# Synthesis and Protein Binding of (4-Carboxybutyl)carbamoyl-Substituted Taxoids

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(4-Carboxybutyl)carbamates **5** and **6**, as well as **10**, derived from 10-*O*-deacetylbaccatin III (**1**) and paclitaxel (**2**), respectively, were synthesized by reaction of unprotected **1** and 2′-*O*-(methoxyacetyl)paclitaxel (**8**), respectively, with trimethylsilyl 5-isocyanatopentanoate in good yields. The carbamoyl-taxoids were conjugated to bovine-serum albumin and analyzed by MALDI-TOF mass spectrometry.

**Introduction.** – Since the discovery and development of paclitaxel (*Taxol*®) as an important anticancer drug [1], a number of attemps have been made to synthesize taxoids carrying potential spacer moieties to conjugate these compounds to proteins for use as haptens in antibody preparation, to attach to fluorescent groups, or to improve their water solubility by binding to hydrophilic molecules. For example, succinoyl-taxoids of 10-*O*-deacetylbaccatin III (**1**) [2] and paclitaxel (**2**) [3][4] were prepared, bound to bovine-serum albumine, and employed in the preparation of antibodies and the development of immunoenzymatic assays. The 2′-*O*-glutarylpaclitaxel served as an intermediate in the synthesis of a hexanediamine derivative, which was treated with fluorescein isothiocyanate as a fluorescent dye [5]. Further taxoid derivatives possessing a free amino group, *e.g.*, 7-*O*-(L-alanyl)taxol [6], were also used to enable coupling to fluorescent [7] or luminescent components [8]. Formation of carbamates is considered another possibility to functionalize taxoid molecules. The (trichloroethoxy)-carbonyl derivative (TROC) of paclitaxel (**2**) has been treated with primary amines to prepare simple carbamates [9]. Polyethylene glycol conjugates at the 7-hydroxy group of paclitaxel linked *via* a carbamate functionality were prepared from an isocyanate precursor [10]. A carbamate side chain has been attached to the 10-*O*-position of 10-deacetyl-7-*O*-(triethylsilyl)baccatin III by reaction with phenyl isocyanate or dimethylcarbamic chloride [11]. Isocyanates carrying a protected $\omega$-carboxy group should be suitable to couple directly to reactive 7- and 10-hydroxy groups of 10-*O*-deacetylbaccatin III (DAB; **1**) or paclitaxel. In this paper, we report the synthesis of taxoid carbamates of that type and demonstrate proper binding to bovine-serum albumin.

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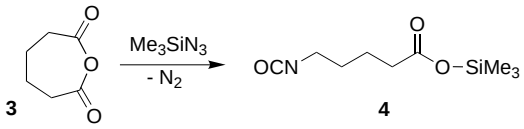
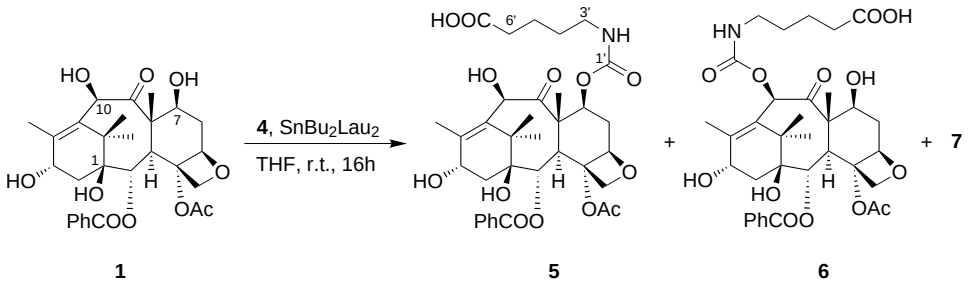
**Results and Discussion.** – Regioselective substitution of 10-*O*-deacetylbaccatin III (**1**) at the 7- or 10-OH group requires selective protection of the 10- or 7-OH group, respectively, generation of the lithium alkoxide, reaction with an electrophile, and finally deprotection [11]. Since we were interested in both 7- and 10-*O*-substituted derivatives, a non-regioselective approach including subsequent separation of the product mixture seemed a reasonable alternative. Therefore, unprotected 10-*O*-deacetylbaccatin III (**1**) was employed in the reaction with trimethylsilyl 5-isocyanatopentanoate (**4**; *Scheme 1*). Starting material **4** was prepared from hexanedioic acid *via* its anhydride **3** and reaction of the latter with azidotrimethylsilane [12]. In comparison with literature data [12], the yield of anhydride **3** was significantly improved to 78%.

*Scheme 1*




As expected, the reaction of **1** with the isocyanato ester **4** in the presence of dibutyltin dilaurate as a catalyst yielded three products: 7-*O*-[(4-carboxybutyl)carbamoyl]-10-*O*-deacetylbaccatin III (**5**), 10-*O*-[(4-carboxybutyl)carbamoyl]-10-*O*-deacetylbaccatin III (**6**), and 7,10-bis-*O*-[(4-carboxybutyl)carbamoyl]-10-*O*-deacetylbaccatin III (**7**; *Scheme 2*). The ratio of monocarbamates **5** and **6**, and dicarbamate **7** was determined by HPLC. The best yield of **5**/**6** and minimum amounts of the undesired dicarbamate **7** were obtained after 16 h reaction at room temperature. Separation of the regioisomers **5** and **6**, and dicarbamate **7** was carried out by prep. HPLC. Mass and NMR specroscopic data (see *Exper. Part*) confirmed their structures.

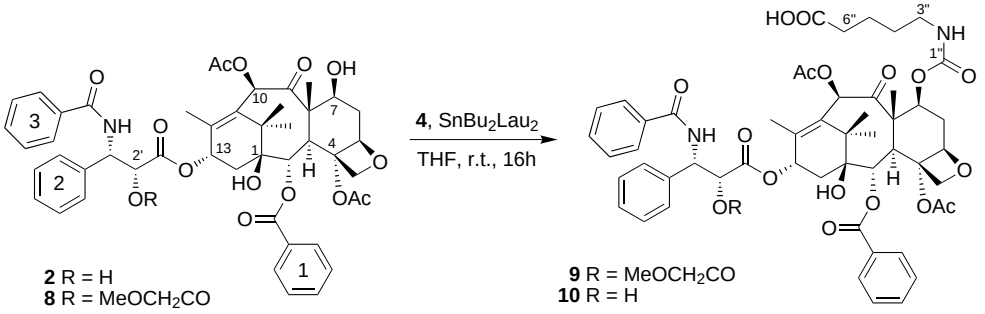
*Scheme 2*




Both carbamates **5** and **6** showed very similar MS and the same $M^+$ at $m/z$ 687. The $^1$H- and $^{13}$C-NMR spectra exhibited additional signals of the (4-carboxybutyl)carbamoyl side chains. The chemical-shift difference for H–C(7) of the parent DAB (**1**) ($\delta$ 4.27) and the corresponding carbamate **5** ($\delta$ 5.46) of *ca.* 1.2 ppm indicated attachment of the carbamate unit at C(7) in compound **5**. A similar shift difference of 1 ppm was observed for H–C(10) of **6** ($\delta$ 6.32) in comparison with that of **1** ($\delta$ 5.32). Attachment of the carbamoyl side chain was further confirmed by long-rang connectivities between *H*–C(7) and O*C*ONH (157.5) of **5** and *H*–C(10) and O*C*ONH (158.1) of **7** in the HMBC spectra.

A similar procedure as for the DAB carbamates **5** and **6** was applied to synthesize paclitaxel carbamate **10** (*Scheme 3*). Paclitaxel (**2**) was protected at the 2′-OH group by methoxyacetyl ($MeOCOCH_2$), a protection group, which has proved useful in taxoid chemistry [13][14]. Reaction of 2′-*O*-(methoxyacetyl)paclitaxel (**8**) with trimethylsilyl 5-isocyanatopentanoate (**4**) yielded 2′-*O*-(methoxyacetyl)paclitaxel carbamate **9**. Separation by means of reversed-phase HPLC (gradient *B*, see *Exper. Part*) revealed two products: a minor one (*ca.* 20%) at $t_R$ 9.7 min and the major component (*ca.* 80%) at $t_R$ 14.2 min. $^1$H-NMR Analysis indicated the major component was the expected compound **9** and the minor one the 2′-*O*-deprotected paclitaxel carbamate **10**. Complete deprotection under basic conditions smoothly gave the desired product **10**, which was purified by prep. HPLC. Mass spectra, 1D and heterocorrelated 2D NMR data confirmed the structure of intermediates **8** and **9**, and of the final product **10**, allowing complete assignment of the $^1$H- and $^{13}$C-NMR chemical shifts.

*Scheme 3*




Attachment of the carbamate moiety caused a downfield shift of H–C(7) from $\delta$ 4.40 in paclitaxel (**2**) or $\delta$ 4.42 in 2′-*O*-(methoxyacetyl)paclitaxel (**8**) to $\delta$ 5.50 in **9** and $\delta$ 5.47 in the final product **10**. A cross-peak between H–C(7) and O*C*ONH in the HMBC spectrum confirmed attachment of the carbamate unit to C(7). $^{13}$C-Chemical-shift differences up to *ca.* 4 ppm were observed for the phenylisoserine side-chain C-atoms C(1′) to C(3′) of **10** in comparison to compound **9** and, surprisingly, up to *ca.* 2.5 ppm for the terminal C-atoms (C(6″) and COOH) of the carbamate side chain. The latter chemical-shift differences may be caused by changes in the environment of the (4-carboxybutyl)carbamate unit: due to the cavity of the paclitaxel skeleton, this unit is located spatially close to the $MeOCH_2CO$ group at the phenylisoserine side chain of **9**.

Conjugation of the carbamate derivatives **5** and **6** to bovine-serum albumin (BSA) as a carrier protein was achieved by the carbodiimide method [15]. MALDI-TOF Mass spectrometry was used to determine the number of [(4-carboxybutyl)carbamoyl]DAB units attached to BSA (*m*/*z* 66504). The average mass of the 7-*O*-[(4-carboxybutyl)-carbamoyl]DAB (**5**)–BSA complex was determined as *m*/*z* 72958, indicating attachment of 9.4 units of **5** to BSA. The *m*/*z* 72348 of 10-*O*-[(4-carboxybutyl)carbamoyl]-DAB (**6**)–BSA complex was due to a 8.5 : 1 ratio of **6**/BSA. The average mass of 7-*O*-[(4-carboxybutyl)carbamoyl]paclitaxel (**10**)–BSA was *m*/*z* 71741, corresponding to a 4.9 : 1 ratio of **10**/BSA. Antigens obtained by this procedure are suitable for the preparation of antibodies against DAB and paclitaxel, respectively.

## Experimental Part

1. *General*. The 10-*O*-deacetylbaccatin III (DAB; **1**) was a gift of *P. Potier*, Gif-sur-Yvette, France. Paclitaxel (**2**), bovine-serum albumine (BSA), and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDAC) were purchased from *Sigma*. Other chemicals and solvents were commercial products from *Aldrich* and *Merck*. All reactions were carried out under a continuous stream of $N_2$. Anal. HPLC: *LiChrospher®-100-RP-18* column (250 × 4 mm); linear gradient 30 → 40% MeCN/$H_2O$ within 25 min, 0.6 ml $min^{-1}$ (gradient *A*); 45% MeCN/$H_2O$ for 5 min, then to 70% MeCN/$H_2O$ within 25 min, 0.8 ml $min^{-1}$ (gradient *B*); detection by *UV-DAD* at 210–400 nm. Prep. HPLC: *Macherey-Nagel Nucleosil®-100-7$C_{18}$* column (250 × 21 mm); linear gradient 20 → 50% MeCN/$H_2O$ within 30 min, 10.0 ml $min^{-1}$; UV detection at 230 nm. NMR Spectra: $^1$H-NMR, $^1$H,$^1$H-COSY, HMBC, and HMQC, *Bruker Avance DRX 500* with a 2.5-mm inverse-detection microprobe head; $^{13}$C-NMR and DEPT, 2.5-mm broadband-detection microprobe head; $^1$H and $^{13}$C routine spectra, *Varian Gemini 2000 300BB*; $CDCl_3$ or $CD_3OD$ as solvent and $Me_4Si$ as an internal standard; $\delta$ in ppm, *J* in Hz. ESI-MS: *TSQ 7000 Finnigan* (electrospray voltage 4.5 kV; heated capillary, temp. 220°; sheath gas $N_2$) coupled with a *Micro-Tech-Ultra-Plus-MicroLC* system (reversed-phase $C_{18}$ colum (4 μm, 1 × 100 mm, *Ultrasep*); gradient $H_2O$/MeCN 4:1 (each containing 0.2% AcOH) → 1:9 within 15 min, then 1:9 for 10 min; flow rate 70 μl $min^{-1}$). HR-MS and/or combustion analysis data are not available. The compounds were used up completely in the preparation of BSA conjugates. MALDI-TOF-MS of the conjugates: *Micromass-TofSpec-2E* time-of-flight mass spectrometer operating in the linear mode (positive-ion detection); delayed ion extraction mode measurements to enhance resolution; matrix, sinapic acid.

2. *Starting Materials. Hexanedioic Anhydride* (= *Oxepane-2,7-dione*; **3**). A mixture of hexanedioic acid (5 g, 34 mmol) and freshly distilled $Ac_2O$ (20 ml) was stirred under reflux for 12 h. AcOH and excess of $Ac_2O$ were evaporated. The crude product was heated to 220° for 10 min and purified by distillation at 84–86°/0.5 Torr: 3.4 g (78%) of **3** ([12]: 23%).

*5-Isocyanatopentanoic Acid Trimethylsilyl Ester* (**4**). Azidotrimethylsilane (3 ml, 20 mmol) was added dropwise to a soln. of freshly prepared **3** (2 g, 16 mmol) in dioxane (12 ml) under continuous stirring at r.t. [12]. The mixture was slowly heated to 75° and, after $N_2$ evolution had ceased, refluxed for additional 20 min. Evaporation and distillation at 80–83°/0.3 Torr gave **4** (2.97 g, 87%). Moisture- and air-sensitive colorless oil. IR ($CHCl_3$): 2256 (N=C=O), 1711 (C=O). $^1$H-NMR (300 MHz, $CDCl_3$): 1.61 (*m*, $CH_2CH_2$); 2.30 (*t*, *J* = 6.8, $CH_2$); 3.28 (*t*, *J* = 6.3, $CH_2$). $^{13}$C-NMR (75.5 MHz, $CDCl_3$): 21.9 ($CH_2$); 30.5 ($CH_2$); 35.0 ($CH_2$); 42.6 ($CH_2$); 122.0 (NCO); 173.6 (COOR).

3. *10-O-Deacetylbaccatin III Carbamates. General Procedure.* An excess of isocyanate **4** (400 μl) and dibutyltin dilaurate (= dibutylbis(1-oxododecyloxy)stannane; 10 μl, 17 μmol) were added dropwise to a soln. of 10-*O*-deacetylbaccatin III (**1**; 30 mg, 55 μmol) in dry THF (5 ml). The mixture was stirred at r.t. for 16 h. Then the reaction was quenched by the addition of MeOH (2 ml), thereby removing the $Me_3Si$ group. The solvent was evaporated and the crude mixture diluted with MeCN/$H_2O$ 60:40. After prepurification (*RP18* cartridge, isocratic MeCN/$H_2O$ 60:40), the isomers were separated by prep. HPLC.

*7-O-[(4-Carboxybutyl)carbamoyl]-10-O-deacetylbaccatin III* (= *5-{{{[(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-(Acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-6,9,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-4-yl]oxy}carbonyl}amino}pentanoic Acid*; **5**). $^1$H-NMR (500 MHz, $CD_3OD$): 5.62 (*d*, *J* = 7.0, H–C(2)); 4.08 (*d*, *J* = 7.0, H–C(3)); 2.28 (*s*, Ac); 5.01 (br. *d*, *J* = 9.1, H–C(5)); 2.50 (*m*, $H_a$–C(6)); 1.85 (*m*, $H_b$–C(6)); 5.46 (*dd*, *J* = 10.3, 7.5, H–C(7)); 3.06 (*m*, $CH_2$(3′)); 1.50 (*m*, $CH_2$(4′)); 1.60 (*m*, $CH_2$(5′)); 2.27 (*m*, $CH_2$(6′)); 5.53 (*s*, H–C(10)); 4.80 (*t*, *J* = 8.8, H–C(13)); 2.38 (*dd*, *J* = 15.6, 7.0, $H_a$–C(14)); 2.25 (*m*, $H_b$–C(14)); 1.08 (*s*, Me(16)); 1.07 (*s*, Me(17)); 2.08 (*s*, Me(18)); 1.78 (*s*, Me(19)); 4.22 (*d*, *J* = 11.3, 1 H, $CH_2$(20)); 4.20 (*d*, *J* = 11.3, 1 H, $CH_2$(20)); 8.12 (*d*, *J* = 8.0, 2 $H_o$); 7.52 (*dd*, *J* = 8.0, 7.6, 2 $H_m$); 7.63 (*t*, *J* = 7.6, $H_p$). $^{13}$C-NMR (125 MHz, $CD_3OD$): 79.4 (C(1)); 76.4 (C(2)); 48.3 (C(3)); 81.7 (C(4)); 172.1 (Me*C*O); 22.7 (*Me*CO), 85.5 (C(5)); 34.8 (C(6)); 73.8 (C(7)); 157.5 (C(1′)); 41.5 (C(3′)); 30.4 (C(4′)); 23.9 (C(5′)); 36.2 (C(6′)); 178.9 (C(7′)); 57.6 (C(8)); 211.7 (C(9)); 76.3 (C(10)); 135.7 (C(11)); 145.1 (C(12)); 68.2 (C(13)); 40.6 (C(14)); 44.0 (C(15)); 20.8 (C(16)); 27.1 (C(17)); 15.4 (C(18)); 11.4 (C(19)); 77.6 (C(20)); 167.8 (Ph*C*O); 131.6 ($C_{ipso}$); 131.2 ($C_o$); 129.7 ($C_m$); 134.5 ($C_p$). ESI-MS: 710 (100, $[M + Na]^+$), 118 (30).

*10-O-[(4-Carboxybutyl)carbamoyl]-10-O-deacetylbaccatin III* (= *5-{{{[(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-(Acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,9,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-6-yl]oxy}carbonyl}amino}pentanoic Acid*; **6**). $^1$H-NMR (500 MHz, $CD_3OD$): 5.62 (*d*, *J* = 6.90, H–C(2)); 3.93 (*d*, *J* = 6.90, H–C(3)); 2.27 (*s*, Ac); 5.02 (br. *d*, *J* = 8.7, H–C(5)); 2.47 (*m*, $H_a$–C(6)); 1.78 (*m*, $H_b$–C(6)); 4.40 (*dd*, *J* = 10.9, 6.9, H–C(7));

6.32 (*s*, H−C(10)); 3.16 (*m*, $CH_2$(3′)); 1.56 (*m*, $CH_2$(4′)); 1.66 (*m*, $CH_2$(5′)); 2.32 (*m*, $CH_2$(6′)); 4.80 (*t*, *J* = 9.0, H−C(13)); 2.38 (*dd*, *J* = 15.5, 7.7, $H_a$−C(14)); 2.26 (*m*, $H_b$−C(14)); 1.12 (*s*, Me(16)); 1.07 (*s*, Me(17)); 2.07 (*s*, Me(18)); 1.64 (*s*, Me(19)); 4.20 (*d*, *J* = 10.9, 1 H, $CH_2$(20)); 4.18 (*d*, *J* = 10.9, 1 H, $CH_2$(20)); 8.12 (*d*, *J* = 7.9, 2 $H_o$); 7.51 (*dd*, *J* = 7.9, 7.4, 2 $H_m$); 7.63 (*t*, *J* = 7.4, $H_p$). $^{13}$C-NMR (125 MHz, $CD_3OD$): 79.5 (C(1)); 76.4 (C(2)); 48.3 (C(3)); 82.0 (C(4)); 172.1 (Me*C*O); 22.7 (*Me*CO); 86.0 (C(5)); 37.3 (C(6)); 72.9 (C(7)); 158.1 (C(1′)); 41.6 (C(3′)); 30.4 (C(4′)); 23.3 (C(5′)); 35.3 (C(6′)); 176.4 (C(7′)); 59.5 (C(8)); 207.5 (C(9)); 77.9 (C(10)); 133.3 (C(11)); 147.9 (C(12)); 68.2 (C(13)); 40.6 (C(14)); 44.0 (C(15)); 21.7 (C(16)); 27.2 (C(17)); 15.6 (C(18)); 10.2 (C(19)); 77.5 (C(20)); 167.8 (Ph*C*O); 131.6 ($C_{ipso}$); 131.1 ($C_o$); 129.7 ($C_m$); 134.5 ($C_p$). ESI-MS: 710 (34, [*M* + Na]$^+$), 118 (100).

*7,10-Bis*-O-*[(4-carboxybutyl)carbamoyl]-10*-O-*deacetylbaccatin III* (=*5,5′-[[(2a*R,*4*S,*4a*S,*6*R,*9*S,*11*S,*12*S,*12a*R,*12*bS*)-12b-(Acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-9,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H-*cyclodeca[3,4]benz[1,2-*b*]oxet-4,6-diyl]bis(oxycarbonylimino)]bis[pentanoic Acid]*; **7**). $^1$H-NMR (500 MHz, $CD_3OD$): 5.63 (*d*, *J* = 6.9, H−C(2)); 4.04 (*d*, *J* = 6.9, H−C(3)); 2.28 (*s*, Ac); 5.02 (br. *d*, *J* = 9.1, H−C(5)); 2.60 (*m*, $H_a$−C(6)); 1.76 (*m*, $H_b$−C(6)); 5.48 (*dd*, *J* = 10.0, 7.5, H−C(7)); 3.11 (*m*, $CH_2$(3′), $CH_2$(3″)); 1.53 (*m*, $CH_2$(4′), $CH_2$(4″)); 1.60 (*m*, $CH_2$(5′), $CH_2$(5″)); 2.30 (*m*, $CH_2$(6′), $CH_2$(6″)); 6.39 (*s*, H−C(10)); 4.80 (*t*, *J* = 8.8, H−C(13)); 2.37 (*dd*, *J* = 15.8, 7.1, $H_a$−C(14)); 2.25 (*m*, $H_b$−C(14)); 1.13 (*s*, Me(16)); 1.06 (*s*, Me(17)); 2.13 (*s*, Me(18)); 1.75 (*s*, Me(19)); 4.21 (*d*, *J* = 8.3, 1 H, $CH_2$(20)); 4.18 (*d*, *J* = 8.3, 1 H, $CH_2$(20)); 8.12 (*d*, *J* = 8.0, 2 $H_o$); 7.52 (*dd*, *J* = 8.0, 7.6, 2 $H_m$); 7.63 (*t*, *J* = 7.6, $H_p$). $^{13}$C-NMR (125 MHz, $CD_3OD$): 79.3 (C(1)); 76.1 (C(2)); 48.9 (C(3)); 81.8 (C(4)); 172.0 (Me*C*O); 22.7 (*Me*CO); 85.6 (C(5)); 34.8 (C(6)); 73.7 (C(7)); 157.3, 157.5 (C(1′), C(1″)); 41.7, 41.6 (C(3′), C(3″)); 30.1, 30.6 (C(4′), C(4″)); 23.6, 23.5 (C(5′), C(5″)); 35.3, 35.2 (C(6′), C(6″)); 178.4, 178.3 (C(7′), C(7″)); 57.6 (C(8)); 206.7 (C(9)); 77.8 (C(10)); 133.1 (C(11)); 147.1 (C(12)); 68.2 (C(13)); 40.4 (C(14)); 44.1 (C(15)); 21.3 (C(16)); 27.0 (C(17)); 15.6 (C(18)); 11.4 (C(19)); 77.5 (C(20)); 167.8 (Ph*C*O); 131.6 ($C_{ipso}$); 131.1 ($C_o$); 129.7 ($C_m$); 134.5 ($C_p$). ESI-MS: 853 (100, [*M* + Na]$^+$), 118 (20).

4. *Paclitaxel Carbamates. 2′*-O-*(Methoxyacetyl)paclitaxel* (=*(*α*R,*β*S)-*β*-(Benzoylamino)-*α*-[(methoxyacetyl)oxy]benzenepropanoic Acid (2a*R,*4*S,*4a*S,*6*R,*9*S,*11*S,*12*S,*12a*R,*12b*S)-6,12b-Bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H-*cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl Ester*; **8**). Protection of paclitaxel (**2**) was carried out according to *Greenwald* and co-workers [10]. The crude product was purified by prep. reversed-phase HPLC (63% yield after HPLC). $^1$H-NMR (500 MHz, $CDCl_3$): 5.67 (*d*, *J* = 7.1, H−C(2)); 3.79 (*d*, *J* = 7.1, H−C(3)); 2.45 (*s*, AcO−C(4)); 4.96 (br. *d*, *J* = 9.3, H−C(5)); 2.52 (*m*, $H_a$−C(6)); 1.87 (*m*, $H_b$−C(6)); 4.42 (*dd*, *J* = 10.3, 6.6, H−C(7)); 6.27 (*s*, H−C(10)); 2.21 (*s*, AcO−C(10)); 6.25 (*t*, *J* = 8.6, H−C(13)); 2.37 (*dd*, *J* = 9.3, 15.4, $H_a$−C(14)); 1.87 (*m*, $H_b$−C(14)); 1.12 (*s*, Me(16)); 1.21 (*s*, Me(17)); 1.92 (*s*, Me(18)); 1.66 (*s*, Me(19)); 4.15 (*d*, *J* = 16.6, 1 H, $CH_2$(20)); 4.08 (*d*, *J* = 16.6, 1 H, $CH_2$(20)); 5.58 (*d*, *J* = 3.0, H−C(2′)); 4.30 (*d*, *J* = 8.6, 1 H, MeOC*H*$_2$CO); 4.18 (*d*, *J* = 8.6, 1 H, MeOC*H*$_2$CO); 3.37 (*s*, *Me*OCH$_2$CO); 6.01 (*dd*, *J* = 9.3, 3.0, H−C(3′)); 6.96 (*d*, *J* = 9.3, NH−C(3′)); 8.13 (*d*, *J* = 7.8, 2 $H_o$ of Ph(1)); 7.58 (*t*, *J* = 7.5, $H_p$ of Ph(1)); 7.50 (*m*, 2 $H_m$ of Ph(1), $H_p$ of Ph (3)); 7.42–7.32 (*m*, 2 $H_o$ of Ph(2), 2 $H_m$ of Ph(3), 2 $H_m$ of Ph(2), $H_p$ of Ph(2)); 7.71 (*d*, *J* = 7.8, 2 $H_o$ of Ph(3)). ESI-MS: 949 ([*M* + Na]$^+$, 3), 926 (100, *M*$^+$).

*7*-O-*[(4-Carboxybutyl)carbamoyl]-2′*-O-*(methoxyacetyl)paclitaxel* (=*(*α*R,*β*S)-*β*-(Benzoylamino)-*α*-[(methoxyacetyl)oxy]benzenepropanoic Acid (2a*R,*4*S,*4a*S,*6*R,*9*S,*11*S,*12*S,*12a*R,*12b*S)-6,12b-Bis(acetyloxy)-12-(benzoyloxy)-4-{[[(4-carboxybutyl)amino]carbonyl}oxy}-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-11-hydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H-*cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl Ester*; **9**). To a soln. of **8** (20 mg, 22 μmol) in dry THF (5 ml) under $N_2$ **4** (48 μl) and dibutyltin dilaurate (5 μl, 8.4 μmol) were added dropwise. The mixture was stirred at r.t. for 12 h. Then, MeOH (2 ml) was added, the mixture evaporated, and the residue diluted with MeCN/$H_2O$ 60:40. After prepurification (*RP 18* cartridge, isocratic MeCN/$H_2O$ 60:40), the final purification was carried out by prep. HPLC. $^1$H-NMR (500 MHz, $CD_3OD$): 5.65 (*d*, *J* = 7.0, H−C(2)); 3.95 (*d*, *J* = 7.0, H−C(3)); 2.44 (*s*, AcO−C(4)); 5.01 (br. *d*, *J* = 9.2, H−C(5)); 2.56 (*m*, $H_a$−C(6)); 1.80 (*m*, $H_b$−C(6)); 5.50 (*dd*, *J* = 10.1, 7.0, H−C(7)); 3.09 (*m*, $CH_2$(3″)); 1.52 (*m*, $CH_2$(4″)); 1.61 (*m*, $CH_2$(5″)); 2.31 (*m*, $CH_2$(6″)); 6.47 (*s*, H−C(10)); 2.12 (*s*, AcO−C(10)); 6.11 (*t*, *J* = 9.0, H−C(13)); 2.27 (*m*, $H_a$−C(14)); 1.95 (*m*, $H_b$−C(14)); 1.14 (*s*, Me(16)); 1.15 (*s*, Me(17)); 1.99 (*s*, Me(18)); 1.77 (*s*, Me(19)); 4.19 (*s*, $CH_2$(20)); 5.63 (*d*, *J* = 5.6, H−C(2′)); 4.21 (*m*, MeOC*H*$_2$CO); 3.37 (*s*, *Me*OCH$_2$CO); 5.93 (*d*, *J* = 5.6, H−C(3′)); 8.13 (*d*, *J* = 8.0, 2 $H_o$ of Ph(1)); 7.58 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(1)); 7.67 (*t*, *J* = 7.4, $H_p$ of Ph(1)); 7.50 (*d*, *J* = 8.0, 2 $H_o$ of Ph(2)); 7.45 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(2)); 7.30 (*t*, *J* = 7.4, $H_p$ of Ph(2)); 7.81 (*d*, *J* = 8.0, 2 $H_o$ of Ph(3)); 7.45 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(3)); 7.54 (*t*, *J* = 7.4, 1 $H_p$ of Ph(3)). $^{13}$C-NMR (125 MHz, $CD_3OD$): 79.0 (C(1)); 76.0 (C(2)); 48.3 (C(3)); 82.0 (C(4)); 171.7 (Me*C*O−C(4)); 23.3 (*Me*CO−C(4)); 85.5 (C(5)); 34.8 (C(6)); 73.3 (C(7)); 157.6 (C(1″)); 41.6 (C(3″)); 30.0 (C(4″)); 23.4 (C(5″)); 34.7 (C(6″)); 177.6 (C(7″)); 57.6 (C(8)); 204.4 (C(9)); 76.8

(C(10)); 170.3 (Me*C*O–C(10)); 20.7 (*Me*CO–C(10)); 134.7 (C(11)); 142.1 (C(12)); 73.2 (C(13)); 36.5 (C(14)); 44.7 (C(15)); 22.2 (C(16)); 26.9 (C(17)); 15.0 (C(18)); 11.9 (C(19)); 77.4 (C(20)); 170.1 (C(1′)); 76.1 (C(2′)); 171.3 (MeOCH$_2$*C*O); 70.3 (MeO*C*H$_2$CO); 59.8 (*Me*OCH$_2$CO); 55.1 (C(3′)); 167.7 (Ph(1)*C*O); 131.4 ($C_{ipso}$ of Ph(1)); 131.3 ($C_o$ of Ph(1)); 130.2 ($C_m$ of Ph(1)); 134.6 ($C_p$ of Ph(1)); 135.6 ($C_{ipso}$ of Ph(2)); 128.5 ($C_o$ of Ph(2)); 128.8 ($C_m$ of Ph(2)); 129.7 ($C_p$ of Ph(2)); 170.7 (Ph(3)*C*O); 138.3 ($C_{ipso}$ of Ph(3)); 128.7 ($C_o$ of Ph(3)); 129.6 ($C_m$ of Ph(3)); 133.0 ($C_p$ of Ph(3)). ESI-MS: 1091 (4, $[M+Na]^+$), 1069 (12, $[M+H]^+$), 1009 (40), 562 (38), 228 (100).

*7-*O*-[(4-Carboxybutyl)carbamoyl]paclitaxel* (=*(α*R,*β*S*)-β-(Benzoylamino)benzenepropanoic Acid (2a*R,*4*S,*4a*S,*6*R,*9*S,*11*S,*12*S,*12a*R,*12b*S*)-6,12b-Bis(acetyloxy)-12-(benzoyloxy)-4-{[[(4-carboxybutyl)amino]carbonyl}oxy}-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-11-hydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1*H*-cyclodeca[3,4]benz[1,2-*b*]oxet-9-yl Ester*; **10**). Deprotection of **9** was done under basic conditions [16]. In a typical experiment, to a soln. of **9** (1.84 mg, 1.72 μmol) in MeOH (1.5 ml), 5% aq. $NH_3$ soln. (0.2 ml, *ca.* 0.59 mmol) was added. The resulting soln. was stirred at r.t. for 1 h and then evaporated without heating. The obtained residue was chromatographed by reversed-phase HPLC (isocratic MeCN/$H_2O$ 45:55): **10** (1.32 mg, 77%). With $^i$Pr$_2$EtN instead of $NH_3$ as base, a similar result was obtained. $^1$H-NMR (500 MHz, $CD_3OD$): 5.64 (*d*, *J* = 6.9, H–C(2)); 3.92 (*d*, *J* = 6.9, H–C(3)); 2.35 (*s*, AcO–C(4)); 4.99 (br. *d*, *J* = 9.5, H–C(5)); 2.53 (*m*, $H_a$–C(6)); 1.79 (*m*, $H_b$–C(6)); 5.47 (*dd*, *J* = 10.3, 6.6, H–C(7)); 3.08 (*t*, *J* = 6.6, $CH_2$(3″)); 1.51 (*m*, $CH_2$(4″)); 1.60 (*m*, $CH_2$(5″)); 2.24 (*m*, $CH_2$(6″)); 6.46 (*s*, H–C(10)); 2.12 (*s*, AcO–C(10)); 6.15 (*dt*, *J* = 9.0, 1.1, H–C(13)); 2.23 (*m*, $H_a$–C(14)); 2.00 (*ddd*, *J* = 14.9, 9.0, 1.1, $H_b$–C(14)); 1.13 (*s*, Me(16)); 1.15 (*s*, Me(17)); 1.96 (*s*, Me(18)); 1.76 (*s*, Me(19)); 4.19 (*s*, $CH_2$(20)); 4.75 (*d*, *J* = 5.3, H–C(2′)); 5.65 (*d*, *J* = 5.3, H–C(3′)); 8.11 (*d*, *J* = 8.0, 2 $H_o$ of Ph(1)); 7.57 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(1)); 7.67 (*t*, *J* = 7.4, $H_p$ of Ph(1)); 7.49 (*d*, *J* = 8.0, 2 $H_o$ of Ph(2)); 7.42 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(2)); 7.29 (*t*, *J* = 7.4, $H_p$ of Ph(2)); 7.85 (*d*, *J* = 8.0, 2 $H_o$ of Ph(3)); 7.46 (*dd*, *J* = 8.0, 7.4, 2 $H_m$ of Ph(3)); 7.54 (*t*, *J* = 7.4, $H_p$ of Ph(3)). $^{13}$C-NMR (125 MHz, $CD_3OD$; starred signals may be interchanged): 79.0 (C(1)); 76.0 (C(2)); 48.3 (C(3)); 82.1 (C(4)); 171.8 (Me*C*O–C(4)); 23.2 (*Me*CO–C(4)); 85.5 (C(5)); 34.8 (C(6)); 73.2 (C(7)); 157.4 (C(1″)); 41.8 (C(3″)); 30.2 (C(4″)); 24.3 (C(5″)); 36.9 (C(6″)); 180.2 (C(7″)); 57.6 (C(8)); 204.3 (C(9)); 76.8 (C(10)); 170.3 (Me*C*O–C(10)); 20.7 (*Me*CO–C(10)); 134.7 (C(11)); 142.0 (C(12)); 72.3 (C(13)); 36.6 (C(14)); 44.7 (C(15)); 22.1 (C(16)); 26.8 (C(17)); 14.8 (C(18)); 11.9 (C(19)); 77.4 (C(20)); 174.5 (C(1′)); 74.8 (C(2′)); 57.7 (C(3′)); 167.7 (Ph(1)*C*O); 131.4 ($C_{ipso}$ of Ph(1)); 131.3 ($C_o$ of Ph(1)); 129.6 ($C_m$ of Ph(1)); 134.7 ($C_p$ of Ph(1)); 135.7 ($C_{ipso}$ of Ph(2)); 129.7 ($C_o$ of Ph(2)); 128.7* ($C_m$ of Ph(2)); 129.1 ($C_p$ of Ph(2)); 170.4 (Ph(3)*C*O); 140.0 ($C_{ipso}$ of Ph(3)); 128.6* ($C_o$ of Ph(3)); 129.6* ($C_m$ of Ph(3)); 132.9 ($C_p$ of Ph(3)). ESI-MS: 1019 (100, $[M+Na]^+$), 997 (48, $[M+H]^+$), 752 (21).

5. *BSA Conjugation. DAB–BSA Conjugate.* To a soln. of **5** or **6** (7.5 mg, *ca.* 11 μmol) in pyridine/$H_2O$ 1:1 (1 ml), a soln. of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDAC; 10 mg) in pyridine/$H_2O$ 1:1 (0.5 ml) was added dropwise within 3 min while stirring. After 10 min at r.t., bovine-serum albumin (BSA; 16.6 mg) in $H_2O$ (1 ml) was added dropwise within 3 min while stirring. The mixture was further stirred for 20 h at r.t. Dialysis for 4 d against $H_2O$ followed by lyophilization yielded the BSA conjugates of compounds **5** and **6**, resp. MALDI-TOF-MS: 72958 (**5**-BSA), 72348 (**6**-BSA); reference, 66504 (BSA).

*Paclitaxel–BSA Conjugate.* According to the same procedure, with **10** (3.1 mg, *ca.* 3.1 μmol) in pyridine/$H_2O$ 1:1 (0.4 ml), EDAC (4.1 mg) in pyridine/$H_2O$ 1:1 (0.2 ml), and BSA (6.9 mg) in $H_2O$ (0.4 ml). MALDI-TOF-MS: 71341 (**10**-BSA).

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