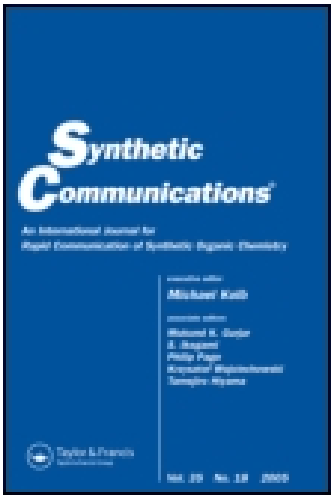

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Synthesis of Novel Heterobifunctional Isocyanato Cross-Linkers and Their Applications for the Preparation of 10-Hydroxycamptothecin and SN-38 Conjugates with Melanotransferrin P97

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Synthesis of Novel Heterobifunctional Isocyanato Cross-Linkers and Their Applications for the Preparation of 10-Hydroxycamptothecin and SN-38 Conjugates with Melanotransferrin P97

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Abstract: Novel heterobifunctional cross-linkers with an isocyanato group, a protected carboxylic group, and a linear chain spacer are synthesized in high yield by coupling monofunctionalized PEG with 1,6-diisocyanatohexane. The isocyanato groups of those linkers are highly reactive and are efficient reagents to couple with the hydroxy groups of 10-hydroxycamptothecin and SN-38 under mild conditions to give a useful precursor for the synthesis of their bioconjugates with proteins such as melanotransferrin p97.

Keywords: bioconjugate, heterobifuncational cross-linker, 10-hydroxycamptothecin, isocyanato, SN-38

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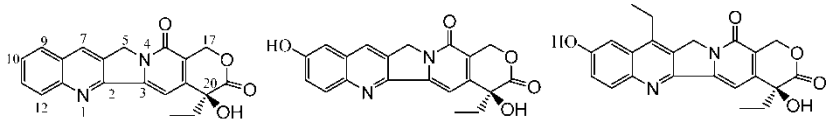
1 INTRODUCTION

Heterobifunctional linkers are one of the most important and widely used cross-linkers in the areas of bioconjugate synthesis and drug delivery for cross-linking small molecule drugs, medical image agents, proteins, enzymes, antibodies, genes, cells, and even living bacteria.[1–3] Heterobifunctional linkers chemically consist of three moieties: a spacer or bridge and two different functional groups that are able to selectively couple with two different target molecules. There are a number of commercially available heterobifuntional linkers, most of which, however, are not efficient for coupling to 10-hydroxycamptothecin (**II**) or 7-ethyl-10-hydroxycamptothecin (SN-38, **III**). (A number of companies are providing heterobifunctional linkers, such as Pierce Inc. Sigma-Aldrich, and Solulink Inc.)

10-Hydroxycamptothecin (**II**) and SN-38 (**III**) are the semisynthesized compounds based on camptothecin (**I**), the natural product first isolated by Wall et al. in 1966 from the Chinese tree *Camptotheca acuminate*.[4] Camptothecin (**I**) and its more potential analogues such as 10-hydroxycamptothecin (**II**) and SN-38 (**III**) have recently undergone extensive clinical evaluation worldwide and display a broad range of clinical activity against solid tumors[5,6] through an unique antitumor mechanism of inhibiting DNA topoisomerase I, an important nuclear enzyme for various DNA functions including transcription and replication.[7]

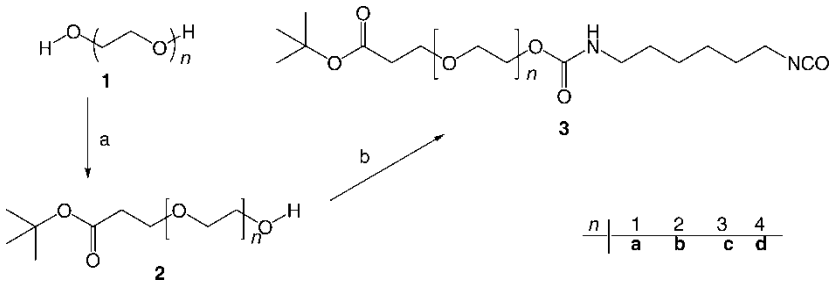


I Camptothecin **II** 10-hydroxycamptothecin **III** SN-38

The 10-hydroxy group of 10-hydroxycamptothecin (**II**) and SN-38 **(III)** had been previously modified through the formation of ester bond,[8] carbamate bond,[9] ether bond,[10] and so forth. In this article, we report the synthesis of heterobifuncational linkers that contain an isocyanato group, a protected carboxylic group, and a linear chain spacer. They are highly reactive to the hydroxyl groups of 10-hydroxycamptothecin and SN-38 to form, in excellent yields, a carbamate bond between the linker and the target drug molecules. The resulting compounds are useful precursors for the synthesis of their conjugates with melanotransferrin p97.

2 RESULTS AND DISCUSSION

We are interested in isocyanato linkers because the isocyanate group is highly reactive to the hydroxyl and amino groups in the target compounds[11] to form a stable carbamate and urea linkage, which can be



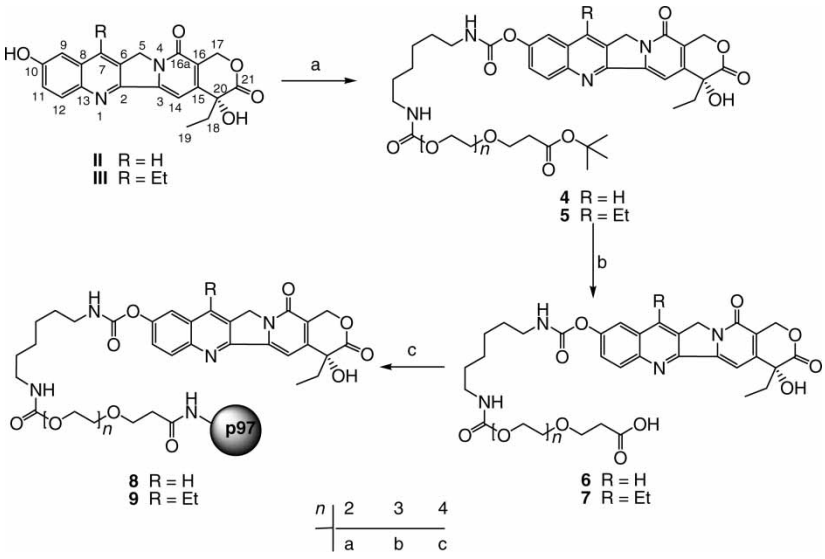
Scheme 1. Reagents and conditions: (a) *tert*-butyl acrylate/Na/THF, rt, 24 h, 40–88%, (b) Et_3N/1,6-diisocyantohexane/dichloromethane, rt, 2 h, 82–95%.

selectively hydrolyzed in some tumor cells.[12,13] The heterobifuncational linkers **3** were synthesized by two steps in high yield as shown in Scheme 1. Polyethylene glycols **1** were mono-alkylated via Michael addition reaction to give compounds **2** in 45–88% by using 1/3 equivalent of *tert*-butyl acrylate and a catalytic amount of sodium in THF, a procedure previously described.[14–16] Compounds **2** were isolated as light yellow oil and fully characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and combustion analysis.

Reaction of the monofunctionalized PEG compounds **2** with equimolar amount of 1,6-diisocyanatohexane gave compounds **3** in 82–90% yield as light yellow oils, which were fully characterized by ^{1}H and ^{13}C NMR, MS, IR, and combustion analysis. ^{13}C spectra of **3** showed the characteristic chemical shifts at ~29 ppm for the methyl groups of the *tert*-butyl group, ~80 ppm for $OCMe_3$, ~121 ppm for the isocyanate, ~156 ppm for the carbamate (NH-CO-O), and ~170 ppm for the $COOBu^t$ ester. Their IR illustrated spectra showed a strong absorption at 2260–2266 cm^{-1}, which was assigned to the isocyanate group. Compounds **3** are sensitive to moisture and will slowly polymerize upon exposure to air or light. They are stable for years in the dark, when kept dry and at low temperature (4°C).

The novel heterobifunctional linkers were used to conjugate melanotransferrin (p97) with anticancer drugs 10-hydroxcamptothecin (**II**) and SN-38 (**III**) (Scheme 2). Melanotransferrin (p97) has been found to localize in capillary endothelial cells of the human brain and to play an important role in the transport of iron across the blood brain barrier (BBB).[17] This provides a base for a vector-mediated approach to delivery of therapeutics to the brain.

The conjugates **8** and **9** are synthesized in three steps as outlined in Scheme 2. Reactions of 10-hydroxycamptothecin (**II**) and SN-38 **(III)** with mono-isocyanato linkers **3** under basic conditions give the expected products **4** and **5** in high yield. Compounds **4** and **5** are semisolid when freshly prepared and slowly solidified upon storing over months at 4°C in the dark. Upon treatment with trifluoroacetic acid, the expected free acids



Scheme 2. Reagents and conditions: (a) **3**/Et_3N/DMF, rt, 2 h. 82–92%, (b) TFA, rt, 20 min, 82–95%, (c) *O*-benzotriazole-1-yl-*N,N,N′,N′*tetramethyluronium tetrafluoroborate(BTTU)/Et_3N/DMF, rt, 60 min, then melanotransferrin (p97), rt, 20 h.

6 and **7** are obtained in 80–95% yield. After activating the carboxy group in compounds **6** and **7** using BTTU (*O*-benzotriazole-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoride), the corresponding intermediates are directly used to react with melanotransferrin p97 to give the expected conjugates **8** and **9**, which were purified by membrane dialysis and characterized by fast protein liquid chromatography (FPLC), SDS-Page, and Western blotting against the p97 antibody (L235 anitbody). The results showed that both compounds **8** and **9** were pure and the conformation of p97 was preserved. The MSR (molar substituted ratio) of the target molecules attached to p97 were estimated (see Table 1) by UV-vis as previously described.[3]

Table 1. Preparation of compounds **10** and **11**

Compound	MSR (molar substituted ratio)	Protein p97 recovery (%)
8a	5.4	88
8b	3.5	90
8c	4.6	95
9a	5.3	89
9b	6.1	91
9c	7.5	86

3 CONCLUSION

We have described an efficient method to synthesize heterobifunctional cross-linkers, which consist of an isocyanto group, a protected craboxylate group, and a linear chain spacer. Those linkers are very efficient reagents to react with the 10-hydroxy group of 10-hydroxycamptotheticin and SN-38 to form a carbamate bond between the target molecules and the linkers. Carbamate bond is chemically stable and hydrolysable in some tumor cells, which suggests that the new heterobifunctional linkers are potentially useful in bio-conjugate synthesis.

4 EXPERIMENTAL

4.1 General

10-Hydroxycamptothecin **(II)** and SN-38 **(III)** were purchased from Qventas, Inc. Dialysis Cassettes Slide-A-Lyzer (10 K, 10,000 MWCO) and Dialysis Tubing SnakeSkin^{TM} (10,000 MWCO) were purchased from Pierce Inc. All other reagents and solvents were purchased from Aldrich, Sigma, and VWR and used as received. The silica gel used in flash chromatography was Merck silica gel 60, 230–400 mesh, and R_f values were measured on Merck silica thin-layer chromatography (TLC) aluminum sheets (silica gel 60 F_{254}). Melting points were determined on a Thomas hot stage or Buchi apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker AC200-, AMX400-, or AMX500-MHz instruments. APT was recorded on the AMX-400 instrument. Part of ^1H and ^{13}C NMR were assigned based on reported data[8] and APT results. UV-vis spectra were recorded on a Beckman DU640 photo-diode-array spectrophotometer in the solvents indicated. IR spectra were recorded on a Shimadzu FTIR 8400. FPLC was run in a AKTA Purifier^{TM} FPLC (using UNICORN^{TM} version 3.10, Amersham Pharmacia Biotech) using Mono Q^R HR 10/10 ion exchange column (from Pharmacia Biotech Inc.) with PBS buffer (0.01 M, pH = 7.4) and 1M NaCl–0.001 M PBS buffers as the mobile phases or using a BIOSEP^{TM} size exclusion column (from Phenomenex, Inc) and 0.01 M PBS buffer (pH = 6.80). Elemental analyses were performed by the micro-analytical laboratory, Department of Chemistry, University of British Columbia (UBC). The high- and low-resolution mass spectra were obtained by mass spectrometer service laboratories, Department of Chemistry, UBC.

4.2 General Procedure for the Preparation of Mono-alkylated PEG Compounds 2

Polyethylene glycol (1.0 mol) was dissolved in THF (500 mL). Sodium (0.212 g, 9.2 mmol) was cut into small pieces and added under stirring. The

mixture was warmed at 40°C until all sodium dissolved. Then tert-butylacrylate (51 mL, 0.34 mmol) was added at room temperature. The mixture was stirred overnight at room temperature. Solvent was removed under vacuum (30–40 mm Hg). The residue oil was mixed with brine (100 mL), and the mixture was extracted with ethyl acetate (6 × 150 mL). The combined extracts were washed with brine and dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure (30–40 mm Hg), the crude product was obtained after drying under vacuum overnight (0.1–0.5 mm Hg), which was pure enough and directly used for the synthesis of compound **3**. Analytical samples were purified through a flash silica-gel chromatographic column using dichloromethane–methanol (95/5, v/v) as eluent.

4.2.1 *Tert*-butyl 3-(ethylene glycol)propanoate (**2a**)

Yield 45%. IR, $\nu = 3450, 2877, 1728, 1369, 1325, 1240, 1178, 1116, 1051, 954, 927, 883, 846, 812, 605, 542, 516\ cm^{-1}$. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.40$ (s, 9H, 3 CH_3), 2.30 (t, $J = 7.0$ Hz, 2H, CH_2), 3.20 (brs, 1H, OH), 3.80 (m, 4H, 2 OCH_2), 4.20 (m, 2H, OCH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 27.5$ (CH_3), 34.2 (CH_2), 61.1, 66.5, 70.1, 80.6 ($OCMe_3$), 170.3 (COO) ppm. LC-MS, $m/z = 213$ $[M + Na]^+$. Anal. calcd. for $C_9H_{18}O_4$ (190.24): C, 56.82; H, 9.54. Found: C, 56.65; H, 9.22.

4.2.2 *Tert*-butyl 3-[di(ethylene glycol)]propanoate (**2b**)

Yield 72%. IR, $\nu = 3452, 2872, 1726, 1452, 1392, 1367, 1332, 1251, 1157, 1112, 1062, 933, 846, 889, 754, 548, 517\ cm^{-1}$. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.35$ (s, 9H, 3 CH_3), 2.35 (t, $J = 7.0$ Hz, 2H, CH_2), 3.80 (m, 11H, 5 OCH_2, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 27.8$ (CH_3), 34.5 (CH_2), 61.4, 66.6, 70.2 (multiple peaks), 72.5, 80.3 ($OCMe_3$), 170.5 (COO) ppm. LC-MS, $m/z = 257$ $[M + Na]^+$. Anal. calcd. for $C_{11}H_{22}O_5$ (234.29): C, 56.39; H, 9.46. Found: C, 56.55; H, 9.32.

4.2.3 *Tert*-butyl 3-[tri(ethylene glycol)]propanoate (**2c**)

Yield: 80.5%. IR, $\nu = 3450, 2870, 1726, 1456, 1392, 1365, 1330, 1251, 1111, 1064, 943, 847, 888, 756, 532\ cm^{-1}$. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.45$ (s, 9H, 3 CH_3), 2.52 (t, $J = 7.0$ Hz, 2H, CH_2), 3.75 (m, 15H, 7 OCH_2, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 27.5$ (CH_2), 28.0 (CH_3), 36.5 (CH_2), 61.3, 65.8, 70.5 (multiple peaks), 72.3, 80.2 ($OCMe_3$), 170.4 (COO) ppm, LSIMS (matrix: thioglycerol), $m/z = 279$ $[M + H]^+$. Anal. calcd. for $C_{13}H_{26}O_6$ (278.34): C, 52.92; H, 9.48. Found: C, 52.55; H, 9.22.

4.2.4 *Tert*-butyl 3-[tetra(ethylene glycol)]propanoate (**2d**)

Yield 88%. IR, $\nu = 3450$, 2870, 1726, 1451, 1365, 1329, 1249, 1105, 1068, 945, 887, 847, 756, 528 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.50$ (s, 9H, 3 CH_3), 2.50 (t, $J = 7.0$ Hz, 2H, CH_2), 3.80 (m, 19H, 9 OCH_2, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 27.5$ (CH_3), 27.8 (CH_2), 36.0 (CH_2), 62.3, 66.6, 70.3 (multiple peaks), 72.3, 76.4, 80.2 ($OCMe_3$), 170.6 (COO) ppm. LSIMS (matrix: thioglycerol), $m/z = 333$ $[M + H]^+$. Anal. calcd. for $C_{15}H_{30}O_7 \cdot H_2O$ (340.41): C, 52.92; H, 9.48. Found: C, 52.88; H, 9.12.

4.3 General Procedure for the Preparation of Mono-isocyanato Heterobifunctional Linkers 3

1,6-Diisocyanatohexane (5 mL, 0.03 mol) was dissolved in anhydrous dichloromethane (50 mL). Then a solution of compound **2** (0.03 mol) in dichoromethane (50 mL) and triethylamine (1.5 mL) was added dropwise under stirring over a period of 30 min. The mixture was stirred at room temperature for 2 h and was protected from moisture. The solvent was removed under vacuum. The oily residue was dried overnight under vacuum to give the crude products **3**, which were purified through flash silica-gel chromatographic column using dichloromethane as eluent.

4.3.1 Compound **3a**

Yield 91%. IR, $\nu = 3458$, 2933, 2862, 2260, 1722, 1526, 1458, 1365, 1240, 1157, 1118, 1053, 954, 846, 777, 756, 582 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.40$ (s, 9H, 3 CH_3), 1.55 (m, 6H, 3 CH_2), 2.43 (t, $J = 7.0$ Hz, 2H, CH_2), 2.55 (t, $J = 7.0$ Hz, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.80 (m, 4H, 2 OCH_2), 4.20 (t, $J = 6.8$ Hz, 2H, OCH_2), 5.10 (s, 1H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 27.1$ (CH_2), 27.3 (CH_2), 28.8 (CH_3), 31.6 (CH_2), 32.0 (CH_2), 34.9 (CH_2), 43.6 (CH_2), 45.4 (CH_2), 70.5 (m, 3 OCH_2), 80.1 ($OCMe_3$), 122.3 (N═C═O), 156.5 (NH-CO-O), 170.3 (COO) ppm. LSIMS (matrix: thioglycerol), $m/z = 358$ $[M + H]^+$. Anal. calcd. for $C_{17}H_{30}N_2O_6$ (358.43): C, 56.97; H, 8.44; N, 7.81. Found: C, 56.88; H, 8.45; N, 7.50.

4.3.2 Compound **3b**

Yield 94%. IR, $\nu = 3485$, 2933, 2866, 2262, 1724, 1523, 1458, 1365, 1392, 1247, 1157, 1114, 1062, 950, 846, 777, 756, 729, 582 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$), $\delta = 1.42$ (s, 9H, 3 CH_3), 1.55 (m, 8H, 4 CH_2), 2.50 (t, $J = 7.0$ Hz, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.80 (m, 8H, 4 OCH_2), 4.20 (t, $J = 6.8$ Hz, 2H, OCH_2), 5.20 (s, 1H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 26.1$ (CH_2), 27.3 (CH_2), 28.1 (CH_3), 29.7

(CH_2), 30.9 (CH_2), 34.9 (CH_2), 40.7 (CH_2), 42.7 (CH_2), 66.8 (OCH_2), 68.8 (OCH_2), 70.5 (m, 3 OCH_2), 80.1 ($OCMe_3$), 121.9 (N═C═O), 156.4 (NH-CO-O), 170.8 (COO) ppm. LSIMS (matrix: thioglycerol), m/z = 403 [M +H]$^+$. Anal. calcd. for $C_{19}H_{34}N_2O_7$ (402.48): C, 56.70; H, 8.51; N, 6.96. Found: C, 56.48; H, 8.35; N, 6.80.

4.3.3 Compound **3c**

Yield 95%. IR, $\nu = 3445, 2933, 2864, 2262, 1722, 1525, 1458, 1392, 1365, 1247, 1109, 1070, 947, 846, 777, 756, 729, 580\ cm^{-1}$. ^{1}H NMR (200 MHz, $CDCl_3$), $\delta = 1.40$ (s, 9H, 3 CH_3), 1.50 (m, 8H, 4 CH_2), 2.50 (t, $J = 7.0$ Hz, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 3.80 (m, 12H, 6 OCH_2), 4.20 (t, $J = 6.8$ Hz, 2H, OCH_2), 5.20 (s, 1H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 26.5$ (CH_2), 27.6 (CH_2), 28.0 (CH_3), 29.7 (CH_2), 31.1 (CH_2), 34.8 (CH_2), 39.8 (CH_2), 42.7 (CH_2), 63.6 (OCH_2), 66.7 (OCH_2), 69.1 (OCH_2), 69.9 (m, 4 OCH_2), 80.2 ($OCMe_3$), 121.8 (N═C═O), 156.4 (NH-CO-O), 170.7 (COO) ppm. LSIMS (matrix: thioglycerol), m/z = 447 [M +H]$^+$. Anal. calcd. for $C_{21}H_{38}N_2O_8$ (446.54): C, 56.48; H, 8.58; N, 6.27. Found: C, 56.28; H, 8.25; N, 6.00.

4.3.4 Compound **3d**

Yield 82%. IR, $\nu = 3337, 2931, 2866, 2266, 1720, 1627, 1535, 1458, 1365, 1247, 1107, 949, 846, 775, 756\ cm^{-1}$. ^{1}H NMR (200 MHz, $CDCl_3$), $\delta = 1.60$ (s, 9H, 3 CH_3), 1.65 (m, 8H, 4 CH_2), 2.45 (t, $J = 7.0$ Hz, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.32 (m, 2H, CH_2), 3.60 (m, 14H, 8 OCH_2), 4.20 (t, $J = 6.8$ Hz, 2H, OCH_2), 5.10 (s, 1H, NH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$), $\delta = 25.7$ (CH_2), 27.7 (CH_2), 29.8 (CH_3), 31.1 (CH_2), 36.2 (CH_2), 40.8 (CH_2), 42.8 (CH_2), 45.4 (CH_2), 63.7 (OCH_2), 66.8 (OCH_2), 70.5 (br, 7 OCH_2), 80.7 ($OCMe_3$), 122.3 (N═C═O), 156.4 (NH-CO-O), 170.8 (COO) ppm. LSIMS (matrix: thioglycerol), m/z = 491 [M +H]$^+$. Anal. calcd. for $C_{23}H_{42}N_2O_9 \cdot 0.5H_2O$ (499.59): C, 55.29; H, 8.68; N, 5.61. Found: C, 54.98; H, 8.45; N, 5.40.

4.4 General Procedure for the Preparation of Compounds 6 and 7: Coupling Reactions of 3 with 10-Hydroxycamptothecin, SN-38

In a 100-mL, round-bottomed flask equipped with a magnetic stirrer bar, 10-hydroxycamptothecin **(II)** [or SN-38 **(III)**, 1.37 mmol], anhydrous DMF (30 mL) and triethylamine (1.0 mL) were added. The flask was placed in an ultrasonic bath until all the solid was completely dissolved. A solution of monocyanato linker **3** (2.75 mmol, 2.0 equivalent) in dichloromethane (10 mL) was added under vigorous stirring. The flask was wrapped with aluminum foil to protect if from light. The reaction was monitored by TLC

(dichloromethane/methanol, 95/5, V/V). After 2 h, TLC confirmed that the reaction was finished. The solvent was removed under vacuum until dry. The residue was taken up by methanol (5 mL) and then mixed with anhydrous ether (40 mL). The resulting suspension mixture was placed in the ultrasonic bath for 30 s and then kept at 4°C for 3 h. The solid was collected by suction filtration to give the expected products **6** (or **7**) as light yellow powder.

4.4.1 Compound **4a** (10-CPT, n = 2)

Yield: 92%, mp 159–164°C. IR, $\nu = 3321$, 2925, 2854, 1733, 1642, 1611, 1523, 1485, 1452, 1354, 1241, 1201, 1134, 1063, 1029, 920, 830, 810, 760 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.83$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.25 (m, 2H, CH_2), 1.29 (m, 4H, 2 CH_2), 1.40 (s, 9H, 3 CH_3), 1.48 (m, 2H, CH_2), 1.79 (t, $J = 7.2$ Hz, 2H, CH_2), 2.38 (t, $J = 6.0$ Hz, 2H, CH_2), 2.78 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 3.40 (obscured with water peak, m, 2H, CH_2) 3.60 (m, 6 H, 3 OCH_2), 4.12 (m, 2H, OCH_2), 5.30 (m, 2H, CH_2), 5.50 (s, 2H, CH_2), 6.60 (s, 1H), 7.10 (m, 1H), 7.33 (m, 1H), 7.61 (m, 1H), 7.88 (m, 1H), 8.45 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.5$ (CH_3), 24.8, 28.1 (CH_3), 28.9, 29.5, 30.3, 30.6, 34.7, 49.8, 62.1, 65.1, 66.0, 69.0 (multiple peaks, 5 OCH_2), 72.0, 79.1, 96.4 (CH), 118.0 (CH), 119.4, 126.4 (CH), 128.5, 119.4 (CH), 130.6, 131.4 (CH), 145.6, 145.6, 149.2, 150.5, 152.8, 154.7 (C═O), 155.8 (C═O), 156.8 (C═O), 170.7 (COO), 172.9 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 289$ (12 500), 333 (14 900), 379 (33 600), 395 (29 700) nm. UV-vis (methanol) $\lambda(\varepsilon) = 294$ (11 600), 335 (14 800), 369 (29 900), 382 (29 300) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 291$ (27 800), 335 (12 500), 371 (27 500), 380 (26 300) nm. LSIMS (matrix: thioglycerol), $m/z = 767$ [$M^+ + H$]. Anal. calcd. for $C_{39}H_{50}N_4O_{12}$: C, 61.08; H, 6.57; N, 7.31. Found: C, 60.95; H, 6.64; N, 7.21.

4.4.2 Compound **4b** (10-CPT, n = 3)

Yield: 89%, mp 175–178°C. IR, $\nu = 3319$, 2922, 2830, 1728, 1658, 1604, 1544, 1484, 1455, 1344, 1225, 1190, 1101, 1042, 996, 920, 837, 801, 763 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.86$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.21 (m, 2H, CH_2), 1.33 (m, 4H, 2 CH_2), 1.45 (s, 9H, 3 CH_3), 1.51 (m, 2H, CH_2), 1.82 (t, $J = 7.2$ Hz, 2H, CH_2), 2.40 (t, $J = 6.0$ Hz, 2H, CH_2), 2.91 (m, 2H, CH_2), 3.12 (m, 2H, CH_2), 3.35 (obscured with water peak, m, 2H, CH_2) 3.56 (m, 10H, 5 OCH_2), 4.10 (m, 2H, OCH_2), 5.28 (m, 2H, CH_2), 5.46 (s, 2H, CH_2), 6.51 (s, 1H), 7.21 (m, 1H), 7.39 (m, 1H), 7.62 (m, 1H), 7.91 (m, 1H), 8.59 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.8$ (CH_3), 25.8, 27.3 (CH_3), 29.0, 29.2, 30.0, 30.1, 35.5, 50.0, 63.1, 65.3, 66.1, 69.4 (multiple peaks, 7 OCH_2), 72.1, 79.3, 96.1 (CH), 117.9 (CH), 119.2, 126.1 (CH), 128.2, 119.3 (CH), 130.1, 131.1 (CH), 146.1, 145.3, 150.2, 150.1, 151.8, 153.7 (C═O), 156.2 (C═O), 157.1 (C═O), 169.9 (COO),

172.2 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 296$ (11 500), 329 (13 900), 371 (32 700), 385 (28 600) nm. UV-vis (methanol) $\lambda(\varepsilon) = 293$ (10 300), 331 (14 500), 363 (29 500), 372 (28 500) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 289$ (27 700), 335 (11 500), 366 (27 400), 379 (25 300) nm. LC-MS (ESI), m/z = 811 [M^+ +H]. Anal. calcd. for $C_{41}H_{54}N_4O_{13}$: C, 60.73; H, 6.71; N, 6.91. Found: C, 60.59; H, 6.64; N, 6.81.

4.4.3 Compound **4c** (10-CPT, n = 4, $C_{43}H_{58}N_4O_{14}$, FW = 854)

Yield: 85%, mp 180–183°C. IR, $\nu = 3313$, 2929, 2860, 1718, 1654, 1600, 1541, 1489, 1446, 1348, 1227, 1195, 1103, 1043, 1001, 916, 835, 800, 760 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.85$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.20 (m, 2H, CH_2), 1.30 (m, 4H, 2 CH_2), 1.40 (s, 9H, 3 CH_3), 1.50 (m, 2H, CH_2), 1.80 (t, $J = 7.2$ Hz, 2H, CH_2), 2.41 (t, $J = 6.2$ Hz, 2H, CH_2), 2.90 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 3.35 (obscured with water peak, m, 2H, CH_2), 3.55 (m, 14H, 7 OCH_2), 4.05 (m, 2H, OCH_2), 5.25 (m, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.50 (s, 1H), 7.20 (m, 1H), 7.38 (m, 1H), 7.65 (m, 1H), 7.95 (m, 1H), 8.62 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.7$ (CH_3), 25.9, 27.5 (CH_3), 29.1, 29.3, 30.0, 30.3, 35.8, 50.2, 62.9, 65.3, 66.2, 69.7 (multiple peaks, 9 OCH_2), 72.4, 79.7, 96.6 (CH), 118.6 (CH), 118.9, 126.2 (CH), 128.4, 130.1 (CH), 130.2, 130.9 (CH), 145.4, 145.5, 149.7, 150.0, 152.1, 154.0 (C═O), 156.1 (C═O), 156.8 (C═O), 170.4 (COO), 172.4 (COO) ppm UV-vis (DMF) $\lambda(\varepsilon) = 295$ (11 400), 332 (13 400), 368 (32 600), 382 (28 400) nm. UV-vis (methanol) $\lambda(\varepsilon) = 292$ (9 350), 330 (14 400), 362 (29 300), 375 (28 200) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 286$ (27 600), 332 (11 200), 368 (27 900), 385 (25 000) nm. LSIMS (matrix: thioglycerol), m/z = 855 [M^+ +H], 799, 365. HRMS (LSIMS, matrix: thioglycerol): found 855.40251, required 855.40278 for $[C_{43}H_{59}N_4O_{14}]^+$. Anal. calcd. for $C_{43}H_{58}N_4O_{14} \cdot 1/2H_2O$: C, 59.78; H, 6.88; N, 6.49. Found: C, 60.04; H, 7.04; N, 6.21.

4.4.4 Compound **5a** (SN-38, n = 2)

Yield: 88%, mp 89–95°C. IR, $\nu = 3311$, 2935, 2864, 1743, 1652, 1621, 1521, 1483, 1442, 1334, 1231, 1213, 1144, 1069, 1035, 930, 836, 823, 790 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.95$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.45 (m, 22 H, 4 CH_3, 5 CH_2), 1.81 (m, 2H, CH_2), 2.40 (m, 4H, 2 CH_2), 3.05 (m, 2H, CH_2), 3.50 (obscured with water peak, m, 8H, CH_2, 3OCH_2), 4.22 (m, 2H, OCH_2), 5.35 (m, 2H, CH_2), 5.45 (s, 2H, CH_2), 7.20 (m, 1H), 7.65 (m, 1H), 7.90 (m, 1H), 8.15 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.7$ (CH_3), 13.7 (CH_3), 22.2, 24.5, 28.4 (CH_3), 28.9, 29.1, 30.2, 30.8, 34.5, 49.9, 62.6, 64.1, 67.3, 70.3 (multiple peaks, 5 OCH_2), 71.8, 79.5, 96.4 (CH), 114.5 (CH), 118.8, 125.8 (CH), 127.0 128.3, 128.4, 131.0 (CH), 145.0, 146.0, 149.8, 151.5, 154.1, 156.8 (C═O), 158.1 (C═O), 156.8 (C═O), 170.7 (COO), 172.9 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 291$ (12 400), 335

(14 700), 380 (34 600), 398 (29 200) nm. UV-vis (methanol) $\lambda(\varepsilon) = 293$ (12 600), 331(14 200), 370 (29 500), 380 (29 500) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 297$ (27 300), 338 (13 400), 377 (28 600), 384 (27 800) nm. LSIMS (matrix: thioglycerol), m/z = 795 [M^+ +H]. Anal. calcd. for $C_{41}H_{54}N_4O_{12}$: C, 61.95; H, 6.85; N, 7.05. Found: C, 61.75; H, 6.64; N, 7.21.

4.4.5 Compound **5b** (SN-38, n = 3)

Yield: 85%, mp 75–80°C. IR, $\nu = 3291, 2945, 2875, 1735, 1642, 1611, 1511, 1451, 1438, 1345, 1233, 1223, 1154, 1069, 1025, 890, 847, 833, 765$ cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.95$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.42 (m, 22 H, 4 CH_3, 5 CH_2), 1.79 (m, 2H, CH_2), 2.44 (m, 4H, 2 CH_2), 3.10 (m, 2H, CH_2), 3.50 (obscured with water peak, m, 12H, CH_2, 5OCH_2), 4.20 (m, 2H, OCH_2), 5.30 (m, 2H, CH_2), 5.50 (s, 2H, CH_2), 7.25 (m, 1H), 7.40 (m, 1H), 7.85 (m, 1H), 8.20 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.8$ (CH_3), 13.6 (CH_3), 23.1, 24.5, 27.9 (CH_3), 28.5, 29.3, 30.1, 30.9, 33.9, 50.3, 61.9, 63.5, 66.8, 70.5 (multiple peaks, 7 OCH_2), 72.1, 80.5, 95.9 (CH), 114.3 (CH), 118.9, 125.2 (CH), 127.3 129.1, 129.5, 131.1 (CH), 145.1, 145.8, 149.3, 150.5, 153.1, 155.9 (C═O), 157.9 (C═O), 158.8 (C═O), 171.7 (COO), 172.5 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 289$ (12 100), 333 (15 200), 385 (35 100), 397 (28 900) nm. UV-vis (methanol) $\lambda(\varepsilon) = 290$ (13 400), 331 (14 500), 371 (29 600), 381 (28 700) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 295$ (28 100), 335 (14 500), 378 (28 700), 386 (26 600) nm. LSIMS (matrix: thioglycerol), m/z = 839 [M^+ +H]. Anal. calcd. for $C_{43}H_{58}N_4O_{13}$: C, 61.56; H, 6.97; N, 6.68. Found: C, 61.75; H, 6.74; N, 6.41.

4.4.6 Compound **5c** (SN-38, n = 4)

Yield: 82%, mp 73–78°C. IR, $\nu = 3285, 2930, 2845, 1729, 1643, 1601, 1521, 1435, 1440, 1340, 1243, 1243, 1165, 1059, 1036, 910, 856, 832, 745$ cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.90$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.46 (m, 22 H, 4 CH_3, 5 CH_2), 1.81 (m, 2H, CH_2), 2.45 (m, 4H, 2 CH_2), 3.12 (m, 2H, CH_2), 3.55 (obscured with water peak, m, 16H, CH_2, 7 OCH_2), 4.25 (m, 2H, OCH_2), 5.35 (m, 2H, CH_2), 5.45 (s, 2H, CH_2), 7.20 (m, 1H), 7.38 (m, 1H), 7.79 (m, 1H), 8.18 (m, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.7$ (CH_3), 13.8 (CH_3), 22.9, 24.1, 28.1 (CH_3), 28.3, 29.0, 29.9, 30.2, 32.3, 49.6, 62.1, 62.9, 67.1, 71.0 (multiple peaks, 9 OCH_2), 72.6, 80.8, 96.1 (CH), 114.4 (CH), 118.2, 125.5 (CH), 126.9, 128.9, 129.1, 131.9 (CH), 146.0, 146.8, 148.3, 151.5, 152.9, 156.1 (C═O), 156.9 (C═O), 159.8 (C═O), 171.3 (COO), 172.1 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 291$ (12 400), 334 (15 500), 380 (36 100), 396 (28 700) nm. UV-vis (methanol) $\lambda(\varepsilon) = 294$ (12 900), 333 (14 900), 379 (31 300), 391 (29 200) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 291$ (28 800), 334 (14 200), 380 (29 700), 391 (27 900) nm. LSIMS (matrix: thioglycerol), m/z = 883 [M^+ +H]. Anal. calcd. for $C_{45}H_{62}N_4O_{14}$: C, 61.21; H, 7.08; N, 6.35. Found: C, 61.15; H, 6.84; N, 6.41.

4.5 General Procedure for Synthesis of Compounds 6 and 7

Compound **4** (or **5**, 0.585 mmol) was placed in a 50-mL, round-bottomed flask equipped with a magnetic stirrer. Trifluoroacetic acid (10 mL) was added. The mixture was stirred at room temperature for 20 min. Then anhydrous ether (60 mL) was added slowly over a period of 5 min. The suspension was then placed over an ultrasonic bath for 2 min. The yellow solid was collected by suction filtration. The crude product is then redissolved in a minimum amount of DMF and precipitated by anhydrous ether. The expected compound **6** (or **7**) was obtained after filtration and drying under vacuum overnight.

4.5.1 Compound **6a** (n = 2)

Yield 88%, mp 132–138°C. IR, $\nu = 3335$, 2932, 2823, 1735, 1642, 1611, 1530, 1450, 1430, 1340, 1240, 1178, 1140, 1120, 1045, 1028, 912, 812 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.85$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.35–1.45 (m, 8H, 4 CH_2), 1.80 (q, J=7.2 Hz, 2H, CH_2), 2.95 (t, $J = 6.9$ Hz, 2H, CH_2), 3.10 (t, $J = 6.9$ Hz, 2H, CH_2), 3.50 (m, 12 H, 4 OCH_2), 4.05 (m, 2H, OCH_2), 5.35 (m, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.60 (s, 1H), 7.25 (m, 1H), 7.55 (m, 1H), 8.15 (m, 1H), 8.20 (m, 1H), 8.60 (m, 1H), 12.20 (brs, COOH, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.8$ (CH_3), 24.7, 28.9, 29.6, 30.3, 34.7, 50.8, 63.5, 64.9, 65.9, 68.8, 70.5 (multiple peaks, 5 OCH_2), 72.8, 96.9 (CH), 118.5 (CH), 119.5, 125.6 (CH), 127.1, 1291 (CH), 130.8, 132.3 (CH), 133.6, 142.1, 146.9, 149.9, 153.8, 154.7 (C═O), 156.4 (C═O), 156.9 (C═O), 170.8 (COO), 172.7 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 283$ (12 100), 333 (12 300), 363 (29 900), 386 (25 300) nm. UV-vis (methanol) $\lambda(\varepsilon) = 281$ (15 700), 334 (13 100), 370 (28 600), 390 (26 800) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 290$ (11, 800), 332 (12 300), 371 (25 900), 391 (25 800) nm. LSIMS (matrix: thioglycerol),]m/z = 711 [M^+ +H]. Anal. calcd. for $C_{35}H_{42}N_4O_{12}$: C, 59.15; H, 5.96; N, 7.88. Found: C, 59.00; H, 6.05; N, 7.75.

4.5.2 Compound **6b** (n = 3)

Yield 95%, mp 148–152°C. IR, $\nu = 3345$, 2930, 2850, 1731, 1652, 1601, 1525, 1449, 1433, 1338, 1236, 1186, 1136, 1121, 1040, 1008, 920, 830 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.90$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.30–1.50 (m, 8H, 4 CH_2), 1.85 (q, $J = 7.2$ Hz, 2H, CH_2), 2.90 (t, $J = 6.9$ Hz, 2H, CH_2), 3.15 (t, $J = 6.9$ Hz, 2H, CH_2), 3.50 (m, 12 H, 6 OCH_2), 4.10 (m, 2H, OCH_2), 5.30 (m, 2H, CH_2), 5.40 (s, 2H, CH_2), 6.55 (s, 1H), 7.20 (m, 1H), 7.60 (m, 1H), 8.10 (m, 1H), 8.25 (m, 1H), 8.50 (m, 1H), 12.15 (brs, COOH, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.9$ (CH_3), 25.4, 29.3, 29.5, 30.8, 34.3, 50.3, 64.5, 65.1, 66.1, 68.2, 69.1 (multiple peaks, 7 OCH_2), 72.3, 96.5 (CH), 118.8 (CH), 119.4, 125.9 (CH),

127.6, 129.5 (CH), 130.1, 132.8 (CH), 133.6, 142.5, 147.9, 150.6, 152.8, 154.1 (C═O), 155.4 (C═O), 156.2 (C═O), 171.8 (COO), 172.6 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 285$ (11 500), 334 (12 500), 365 (29 500), 386 (25 900) nm. UV-vis (methanol) $\lambda(\varepsilon) = 284$ (15 900), 335 (12 800), 369 (29 900), 389 (25 700) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 289$ (17 800), 334 (11 300), 370 (24 900), 389 (25 100) nm. LSIMS (matrix: thioglycerol), $m/z = 755$ [M^+ +H]. Anal. calcd. for $C_{37}H_{46}N_4O_{13}$: C, 58.88; H, 6.14; N, 7.42. Found: C, 58 60; H, 6.05; N, 7.25.

4.5.3 Compound **6c** (n = 4)

Yield 92%, mp 155–159°C. IR, $\nu = 3311$, 2920, 2858, 1730, 1655, 1600, 1539, 1498, 1443, 1348, 1226, 1195, 1151, 1103, 1043, 1001, 914, 833 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.85$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.25–1.50 (m, 8H, 4 CH_2), 1.84 (q, J =7.2 Hz, 2H, CH_2), 2.95 (t, $J = 6.9$ Hz, 2H, CH_2), 3.13 (t, $J = 6.9$ Hz, 2H, CH_2), 3.55 (m, 16H, 8 OCH_2), 4.05 (m, 2H, OCH_2), 5.25 (m, 2H, CH_2), 5.44 (s, 2H, CH_2), 6.55 (s, 1H), 7.26 (m, 1H), 7.60 (m, 1H), 8.08 (m, 1H), 8.16 (m, 1H), 8.67 (m, 1H), 12.10 (brs, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.8$ (CH_3), 25.9, 29.1, 29.3, 30.2, 34.7, 50.2, 64.9, 65.2, 66.2, 68.9, 69.7 (multiple peaks, 9 OCH_2), 72.4, 96.6 (CH), 118.6 (CH), 119.0, 126.2 (CH), 128.4, 130.2 (CH), 130.8, 131.1 (CH), 131.6, 145.4, 149.7, 150.0, 152.1, 154.0 (C═O), 156.1 (C═O), 156.8 (C═O), 172.4 (COO), 172.6 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 283$ (10 600), 332 (11 800), 367 (28 900), 385 (25 000) nm. UV-vis (methanol) $\lambda(\varepsilon) = 283$ (15 800), 332 (11 800), 367 (28 900), 385 (25 000) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 286$ (17 100), 332 (9 300), 368 (23 700), 386 (21 100) nm. LSIMS (matrix: thioglycerol), $m/z = 799$ [M^+ +H], 429, 365. HRMS (LSIMS, matrix: thioglycerol): found 799.34053, required 799.34018 for $[C_{39}H_{51}N_4O_{14}]^+$. Anal. calcd. for $C_{39}H_{50}N_4O_{14} \cdot 1/3H_2O$: C, 58.20; H, 6.35; N, 6.96. Found: C, 58.10; H, 6.27; N, 7.05.

4.5.4 Compound **7a** (n = 2)

Yield 87%, mp 122–127°C. IR, $\nu = 3343$, 2935, 2866, 1734, 1635, 1620, 1545, 1489, 1433, 1332, 1234, 1175, 1143, 1123, 1025, 1032, 915, 746 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.93$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.35–1.50 (m, 11 H, CH_3, 4 CH_2), 1.90 (q, $J = 7.2$ Hz, 2H, CH_2), 2.55 (m, 4H, 2 CH_2), 3.10 (m, 4H, 2 CH_2), 3.85 (m, 8H, 4 OCH_2), 4.20 (m, 2H, OCH_2), 5.35 (m, 2H, CH_2), 5.45 (s, 2H, CH_2), 7.25 (m, 1H), 7.50 (m, 1H), 7.85 (m, 1H), 8.20 (m, 1H), 11.25 (brs, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.9$ (CH_3), 13.2 (CH_3), 22.8, 26.5, 26.8, 29.2, 29.9, 30.4, 34.8, 62.0, 63.1, 66.4, 67.3, 68.7 (multiple peaks, 5 OCH_2), 72.6, 96.5 (CH), 118.2, 119.3, 127.1 (CH), 128.6, 130.4 (CH), 130.7, 131.6 (CH), 132.7, 146.2, 149.4, 150.7, 152.1, 153.9 (C═O), 157.1 (C═O), 157.9

(C═O), 170.2 (COO), 172.1 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 282$ (13 700), 330 (13 500), 370 (26 600), 385 (27 300) nm. UV-vis (methanol) $\lambda(\varepsilon) = 289$ (12 900), 340 (13 100), 365 (25 200), 382 (23 400) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 284$ (13 400), 340 (13 900), 374 (24 400), 390 (22 300) nm. LSIMS (matrix: thioglycerol), m/z = 739 [M^+ +H]. Anal. calcd. for $C_{37}H_{46}N_4O_{12}$: C, 60.15; H, 6.28; N, 7.58. Found: C, 59.90; H, 6.15; N, 7.45.

4.5.5 Compound **7b** (n = 3)

Yield 85%, mp 143–147°C. IR, $\nu = 3332$, 2945, 2875, 1724, 1634, 1626, 1535, 1490, 1422, 1312, 1245, 1185, 1153, 1120, 1020, 1036, 910, 736 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.90$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.35–1.50 (m, 11 H, CH_3, 4 CH_2), 1.85 (q, $J = 7.2$ Hz, 2H, CH_2), 2.50 (m, 4H, 2 CH_2), 3.15 (m, 4H, 2 CH_2), 3.70 (m, 12H, 6 OCH_2), 4.10 (m, 2H, OCH_2), 5.30 (m, 2H, CH_2), 5.40 (s, 2H, CH_2), 7.35 (m, 1H), 7.55 (m, 1H), 7.80 (m, 1H), 8.25 (m, 1H), 11.20 (brs, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.8$ (CH_3), 13.5 (CH_3), 23.0, 26.4, 26.7, 29.6, 29.8, 30.2, 35.4, 62.5, 63.4, 66.1, 67.8, 68.9 (multiple peaks, 7 OCH_2), 72.9, 96.8 (CH), 118.5, 119.4, 127.3 (CH), 128.7, 130.1 (CH), 130.5, 131.8 (CH), 132.6, 146.0, 149.1, 150.1, 151.8, 154.8 (C═O), 156.9 (C═O), 157.8 (C═O), 170.8 (COO), 172.9 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 283$ (12 600), 336 (12 300), 375 (27 300), 390 (28 100) nm. UV-vis (methanol) $\lambda(\varepsilon) = 283$ (14 500), 344 (14 500), 369 (27 200), 392 (24 400) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 280$ (17 400), 341 (15 300), 370 (24 000), 395 (22 900) nm. LSIMS (matrix: thioglycerol), m/z = 783 [M^+ +H]. Anal. calcd. for $C_{39}H_{50}N_4O_{13}$: C, 59.84; H, 6.44; N, 7.16. Found: C, 59.70; H, 6.55; N, 7.35.

4.5.6 Compound **7c** (n = 4)

Yield 82%, mp 132–137°C. IR, $\nu = 3322$, 2925, 2866, 1727, 1652, 1610, 1542, 1492, 1434, 1326, 1237, 1175, 1148, 1109, 1048, 1011, 912, 836 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), $\delta = 0.95$ (t, $J = 7.2$ Hz, 3H, CH_3), 1.25–1.50 (m, 11 H, CH_3, 4 CH_2), 1.90 (q, $J = 7.2$ Hz, 2H, CH_2), 2.55 (m, 4H, 2 CH_2), 3.10 (m, 4H, 2 CH_2), 3.75 (m, 16H, 8 OCH_2), 4.05 (m, 2H, OCH_2), 5.25 (m, 2H, CH_2), 5.44 (s, 2H, CH_2), 7.25 (m, 1H), 7.65 (m, 1H), 7.90 (m, 1H), 8.20 (m, 1H), 11.10 (brs, 1H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz), $\delta = 7.7$ (CH_3), 13.8 (CH_3), 22.33, 26.1, 26.4, 29.5, 29.4, 30.0, 35.8, 62.9, 63.7, 66.2, 68.8, 69.7 (multiple peaks, 9 OCH_2), 72.4, 96.5 (CH), 118.8, 119.0, 127.0 (CH), 128.4, 130.2 (CH), 130.8, 131.1 (CH), 131.6, 145.0, 149.9, 150.0, 151.5, 154.1 (C═O), 156.1 (C═O), 156.8 (C═O), 170.4 (COO), 172.6 (COO) ppm. UV-vis (DMF) $\lambda(\varepsilon) = 285$ (11 800), 340 (12 900), 374 (29 300), 394 (28 200) nm. UV-vis (methanol) $\lambda(\varepsilon) = 290$ (14 700), 354 (13 800), 379 (26 200), 399 (25 800) nm. UV-vis (DMSO) $\lambda(\varepsilon) = 279$ (16 400), 342 (14 300), 376 (24 600), 390 (23 100)

nm. LSIMS (matrix: thioglycerol), $m/z = 827$ $[M^+ + H]$. Anal. calcd. for $C_{41}H_{54}N_4O_{14}$: C, 59.55; H, 6.58; N, 6.78. Found: C, 59 30; H, 6.75; N, 6.55.

4.6 General Procedure for Synthesis of Conjugates 8 and 9

A mixture of compounds **6** (or **7**, 0.007 mmol), *O*-benzotriazole-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (BTTU, 6.9 mg, 0. 021 mmol, 3.0 equiv.), triethylamine (0.01 mL, 0.07 mmol, 10 equiv.), and DMF (0.5 mL) was stirred at room temperature for 60 min. The solution was saved for the following reaction.

Melanotransferrin p97 (in PBS, pH 7.4, $c = 1.23$ mg/mL, 11 mL, 1.433×10^{-4} mM) was placed in a 50-mL, round-bottomed flask. To this solution, BTTU-activated camptothecin compound prepared from the previous mixture (0.007 mmol, 50 equiv., mixed with 1.5 mL of DMF) was added dropwise over a period of 5 min under vigorous stirring. The mixture was stirred at room temperature for 20 h, then purified by dialysis using Slide-A-Lyzer dialysis cassette (WMCO = 10 K) against PBS (10 mM, pH = 7.4) to give the expected conjugates **8** and **9**.

REFERENCES

1. Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, 1996.
2. Wong, S. S. *Chemistry of Protein Conjugation and Cross-Linking*; CRC Press: Boca Raton, FL, 1991.
3. (a) Chen, Q.; Sowa, D. A.; Cai, J.; Gabathuler, R. Efficient one-pot synthesis of doxorubicin conjugates through its amino group to melanotransferrin P97. *Synth. Commun.* **2003**, *33* (14), 2399–2419; (b) Chen, Q.; Sowa, D. A.; Cai, J.; Gabathuler, R. Synthesis of doxorubicin conjugates through hydrazone bonds to melanotransferrin P97. *Synth. Commun.* **2003**, *33* (14), 2375–2388; (c) Chen, Q.; Sowa, D. A.; Gabathuler, R. Synthesis of doxorubicin conjugates through 14-hydroxy group to melanotransferrin P97. *Synth. Commun.* **2003**, *33* (14), 2389–2398; (d) Chen, Q.; Gabathuler, R. Synthesis of camptothecin melantotransferrin (P97) conjugate through a carbamate bond. *Synth. Commun.* **2004**, *34* (13), 2415–2424; (e) Chen, Q.; Gabathuler, R. Efficient synthesis of polyethylene glycol mono-carboxylate via michael conjugate addition. *Synth. Commun.* **2004**, *3* (13), 2425–2432; (f) Chen, Q.; Gabathuler, R. Efficient Synthesis of doxorubicin Melanotransferrin P97 conjugates through SMCC linker. *Synth. Commun.* **2004**, *34* (13), 2407–2414.
4. (a) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. Plant antitumor agents, I: The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata. *J. Am. Chem. Soc.* **1966**, *88* (16), 3888–3890; (b) Wall, M. E.; Wani, M. C. Camptothecin and taxol: Discovery to clinic: 13th Bruce F. Cain-Memorial Award Lecture. *Cancer Res.* **1995**, *55*, 753–760.
5. For reviews, see (a) Potmesil, M.; Pinedo, H. *Camptothecins: New Anticancer Agents*; CRC Press: Ann Arbor, MI, 1995; (b) Pantazis, P.; Giovanella, B. C.; Rothenberg, M. L. (Eds.). *The Camptothecins: From Discovery to the Patient*;

Academy of Sciences: New York, 1996; (c) Liehr, J G.; Giovanella, B. C.; Verschraegen, C. F. (Eds.). *The Camptothecins: Unfolding Their Anticancer Potential*; Academy of Sciences: New York, 2000.

6. (a) Zhang, R. W.; Li, Y. F.; Cai, Q. Y.; Liu, T. P.; Sun, H.; Chambless, B. Preclinical pharmacology of the natural product anticancer agent 10-hydroxycamptothecin, an inhibitor of topoisomerase I. *Cancer Chemth. Pharm.* **1998**, *41* (4), 257–267; (b) Erlichman, C.; Boerner, S. A.; Hallgren, C. G.; Spieker, R.; Wang, X. Y.; James, C. D.; Scheffer, G. L.; Maliepaard, M.; Ross, D. D.; Bible, K. C.; Kaufmann, S. H. The HER tyrosine kinase inhibitor CI1033 enhances cytotoxicity of 7-ethyl-10-hydroxycamptothecin and topotecan by inhibiting breast cancer resistance protein-mediated drug efflux. *Cancer Res.* **2001**, *61* (2), 739–748; (c) Kohara, H.; Tabata, M.; Kiura, K.; Ueoka, H.; Kawata, K.; Chikamori, M.; Aoe, K.; Chikamori, K.; Matsushita, A.; Harada, M. Synergistic effects of topoisomerase i inhibitor, 7-ethyl-10-hydroxycamptothecin, and irradiation in a cisplatin-resistant human small cell lung cancer cell line. *Clin. Cancer Res.* **2002**, *8* (1), 287–292.
7. For reviews, see (a) Pantazis, P. Preclinical studies of water-insoluble camptothecin congeners: Cytotoxicity, development of resistance, and combination treatments. *Clin. Cancer Res.* **1995**, *1* (11), 1235–1244; For reviews, see; (b) Liu, L. F. Biochemistry of camptothecin. In *Camptothecins: New Anticancer Agents*; Potmesol, M., Pinedo, H., (Eds.) CRC Press: Ann Arbor, MI, 1995, pp. 9–10.
8. (a) Greenwald, R. B.; Choe, Y. H.; Wu, D. Selective phenolic acylation of 10-hydroxycamptothecin using poly(ethylene glycol) carboxylic acid. *Bioorg. Med. Chem. Lett.* **2003**, *13* (3), 577–580.
9. (a) Bourzat, J. D.; Vuilhorgne, M.; Rivory, L. P.; Robert, J.; Commercon, A. Semisynthesis of RPR 121056A, a major metabolite of irinotecan (CPT-11). *Tetrahedron Lett.* **1996**, *37* (35), 6327–6330; (b) Angenault, S.; Thirot, S.; Schmidt, F.; Monneret, C.; Pfeiffer, B.; Renard, P. Cancer chemotherapy: A SN-38 (7-ethyl-10-hydroxycamptothecin) glucuronide prodrug for treatment by a PMT (prodrug monotherapy) strategy. *Bioorg. Med. Chem. Lett.* **2003**, *13* (5), 947–950.
10. Wang, C. C. C.; Dervan, P. B. Sequence-specific trapping of topoisomerase I by DNA binding polyamide-camptothecin conjugates. *J. Am. Chem. Soc.* **2001**, *123* (36), 8657–8661.
11. Ulrich, H. *Chemistry and Technology of Isocyanate*; John Wiley & Sons: New York, 1996.
12. Heymann, E. *Metabolic Basis of Detoxication*; Jakoby, W. B., Bend, J. R., and Caldwell, J., (Eds.) In *Metabolic Basis of Detoxication: Metabolism of Functional Groups*. Academic Press: New York, 1982, pp. 229–231.
13. Sawada, S.; Okajima, S.; Aiyama, R.; Nokata, K. I.; Furuta, T.; Yokokura, T.; Suguno, E.; Yamaguchi, K.; Miyasaka, T. Synthesis and antitumor activity of 20(s)-camptothecin dervatives—Carbamated-linked, water-soluble derivatives of 7-ethyl-10-hydroxycamptothecin. *Chem. Pharm. Bull.* **1991**, *39* (6), 1446–1454.
14. (a) Seitz, O.; Kunz, H. HYCRON, an allylic anchor for high-efficiency solid phase synthesis of protected peptides and glycopeptides. *J. Org. Chem.* **1997**, *62* (4), 813–826; (b) Chen, Q.; Gabathuler, R. Efficient synthesis of polyethylene glycol mono-carboxylate via Michael conjugate addition. *Synth. Commun.* **2004**, *3* (13), 2425–2432.
15. Warnecke, A.; Kratz, F. Maleimide-oligo(ethylene glycol) derivatives of camptothecin as albumin-binding prodrugs: Synthesis and antitumor efficacy. *Bioconjugate Chem.* **2003**, *14* (2), 377–387.

16. Delgado, C.; Francis, G. F.; Fisher, D. The uses and properties of PEG-linked proteins. *Crit. Rev. Ther. Drug Carrier Syst.* **1992**, *9* (3), 249–304.
17. (a) Rothenberger, S.; Food, M. R.; Gabathuler, R.; Kennard, M. L.; Yamada T.; Yasuhara, O.; McGeer, P. L.; Jefferies, W. A. Coincident expression and distribution of melanotransferrin and transferrin receptor in human brain capillary endothelium. *Brain Res.* **1996**, *712*, 117–121; (b) Yamada, T.; Tsujioka, Y.; Taguchi, J.; Takahashi, M.; Tsuboi, Y.; Moroo, I.; Yang, J.; Jefferies, W. A. Melanotransferrin is produced by senile plaque-associated reactive microglia in Alzheimer's disease. *Brain Res.* **1999**, *845*, 1–5.