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Synthesis of Novel Paclitaxel Prodrugs Designed for Bioreductive Activation in Hypoxic Tumour Tissue

Eric W.P. Damen,[a] Tapio J. Nevalainen,[b] Toine J.M. van den Bergh,[a] Franciscus M.H. de Groot[a] and Hans W. Scheeren[a,*]

[a]*Department of Organic Chemistry, NSR Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands*

[b]*Department of Pharmaceutical Chemistry, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland*

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Abstract—The syntheses and preliminary evaluation of the first potential bioreductive paclitaxel prodrugs are described. These prodrugs were designed as potential candidates in more selective chemotherapy by targeting hypoxic tumour tissue. Aromatic nitro and azide groups were used as the bioreductive trigger. Generation of paclitaxel occurs after reduction and subsequent 1,6-elimination or 1,8-elimination. All prodrugs are stable in buffer and indeed give paclitaxel after chemical reduction of the aromatic nitro or azide functionality. In aerobic cytotoxicity assays several prodrugs exhibit diminished cytotoxicity. These compounds are interesting candidates for further biological evaluation.

Introduction

Many solid tumours are inefficient in the development of their own blood supply, resulting in hypoxic regions within the tumour cell population.[1] In recent years, a number of low toxic (pro)drugs have been developed, capable of selective activation in hypoxic tissue.[2] Many of these compounds use the reduction of an aromatic nitro compound as a bioreductive switch to generate the active species.[3] Among these, analogues of nitroimidazoles,[4] *N*-oxides[5] and nitrobenzyl carbamates[6] have been used as reductive trigger. We now report the first synthesis of bioreductive prodrugs of paclitaxel **1**, employing the aromatic nitro and azido group as the bioreductive trigger. Paclitaxel is one of the most promising anti-cancer agents used in the clinic today. However, also paclitaxel exhibits dose limiting side effects, a common problem with cytotoxic agents. Prodrug strategies can reduce these side effects by selective release of the active drug in tumour tissue. Because of the potency of the parent drug, paclitaxel prodrugs can be very powerful chemotherapeutic agents. Low toxicity of paclitaxel prodrugs can be achieved by blocking the C2′–OH group, which is important for activity.[7] The paclitaxel prodrugs are designed to release paclitaxel after reduction of the nitro group to a hydroxylamine or amine group and subsequent 1,6-elimination of a 4-hydroxylamino or 4-amino benzyloxycarbonyl moiety, a concept that has been used successfully in prodrug chemistry.[8]

Synthesis

In a proof of principal study, prodrug **1a** was prepared via reaction of paclitaxel with 4-nitrobenzyl chloroformate (PNBC). The compound was stable in buffer solution (pH = 7.4, 37 °C) and upon chemical reduction of the nitro group formation of paclitaxel was indeed observed. Next, a series of prodrugs was prepared with additional electron withdrawing substituents on the aromatic ring and with nitro heteroaromatic moieties (which both increase the reduction potential of the nitro group). Finally some paclitaxel prodrugs were synthesised with an aromatic azide group as the bioreductive trigger. Azides are known to undergo oxygen-inhibited enzymatic reduction[9] and can therefore potentially serve to trigger hypoxia selective prodrugs.[10]

*Corresponding author. Tel.: +31-24-365-2331; fax: +31-24-365-2929; e-mail: jsch@sci.kun.nl

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The prodrugs **1b–1i** were prepared in good yield by activation of the alcohols (**3b–3i**)[11] with 4-nitrophenyl chloroformate and subsequent coupling of the activated carbonates (**2b–i**) with paclitaxel (Scheme 1).

Prodrug **1c** contains of a novel self-eliminating moiety, that after chemical reduction of the nitro group (Zn, AcOH/MeOH) fragmented to give the parent drug in quantitative yield via 1,8-elimination (Scheme 2). The other prodrugs also yielded paclitaxel after chemical reduction.

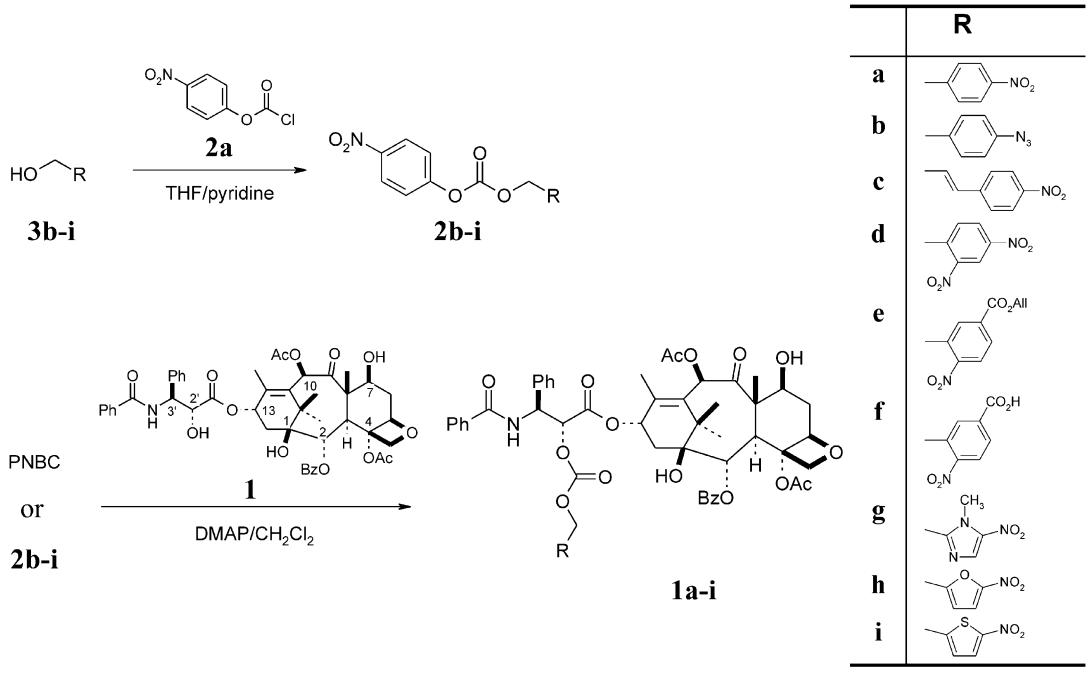
A different strategy was applied in the design and synthesis of azide prodrug **1k** in which the 3′NH-position of paclitaxel is functionalised with the self eliminating moiety and the C2′–OH position is blocked by a benzoyl group. This prodrug was prepared through semisynthesis by treating optically active phenylisoserine methyl ester with 4-azidobenzyl-4′-nitrophenyl carbonate. The modified side chain was protected as an oxazolidine[12] prior to coupling. Saponification of the methyl ester and coupling to 7-Aloc baccatin III[13] yielded the protected prodrug. After deprotection and benzoylation prodrug **1k** was obtained (Scheme 3).

After reduction ($NaBH_4$, EtOH) and elimination, a primary amine was formed at C3′. Migration of the benzoyl[14] at C2′ to the C3′–NH_2 resulted in the formation of paclitaxel.

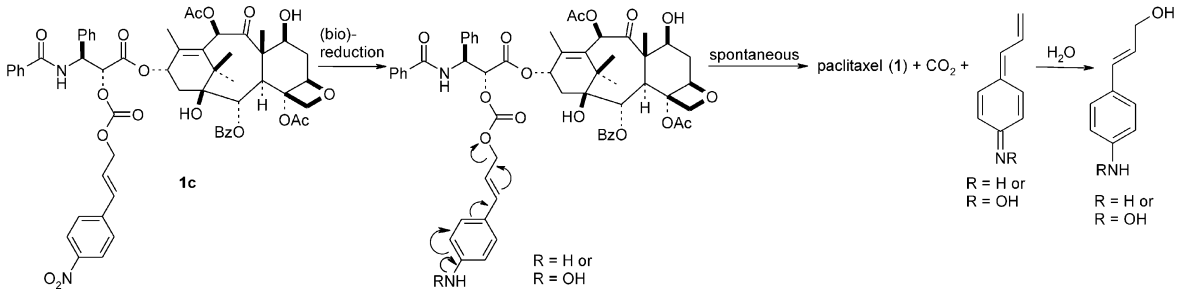
Biological Activity

All prodrugs proved to be stable in Tris–buffer (pH = 7.4, 37 °C) for at least 24 h. Cytotoxicity assays against seven well defined human tumour cell lines (Table 1) showed that prodrugs **1a–b** and **1d–i** possess only a slightly reduced cytotoxicity. This is probably due to enzymatic hydrolysis of the benzyl carbonate by esterases present in the assay. This results in nonspecific release of paclitaxel. Lack of stability is a major problem for many paclitaxel prodrugs.[15]

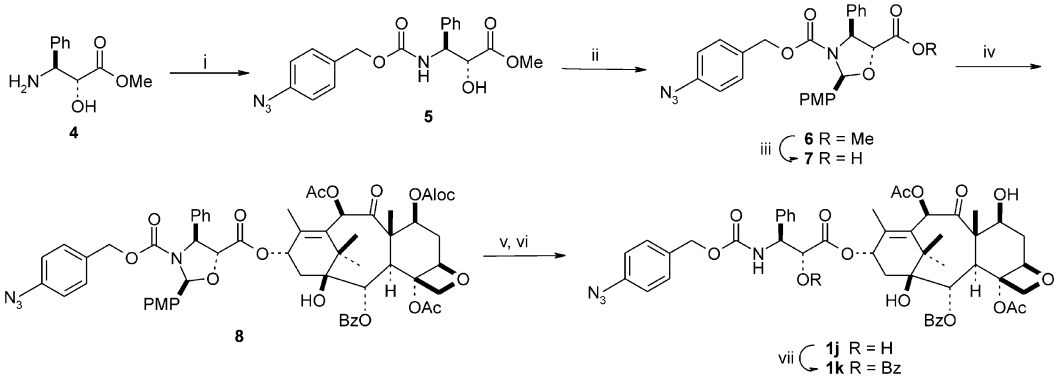
Prodrug **1c** displayed a significantly reduced cytotoxicity and therefore a greater stability towards hydrolytic enzymes. The novel linker of prodrug **1c** might be very useful as a spacer in the preparation of paclitaxel prodrugs with targets other than hypoxia, since prodrug lability is often a problem with tumour selective



Scheme 1.



Scheme 2.



Scheme 3. (i) 4-Azidobenyl-4′-nitrophenyl carbonate, pyridine, THF, 3 hm rtm 70%; (ii) 4-methoxybenzaldehyde dimethyl acetal, PTS, toluene, reflux, 3 h, 97%; (iii) K_2CO_3, MeOH, H_2O, 16 h, rt, 76%; (iv) 7-Aloc baccatin III DIC, DMAP, CH_2Cl_2, rt, 3 h 59%; (v) $Pd(PPh_3)_4$, morpholine, THF, rt 15 min, 78%; (vi) PTS, MeOH, rt 24 h, 87%; (vii) benzoic acid, DIC, DMAP, CH_2Cl_2, rt, 4 h, 76%.

Table 1. Cytotoxicity assay of paclitaxel prodrugs against seven well defined human tumour cell lines

Compound	IC_{50}[a] (ng/ mL)						
	H226	MCF7	EVSA-T	WIDR	IGROV	M19	A498
Paclitaxel	<3	<3	<3	<3	10	<3	<3
1a	8	4	6	4	36	9	52
1b	6	<3	5	3	36	7	50
1c	187	186	221	308	620	384	1387
1d	7	10	19	19	83	101	83
1e	7	11	21	18	114	151	141
1f	19	5	5	6	19	25	66
1g	13	<3	<3	<3	13	14	36
1h	5	<3	<3	<3	5	7	22
1i	6	<3	<3	<3	6	9	21
1j	14	<3	6	4	23	14	41
1k	67	25	53	41	158	68	193

[a]IC_{50}, drug concentration required to inhibit cell proliferation to 50% versus untreated cells (37 °C, 5 days).

paclitaxel prodrugs. Prodrug **1k** showed a lower toxicity as well, although less apparently than prodrug **1c**. Compound **1j**, which can be considered a new paclitaxel derivative modified at C3′-NH, showed a slightly lower cytotoxicity compared to paclitaxel.

Conclusions

In conclusion, we have prepared the first bioreductive paclitaxel prodrugs. All prodrugs were stable in buffer and released paclitaxel after reduction. Unfortunately, benzyl carbonate prodrugs **1a–b** and **1d–i** did not show a much reduced cytotoxicity under aerobic conditions. Prodrugs **1c** and **1k** did display a lower cytotoxicity and these are selected for further evaluation in anaerobic environment.

Experimental

^{1}H NMR spectra were recorded on a Bruker AM-300 (300 MHz) or on a Bruker AC100 (100 MHz) spectrometer. Chemical shift values are given in ppm (δ) relative to TMS as internal standard. For numbering of the atoms in paclitaxel, see Scheme 1. Mass spectra were obtained with VG Micromass 7070E spectrometer. Elemental analyses were carried out in triplicate on a Carlo Erba EA 1108 element analyzer. Melting points were measured on a Reichert Thermopan microscope and are uncorrected. TLC analysis was performed on Merck precoated silica gel 60 F-254 plates. Spots were visualized with UV light and 10% H_2SO_4 (1 L) solution containing ammonium molybdate (25 g) and ceric ammonium sulfate (10 g) followed by charring. Column chromatography was preformed with silica 60 (Baker). THF was dried by refluxing over sodium and was distilled prior to use. Dichloromethane was distilled from calcium hydride prior to use. When necessary, all reactions were carried out under an argon atmosphere. Unless stated otherwise, materials were obtained from commercial sources and used without further purification. Paclitaxel was generous gift of Pharmachemie, Haarlem, The Netherlands.

2′-(4-Nitrobenzyl carbonate) paclitaxel (1a). To a solution of paclitaxel (500 mg, 0.60 mmol) in CH_2Cl_2 (75 mL) was added diisopropyl ethylamine (220 μL, 1.21 mmol), 4-nitrobenzyl chloroformate (156 mg, 0.72 mmol) and a few crystals of DMAP. After stirring for 3 h, with not all the starting material consumed, the mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution, demineralised water, 0.5 N $KHSO_4$ solution and brine. The solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via column chromatography (EtOAc/hexane 1:1), affording the title compound **1a** (405 mg, 0.39 mmol, 67% (conversion yield 89%) and unreacted paclitaxel (138 mg, 0.16 mmol). Mp 170–171 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, 2H, J = 8.7 Hz, nitrophenyl), 8.15 (d, 2H, J = 7.2 Hz, H–Ph), 7.72 (d, 2H, J = 7.5 Hz, H–Ph), 7.46 (m, 13H, H–Ph, nitrophenyl), 6.86 (d, 1H, J = 9.4 Hz, NH), 6.29 (m, 2H, H10, H13), 6.02 (dd, 1H, J = 9.3, 2.7 Hz, H3′), 5.69 (d, 1H, J = 7.1 Hz, H2), 5.46 (d, 1H, J = 2.8 Hz, H2′), 5.30 (d, 1H, J = 13.2 Hz, Ar–CH_2), 5.23 (d, 1H, J = 13.2 Hz, Ar–CH_2), 4.98 (d, 1H, J = 7.7 Hz, H5), 4.44 (m, 1H, H7), 4.33 (d, 1H, J = 8.5 Hz, H20a), 4.21 (d, 1H, J = 8.4 Hz, H20b), 3.82 (d, 1H, J = 7.0 Hz, H3), 2.53 (m, 1H, H6a), 2.47 (s, 3H, 4-OAc), 2.35 (m, 2H, H14), 2.24 (s, 3H, 10-OAc), 1.92 (s, 3H, H18), 1.90 (m, 1H, H6b), 1.69 (s, 3H, H19), 1.26 (s, 3H, H16), 1.14 (s, 3H, H17); FAB-MS, 1033 $[M+H]^+$, 1055 $[M+Na]^+$. Anal. ($C_{55}H_{56}N_2O_{18}$)

calcd C 63.95%, H 5.46%, N 2.71%, measured C 64.11%, H 5.42%, N 2.64%.

2′-(4-Azidobenzyl carbonate) paclitaxel (1b). A solution of 4-azidobenzyl alcohol (22.9 mg, 0.154 mmol), pyridine (16.0 μL, 0.200 mmol), 4-nitrophenyl chloroformate (27.8 mg, 0.138 mmol) and a few crystals of DMAP in CH_2Cl_2 (5 mL) was left stirring for 40 min. After no 4-azidobenzyl alcohol could be detected by means of TLC (SiO_2, CH_2Cl_2/MeOH 95:5), paclitaxel (100.4 mg, 0.118 mmol) and DMAP (16.2 mg, 0.133 mmol) were added and stirring was continued for 70 h. The mixture was diluted with EtOAc and subsequently washed with a saturated $NaHCO_3$ solution, demineralised water, aqueous 0.5 N $KHSO_4$ solution and brine. The solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/hexane 1:1), to yield prodrug **1b** (121.0 mg, 0.118 mmol, 100%); mp 139–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, 2H, J = 7.1 Hz, H–Ph), 7.72 (d, 2H, J = 7.1 Hz, H–Ph), 7.61 (t, 1H, J = 7.3 Hz, H–Ph), 7.47 (m, 12H, H–Ph, azidophenyl), 7.01 (d, 2H, J = 8.4 Hz, azidophenyl), 6.88 (d, 1H, J = 9.3 Hz, NH), 6.30 (m, 2H, H10, H13), 5.98 (dd, 1H, J = 9.2 Hz, 2.7 Hz, H3′), 5.69 (d, 1H, J = 7.1 Hz, H2), 5.44 (d, 1H, J = 2.7 Hz, H2′), 5.30 (d, 1H, J = 13.2 Hz, Ar–CH_2a), 5.23 (d, 1H, J = 13.2 Hz, Ar–CH_2b), 4.98 (d, 1H, J = 7.7 Hz, H5), 4.45 (m, 1H, H7), 4.33 (d, 1H, J = 8.5 Hz, H20a), 4.21 (d, 1H, J = 8.4 Hz, H20b), 3.82 (d, 1H, J = 7.1 Hz, H3), 2.57 (m, 1H, H6a), 2.46 (s, 3H, 4-OAc), 2.35 (m, 2H, H14), 2.24 (s, 3H, 10-OAc), 1.92 (s, 3H, H18), 1.89 (m, 1H, H6b), 1.69 (s, 3H, H19), 1.24 (s, 3H, H16), 1.14 (s, 3H, H17); FAB-MS, 1029 $[M+H]^+$, 1051 $[M+Na]^+$. Anal. ($C_{55}H_{56}N_4O_{16}$) calcd C 64.19%, H 5.49%, N 5.44%, measured C 64.25%, H 5.56%, N 5.26%.

(2*R*,3*S*)-*N*-(4-Azidobenzyloxycarbonyl) phenylisoserine methyl ester (5). A solution of amino alcohol **4**[16] (0.50 g, 2.6 mmol) in THF (15 mL), pyridine (337 μL, 4.2 mmol) and 4-azidobenzyl,4′-nitrophenyl carbonate (0.82 g, 2.6 mmol) was stirred for 72 h in the absence of light. The mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution, demineralised water, 0.5 N $KHSO_4$ solution and brine. The solution was dried over Na_2SO_4 and concentrated in vacuo. Crystallisation in a hexane/EtOAc mixture yielded compound **5** (0.67 g, 1.8 mmol, 69%); mp 67–70 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.35 (m, 7H), 6.96 (m, 3H), 5.69 (d, 1H, J = 9.0 Hz), 5.25 (d, 1H, J = 9.0 Hz), 5.04 (s, 2H), 4.48 (br s, 1H), 3.81 (s, 3H).

***N*-(4-Azidobenzyloxycarbonyl) oxazolidine methyl ester (6).** To a solution of **5** (502 mg, 1.36 mmol) in anhydrous toluene (20 mL) was added 4-methoxybenzaldehyde dimethyl acetal (1.25 mL, 6.80 mmol) and a few crystals of PTS. The reaction was heated to reflux and stirring was continued for 30 min. The reaction was allowed to cool to ambient temperature and the reaction mixture was quenched with $NaHCO_3$ and diluted with Et_2O. The organic layer was washed with washed with a saturated $NaHCO_3$ solution, demineralised water and brine. The resulting yellow oil was purified via column chromatography (EtOAc/heptane 2:5) to yield oxazolidine **6** (642 mg, 1.32 mmol, 97%); mp 123–126 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 7.34 (m, 7H), 6.80 (m, 6H), 6.40 (s, 1H), 5.48 (d, 1H, J = 4.1 Hz), 4.93 (d, 1H, J = 12.2 Hz), 4.66 (d, 1H, J = 12.2 Hz), 4.58 (d, 1H, J = 4.1 Hz), 3.81 (s, 3H), 3.58 (s, 3H).

***N*-(4-Azidobenzyloxycarbonyl) oxazolidine carboxylic acid (7).** The oxazolidine methyl ester **6** (639 mg, 1.31 mmol) was dissolved in MeOH (15 mL). Next, a solution of K_2CO_3 (452 mg, 3.28 mmol) in demineralised water (5 mL) was added. The reaction was stirred for 16 h at room temperature. The reaction mixture was diluted with water and washed with Et_2O to remove organic impurities. Acetate buffer (pH = 5) was added to the water layer, which was extracted 3 times with CH_2Cl_2. The solution was dried over Na_2SO_4 and concentrated in vacuo to yield the title compound, which is used in the following reaction without further purification. The yield of compound **7** was 472 mg (1.00 mmol, 76%); mp 162–166 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 7H), 6.87 (m, 2H), 6.79 (d, 2H, J = 8.5 Hz), 6.71 (d, 2H, J = 8.5 Hz), 6.41 (s, 1H), 5.47 (d, 1H, J = 4.1 Hz), 4.91 (d, 1H, J = 12.2 Hz), 4.68 (d, 1H, J = 12.3 Hz), 4.63 (d, 1H, J = 4.2 Hz), 3.81 (s, 3H).

7-Aloc-2′,3′-oxazolidine protected derivative 8. To a solution of 7-Aloc baccatin III (195 mg, 0.29 mmol) in CH_2Cl_2 (10 mL) at 0 °C was subsequently added: oxazolidine carboxylic acid **7** (276 mg, 0.58 mmol), DIC (112 μL, 0.73 mmol) and a few crystals of DMAP. The reaction was stirred at room temperature. After all the 7-Aloc baccatin III was consumed, as indicated on TLC, the mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution, demineralised water, 0.5 N $KHSO_4$ solution and brine. The solution was dried over Na_2SO_4 and evaporated to dryness. Column chromatography (EtOAc/heptane 1:1) yielded compound **8** (194 mg, 0.17 mmol, 59%); mp 104–106 °C; This compound was used without further purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, 2H, J = 7.4 Hz, H–Ph), 7.63 (t, 1H, J = 7.4 Hz, H–Ph), 7.40–7.12 (m, 9H, H–Ar), 6.89 (d, 2H, J = 6.7 Hz, azidophenyl), 6.78 (d, 2H, J = 8.4 Hz, H–Ph), 6.69 (d, 2H, J = 8.0 Hz, H–Ph–OMe), 6.41 (s, 1H, oxazolidine), 6.19 (s, 1H, H10), 6.10 (t, 1H, J = 8.3 Hz, H13), 5.95 (m, 1H, H3′), 5.61 (d, 1H, J = 7.0 Hz, H2), 5.42 (m, H, H2′, allyl), 5.30 (m, 2H, Ar–CH_2), 4.88 (m, 2H, allyl), 4.63 (m, 3H, allyl, H7), 4.24 (d, 1H, J = 8.4 Hz, H20a), 4.08 (d, 1H, J = 8.3 Hz, H20b), 3.84 (d, 1H, J = 4.4 Hz, H3), 3.83 (s, 3H, MeO), 2.56 (m, 1H, H6a), 2.46 (s, 3H, 4-OAc), 2.33 (m, 2H, H14), 2.13 (s, 3H, 10-OAc), 1.90 (m, 1H, H6b), 1.82 (s, 3H, H18), 1.62 (s, 3H, H19), 1.20 (s, 3H, H16), 1.13 (s, 3H, H17).

3′*N*-(4-Azidobenzyloxycarbonyl)-3′*N*-debenzoyl paclitaxel (1j). To a solution of **8** (147 mg, 0.13 mmol) and morpholine (64 μL, 0.73 mmol) in THF (5 mL), a few crystals of $Pd(PPh_3)_4$ were added. When the reaction was complete, as was demonstrated by TLC (EtOAc/hexane 1:1), the mixture was filtered over Hyflo. The filtrate was concentrated in vacuo. The residue was taken up in EtOAc and successively washed with a saturated $NaHCO_3$ solution, demineralised water, 0.5 N

$KHSO_4$ solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified via column chromatography (EtOAc/hexane 1:1). The total yield of this reaction was dissolved in MeOH (15 mL). To this solution PTS (19.8 mg, 0.104 mmol) was added. The mixture was stirred in the absence of light in for 24 h, concentrated, diluted with EtOAc and washed with a $NaHCO_3$ solution and brine. The solution was dried over Na_2SO_4 and evaporated to dryness. Column chromatography (CH_2Cl_2/MeOH 95:5) and freeze drying in dioxane afforded **1j** (70.1 mg, 0.075 mmol, 87%) as a fluffy white solid; mp 146–151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 2H, J = 7.6 Hz, H–Ph), 7.51 (t, 1H, J = 7.6 Hz, H–Ph), 7.41 (m, 8H, H–Ar), 7.17 (d, 2H, J = 8.3 Hz, H–Ar), 6.92 (d, 2H, J = 6.7 Hz, azidophenyl), 6.27 (s, 1H, H10), 6.23 (t, 1H, J = 8.5 Hz, H13), 5.65 (m, 2H, H3′, H2), 5.03 (d, 1H, J = 12.2 Hz, Ar–CH_2a), 4.94 (d, 1H, J = 7.2 Hz, H5), 4.91 (d, 1H, J = 12.1 Hz, Ar–CH_2b), 4.66 (br s, 1H, H2′), 4.40 (m, 1H, H7), 4.30 (d, 1H, J = 8.4 Hz, H20a), 4.18 (d, 1H, J = 8.3 Hz, H20b), 3.79 (d, 1H, J = 7.1 Hz, H3), 2.56 (m, 1H, H6a), 2.45 (s, 3H, 4-OAc), 2.17 (s, 3H, 10-OAc), 2.12 (m, 2H, H14), 1.92 (m, 1H, H6b), 1.88 (s, 3H, H18), 1.65 (s, 3H, H19), 1.24 (s, 3H, H16), 1.15 (s, 3H, H17); FAB-MS, 947 $[M+Na]^+$. Anal. ($C_{48}H_{52}N_4O_{15}$) calcd C 62.33%, H 5.67%, N 6.06%, measured C 62.34%, H 5.82%, N 5.36%.

Azido prodrug 1k. To a solution of **1j** (55.0 mg, 0.060 mmol) in CH_2Cl_2 (10 mL) were added DIC (20 μL, 0.13 mmol) and a few crystals of DMAP. After stirring for 4 h, the mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution, demineralised water, 0.5 N $KHSO_4$ solution, again with demineralised water and brine. The solution was dried over Na_2SO_4 and evaporated to dryness. The residue was purified via column chromatography (EtOAc/hexane 2:3), affording prodrug **1k** (47.8 mg, 0.046 mmol, 77%); mp 150–152 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.11 (d, 2H, J = 7.4 Hz, H–Ph), 7.96 (d, 2H, J = 8.2 Hz, azidophenyl), 7.51–7.21 (m, 11H, H–Ar), 7.17 (d, 2H, J = 7.9 Hz, H–Ph), 6.89 (d, 1H, J = 8.3 Hz, NH), 6.28 (m, 2H, H10, H13), 5.65 (m, 4H, H2, H2′, Ar–CH_2), 5.05 (d, 1H,J = 12.4 Hz, Ar–CH_2a), 4.96 (d, 1H, J = 6.5 Hz, H5), 4.93 (d, 1H, J = 12.2 Hz, ArCH_2b), 4.48 (m, 1H, H7), 4.29 (d, 1H, J = 8.4 Hz, H20a), 4.18 (d, 1H, J = 8.4 Hz, H20b), 3.79 (d, 1H, J = 7.1 Hz, H3), 2.56 (m, 1H, H6a), 2.46 (s, 3H, 4-OAc), 2.33 (m, 2H, H14), 2.23 (s, 3H, 10-OAc), 1.88 (s, 3H, H18), 1.90 (m, 1H, H6b), 1.67 (s, 3H, H19), 1.23 (s, 3H, H16), 1.13 (s, 3H, H17); FAB-MS, 1029 $[M+H]^+$, 1051 $[M+Na]^+$. Anal. ($C_{55}H_{56}N_4O_{16}\cdot H_2O$) calcd C 63.10%, H 5.55%, N 5.34%, measured C 63.56%, H 5.59%, N 5.32%.

2′-(4-Nitrocinnamylcarbonate) paclitaxel (1c). The procedure identical to the preparation of prodrug **1b** was followed to yield **1c** (58%); mp 151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.19 (d, 2H, J = 8.7 Hz, nitrophenyl), 8.15 (d, 2H, J = 7.2 Hz, H–Ph), 7.75 (d, 2H, J = 7.2 Hz, H–Ph), 7.35–7.67 (m, 13H, H–Ph), 6.75 (d, 1H, J = 16.0 Hz, *H*C = CH–CH_2), 6.43 (dt, 1H, J = 16.0 Hz, HC = C*H*–CH_2), 6.34 (s, 1H, H10), 6.26 (bt, 1H, H13), 6.01 (m, 1H, H3′), 5.72 (d, 1H, J = 7.1 Hz, H2), 5.46 (d, 1H, J = 2.8 Hz, H2′), 4.99 (bd, 1H, J = 7.9 Hz, H5), 4.87 (bt, 2H, CH_2-spacer), 4.39 (m, 1H, H7), 4.32 (d, 1H, J = 8.4 Hz, H20a), 4.26 (d, 1H, J = 8.4 Hz, H20b), 3.82 (d, 1H, J = 7.0 Hz, H3), 2.55 (m, 1H, H6a), 2.46 (s, 3H, 4-OAc), 2.22 (s, 3H, 10-OAc), 1.96 (s, 3H, H18), 1.70 (s, 3H, H19), 1.22 (s, 3H, H16), 1.17 (s, 3H, H17); FAB-MS, 1059 $[M+H]^+$, 1081 $[M+Na]^+$. Anal. ($C_{57}H_{58}N_2O_{18}\cdot 2^1/_2H_2O$) calcd C 62.01%, H 5.75%, N 2.54%, measured C 62.06%, H 5.31%, N 2.60%.

Proof of principle of 1,8-elimination: chemical reduction of the nitrocinnamyl carbonate 1c. 36 mg of the prodrug 2′-[4-nitrocinnamyl carbonate]-paclitaxel **1c** was dissolved in 8 mL methanol and 2 mL acetic acid. A catalytic amount of Zinc powder was added and the red mixture was stirred for 12 h. Dichloromethane was added and the organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate, brine, and water and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (ethyl acetate/hexane; 2:1), to yield 28 mg of paclitaxel (confirmed by 300 MHz ^{1}H NMR) and 4 mg of unreacted starting compound. When the compound was stirred in the absence of zinc powder under the same conditions, no paclitaxel was formed, indicating that reduction of the nitro group by zinc induced the release of paclitaxel.

2′-*O*-(2,4-Dinitrobenzyloxycarbonyl) paclitaxel (1d). The procedure identical to the preparation of prodrug **1b** from **3d**[17,18] was followed to yield **1d** (101 mg, 0.09 mmol, 78%) as a white solid; mp 161–163 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (1H, d, J = 1.8 Hz, H3–Ph), 8.47 (1H, dd, J = 2.6, J = 8.8 Hz, H5–Ph), 8.16 (2H, d, J = 7.3 Hz, H–Ph), 7.83 (1H, d, J = 8.6 Hz, H–Ph), 7.74 (2H, d, J = 7.5 Hz, H–Ph), 7.61 (1H, m, H–Ph), 7.54–7.36 (10H, m, H–Ph), 6.89 (1H, d, J = 9.7 Hz, NH), 6.28 (1H, s, H10), 6.28 (1H, m, H13), 6.01 (1H, dd, J = 2.6, 9.7 Hz, H3′), 5.70 (1H, d, J = 7.2 Hz, H2), 5.44 (1H, d, J = 2.6 Hz, H2′), 5.17 (2H, s, Ar–CH_2), 4.98 (1H, dd, J = 9.7, 2.6 Hz, H5), 4.44 (1H, dd, J = 11.4, 7.0 Hz, H7), 4.32 (1H, d, J = 8.8 Hz, H20a), 4.21 (1H, d, J = 8.8 Hz, H20b), 3.82 (1H, d, J = 6.1 Hz, H3), 2.56 (1H, ddd, J = 6.1, 9.7, 14.9 Hz, H6a), 2.47 (3H, s, 4-OAc), 2.43 (1H, m, H14a), 2.40 (1H, m, C7OH), 2.23 (2H, m, H14b, H6b), 2.22 (3H, s, 10-OAc), 1.93 (3H, s, H18), 1.87 (1H, m, H6b), 1.69 (3H, s, H19), 1.25 (3H, s, H16), 1.15 (3H, s, H17); FAB-MS, 1100 $[M+Na]^+$. Anal. ($C_{55}H_{55}N_3O_{20}$) calcd C 61.3%, H 5.1%, N 3.9%, measured C 61.1%, H 4.6%, N 3.7%.

3-Bromomethyl-4-nitrobenzoic acid ethyl ester. The mixture of 3-methyl-4-nitrobenzoic acid ethyl ester[19] (5.0 g, 24 mmol), NBS (6.4 g, 36 mmol), $(BzO)_2$, in CCl_4 (100 mL) was refluxed and irradiated with 250 W lamp for 16 h. The solvent was evaporated and the residue was chromatographed on silica gel eluting with EtOAc/heptane 1:8 to afford the title compound as an oil (4.87 g, 17 mmol, 70%), which was used as such in the next step; ^{1}H NMR (100 MHz, $CDCl_3$) δ 8.22 (1H, m, H3), 8.10 (2H, m, H4, H6), 4.83 (2H, s), 4.44 (2H, q, J = 7.1 Hz), 1.43 (3H, t, J = 7.1 Hz).

3-Hydroxymethyl-4-nitrobenzoic acid ethyl ester. The mixture of 3-bromomethyl-4-nitrobenzoic acid ethyl ester (1.6 g, 5.5 mmol), H_2SO_4 (3 mL) and water (50 mL) was refluxed overnight. The solution was made basic with 5 N NaOH solution and extracted with diethyl ether. The aqueous layer was acidified with 1 N HCl, extracted into EtOAc, dried (Na_2SO_4) and evaporated. The residue was refluxed in EtOH (150 mL) with H_2SO_4 (1 mL) overnight. EtOH was evaporated and the residue was chromatographed on silica gel eluting with EtOAc/heptane 2:5 to afford the title compound as an oil (1.1 g, 89%), which was directly used in the following step; ^{1}H NMR (100 MHz, $CDCl_3$) δ 8.43 (1H, s, H3), 8.12 (2H, s, H4, H6), 5.03 (2H, s), 4.44 (2H, q, J = 7.2 Hz), 2.00 (1H, bs, OH), 1.43 (3H, t, J = 7.2 Hz).

3-Hydroxymethyl-4-nitrobenzoic acid allyl ester (3e). The mixture of 3-hydroxymethyl-4-nitrobenzoic acid ethyl ester (1.0 g, 4.44 mmol), allyl alcohol (100 mL) and concd H_2SO_4 (3 mL) was refluxed for 24 h. Allyl alcohol was evaporated and the residue was purified by silica gel column chromatography eluting with EtOAc/heptane 2:5 to afford yellow solid, which was crystallised from isopropanol/heptane to yield **3e** as yellow crystals (270 mg, 1.14 mmol, 26%), which were used as such in the next step; mp 73–75 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 8.46 (1H, s, H3), 8.14 (2H, s, H4, H6), 5.52–5.29 (2H, m, allyl), 6.20–5.87 (1H, m, allyl), 5.02 (2H, bs), 5.85 (2H, m, allyl), 2.51 (1H, bs, OH).

2-Nitro-5-(allyloxycarbonyl)benzyl 4-nitrophenyl carbonate (2e). To a mixture of **3e** (170 mg, 0.72 mmol), pyridine (0.28 mL) in dry THF (10 mL) was added dropwise 4-nitrophenyl chloroformate (217 mg, 1.07 mmol) in dry THF (10 mL) at 20 °C and the mixture was stirred for 42 h. The reaction mixture was filtered and evaporated under reduced pressure. The oily residue was redissolved in EtOAc (20 mL), washed with 10% citric acid solution and brine, dried (Na_2SO_4) and evaporated to give oil, which was purified by silica gel column chromatography eluting with CH_2Cl_2/heptane 3:1 to afford **2e** as white solid (171mg, 0.42 mmol, 59%), which was used directly in the subsequent reaction step; mp 102–104 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 8.39 (1H, s, H3), 8.31 (2H, m, H3′,5′), 8.31 (2H, s), 8.23 (2H, s, H4, H6), 7.42 (2H, m, H2′,6′), 4.90 (2H, d, J = 5.7 Hz), 5.52–5.29 (2H, m, allyl), 5.74 (2H, s, CH_2O), 6.20–5.87 (1H, m, allyl), 5.02 (2H, bs), 5.85 (2H, m, allyl) 2.51 (1H, bs, OH).

2′-*O*-[2-Nitro-5-(allyloxycarbonyl)benzyloxycarbonyl]paclitaxel (1e). Analogously to the preparation of **1b** to afford **1e** as white solid (159 mg, 90%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (1H, s, H–Ph), 8.17 (2H, s, H–Ph), 8.15 (2H, d, J = 7.3 Hz, H–Ph), 7.76 (2H, d, J = 7.4 Hz, H–Ph), 7.60 (1H, m, H–Ph), 7.54–7.34 (10H, m, H–Ph), 6.94 (1H, d, J = 9.7 Hz, NH), 6.28 (1H, s, H10), 6.28 (1H, m, H13), 6.03 (1H, dd, J = 2.6, 8.7 Hz, H3′), 5.97 (1H, m, allyl), 5.69 (1H, d, J = 7.2 Hz, H2), 5.60 (2H, s, Ar–CH_2), 5.48 (1H, d, J = 2.6 Hz, H2′), 5.43–5.27 (2H, m, allyl), 4.98 (1H, dd, J = 9.7, 2.6 Hz, H5), 4.85 (2H, d, J = 6.5 Hz, allyl), 4.43 (1H, m, H7), 4.32 (1H, d, J = 8.3 Hz, H20a), 4.20 (1H, d, J = 8.3 Hz, H20b), 3.81 (1H, d, J = 7.0 Hz, H3), 2.55 (1H, m, H14a), 2.46 (3H, s, 4-OAc), 2.52–2.36 (3H, m, C7OH, H6a and H14b), 2.23 (3H, s, 10-OAc), 1.91 (3H, s, H18), 1.89 (1H, m, H6b), 1.68 (3H, s, H19), 1.24 (3H, s, H16), 1.14 (3H, s, H17); FAB-MS, 1139 $[M+Na]^+$. Anal. ($C_{59}H_{60}N_2O_{20}$) calcd C 63.4%, H 5.4%, N 2.5%, measured C 63.2%, H 5.1%, N 2.7%.

2′-*O*-(2-Nitro-5-carboxybenzyloxycarbonyl)paclitaxel (1f). To a solution of (78 mg, 0.071 mmol) in dry THF under an argon was added glacial acetic acid (10 μL, 0.18 mmol, 2.5 equiv) and $(C_4H_9)_3SnH$ and few crystals of $Pd(PPh_3)_4$. After 30 min 1 mL of 0.5 M HCl in EtOAc was added carefully with stirring. 10 mL ethyl ether and 20 mL EtOAc were added and the organic phase was washed with water, brine, dried (Na_2SO_4) and evaporated. The crude compound was chromatographed with silica gel eluting first with CH_2Cl_2 and then MeOH/CH_2Cl_2 (1:9) to afford **1f** as white solid (49.2 mg, 0.046 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ 8.31 (1H, s, H–Ph), 8.17 (2H, s, H–Ph), 8.14 (2H, d, J = 7.8 Hz, H–Ph), 7.75 (2H, d, J = 6.8 Hz, H–Ph), 7.60 (1H, m, H–Ph), 7.52–7.36 (10H, m, H–Ph), 6.31 (1H, s, H10), 6.22 (1H, bt, J = 8.7 Hz, H13), 6.01 (1H, dd, J = 3.0, 9.2 Hz, H3′), 5.70 (1H, d, J = 7.1 Hz, H2), 5.65 (2H, d, J = 15.1 Hz, Ar–CH_2a), 5.57 (2H, d, J = 15.1 Hz, Ar–CH_2b), 5.50 (1H, d, J = 3.5 Hz, H2′), 4.97 (1H, bd, J = 7.1 Hz, H5), 4.39 (1H, dd, J = 6.2 Hz, 10.5 Hz, H7), 4.32 (1H, d, J = 8.5 Hz, H20a), 4.23 (1H, d, J = 8.5 Hz, H20b), 3.79 (1H, d, J = 7.1 Hz, H3), 2.53 (1H, m, H6a), 2.41 (3H, s, 4-OAc), 2.41–2.34 (2H, m, H14a and C7OH), 2.27 (3H, m, H6 and H14b), 2.23 (3H, s, 10-OAc), 1.89 (3H, s, H18), 1.89 (1H, m, H6b), 1.68 (3H, s, H19), 1.22 (3H, s, H16), 1.15 (3H, s, H17); FAB-MS, 1100 $[M+Na+1]^+$. Anal. ($C_{55}H_{56}N_2O_{20}\cdot 2H_2O$) C 60.4%, H 5.4%, N 2.5%, measured C 60.2%, H 5.4%, N 2.4%.

2′-*O*-(5-Methyl-nitro-1H-imidazol-2-yl)methyloxycarbonyl)paclitaxel (1g). Analogously to the preparation of **1b** from **3g**[20] to afford **1g** as a white powder (101 mg, 91%); mp 150–152 °C; ^{1}H NMR (300 MHz, $CDCl_3$) d = 8.15 (2H, d, J = 7.2 Hz, H–Ph), 7.72 (2H, d, J = 7.3 Hz, H–Ph), 7.61 (1H, m, H–Ar), 7.54–7.35 (10H, m, H–Ph), 7.26 (1H, d, J = 3.5 Hz, H–Ar), 6.88 (1H, d, J = 8.8 Hz, NH), 6.66 (1H, d, J = 3.5 Hz, H–Ar), 6.30 (1H, s, H10), 6.28 (1H, d, J = 9.7 Hz, H13), 6.01 (1H, dd, J = 2.6 Hz, 9.7 Hz, H3′), 5.70 (1H, d, J = 7.2 Hz, H2), 5.44 (1H, d, J = 2.6 Hz, H2′), 5.17 (2H, s, Ar–CH_2), 4.98 (1H, dd, J = 9.7 Hz, 2.6 Hz, H5), 4.44 (1H, dd, J = 11.4 Hz, 7.0 Hz, H7), 4.32 (1H, d, J = 8.8 Hz, H20a), 4.21 (1H, d, J = 8.8 Hz, H20b), 3.82 (1H, d, J = 6.1 Hz, H3), 2.56 (1H, ddd, J = 6.1 Hz, 9.7 Hz, 14.9 Hz, H6a), 2.47 (3H, s, 4-OAc), 2.47–2.35 (2H, m, C7OH, H14b), 2.25 (1H, m, H14a) 2.24 (3H, s, 10-OAc), 1.93 (3H, s, H18), 1.83 (1H, m, H6b), 1.69 (3H, s, H19), 1.25 (3H, s, H16), 1.15 (3H, s, H17); FAB-MS, 1037$[M+H]^+$, 1059 $[M+Na]^+$. Anal. ($C_{53}H_{56}N_4O_{18}\cdot H_2O$) calcd C 60.34%, H 5.54%, N 5.31%, measured C 60.23%, H 5.68%, N 4.95%.

2′ - *O* - (5 - Nitrofuran - 2 - ylmethyloxycarbonyl)paclitaxel (1h). Analogously to the preparation of **1b** from **3h**[21] to yield **1h** as a white powder (90 mg, 74%); mp 156–158 °C.

^{1}H NMR (300 MHz, $CDCl_3$) d = 8.15 (2H, d, J = 7.2 Hz, H–Ph), 7.72 (2H, d, J = 7.3 Hz, H–Ph), 7.61 (1H, m, H–Ar), 7.54–7.35 (10H, m, H–Ph), 7.26 (1H, d, J = 3.5 Hz, H–Ar), 6.88 (1H, d, J = 8.8 Hz, NH), 6.66 (1H, d, J = 3.5 Hz, H–Ar), 6.30 (1H, s, H10), 6.28 (1H, d, J = 9.7 Hz, H13), 6.01 (1H, dd, J = 2.6 Hz, 9.7 Hz, H3′), 5.70 (1H, d, J = 7.2 Hz, H2), 5.44 (1H, d, J = 2.6 Hz, H2′), 5.17 (2H, s, Ar–CH_2), 4.98 (1H, dd, J = 9.7, 2.6 Hz, H5), 4.44 (1H, dd, J = 11.4, 7.0 Hz, H7), 4.32 (1H, d, J = 8.8 Hz, H20a), 4.21 (1H, d, J = 8.8 Hz, H20b), 3.82 (1H, d, J = 6.1 Hz, H3), 2.56 (1H, ddd, J = 6.1, 9.7, 14.9 Hz, H6a), 2.47 (3H, s, 4-OAc), 2.49–2.35 (2H, m, H14a, C7OH), 2.22 (1H, m, H14b), 2.24 (3H, s, 10-OAc), 1.93 (3H, s, H18), 1.83 (1H, m, H6b), 1.69 (3H, s, H19), 1.25 (3H, s, H16), 1.15 (3H, s, H17); FAB-MS, 1023 $[M+H]^+$, 1045 $[M+Na]^+$. Anal. ($C_{53}H_{54}N_2O_{19}$) calcd C 62.23%, H 5.32%, N 2.74%, measured C 62.32%, H 5.64%, N 2.64%.

2′-*O*-(5-Nitrothiophene-2-ylmethyloxycarbonyl)paclitaxel (1i). Analogously to the preparation of **1b** from **3i**[22] to afford **1i** as a white powder (105 mg, 94%); mp 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (2H, d, J = 7.2 Hz, H–Ph), 7.79 (1H, d, J = 3.9 Hz, H–Ar), 7.72 (2H, d, J = 7.5 Hz, H–Ph), 7.61 (1H, m, H–Ph), 7.54–7.35 (10H, m, H–Ph), 7.04 (1H, d, J = 3.5 Hz, H–Ar), 6.86 (1H, d, J = 9.2 Hz, NH), 6.66 (1H, d, J = 3.5 Hz, H–Ar), 6.30 (1H, s, H10), 6.27 (1H, d, J = 9.2 Hz, H13), 6.01 (1H, dd, J = 3.1Hz, 9.7 Hz, H3′), 5.69 (1H, d, J = 7.0 Hz, H2), 5.46 (1H, d, J = 2.6 Hz, H2′), 5.27 (2H, s, Ar–CH_2), 4.98 (1H, dd, J = 9.7, 1.7 Hz, H5), 4.45 (1H, ddd, J = 10.1, 6.6, 3.9 Hz, H7), 4.33 (1H, d, J = 8.8 Hz, H20a), 4.21 (1H, d, J = 8.8 Hz, H20b), 3.82 (1H, d, J = 7.0 Hz, H3), 2.57 (1H, ddd, J = 6.6, 9.7, 14.9 Hz, H6a), 2.47 (3H, s, 4-OAc), 2.50–2.36 (2H, m, H14a, C7OH), 2.24 (1H, m, H14b), 2.24 (3H, s, 10-OAc), 1.94 (3H, s, H18), 1.89 (1H, m, H6b), 1.69 (3H, s, H19), 1.24 (3H, s, H16), 1.14 (3H, s, H17); FAB-MS, 1039 $[M+H]^+$, 1061$[M+Na]^+$. Anal. ($C_{53}H_{54}N_2O_{18}S$) calcd C 61.26%, H 5.24%, N 2.70%, S 3.09%, measured C 62.32%, H 5.64%, N 2.64%, S 2.36%.

In vitro cytotoxicity assay. The following human tumor cell lines were used: MCF-7 and EVSA breast carcinoma cells, WIDE colon carcinoma cells, IGROV ovarian carcinoma cells, M19 MEL melanoma cells, A498 renal carcinoma cells and H226 non-small cell lung carcinoma cells. The antiproliferative effects were determined applying the microculture sulforhodamine B (SRB) test[23] and are expressed as ID50 values. Results were averaged from experiments that were performed in quadruplicate.

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