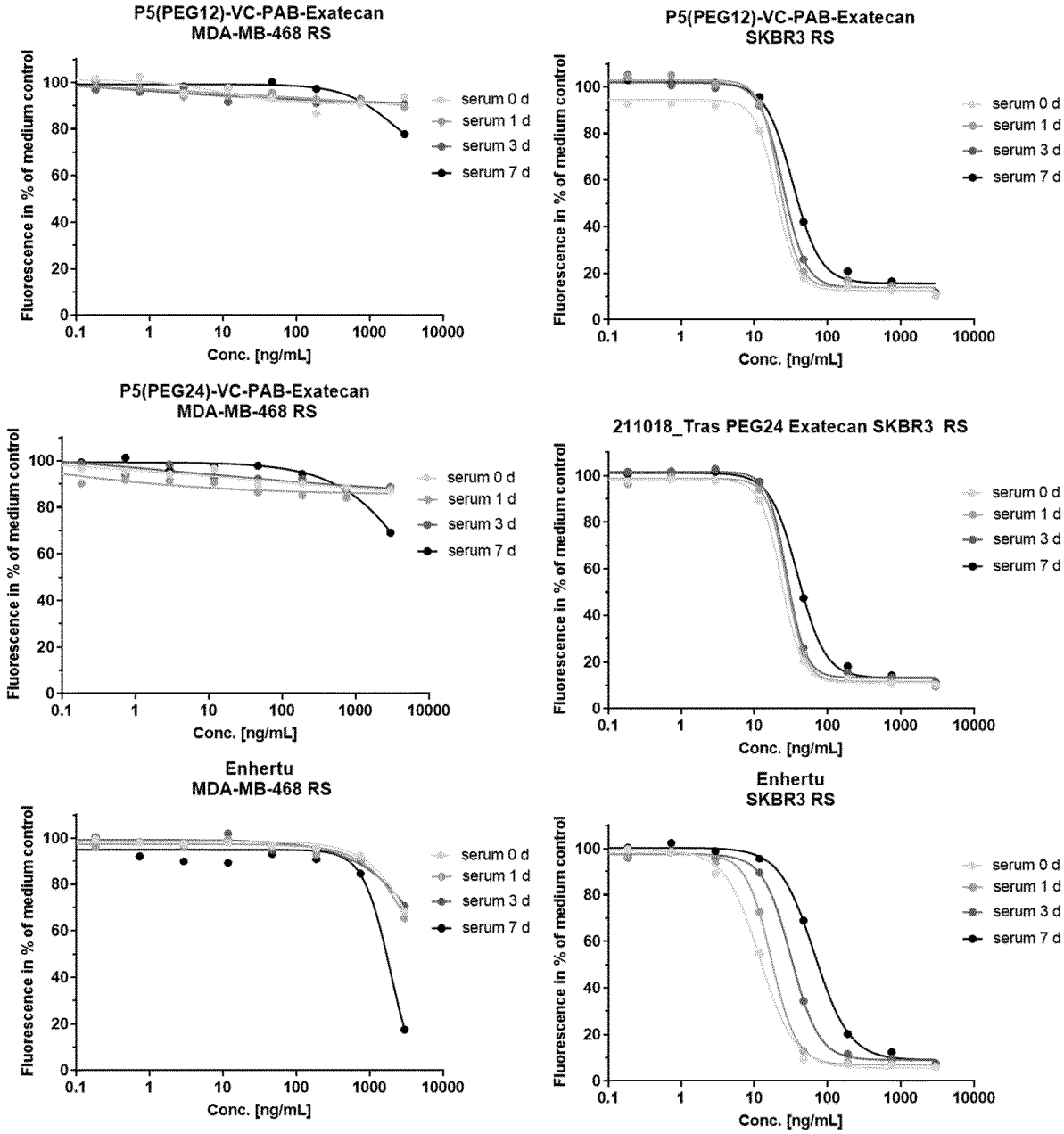

P5(PEG12)-VC-PAB-Exatecan
MDA-MB-468 RS
P5(PEG12)-VC-PAB-Exatecan
SKBR3 RS
P5(PEG24)-VC-PAB-Exatecan
MDA-MB-468 RS
211018_Tras PEG24 Exatecan SKBR3 RS
Enhertu
MDA-MB-468 RS
Enhertu
SKBR3 RS
Fluorescence in % of medium control
Conc. [ng/mL]
serum 0 d
serum 1 d
serum 3 d
serum 7 d

Figure 51

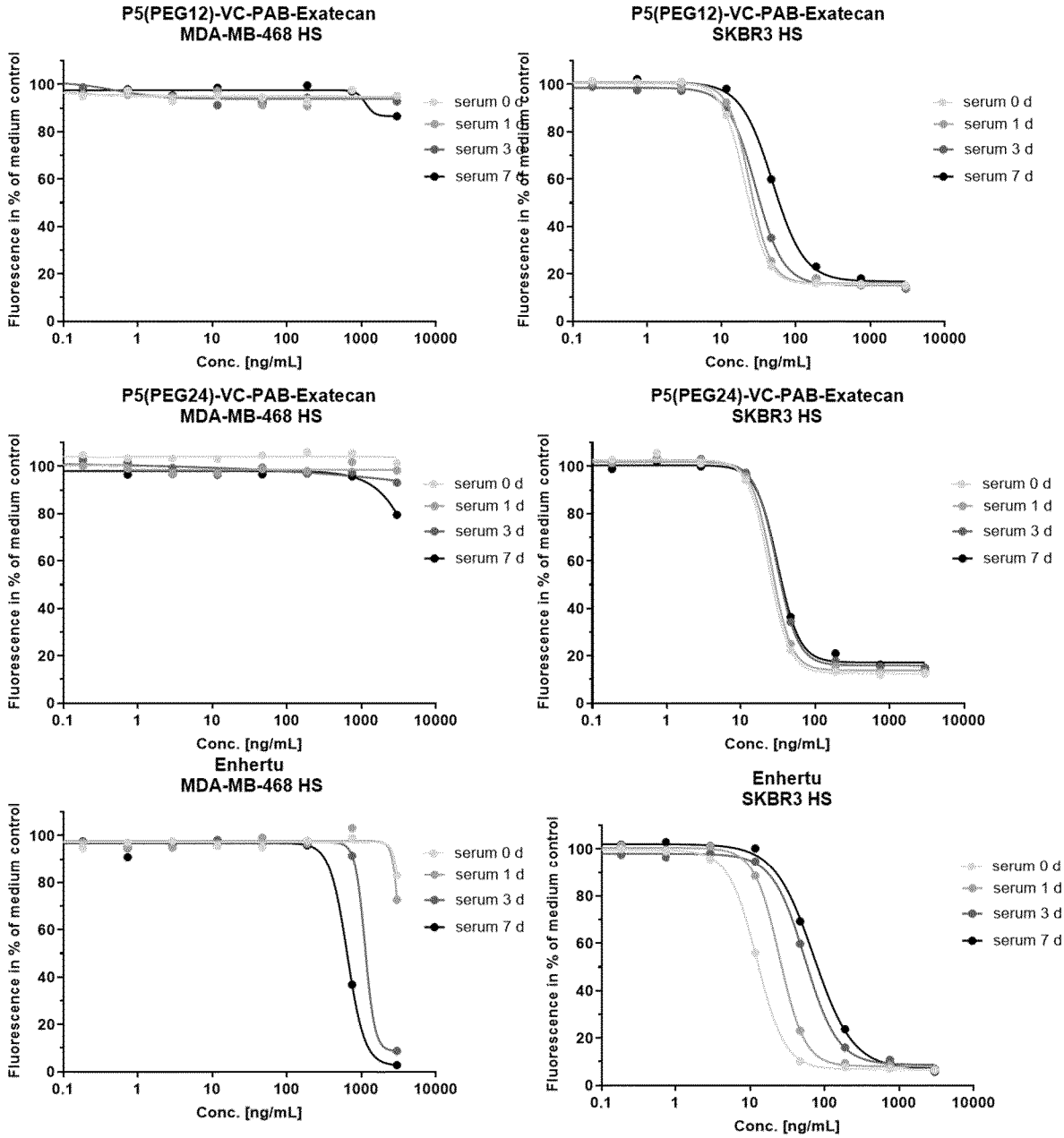

P5(PEG12)-VC-PAB-Exatecan
MDA-MB-468 HS
P5(PEG12)-VC-PAB-Exatecan
SKBR3 HS
P5(PEG24)-VC-PAB-Exatecan
MDA-MB-468 HS
P5(PEG24)-VC-PAB-Exatecan
SKBR3 HS
Enhertu
MDA-MB-468 HS
Enhertu
SKBR3 HS
Fluorescence in % of medium control
Conc. [ng/mL]
serum 0 d
serum 1 d
serum 3 d
serum 7 d

Figure 52

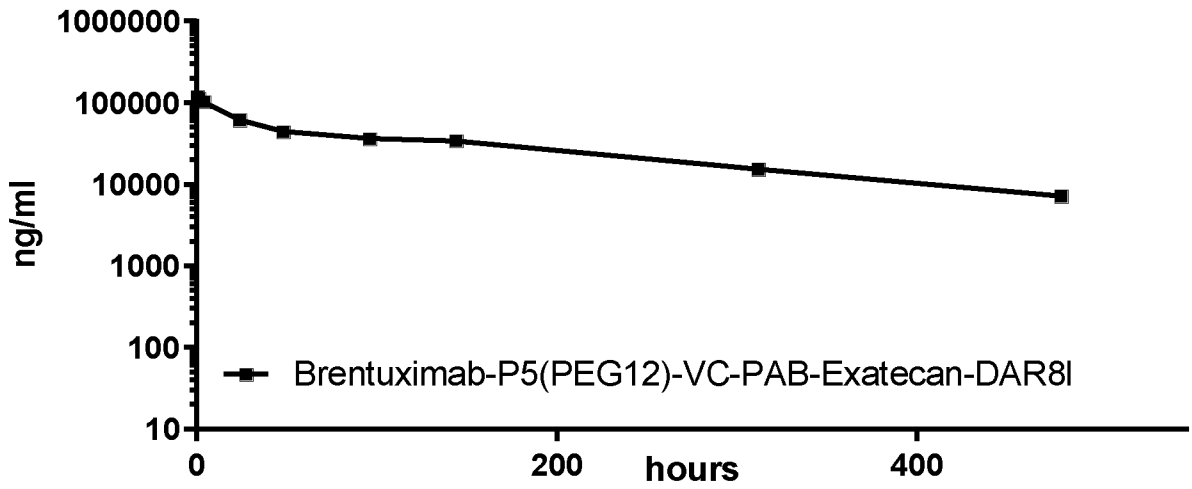


Figure 53

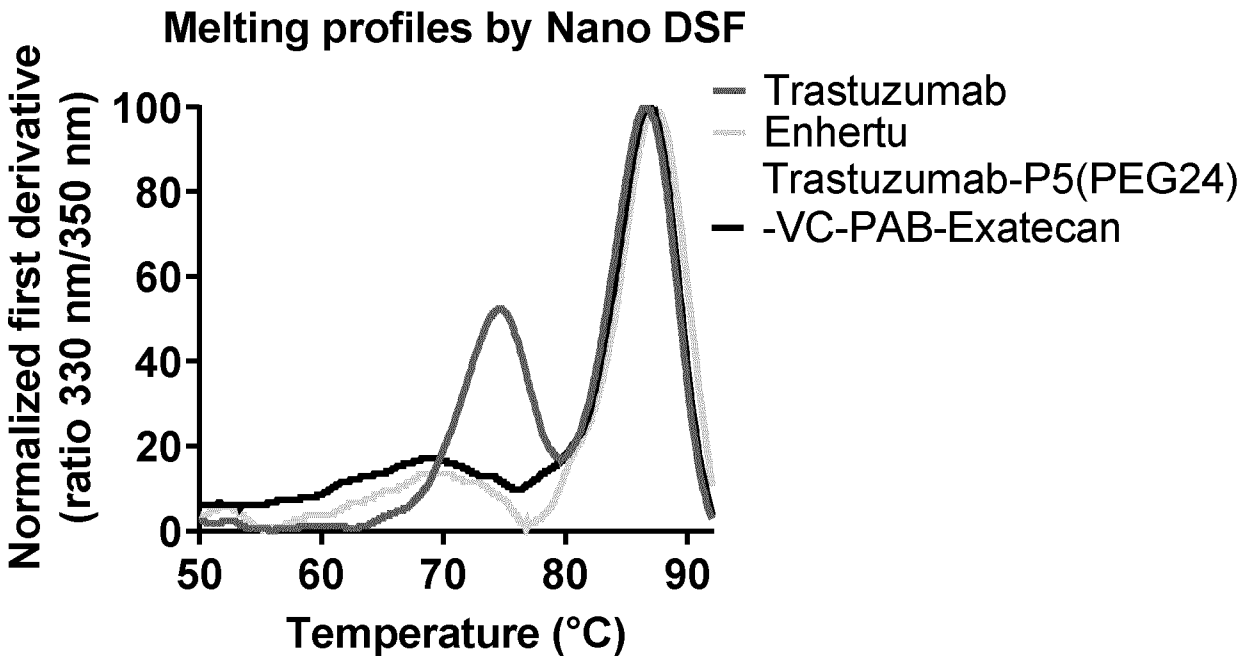
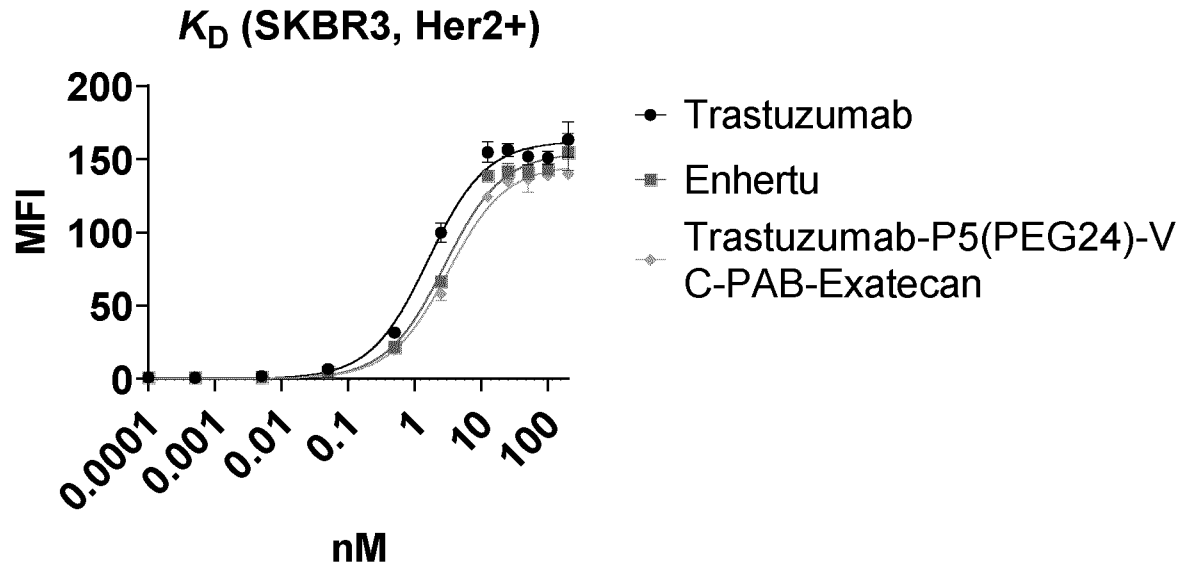


Figure 54



Trastuzumab	Enhertu	Tras-P5-Tubutecan
1.600	2.804	3.167

Figure 55

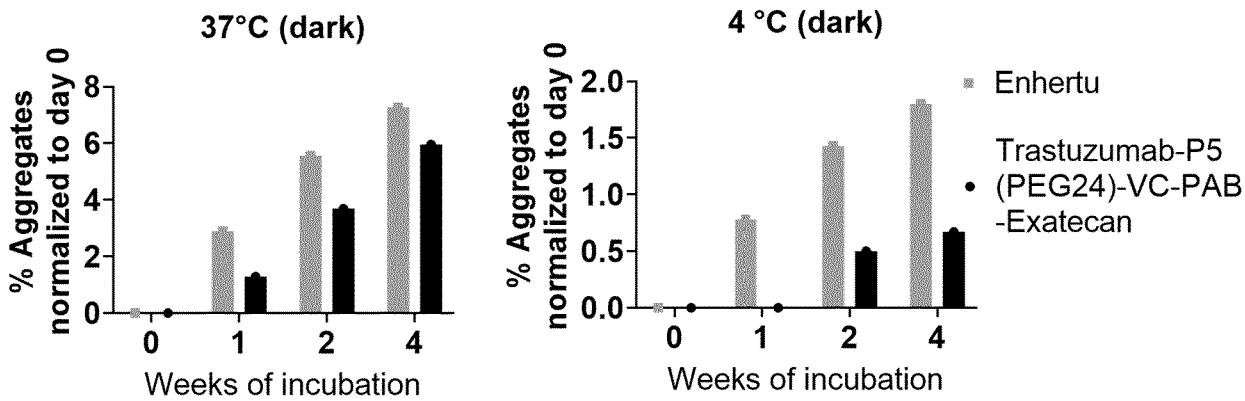


Figure 56

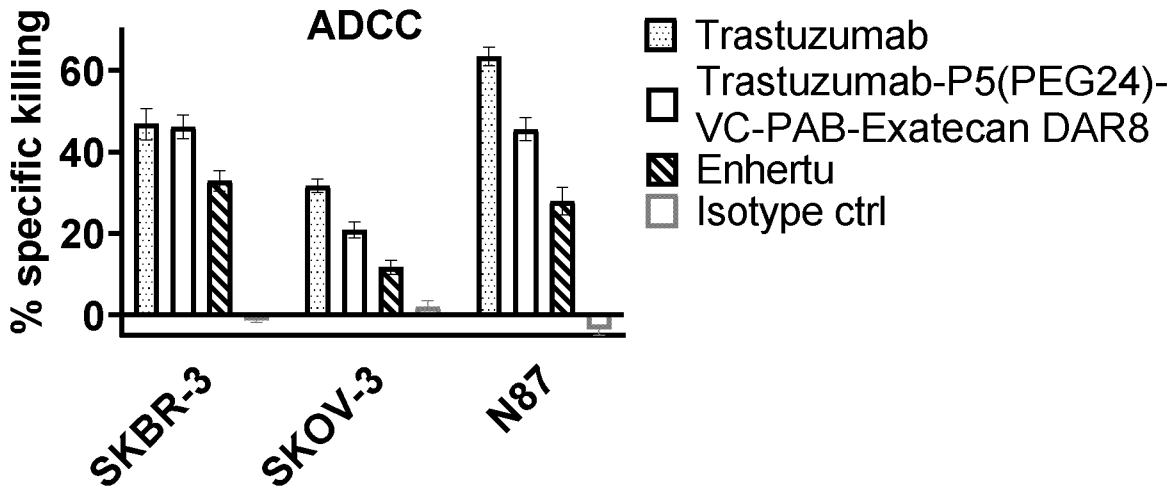


Figure 57

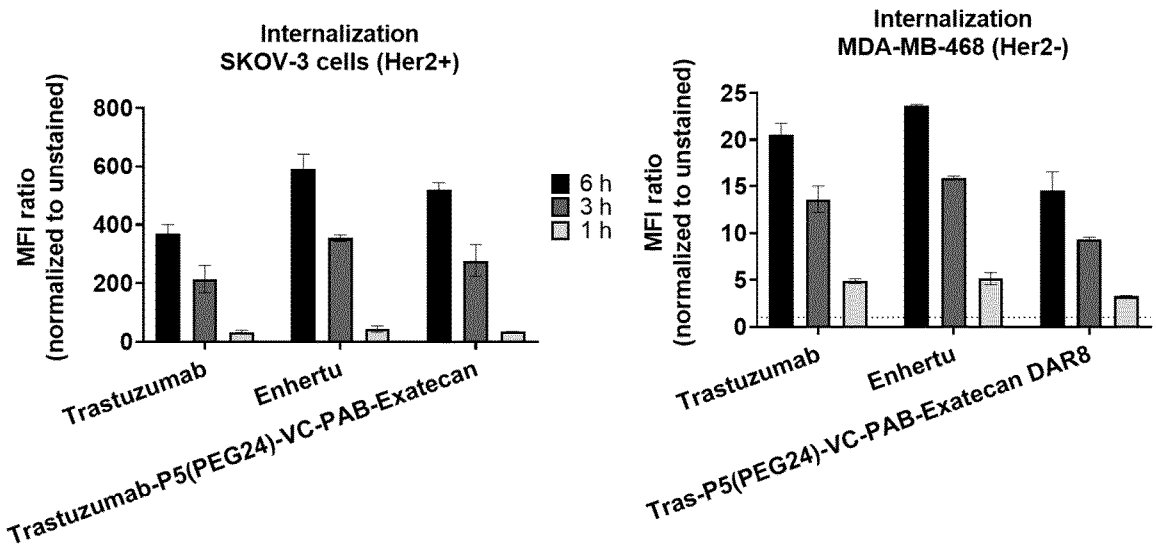


Figure 58

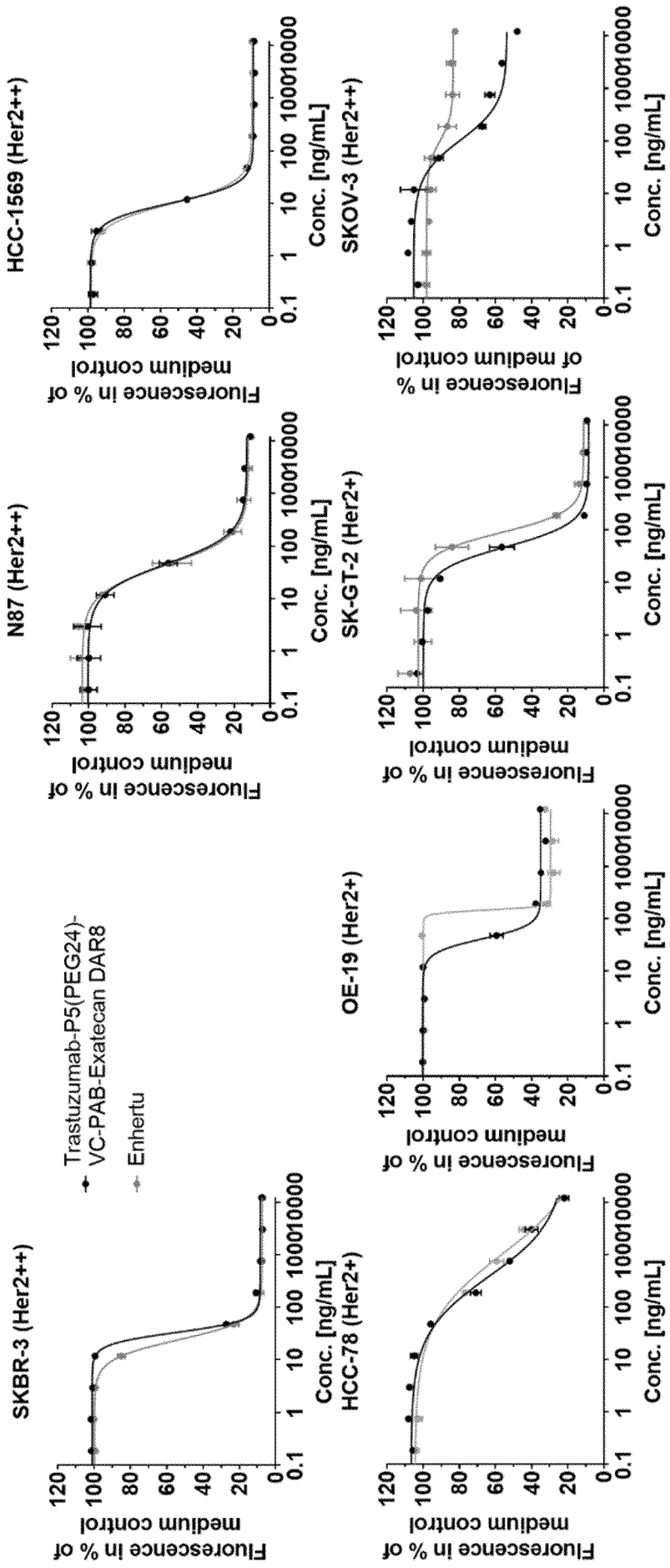

Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR8
Enhertu
SKBR-3 (Her2++)
N87 (Her2++)
HCC-1569 (Her2++)
HCC-78 (Her2+)
OE-19 (Her2+)
SK-GT-2 (Her2+)
SKOV-3 (Her2++)
Fluorescence in % of medium control
Conc. [ng/mL]
0
20
40
60
80
100
120
0.1
1
10
100
1000
10000

Figure 59

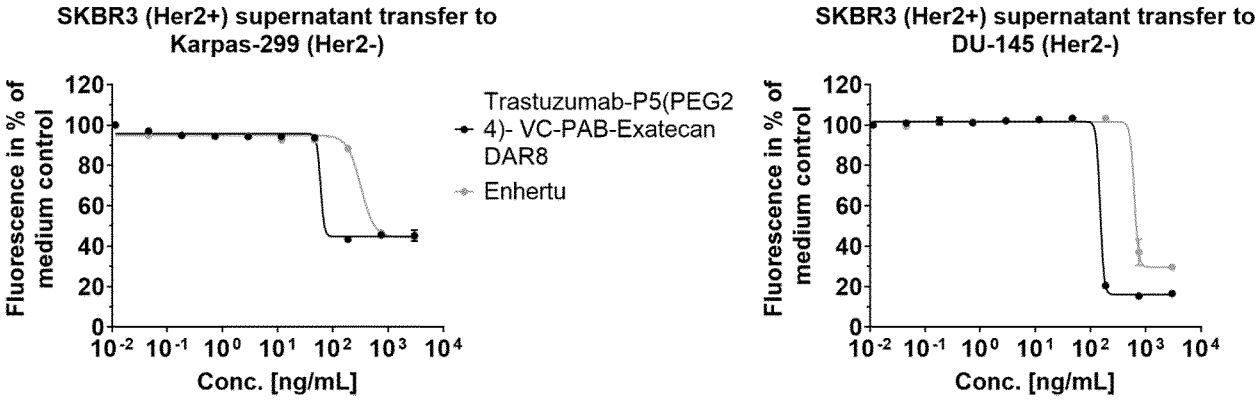


Figure 60

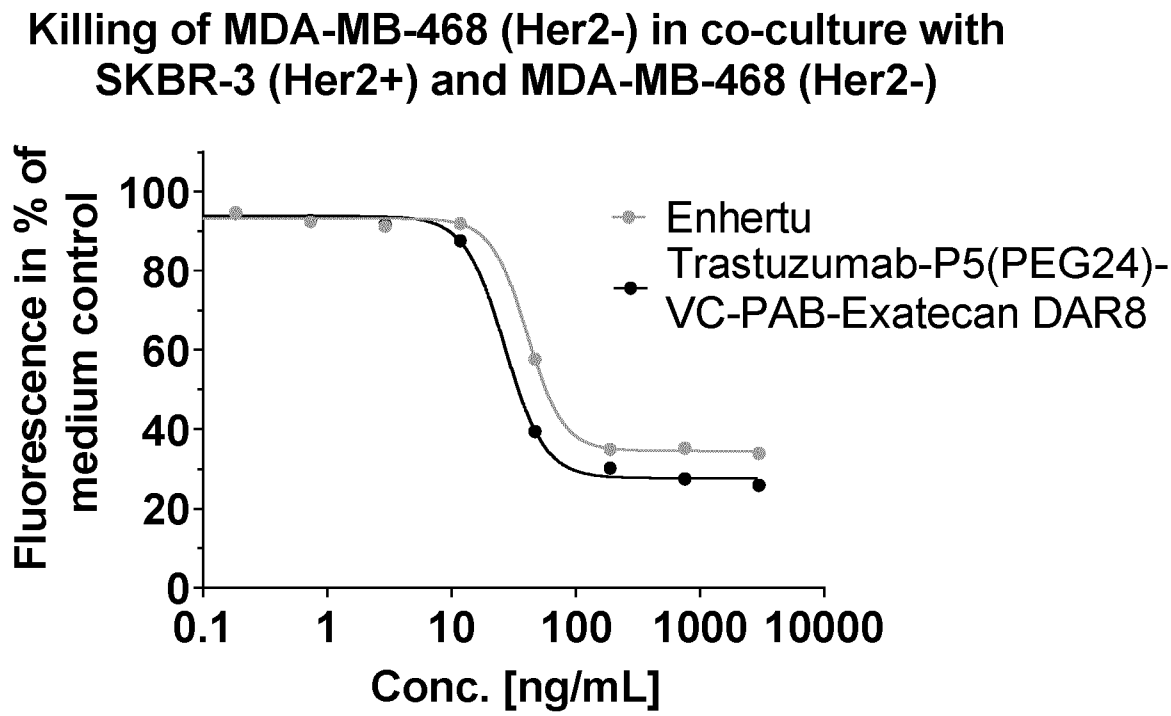


Figure 61

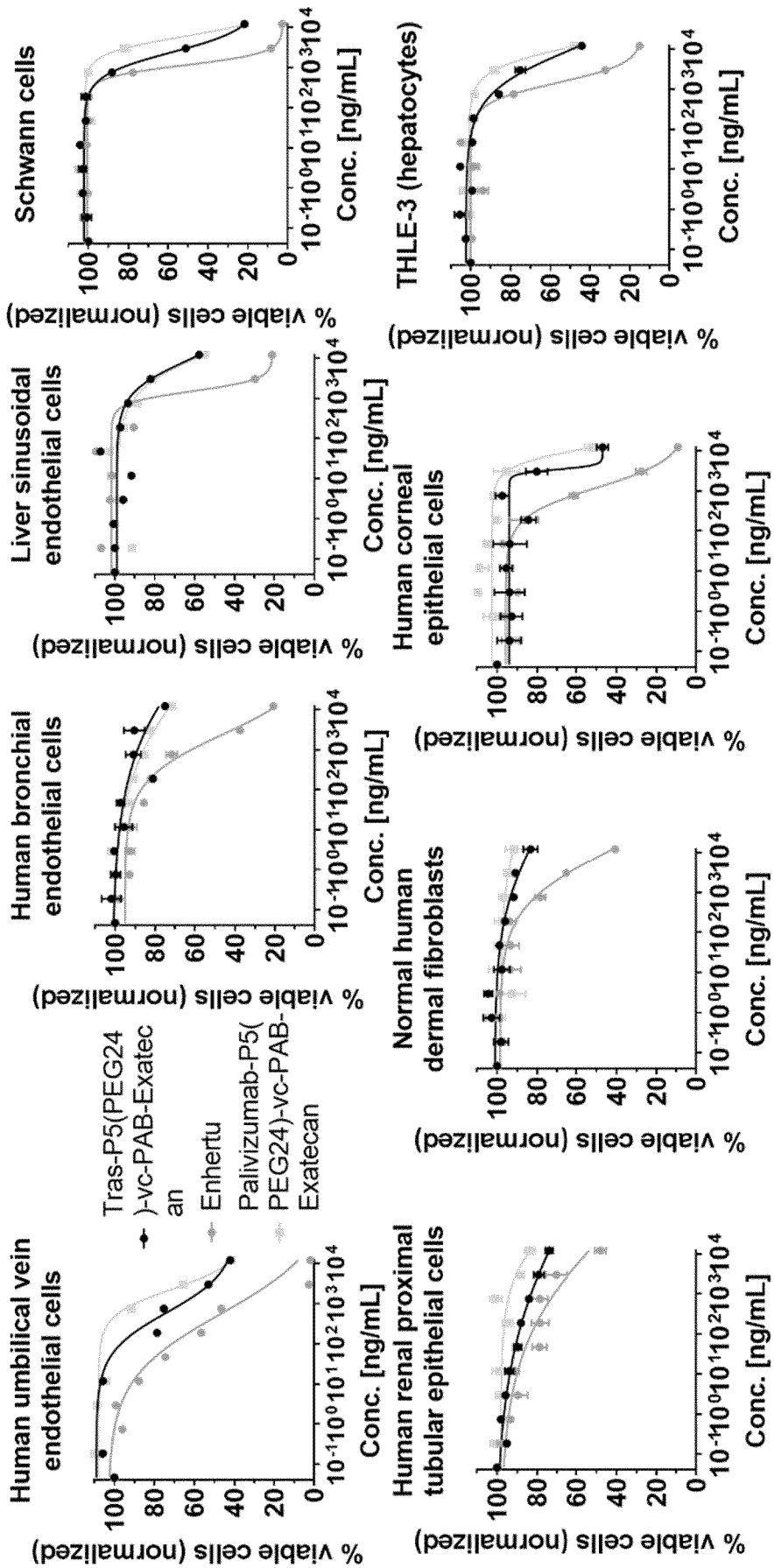

Human umbilical vein endothelial cells
Human bronchial endothelial cells
Liver sinusoidal endothelial cells
Schwann cells
Normal human dermal fibroblasts
Human corneal epithelial cells
THLE-3 (hepatocytes)
Human renal proximal tubular epithelial cells
% viable cells (normalized)
Conc. [ng/mL]
Tras-P5(PEG24)-vc-PAB-Exatecan
Enhertu
Palivizumab-P5(PEG24)-vc-PAB-Exatecan

Figure 62

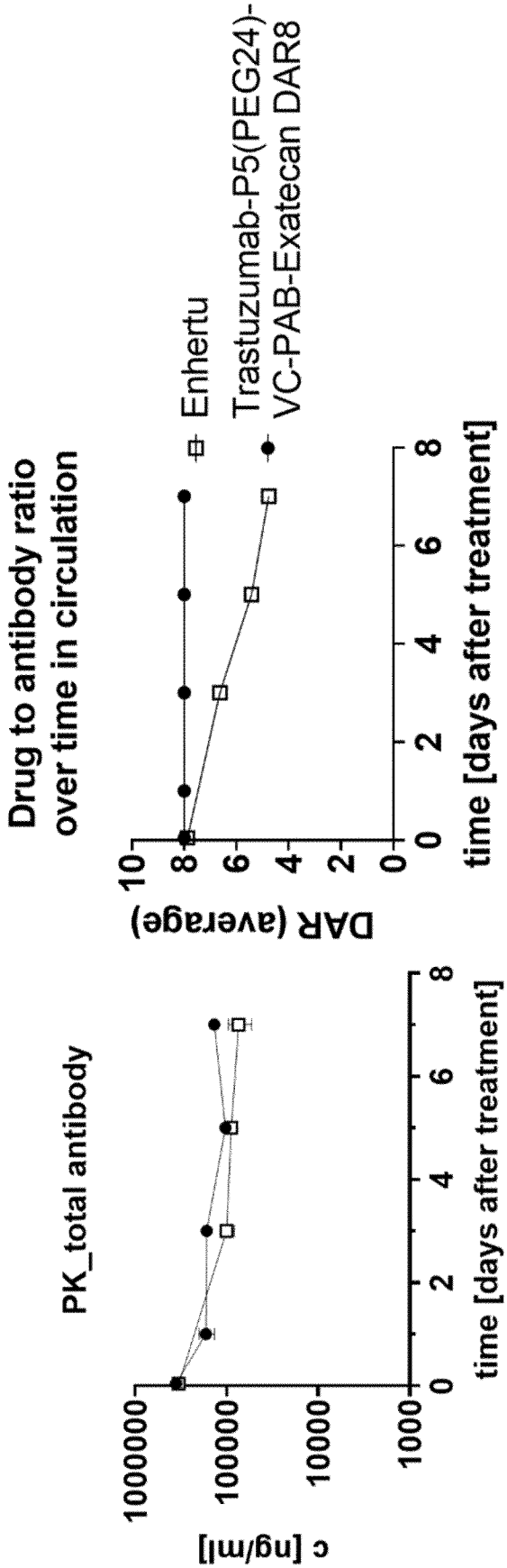

Drug to antibody ratio
over time in circulation
DAR (average)
time [days after treatment]
Enhertu
Trastuzumab-P5(PEG24)-
VC-PAB-Exatecan DAR8
PK_total antibody
c [ng/ml]
time [days after treatment]

Figure 63

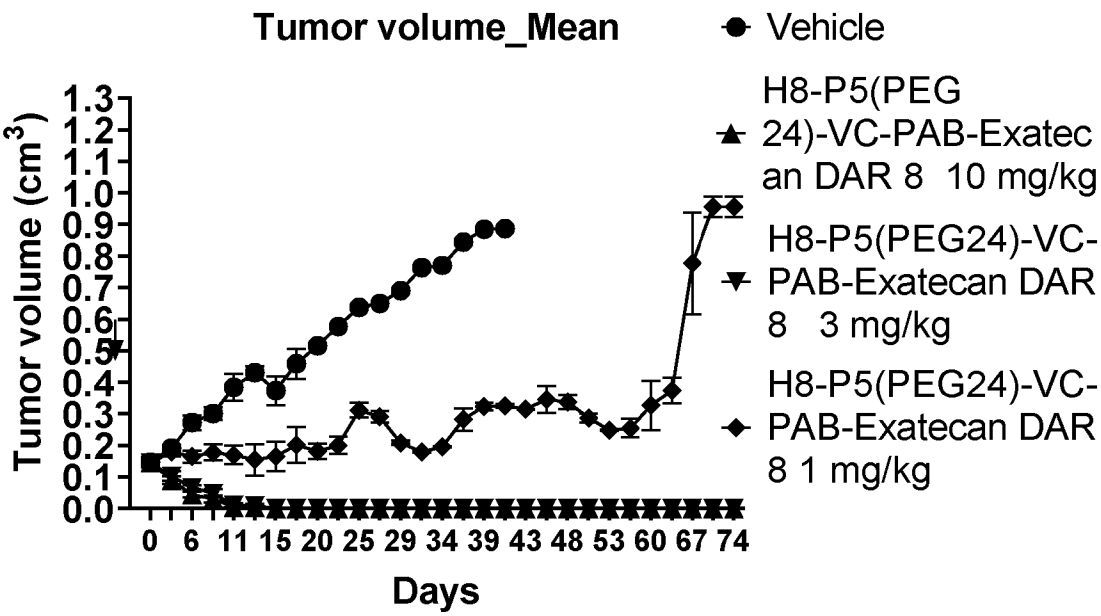


Figure 64

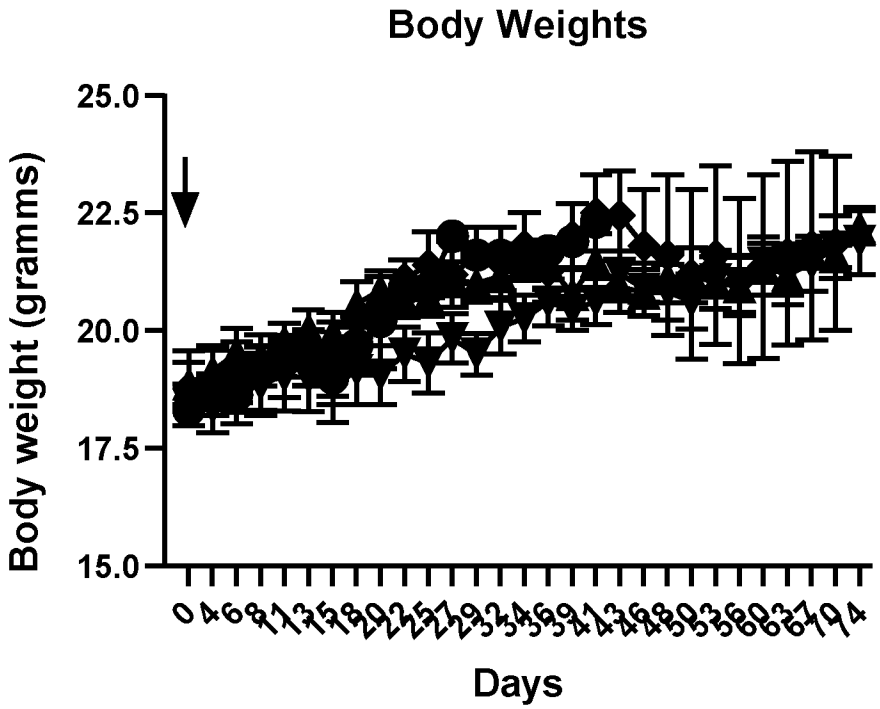


Figure 65

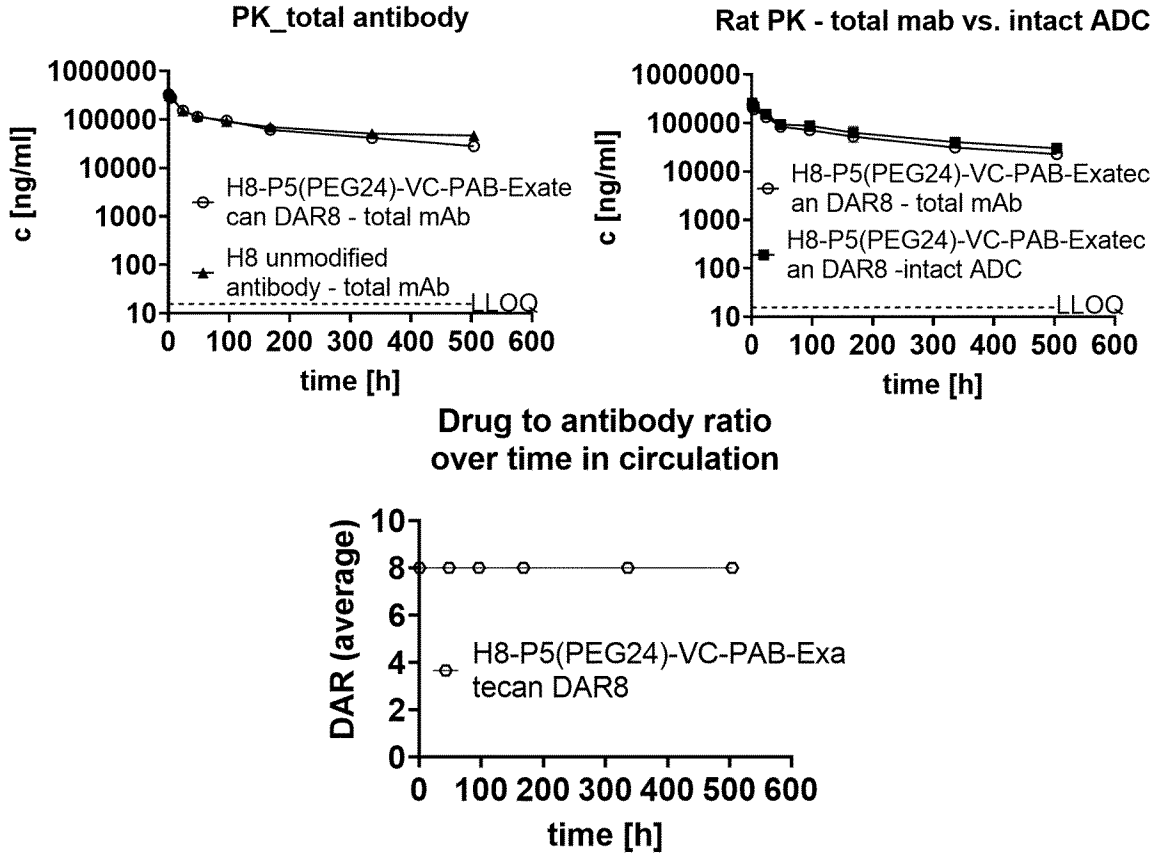
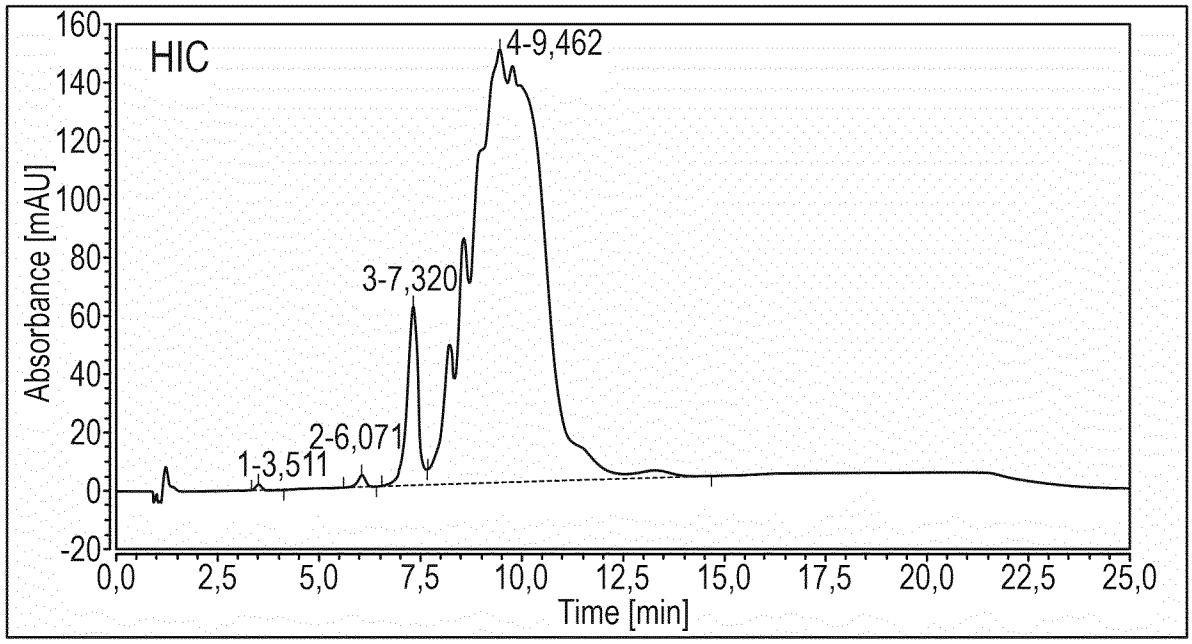

PK_total antibody
c [ng/ml]
1000000
100000
10000
1000
100
10
H8-P5(PEG24)-VC-PAB-Exatecan DAR8 - total mAb
H8 unmodified antibody - total mAb
LLOQ
0 100 200 300 400 500 600
time [h]
Rat PK - total mab vs. intact ADC
c [ng/ml]
H8-P5(PEG24)-VC-PAB-Exatecan DAR8 - total mAb
H8-P5(PEG24)-VC-PAB-Exatecan DAR8 -intact ADC
LLOQ
time [h]
Drug to antibody ratio over time in circulation
DAR (average)
10
8
6
4
2
0
H8-P5(PEG24)-VC-PAB-Exatecan DAR8
time [h]

Figure 66


HIC
4-9,462
3-7,320
2-6,071
1-3,511
Absorbance [mAU]
Time [min]

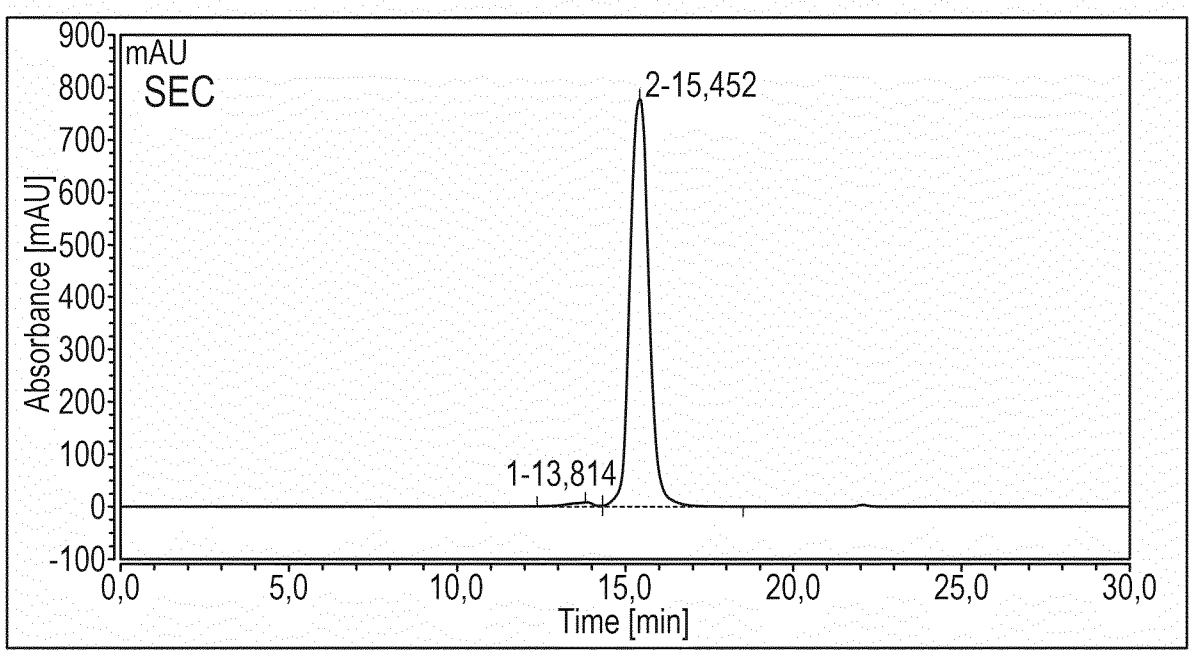

mAU
SEC
2-15,452
1-13,814
Absorbance [mAU]
Time [min]

Figure 67

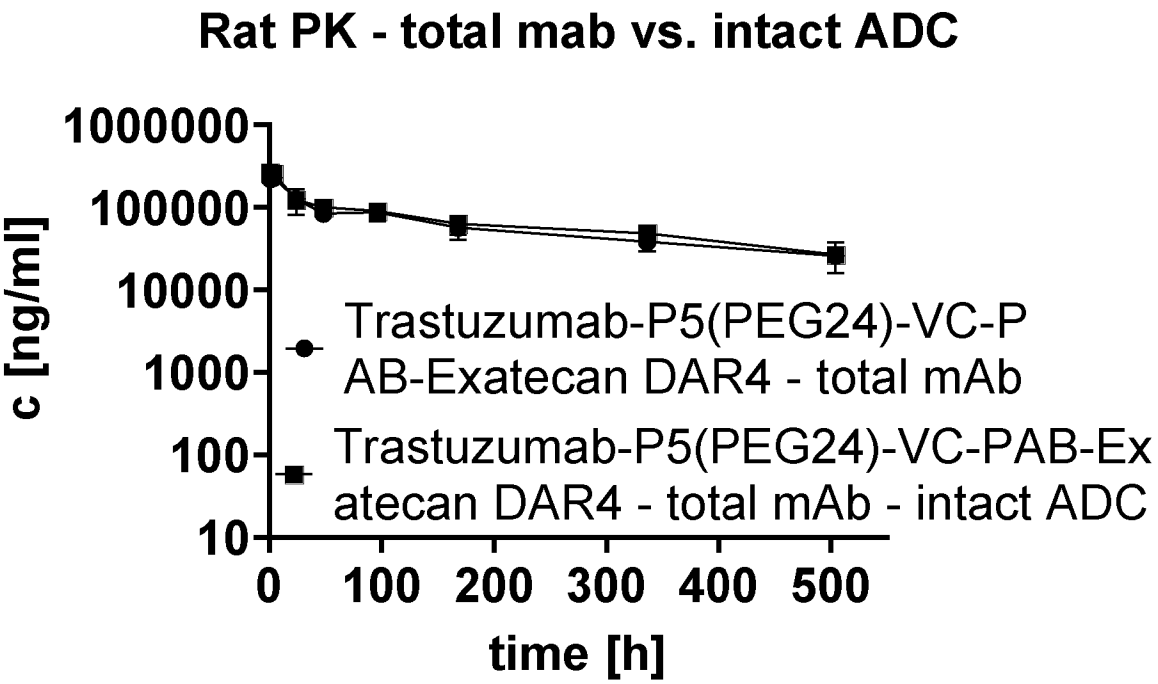
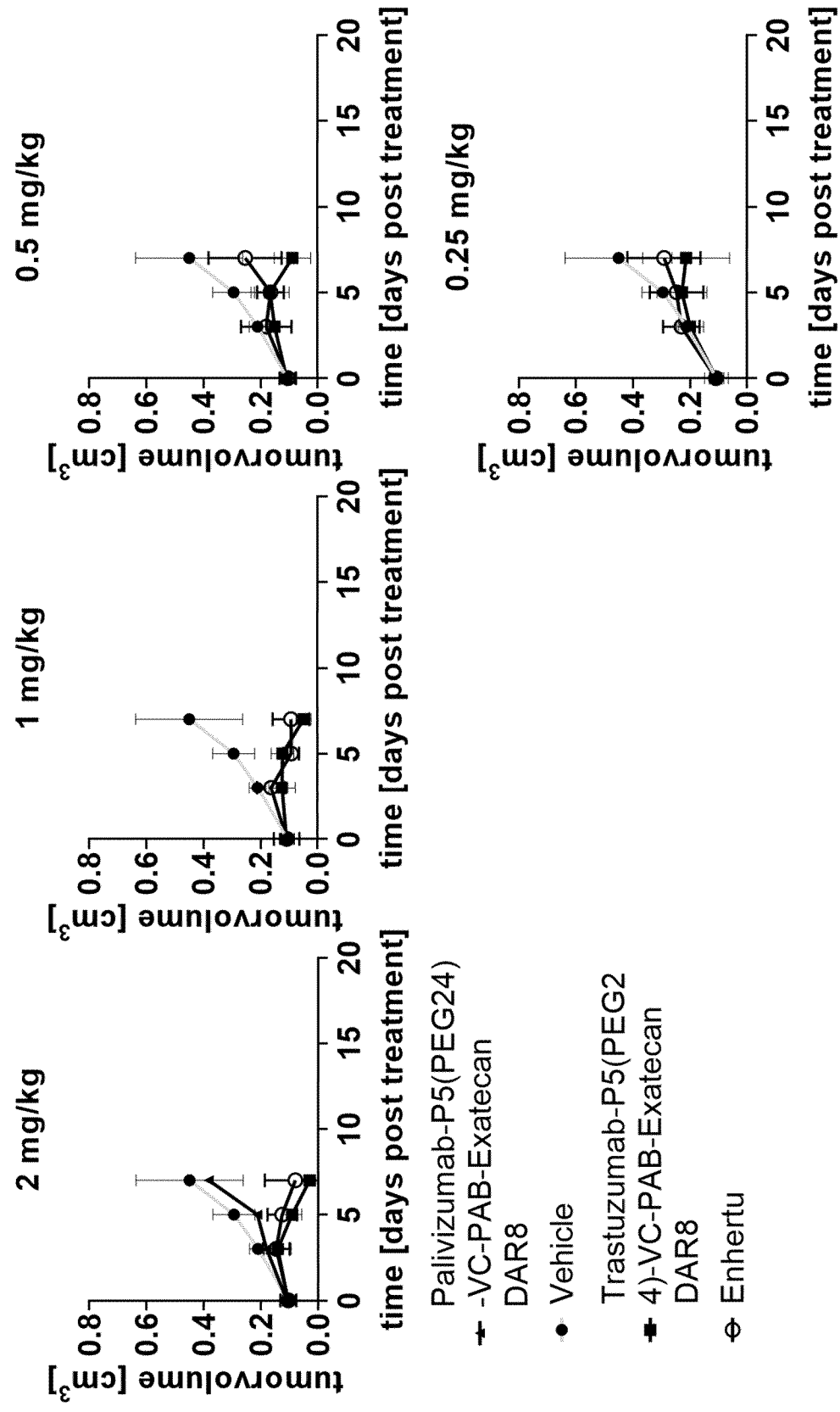

Rat PK - total mab vs. intact ADC
1000000
100000
10000
1000
100
10
c [ng/ml]
Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR4 - total mAb
Trastuzumab-P5(PEG24)-VC-PAB-Exatecan DAR4 - total mAb - intact ADC
0
100
200
300
400
500
time [h]

Figure 68


2 mg/kg
1 mg/kg
0.5 mg/kg
0.25 mg/kg
tumorvolume [cm³]
time [days post treatment]
0.0
0.2
0.4
0.6
0.8
0
5
10
15
20
Palivizumab-P5(PEG24)
-VC-PAB-Exatecan
DAR8
Vehicle
Trastuzumab-P5(PEG2
4)-VC-PAB-Exatecan
DAR8
Enhertu

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/081371

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/68
ADD.

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)
A61K

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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KASPER MARC-ANDRÉ ET AL: "Ethynylphosphonamidates for the Rapid and Cysteine-Selective Generation of Efficacious Antibody-Drug Conjugates", ANGEWANDTE CHEMIE INTERNATIONAL EDITION , vol. 58, no. 34 19 August 2019 (2019-08-19), pages 11631-11636, XP055911394, ISSN: 1433-7851, DOI: 10.1002/anie.201904193 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/anie.201904193	1-6, 8-27, 35-42
A	figures 1-3 the whole document	7,28-34

-/--

[x] Further documents are listed in the continuation of Box C.

[x] 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

"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
2 February 2023	13/02/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Langer, Miren

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KASPER MARC-ANDRÉ ET AL: "N -Hydroxysuccinimide-Modified Ethynylphosphonamidates Enable the Synthesis of Configurationally Defined Protein Conjugates", CHEMBIOCHEM , vol. 21, no. 1-2 15 January 2020 (2020-01-15), pages 113-119, XP055911400, ISSN: 1439-4227, DOI: 10.1002/cbic.201900587 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/cbic.201900587 -----	1-42
A	WO 2018/041985 A1 (FORSCHUNGSVERBUND BERLIN EV [DE] ET AL.) 8 March 2018 (2018-03-08) cited in the application -----	1-42
A	US 2019/343828 A1 (JEFFREY SCOTT [US] ET AL) 14 November 2019 (2019-11-14) claims 1-13 -----	1-42
A	WO 2010/033733 A1 (ENDOCYTE INC [US]; LEAMON CHRISTOPHER PAUL [US] ET AL.) 25 March 2010 (2010-03-25) -----	1-42
A	US 2009/099224 A1 (NARKUNAN KESAVARAM [US] ET AL) 16 April 2009 (2009-04-16) -----	1-42
A	WO 94/11377 A2 (GLAXO INC [US]; BESTERMAN JEFFREY MARK [US] ET AL.) 26 May 1994 (1994-05-26) -----	1-42
A	WO 2020/256544 A1 (TAGWORKS PHARMACEUTICALS B V [NL]) 24 December 2020 (2020-12-24) -----	1-42
A	KASPER MARC-ANDRÉ ET AL: "Cysteine-Selective Phosphonamidate Electrophiles for Modular Protein Bioconjugations", ANGEWANDTE CHEMIE INTERNATIONAL EDITION , vol. 58, no. 34 19 August 2019 (2019-08-19), pages 11625-11630, XP055911580, ISSN: 1433-7851, DOI: 10.1002/anie.201814715 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/anie.201814715 ----- -/--	1-42

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/081371

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2020/256546 A1 (TAGWORKS PHARMACEUTICALS B V [NL]; UNIV WIEN TECH [AT]) 24 December 2020 (2020-12-24)	1-42
A	OCHTROP PHILIPP ET AL: "Recent advances of thiol-selective bioconjugation reactions", CURRENT OPINION IN CHEMICAL BIOLOGY, CURRENT BIOLOGY LTD, LONDON, GB, vol. 58, 7 July 2020 (2020-07-07), pages 28-36, XP086377109, ISSN: 1367-5931, DOI: 10.1016/J.CBPA.2020.04.017 [retrieved on 2020-07-07]	1-42

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/081371

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		TW 202010498 A	16-03-2020
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		US 2022193069 A1	23-06-2022
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		CA 2737496 A1	25-03-2010
		CN 102215844 A	12-10-2011
		EP 2349274 A1	03-08-2011
		IL 211763 A	31-07-2014
		JP 5675619 B2	25-02-2015
		JP 2012502921 A	02-02-2012
		NZ 592255 A	26-07-2013
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		US 2014213760 A1	31-07-2014
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		MY 109296 A	31-12-1996
		SI 9300591 A	30-06-1994
		US 6100273 A	08-08-2000
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		CA 3143921 A1	24-12-2020
		EP 3983403 A1	20-04-2022
		IL 289094 A	31-01-2022

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Information on patent family members

International application No
PCT/EP2022/081371

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		CA 3143925 A1	24-12-2020
		CN 115135628 A	30-09-2022
		EP 3983363 A1	20-04-2022
		IL 289093 A	01-02-2022
		JP 2022537543 A	26-08-2022
		WO 2020256546 A1	24-12-2020