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Antitumor Agents 216. Synthesis and Evaluation of Paclitaxel–Camptothecin Conjugates as Novel Cytotoxic Agents[1]

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Abstract—Five conjugates (**16**–**20**) composed of a paclitaxel and a camptothecin derivative joined by an imine linkage were synthesized and evaluated as cytotoxic agents and as inhibitors of DNA topoisomerase I. All of the conjugates were potent inhibitors of tumor cell replication with improved activity relative to camptothecin. Significantly, compounds **16**–**18** were more active than paclitaxel and camptothecin against HCT-8 (colon adenocarcinoma) cell replication, and the spectrum of activity was different from a simple mixture of paclitaxel and camptothecin. All of the conjugates were significantly less potent than camptothecin as inhibitors of human topoisomerase I in vitro with **16**, **18**, and **19** showing only marginal activity at 50 μM. Based on activity against drug-resistant cell line replication, one could conclude that the conjugates are simply acting as 'weak taxanes', but the spectrum of activity, particularly against MCF-7 and HCT-8, strongly suggests that a novel mechanism of action has been achieved through conjugation.
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Introduction

Paclitaxel (**1**)[2] and camptothecin (**2**)[3] are both plant-derived antitumor agents currently in clinical use. Paclitaxel was approved by the FDA for treatment of advanced ovarian cancer in 1992 and for treatment of breast cancer in 1994. The mechanism of antitumor effect of paclitaxel is antimitotic, specifically promoting the irreversible assembly of tubulin into microtubules.[4] The natural alkaloid, camptothecin (**2**) is another compound that has undergone continual structural modification aimed at developing more useful chemotherapeutic agents. Its significant antitumor activity is attributable to inhibition of DNA topoisomerase I (DNA topo I).[5,6] Although both paclitaxel and camptothecin possess potent antitumor activity, recent reports have shown that treatment with these drugs often results in a number of undesired side effects as well as multi-drug resistance. Therefore, it remains essential to develop new anticancer agents with fewer side effects and improved activity against various classes of tumors.

Previously, we reported the synthesis and evaluation of two 4′-*O*-demethyl epipodophyllotoxin-camptothecin conjugates (**3** and **4**) as inhibitors of mammalian DNA topoisomerases I and II.[7] The most active conjugate inhibited cell growth similarly to both topo I- and II-inhibitory components (Fig. 1). These conjugates were more cytotoxic than epipodophyllotoxin in several cancer cell lines including HOP-62 leukemia, SW-620 colon cancer, MCF/ADR adriamycin-resistant breast cancer, and A-498 renal cancer. One conjugate was more active than either etoposide or **2** against human KB (nasopharnyx) and DU-145 (prostate) tumor cell growth in nude mice. This novel topo I and topo II dual inhibitory activity and the unique antitumor action of these camptothecin-etoposide analogue conjugates prompted us to investigate the bis-molecular model for generating novel types of antitumor agents. Currently, there are two reports describing conjugates between paclitaxel and either daunorubicin or chlorambucil.[8,9] DNA topo inhibitor-paclitaxel hybrids have not been investigated.

The aim of this work was to investigate whether an antitumor agent displaying multiple antitumor activity or improved activity against drug resistant cells could be prepared through conjugation between paclitaxel and

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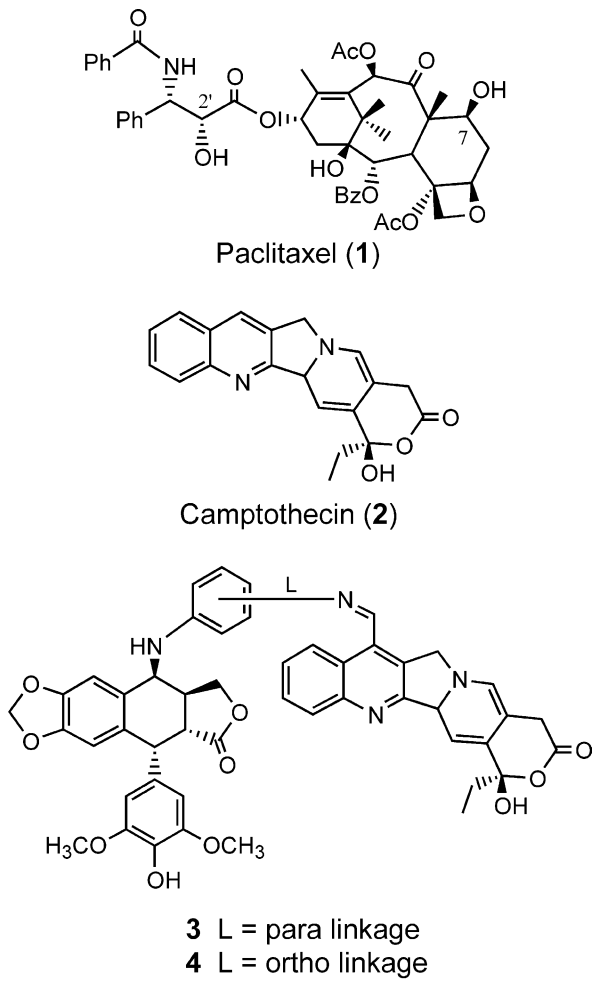


Figure 1.

camptothecin derivatives, and how their antitumor effects are affected by the conjugation. We report in this paper the synthesis and evaluation of paclitaxel-camptothecin conjugates as cytotoxic agents and DNA topo I inhibitors.

Chemistry

The choice of the linkage position was based on the known tolerance of the C-7 ester group in the taxane nucleus[10,11] and recent SAR study of 7-substituted camptothecin analogues.[12,13] However, the C-2′ hydroxyl group of the paclitaxel side chain is normally the most reactive and selective C-7 acylation can be achieved only when C-2′ is protected. The procedure of Nicolaou et al.[11] was used for the preparation of C-7 amino-functionalized paclitaxels (**11–15**). Thus, 2′-carboxybenzyl (Cbz)-paclitaxel was treated with *N*-Cbz-amino acids in the presence of DCC and DMAP in CH_2Cl_2 to give **6–10** in good yields. Subsequent reductive removal of both Cbz groups by hydrogenation gave the desired C-7 amino-functionalized paclitaxels (**11–15**). Five paclitaxel-camptothecin conjugates (**16–20**) were obtained by conjugation of **11–15** with 7-formyl-camptothecin (**5**) in benzene as shown in Scheme 1. Compounds **16–20** were unstable on silica gel (the parental compounds were regenerated), but could be purified by Sephadex LH-20 column chromatography. All conjugates (**16–20**) showed the characteristic imine proton signal at δ 9.30–9.55 in their ^{1}H NMR spectra.

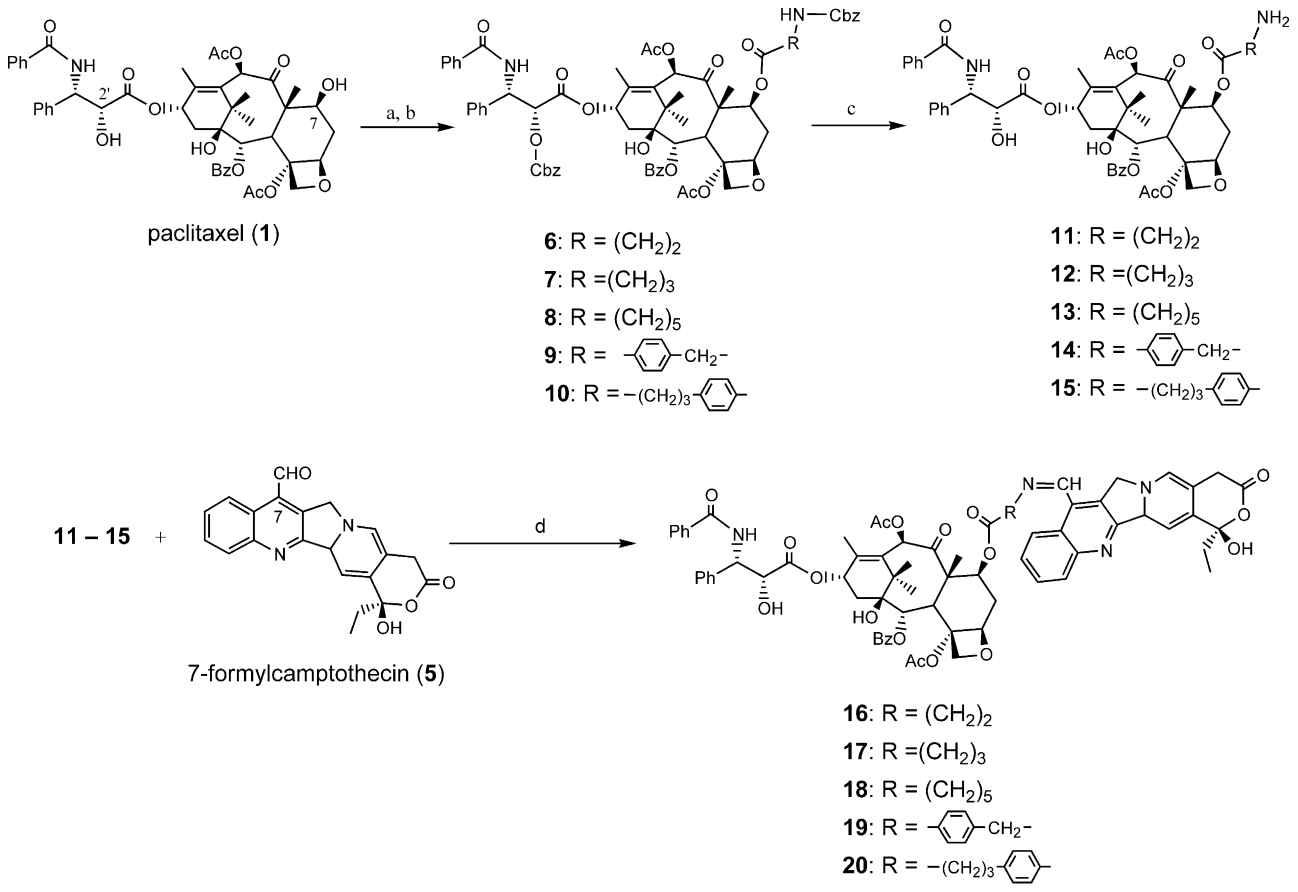
Results and Discussion

The five conjugates (**16–20**) were tested for cytotoxicity against a panel of human tumor cell lines (HTCL) including paclitaxel-resistant and camptothecin-resistant derivatives.[14] The results are shown in Table 1 with the values for paclitaxel (**1**) and camptothecin (**2**) and a combination of both drugs given for comparison. Activity profiles for the conjugates against MCF-7 (breast cancer) and HCT-8 (colon adenocarcinoma) cells are shown in Figure 2A and B, respectively. All of the conjugates were potent inhibitors of tumor cell replication with improved activity relative to **2**, and also showed better activity than **2** against camptothecin-resistant KB-CPT cells. Significantly, **16–18** were more active against HCT-8 cell replication than both **1** and **2**, and their potency and spectrum of activity were different from a simple mixture of **1** and **2** (Table 1 and Fig. 2). SAR consideration of the linker between the paclitaxel and camptothecin moieties showed that **16–18**, in which paclitaxel and camptothecin were linked by aliphatic amino acids, showed better cytotoxic activity than **19** and **20**, in which paclitaxel and camptothecin were linked by aromatic amino acids.

Table 1. Activity of paclitaxel–camptothecin conjugates **16–20** as inhibitors of cancer cell replication[a]

Compd	Cell line/ED_{50}							
	KB	KB-CPT	1A9	1A9-PTX10	HCT8	MCF-7	PC-3	Mean ED_{50}[b]
Paclitaxel	−12.1	−12.2	−12.2	−11.7	−7.1	−13.9	−10.3	−11.1
Camptothecin	−10.8	−7.5	−10.2	−9.4	−9.3	−7.1	−7.1	−8.9
16	< −11.0 (67)	−11.1	< −11.0 (76)	−10.9	−10.6	−7.1	−7.0	< −9.3
17	−10.4	−9.7	−10.2	−10.5	−10.0	−7.1	−7.1	−9.0
18	−9.3	−8.1	−10.0	−10.5	−10.6	−7.5	−7.5	−9.0
19	−8.5	−8.9	−8.1	−8.4	−8.1	−6.0	−7.5	−7.6
20	−10.8	−8.1	−8.3	−7.1	−7.4	> −4.0 (48)	> −5.0 (45)	> −7.1
Paclitaxel + Camptothecin (1:1)	−6.9	−10.6	−10.5	−9.0	−7.9	−6.4	−6.6	−7.7

[a]Effects on tumor cell line replication were determined using a standard method.[15] Cell line/ED_{50} in $\log_{10}$ M (replicates varied no more than 5%). If inhibition < 50% at highest test concentration or > 50% at lowest, the observed percentage inhibition observed is the bracketed value.
[b]Mean value is taken from HTCL data and does not include values for drug-resistant variants.



Scheme 1. (a) Benzylchroloformate, pyridine, CH_2Cl_2, 83%; (b) *N*-Cbz-NH-R-COOH, DCC, DMAP, CH_2Cl_2, 84–97%; (c) H_2, 5% Pd–C, MeOH, 81–97%; (d) benzene, MS 4 Å, reflux, 55–85%.

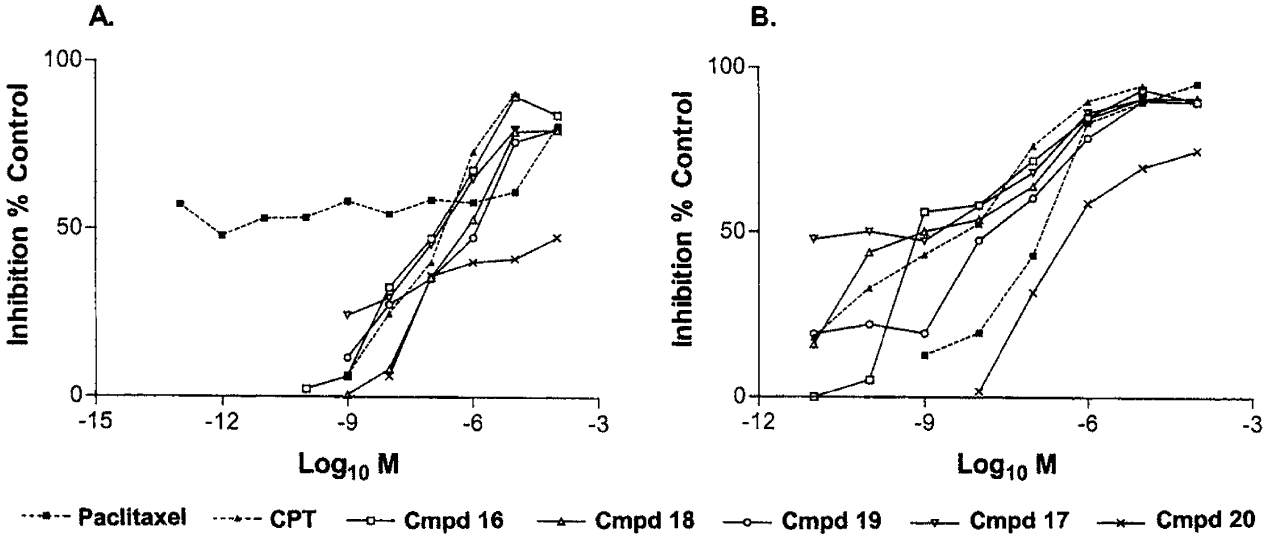


Figure 2. Compounds were tested as inhibitors of human tumor cell line replication and ED_{50} values interpolated from dose–response activity data are given in Table 1. Graphs show activity profiles of compounds against MCF-7 (A) and HCT-8 cell replication (B).

Compounds **16**–**20** were also tested as inhibitors of human DNA topo I activity in vitro (Fig. 3).[15] All of the conjugates were significantly less potent than **2** in this assay. Compounds **16**, **18**, and **19** showed only marginal activity at 50 μM, while **17** and **20** were inactive. None of the conjugates inhibited DNA relaxation.

Based on activity against drug-resistant cell line replication, one could conclude that the conjugates are simply acting as 'weak taxanes', but the spectrum of activity, particularly against MCF-7 and HCT-8 (Fig. 2), is not consistent with this conclusion. The latter results indicate that, compared with **1** and **2**, the cytotoxicity

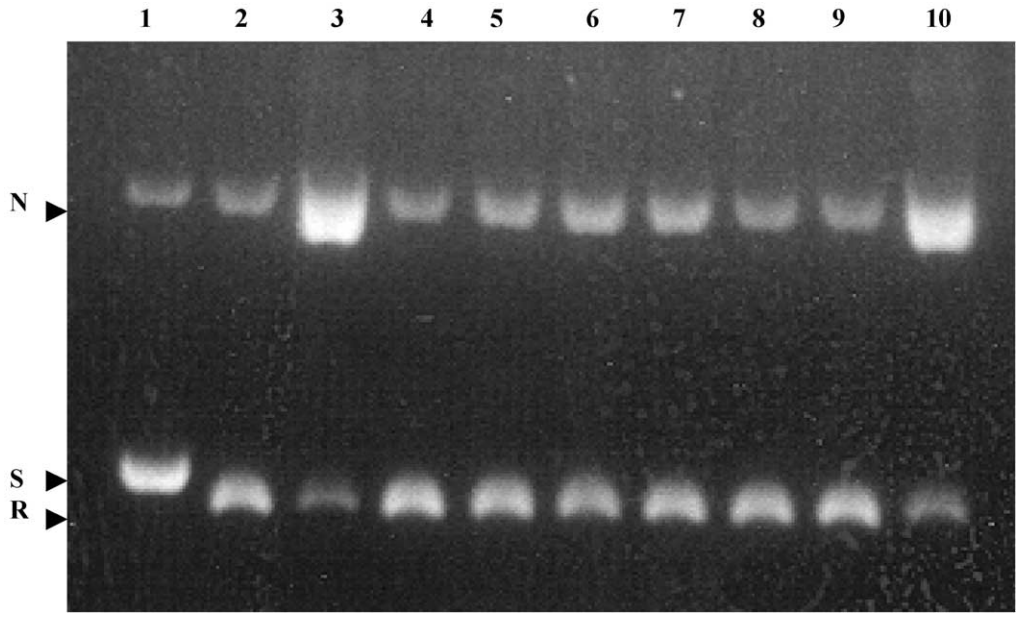


Figure 3. Compounds were tested as human DNA topoisomerase I inhibitors and analyzed using an ethidium bromide-containing agarose gel as described.[15] Lane 1 is the pBR322 DNA substrate. Lane 2 is enzyme control. Lanes 3–10 are compound treated (50 μM) as follows: lanes 3 and 10, campthothecin; lane 4, paclitaxal; lane 5, compound **16**; lane 6, compound **18**; lane 7, compound **19**; lane 8, compound **17**; lane 9, compound **20**.

profiles of **16**–**18** are quite distinctive. In addition, because the results from the DNA topo I inhibition assay show conclusively that the conjugates, unlike **2**, are not DNA topo I inhibitory in vitro, it is likely that a novel mechanism of action has been achieved through conjugation.

Conclusions

In summary, we have synthesized five paclitaxel-camptothecin conjugates joined by an ester and an imine linkage. When evaluated for cytotoxicity against a panel of human tumor cell lines, all of the conjugates showed potent cytotoxic activity against tumor cell replication. Interestingly, compounds **16**–**18** showed improved activity against HCT-8 cell replication compared with either **1** or **2**. In-depth mechanistic studies and the development of new paclitaxel-camptothecin conjugates are actively underway in our laboratory.

Experimental

General procedures

Paclitaxel was obtained from Yung Shin Pharmaceutical Ind. Co., Ltd., Taiwan. Preparation of 7-formyl-camptothecin was reported in our previous paper.[7] All reagents and solvents were reagent grade or were purified by standard methods before use. ^{1}H NMR spectra were obtained on a Varian Gemini-300 spectrometer using TMS as the internal standard. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and Hz, respectively. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Column chromatography was carried out on silica gel 60 (Merck 230–400 mesh), and thin layer chromatography (TLC) was performed using pre-coated silica gel on aluminum plates (Aldrich, Inc.). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. *N*-Cbz-animo acids were prepared from the appropriate amino acids using standard methodology[16] using benzyl-chloroformate, except for *N*-Cbz-β-alanine (Sigma). 2′-*O*-Cbz-paclitaxel was synthesized by a literature method.[11]

General procedure for 7-*O*-acylation of 2′-*O*-Cbz-paclitaxel. A solution of 2′-*O*-Cbz-paclitaxel and 4-(*N*,*N*-dimethylamino)pyridine (1.0 equiv) in dry dichloromethane (5 mL) under nitrogen was treated with the appropriate *N*-Cbz-amino acid (5.0 equiv) and dicyclohexylcarbodiimide (DCC, 5.0 equiv) overnight at rt. The mixture was diluted with dichloromethane (5 mL) and filtered to remove the urea precipitate. The solvent was evaporated in vacuo and the resulting residue was purified by silica gel column chromatography using a *n*-hexane–EtOAc solvent system (2:1 to 1:1) to afford the desired 2′-*O*-Cbz-7-*O*-acyl paclitaxel as the sole product.

2′-*O*-Cbz-7-(*N*-Cbz-β-alanyl)-paclitaxel (6). Yield 90.5% (starting with 59.3 mg of 2′-*O*-Cbz-paclitaxel); white powder, $[\alpha]_D$ −44.7° (*c* 0.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.16 (3H, s), 1.22 (3H, s), 1.81 (3H, s), 2.00 (3H, s), 2.06 (3H, s), 2.46 (3H, s), 3.46 (2H, m), 3.96 (1H, d. J = 7.0 Hz), 4.20 (1H, d, J = 8.5 Hz), 4.33 (1H, d, J = 8.5 Hz), 4.95 (1H, d, J = 8.5 Hz), 5.11 (2H, m), 5.17 (2H, m), 5.46 (1H, d. J = 2.5 Hz), 5.64 (1H, dd, J = 7.2, 9.8 Hz), 5.70 (1H. d. J = 7.0 Hz), 5.77 (1H, t, J = 5.1 Hz), 5.98 (1H, d, J = 9.0 Hz), 6.26 (1H, t, J = 9.3 Hz), 6.29 (1H, s), 6.94 (1H, d, J = 9.0 Hz), 7.30–7.42 (band, 17H), 7.44–7.54 (3H, m), 7.62 (1H, m), 7.74 (2H, d, J = 8.5 Hz), 8.13 (2H, d, J = 8.5 Hz); FAB-MS m/z 1193 $[M+1]^+$. All data are in agreement with reported literature values.[3]

2′-*O*-Cbz-7-(*N*-Cbz-4-aminobutyroyl)-paclitaxel (7). Yield 97.2% (starting with 69.1 mg of 2′-*O*-Cbz-paclitaxel); white powder, $[\alpha]_D$ −31.5° (*c* 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.16 (3H, s), 1.21 (3H, s), 1.81 (3H, s), 1.99 (3H, s), 2.09 (3H, s), 2.45 (3H, s), 3.19–3.40 (2H, m), 3.94 (1H, d. *J* = 6.9 Hz), 4.20 (1H, d, *J* = 8.5 Hz), 4.33 (1H, d, *J* = 8.5 Hz), 4.95 (1H, d, *J* = 8.5 Hz), 5.08 (2H, s), 5.17 (2H, m), 5.45 (1H, d. *J* = 2.4 Hz), 5.57 (1H, dd, *J* = 6.9, 10.2 Hz), 5.71 (1H. d, *J* = 6.9 Hz), 5.73 (1H, t, *J* = 5.1 Hz), 5.98 (1H, dd, *J* = 2.4, 9.0 Hz), 6.24 (1H, s), 6.26 (1H, t, *J* = 9.3 Hz), 6.92 (1H, d, *J* = 9.0 Hz), 7.27–7.46 (band, 16H), 7.48–7.54 (3H, m), 7.62 (1H, m), 7.73 (2H, d, *J* = 8.5 Hz), 8.13 (2H, d, *J* = 8.5 Hz); ESI-MS *m*/*z* 1230 $[M + Na]^+$.

2′-*O*-Cbz-7-(*N*-Cbz-6-aminohexanoyl)-paclitaxel (8). Yield 94.7% (starting with 78.3 mg of 2′-*O*-Cbz-paclitaxel); white powder, $[\alpha]_D$ −44.0° (*c* 1.04, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.16 (3H, s), 1.21 (3H, s), 1.81 (3H, s), 2.00 (3H, s), 2.13 (3H, s), 2.45 (3H, s), 3.19 (2H, dd, *J* = 6.3, 12.9 Hz), 3.96 (1H, d. *J* = 6.6 Hz), 4.19 (1H, d, *J* = 8.5 Hz), 4.32 (1H, d, *J* = 8.5 Hz), 4.96 (1H, d, *J* = 8.7 Hz), 5.08 (2H, s), 5.16 (2H, m), 5.45 (1H, d. *J* = 2.4 Hz), 5.60 (1H, dd, *J* = 7.2, 10.5 Hz), 5.71 (1H. d, *J* = 6.9 Hz), 5.98 (1H, dd, *J* = 3.0, 9.5 Hz), 6.26 (1H, t, *J* = 9.0 Hz), 6.28 (1H, s), 6.95 (1H, d, *J* = 9.3 Hz), 7.26–7.43 (band, 16H), 7.46–7.53 (3H, m), 7.61 (1H, m), 7.73 (2H, d, *J* = 8.5 Hz), 8.13 (2H, d, *J* = 8.5 Hz); FAB-MS *m*/*z* 1236 $[M + 1]^+$.

2′-*O*-Cbz-7-(*N*-Cbz-4-methylaminobenzoyl)-paclitaxel (9). Yield 89.1% (starting with 70.4 mg of 2′-*O*-Cbz-paclitaxel); white powder, $[\alpha]_D$ −28.2° (*c* 0.99, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.19 (3H, s), 1.21 (3H, s), 1.95 (3H, s), 1.97 (3H, s), 2.04 (3H, s), 2.48 (3H, s), 4.05 (1H, d. *J* = 6.9 Hz), 4.24 (1H, d, *J* = 8.5 Hz), 4.37 (1H, d, *J* = 8.5 Hz), 4.42 (1H, d, *J* = 6.0 Hz), 5.01 (1H, d, *J* = 8.4 Hz), 5.10–5.22 (6H, m), 5.48 (1H, d. *J* = 2.7 Hz), 5.74–5.81 (2Hm), 5.99 (1H, dd, *J* = 2.7, 9.2 Hz), 6.27 (1H, t, *J* = 9.0 Hz), 6.41 (1H, s), 6.97 (1H, d, *J* = 9.3 Hz), 7.29–7.42 (band, 19H), 7.46–7.54 (3H, m), 7.62 (1H, m), 7.74 (2H, d, *J* = 8.5 Hz), 7.89 (2H, d, *J* = 8.4 Hz). 8.15 (2H, d, *J* = 8.5 Hz); ESI-MS *m*/*z* 1277 $[M + Na]^+$.

2′-*O*-Cbz-7-(*N*-Cbz-4-(4-aminophenyl)-butyroyl)-paclitaxel (10). Yield 83.8% (starting with 66.8 mg of 2′-*O*-Cbz-paclitaxel); white powder, $[\alpha]_D$ −46.5° (*c* 0.40, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.17 (3H, s), 1.21 (3H, s), 1.81 (3H, s), 2.01 (3H, s), 2.13 (3H, s), 2.45 (3H, s), 3.96 (1H, d. *J* = 6.9 Hz), 4.19 (1H, d, *J* = 8.5 Hz), 4.33 (1H, d, *J* = 8.5 Hz), 4.96 (1H, d, *J* = 8.1 Hz), 5.12–5.21 (4H, m), 5.46 (1H, d. *J* = 2.7 Hz), 5.60 (1H, dd, *J* = 7.2, 10.5 Hz), 5.70 (1H. d, *J* = 7.2 Hz), 5.98 (1H, dd, *J* = 2.7, 9.2 Hz), 6.26 (1H, t, *J* = 9.0 Hz), 6.29 (1H, s), 6.66 (1H, s), 6.94 (1H, d, *J* = 9.3 Hz), 7.12 (2H, d, *J* = 8.7 Hz), 7.26–7.42 (band, 19H), 7.46–7.53 (3H, m), 7.61 (1H, m), 7.73 (2H, d, *J* = 8.5 Hz), 8.13 (2H, d, *J* = 8.5 Hz); FAB-MS *m*/*z* 1306 $[M + Na]^+$.

General procedure for removal of the benzyloxycarbonyl (Cbz) protecting group. A solution of taxoid (**6–10**) in methanol under nitrogen was treated with 50 wt.% of 5% palladium on activated carbon (Degussa type E101 NO/W) and placed under hydrogen (40 psi). The mixture was shaken in a Parr apparatus for 7 h then the content was filtered to remove the catalyst and evaporated to dryness in vacuo to give the desired deprotected compounds **11–15**.

7-*O*-β-Alanylpaclitaxel (11). Yield 81.2% (starting with 47.2 mg of **6**); white film, $[\alpha]_D$ −39.1° (*c* 0.34, MeOH); ^1H NMR (CD_3OD): δ 1.10 (3H, s), 1.16 (3H, s), 1.78 (3H, s), 1.88 (3H, s), 2.16 (3H, s), 2.37 (3H, s), 3.12–3.22 (2H, m), 3.90 (1H, d, *J* = 7.0 Hz), 4.20 (2H, dd, *J* = 7.0, 14.0 Hz), 4.73 (1H, d, *J* = 6.0 Hz), 5.00 (1H, m), 5.64 (3H, m), 6.15 (1H, br t, *J* = 6.2 Hz), 6.23 (1H, s), 7.28–7.68 (band, 11H), 7.85 (2H, d, *J* = 8.5 Hz), 8.10 (2H, d, *J* = 8.5 Hz); FAB-MS *m*/*z* 925 $[M + 1]^+$. All data are in agreement with reported literature values.[3]

7-*O*-(4-Aminobutyroyl)paclitaxel (12). Yield 92.7% (starting with 59.1 mg of **7**); white film, $[\alpha]_D$ −29.7° (*c* 0.37, MeOH); ^1H NMR (CD_3OD): δ 1.11 (3H, s), 1.16 (3H, s), 1.78 (3H, s), 1.87 (3H, s), 2.16 (3H, s), 2.37 (3H, s), 3.01 (2H, t, *J* = 7.5 Hz), 3.90 (1H, d, *J* = 6.9 Hz), 4.20 (2H, br t, *J* = 8.7 Hz), 4.75 (1H, d, *J* = 5.4 Hz), 5.00 (1H, d, *J* = 9.3 Hz), 5.59–5.66 (3H, m), 6.15 (1H, br t, *J* = 6.2 Hz), 6.21 (1H, s), 7.26–7.69 (band, 11H), 7.85 (2H, d, *J* = 8.5 Hz), 8.10 (2H, d, *J* = 8.5 Hz); ESI-MS *m*/*z* 939 $[M + 1]^+$.

7-*O*-(6-Aminohexanoyl)paclitaxel (13). Yield 81.1% (starting with 67.1 mg of **8**); white film, $[\alpha]_D$ −26.0° (*c* 0.53, MeOH); ^1H NMR (CD_3OD): δ 1.11 (3H, s), 1.16 (3H, s), 1.77 (3H, s), 1.88 (3H, s), 2.14 (3H, s), 2.37 (3H, s), 2.92 (2H, m), 3.90 (1H, d, *J* = 6.9 Hz), 4.20 (2H, m), 4.75 (1H, d, *J* = 5.4 Hz), 4.99 (1H, d, *J* = 9.0 Hz), 5.58 (1H, dd, *J* = 7.8, 10.5 Hz), 5.60–5.70 (2H, m), 6.15 (1H, br t, *J* = 6.2 Hz), 6.24 (1H, s), 7.26–7.72 (band, 11H), 7.85 (2H, d, *J* = 8.5 Hz), 8.10 (2H, d, *J* = 8.5 Hz); FAB-MS *m*/*z* 968 $[M + 1]^+$.

7-*O*-(4-Methylaminobenzoyl)paclitaxel (14). Yield 96.7% (starting with 58.1 mg of **9**); white film, $[\alpha]_D$ −12.9° (*c* 0.38, MeOH); ^1H NMR (CD_3OD): δ 1.13 (3H, s), 1.15 (3H, s), 1.91 (9H, s), 2.40 (3H, s), 3.90 (1H, d, *J* = 6.9 Hz), 4.18–4.30 (4H, m), 4.76 (1H, d, *J* = 5.7 Hz), 5.05 (1H, m), 5.65 (1H, d, *J* = 5.1 Hz), 5.70 (1H, d, *J* = 7.2 Hz), 5.76 (1H, dd, *J* = 7.2, 10.5 Hz), 6.16 (1H, br t, *J* = 6.2 Hz), 6.35 (1H, s), 7.28–7.71 (band, 13H), 7.85 (2H, d, *J* = 8.5 Hz), 7.94 (2H, d, *J* = 8.5 Hz), 8.12 (2H, d, *J* = 8.5 Hz); ESI-MS *m*/*z* 1009 $[M + Na]^+$.

7-*O*-(4-(4-Aminophenyl)butyroyl)paclitaxel (15). Yield 81.3% (starting with 67.1 mg of **10**); white film, $[\alpha]_D$ −45.3° (*c* 0.15, MeOH); ^1H NMR (CD_3OD): δ 1.11 (3H, s), 1.14 (3H, s), 1.77 (3H, s), 1.89 (3H, s), 2.09 (3H, s), 2.36 (3H, s), 2.46 (2H, t, *J* = 5.7 Hz), 3.89 (1H, d, *J* = 6.9 Hz), 4.19 (2H, brs), 4.75 (1H, d, *J* = 5.4 Hz), 4.97 (1H, d, *J* = 9.6 Hz), 5.56 (1H, dd, *J* = 7.8, 10.5 Hz), 5.63–5.66 (2H, m), 6.15 (1H, br t, *J* = 6.2 Hz), 6.26 (1H, s), 6.67 (2H, d, *J* = 8.4 Hz), 6.93 (2H, d, *J* = 8.4 Hz), 7.28 (1H, m), 7.38–7.68 (band, 10H), 7.84 (2H, d, *J* = 8.5 Hz), 8.10 (2H, d, *J* = 8.5 Hz); FAB-MS *m*/*z* 1038 $[M + Na]^+$.

General procedure for the synthesis of paclitaxel–camptothecin conjugates (16–20). A solution of taxoid (**11–15**) and 7-formylcamptothecin (1.2 equiv) in dry ben-

zene (10 mL) was refluxed over 4 Å molecular sieves overnight. The mixture was filtered and evaporated in vacuo to give crude paclitaxel–camptothecin conjugates (**16–20**). The product was purified by Sephadex LH-20 column chromatography with $CHCl_3$–MeOH (1:1) as eluent.

16: Yield 85.4% [starting with 14.2 mg (0.015 mmol) of **11**]; pale yellow amorphous solid, $[\alpha]_D$ −33.9° (*c* 0.18, $CHCl_3$), ^1H NMR ($CDCl_3$): δ 1.02 (3H, t, *J* = 7.5 Hz), 1.18 (3H, s), 1.25 (3H, s), 1.80 (3H, s), 1.85 (3H, s), 2.19 (3H, s), 2.37 (3H, s), 3.92 (1H, d, *J* = 6.6 Hz), 4.14 and 4.29 (each 1H, d, *J* = 8.4 Hz), 4.80 (1H, brs), 4.90 (1H, d, *J* = 9.3 Hz), 5.21 (1H, d, *J* = 16.5 Hz), 5.40 (2H, s), 5.58–5.70 (3H, m), 5.80 (1H, dd, *J* = 8.9, 2.1 Hz), 6.19 (1H, t, *J* = 9.0 Hz), 6.24 (1H, s), 7.27–7.86 (16H, m), 8.09 (2H, d, *J* = 7.2 Hz), 8.27 (1H, d, *J* = 8.4 Hz), 8.45 (1H, d, *J* = 8.4 Hz), 9.32 (1H, s); FAB-MS *m*/*z* (rel. int.) 1284 $[M+1]^+$ (35), 1224 (15), 940 (51), 449 (77), 289 (98), 240 (100). Anal. ($C_{71}H_{72}N_4O_{18}$): theory: C, 67.18; H, 5.72. Found C, 67.12; H, 5.69.

17: Yield 60.7% [starting with 27.0 mg (0.029 mmol) of **12**]; pale yellow amorphous solid, $[\alpha]_D$ −17.2° (*c* 0.47, $CHCl_3$), ^1H NMR ($CDCl_3$): δ 1.04 (3H, t, *J* = 7.5 Hz), 1.16 (3H, s), 1.20 (3H, s), 1.81 (3H, s), 1.83 (3H, s), 2.04 (3H, s), 2.38 (3H, s), 3.81–3.93 (4H, m), 4.18 and 4.31 (each 1H, d, *J* = 8.4 Hz), 4.81 (1H, dd, *J* = 6.2, 2.7 Hz), 4.93 (1H, d, *J* = 8.7 Hz), 5.30 (1H, d, *J* = 16.5 Hz), 5.50 (2H, s), 5.58 (1H, dd, *J* = 10.2, 6.9 Hz), 5.67 (1H, d, *J* = 6.9 Hz), 5.74 (1H, d, *J* = 16.5 Hz), 5.80 (1H, dd, *J* = 8.9, 2.4 Hz), 6.17 (1H, t, *J* = 9.0 Hz), 6.23 (1H, s), 7.14 (1H, d, *J* = 9.3 Hz), 7.30–7.87 (15H, m), 8.10 (2H, d, *J* = 7.2 Hz), 8.28 (1H, d, *J* = 8.4 Hz), 8.50 (1H, d, *J* = 8.4 Hz), 9.32 (1H, s); ESI-MS *m*/*z* 1320 $[M+Na]^+$. Anal. ($C_{72}H_{74}N_4O_{18}$): theory: C, 67.38; H, 5.81. Found C, 67.30; H, 5.79.

18: Yield 69.3% [starting with 27.8 mg (0.029 mmol) of **13**]; pale yellow amorphous solid, $[\alpha]_D$ −41.3 (*c* 0.15, $CHCl_3$), ^1H NMR ($CDCl_3$): δ 1.03 (3H, t, *J* = 7.5 Hz), 1.15 (3H, s), 1.25 (3H, s), 1.57 (3H, s), 1.76 (3H, s), 2.10 (3H, s), 2.28 (3H, s), 3.80 (1H, d, *J* = 6.6 Hz), 4.13 and 4.27 (each 1H, d, *J* = 8.4 Hz), 4.79 (1H, d, *J* = 2.7 Hz), 4.85 (1H, d, *J* = 8.1 Hz), 5.31 (1H, d, *J* = 16.5 Hz), 5.46 (1H, dd, *J* = 10.2, 7.2 Hz), 5.55 (2H, s), 5.61 (1H, d, *J* = 6.9 Hz), 5.74 (2H, m), 6.07 (1H, t, *J* = 9.0 Hz), 6.12 (1H, s), 7.18 (1H, d, *J* = 9.3 Hz), 7.32–7.88 (15H, m), 8.08 (2H, d, *J* = 7.2 Hz), 8.30 (1H, d, *J* = 8.4 Hz), 8.52 (1H, d, *J* = 8.4 Hz), 9.30 (1H, s); ESI-MS *m*/*z* 1348 $[M+Na]^+$. Anal. ($C_{74}H_{78}N_4O_{18}$): theory: C, 67.77; H, 5.99. Found C, 67.69; H, 5.98.

19: Yield 64.3% [starting with 28.2 mg (0.029 mmol) of **14**]; pale yellow amorphous solid, $[\alpha]_D$ −8.0° (*c* 0.40, $CHCl_3$), ^1H NMR ($CDCl_3$): δ 1.02 (3H, t, *J* = 7.5 Hz), 1.19 (3H, s), 1.25 (3H, s), 1.95 (3H, s), 2.17 (3H, s), 2.40 (3H, s), 2.63 (3H, s), 4.00 (1H, d, *J* = 6.3 Hz), 4.23 and 4.33 (each 1H, d, *J* = 8.1 Hz), 4.82 (1H, d, *J* = 2.7 Hz), 4.98 (1H, d, *J* = 8.7 Hz), 5.14 (2H, brs), 5.28 (1H, d, *J* = 16.2 Hz), 5.52 (2H, s), 5.67–5.76 (2H, m), 5.81 (1H, dd, *J* = 8.7, 2.1 Hz), 6.19 (1H, t, *J* = 9.0 Hz), 6.37 (1H, s), 7.21–7.96 (20H, m), 8.12 (2H, d, *J* = 7.2 Hz), 8.27 (1H, d, *J* = 8.4 Hz), 8.42 (1H, d, *J* = 8.4 Hz), 9.39 (1H, s); ESI-MS *m*/*z* 1396 $[M+Na]^+$. Anal. ($C_{76}H_{74}N_4O_{18}$): theory: C, 68.56; H, 5.60. Found C, 68.40; H, 5.56.

20: Yield 55.1% [starting with 23.3 mg (0.023 mmol) of **15**]; pale yellow amorphous solid, $[\alpha]_D$ −11.5 (*c* 0.27, $CHCl_3$), ^1H NMR ($CDCl_3$): δ 1.06 (3H, t, *J* = 7.5 Hz), 1.18 (3H, s), 1.25 (3H, s), 1.84 (3H, s), 1.85 (3H, s), 2.17 (3H, s), 2.39 (3H, s), 3.94 (1H, d, *J* = 6.6 Hz), 4.20 and 4.33 (each 1H, d, *J* = 8.4 Hz), 4.81 (1H, brs), 4.96 (1H, d, *J* = 9.3 Hz), 5.33 (1H, d, *J* = 16.5 Hz), 5.58 (1H, m), 5.70 (1H, d, *J* = 5.7 Hz), 5.73 (2H, s), 5.81(1H, d, *J* = 5.7 Hz), 6.19 (1H, t, *J* = 9.0 Hz), 6.26 (1H, s), 7.08 (1H, d, *J* = 9.3 Hz), 7.27–7.80 (18H, m), 7.88 (1H, t, *J* = 7.2 Hz), 8.12 (2H, d, *J* = 7.2 Hz), 8.33 (1H, d, *J* = 8.4 Hz), 8.56 (1H, d, *J* = 8.4 Hz), 9.55 (1H, s); ESI-MS *m*/*z* 1368 $[M+Na]^+$. Anal. ($C_{78}H_{78}N_4O_{18}$): theory: C, 68.91; H, 5.78. Found C, 68.86; H, 5.77.

Human tumor cell replication assay. Compounds were tested as inhibitors of cell growth against a limited panel of solid tumor lines including KB (epidermoid carcinoma, CL 17) and a camptothecin-resistant sub-line called KB-CPT; HCT-8 (ileocecal adenocarcinoma, CCL 244); MCF-7 (breast adenocarcinoma, HTB 22); PC-3 (prostate adenocarcinoma, CRL 1435); 1A9 (ovarian carcinoma) and a plactixel resistant sub-line called PTX10 with mutated β-tubulin. Drug-resistant cell lines were generous gifts of Dr. Y. C. Cheng (Yale) and Dr. P. Giannakakou (NIH, Bethesda, MD). Cell lines were adapted for growth in RPMI-1640 medium supplemented with 25 mM Hepes, 2% $NaHCO_3$, 10% (v/v) fetal calf serum and 100 μg/mL kanamycin. Cultures were maintained in a 5% CO_2 atmosphere at 37 °C. The sulforhodamine B microtiter plate assay described by Rubenstein et al.[14] was used to evaluate compounds as inhibitors of cell replication. The ED_{50} value, the concentration of compound that inhibited cell line replication by 50% relative to control after three days of continuous exposure was interpolated from dose–response graphs fitted to data using Prizm (GraphPad software, San Diego, CA).

DNA topoisomerase I activity assay. Compounds were tested as inhibitors of enzyme catalyzed plasmid DNA relaxation. Reactions contained 0.5 μg pBR322 DNA and 0.5 U human DNA topoisomerase I (TopoGen, Columbus, OH), compounds at 50 μM and buffer components described previously.[15] After 15 min at 37 °C, reactions were terminated by adding proteinase K (0.1 mg/mL) and 1% (w/v) SDS. After 1 h at 50 °C, DNA was resolved on a 1% (w/v) agarose gel containing 0.5 mg/mL ethidium bromide in standard TBE buffer, and photographed under UV light using polaroid 667 film.

Elemental analysis data for compounds 16–20

Compd	Formula	Calculated		Found	
		C	H	C	H
16	$C_{71}H_{72}N_4O_{18}$	67.18	5.72	67.12	5.69
17	$C_{72}H_{74}N_4O_{18}$	67.38	5.81	67.30	5.79
18	$C_{74}H_{78}N_4O_{18}$	67.77	5.99	67.69	5.98
19	$C_{76}H_{74}N_4O_{18}$	68.56	5.60	68.40	5.56
20	$C_{78}H_{78}N_4O_{18}$	68.91	5.78	68.86	5.77

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