Anti-Cancer Drugs 2001, **12,** pp. 305–313

Report

A new prodrug of paclitaxel: synthesis of Protaxel

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2′ and 7 Polyol carbonates of paclitaxel were synthesized and screened as potential paclitaxel prodrugs. Paclitaxel is released from 7-(2″,3″-dihydroxypropylcarbonato) paclitaxel (Protaxel) at rates inversely proportional to pH, by an intramolecular cyclization. Compared to paclitaxel, maximum tolerated i.v. or i.p. doses (MTD) of Protaxel are about 2.5- to 3-fold higher; its efficacy is substantially higher in human cancer line xenografts in athymic mice, especially in prostate PC-3, breast MDA-MB 468 and ovary OVCAR-1. [© 2001 Lippincott Williams & Wilkins.]

Key words: Cancer, derivative, paclitaxel, prodrug, Protaxel, Taxol.

Introduction

Paclitaxel (Taxol®, **1**) is a useful drug for numerous cancers[1] as well as for malaria[2] and multiple sclerosis.[3] The therapeutic potential of **1** is limited by hemato-toxicity, neuro-toxicity[4] and development of resistance.[5] The span between the maximal tolerated therapeutic dose and intolerable toxic levels is narrow,[6] possibly reflecting the high hydrophobicity and thus chemotoxicity of **1**. Poor aqueous solubility necessitates formulation of emulsions containing Cremophor EL®, an oil, of considerable toxicity.[4]

Many attempts were made to improve on **1** by derivatization with ester, carbamate and carbonate linkages to produce non-ionic or charged moieties.[7–14] Enzymatically cleavable groups,[10,12] tumor targeting moieties,[13] sugars[11b] and polymeric chains[11,14] were attached at the C-2′ and/or C-7 hydroxyl, with the idea of increasing aqueous/ethanolic solubility and sustained slow release of **1**. None of these taxoids are currently in clinical use.

2′-Alkylcarbonate derivatives showed poor aqueous solubility and only marginal therapeutic improvement over **1** *in vivo*.[10a] The mechanism of release of **1** from these prodrugs is simple hydrolysis. We attempted to apply our previously successful approach to the detoxification of triiodinated benzenes, the radiographic contrast media, by using polyols in non-ionic species.[15] By exploring several sites and modes of their attachment to **1**, we propose a novel prodrug of **1** with increased regional hydrophilicity, a stable formulation and improved systemic tolerance, while providing a sustained release of the parent compound. We suspected that an intramolecular nucleophilic cyclization pathway rather than simple hydrolysis and increased stability with lower pH (which is characteristic of most tumors[16]) should decelerate release of **1** in that milieu. Here we report the synthesis, characterization and kinetics of decomposition of several paclitaxel derivatives (Figure 1) as well as preliminary *in vivo* evaluation of Protaxel, a prodrug of paclitaxel.

Materials and methods

General methods

^{1}H- and ^{13}C{^{1}H}-NMR were recorded on a Bruker 500 MHz instrument in $CDCl_3$ by NuMega Resonance Labs (San Diego CA). Mass Spectrometry analysis was performed by the Center for Mass Spectrometry (La Jolla CA). Purity and identification of taxoids **1–6** were determined by high-performance liquid chromato-graphy (HPLC) using a C-18 Waters Symmetry column in a 60/40 acetonitrile/5 mM KH_2PO_4 (pH 3.5) mobile phase at 230 nm [for non-polar intermediates a 75/25 acetonitrile/5 mM KH_2PO_4 (pH 3.5) mobile phase was used]. Paclitaxel (**1**) was purchased from Handetech (Houston, TX). 1-*O*-Chlor-

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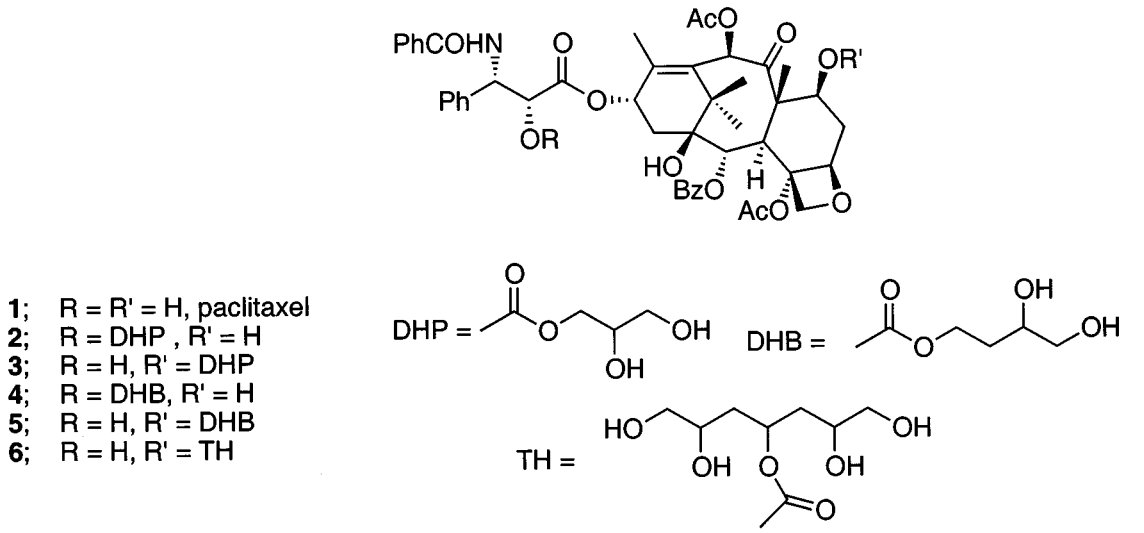


Figure 1. Structure of novel paclitaxel–polyol-carbonate adducts.

oformyl-2,3-*O*-isoproylidene-DL-glycerol was synthesized according to a slightly modified procedure described previously.[19b] Synthesis and characterization of taxoids **2**, **4**, **5** and **6** (including intermediates) are shown graphically in Schemes 1 and 2 and Table 1, and described in depth elsewhere.[17b]

Compounds

2′,7-[Bis-(2″,3″-isopropylideneglycerlcarbonoxy)] paclitaxel (11). To a solution of **1** (2.0 g, 2.34 mmol) and 1-*O*-chloroformyl-2,3-*O*-isoproylidene-DL-glycerol (9.12 mg, 46.84 mmol) in CH_2Cl_2 (40 ml) at −70°C under an inert atmosphere was added pyridine (2.84 ml, 35.13 mmol). The reaction was removed from the cold bath and allowed to stir at room temperature for 4.5 h. The reaction mixture was washed with water (3 × 40 ml), the organic layer dried over $MgSO_4$, filtered and then reduced in volume to about 5 ml. The resulting solution was purified by silica gel chromatography (CH_2Cl_2/acetone) to yield 2.63 g **11** (84.1%). Purity by HPLC was greater than 98%. ^{1}H-NMR ($CDCl_3$): δ 8.13 (d, 2H, OC(O) *o*-ArH), 7.75 (d, 2H, NC(O) *o*-ArH), 7.62 (t, 1H, OC(O) *p*-ArH), 7.53–7.49 (band, 3H), 7.43–7.35 (band, 7H), 6.91 (t, 1H, NH), 6.35 (s, 1H, C(10)-H), 6.27 (t, 1H, C(13)-H), 5.99 (t, 1H, C(3′)-H), 5.69 (d, 1H, C(2)-H), 5.51 (dd, 1H, C(7)-H), 5.44 (dd, 1H, C(2′-H), 4.97 (d, 1H, C(5)-H) 4.39–4.25 (band, 3H), 4.24–4.10 (band, 7H), 3.96 (d, 1H), 3.85 (dd, 1H), 3.75 (m, 1H), 2.61 (p, 1H, C(6)-H), 2.46 (d, 3H, C(4)-OAc), 2.41 (m, 1H, C(14)-H), 2.23 (m, 1H, C(14)-H), 2.14 (d, 3H, C(10)-OAc), 2.01 (s, 3H, C(12)-CH3), 1.97 (m, 1H, C(6)-H), 1.81 (s, 3H, C(8)-CH3), 1.41 (d, 3H), 1.38 (s, 3H), 1.35 (d, 6H), 1.21 (s, 3H, C(15)-CH_3), 1.16 (s, 3H, C(15)-CH_3).

2′,7-(Bis-alloc)-Paclitaxel (9). To a solution of **1** (4.00 g, 4.68 mmol) and allyl chloroformate (9.94 ml, 93.68 mmol) in CH_2Cl_2 (40.0 ml) at −70°C was added 1.0 M LiN[Si(CH_3)$_3$]$_2$ in THF (18.72 ml). The reaction was removed from the cold bath and stirred at room temperature for 30 min. The mixture was washed with H_2O (2 × 50 ml) and brine (2 × 50 ml), the organic layer dried over $MgSO_4$, filtered, and concentrated to 20 ml. The resulting solution was purified by preparative HPLC to yield 3.96 g (82.8%) of **9**. Purity by HPLC was greater than 99%. ^{1}H-NMR ($CDCl_3$): δ 8.13 (d, 2H, OC(O) *o*-ArH), 7.75 (d, 2H, NC(O) *o*-ArH), 7.62 (t, 1H, OC(O) *p*-ArH), 7.53–7.49 (band, 3H), 7.45–7.36 (band, 7H), 6.92 (d, 1H, NH), 6.39 (s, 1H, C(10)-H), 6.27 (t, 1H, C(13)-H), 5.99–5.87 (band, 3H), 5.70 (d, 1H, C(2)-H), 5.51 (dd, 1H, C(7)-H), 5.43 (d, 1H, C(2′)-H), 5.38–5.23 (band, 4H), 4.97 (d, 1H, C(5)-H), 4.73–4.60 (band, 4H), 4.34 (d, 1H, C(20)-H), 4.19 (d, 1H, C(20)-H), 3.97 (d, 1H, C(3)-H), 2.63 (m, 1H, C(6)-H), 2.46 (s, 3H, C(4)-OAc), 2.40 (dd, 1H), 2.22 (dd, 1H), 2.15 (s, 3H, C(10)-OAc), 2.03 (s, 3H, C(10)-CH_3), 1.97 (t, 1H), 1.82 (s, 3H, C(11)-CH_3), 1.21 (s, 3H, C(15)-CH_3), 1.17 (s, 3H, C(15)-CH_3); ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 201.96, 169.90, 169.07, 168.22, 167.45, 167.13, 154.13, 154.06, 141.45, 136.97, 133.94, 133.79, 132.88, 132.25, 132.13, 130.96, 130.43, 129.35, 128.96, 128.93, 128.73, 127.37, 126.81, 120.08, 118.98, 84.15, 81.07, 78.95, 77.49, 77.23,

76.98, 76.59, 75.56, 75.49, 74.72, 72.28, 69.76, 69.32, 56.30, 52.98, 47.18, 43.51, 35.59, 33.56, 26.68, 22.87, 21.58, 20.96, 14.76, 10.95; FAB LRMS *m/e* 1022, M + H^+ calcd for $C_{55}H_{59}NO_{18}$ 1022.09; UV max 230 nm (ε 28556), min 272 nm (ε 1564).

7-(2″,3″-Dihydroxypropyl carbonoxy)paclitaxel (3). Method A. To a solution of **11** (3.75 g, 3.20 mmol) in THF (75 ml) was added 1 N HCl (75 ml). The reaction was stirred at 35°C for 3 h to give complete conversion to 2′,7-[bis-2″,3″-dihydroxypropylcarbonoxy)]paclitaxel (not isolated). After the reaction reached ambient temperature it was diluted with THF (75 ml), washed with brine (75 ml), 50/50 brine/water (75 ml), 0.1 M KH_2PO_4 at pH 6.5 (2 × 75 ml) and again with brine (75 ml). The organic layer was treated with 0.1 M KH_2PO_4 at pH 6.5 (75 ml) and stirred at 35°C for 2 h. The reaction mixture was then washed with brine (75 ml), 50/50 brine/water (75 ml) and again with brine (75 ml). The organic layer was dried over $MgSO_4$, filtered and then concentrated to approximately 15 ml. The resulting solution was purified by preparative HPLC to yield **3** 2.76 g (88.7%). Purity by HPLC was greater than 99%. Method B. To a rapidly stirred solution of **9** (2.70 g, 2.64 mmol) in THF (135.00 ml) was added H_2O (135.00 ml), 70% *tert*-BuOOH in H_2O (9.04 ml, 66.04 mmol) and 0.16 M OsO_4 in *tert*-BuOH (18.08 ml, 2.64 mmol). This resulted in a homogeneous light yellow solution which was allowed to stir at 35°C for 6 h and yielded complete oxidation to 2,7-[(bis-2″,3″-dihydroxypropylcarbonate)]paclitaxel (not isolated). The reaction was allowed to reach ambient temperature over the course of 1 h and then the pH was adjusted to about 7 with 0.1 M $NaHCO_3$ (2.08 ml). After 0.5 h the solution was diluted with THF (135.00 ml), the reaction mixture washed with brine (1 × 70 ml then 2 × 135 ml), the organic layer dried over $MgSO_4$, filtered and then concentrated to 14 ml. The resulting yellow solution was purified by preparative HPLC to yield 2.12 g (82.8%) **3**. Purity by HPLC was greater than 99%. ^{1}H-NMR ($CDCl_3$): δ 8.11 (d, 2H, OC(O) *o*-ArH), 7.76 (d, 2H, NC(O) *o*-ArH), 7.62 (t, 1H, OC(O) *p*-ArH), 7.52–7.48 (band, 5H), 7.43–7.34 (band, 5H), 7.03 (d, 1H, NH), 6.26 (s, 1H, C(10)-H), 6.19 (t, 1H, C(13)-H), 5.80 (dd, 1H, C(3′)-H), 5.67 (d, 1H, C(2)-H), 5.42 (dd, 1H, C(7)-H), 4.95 (d, 1H, C(5)-H), 4.80 (d, 1H, C(2')-H), 4.58–4.39 (dd, 1H, C(2″)-H) 4.32 (d, 1H, C(20)-H), 4.18 (d, 1H, C(20)-H), 4.06–3.94 (band, 4H), 3.90 (d, 1H, C(3)-H), 3.72–3.59 (band, 5H), 2.67 (m, 1H, C(6)-H), 2.39 (s, 3H, C(4)-OAc), 2.36–2.29 (band, 3H), 2.22 (d, 4H), 1.98–1.93 (band, 2H), 1.83 (s, 3H, C(16)-OAc), 1.82 (s, 3H, C(12)-CH_3), 1.23 (s, 3H, C(15)-CH_3), 1.16 (s, 3H, C(15)-CH_3); ^{13}C{^{1}H}-NMR ($CDCl_3$): δ 201.28, 201.18, 172.85, 171.58, 171.44, 170.70, 167.48, 166.95, 154.46, 154.34, 141.41, 141.34, 138.18, 134.00, 133.84, 132.73, 132.20, 130.37, 129.27, 129.21, 128.93, 128.54, 127.26, 84.02, 81.15, 78.62, 77.48, 77.23, 76.97, 76.57, 76.24, 76.19, 76.12, 74.40, 73.31, 72.39, 70.02, 69.06, 62.96, 62.77, 56.19, 56.16, 55.21, 47.25, 43.47, 35.76, 33.42, 26.80, 22.72, 21.18, 21.12, 21.07, 14.82, 10.92; FAB LRMS (NBA/CsI) *m/e* 1104, M + Cs^+ calcd for $C_{51}H_{57}NO_{18}$ 1104.30; UV max 230 nm (ε 27891), min 274 nm (ε 1489).

Identification of **1** and 4-(hydroxymethyl)-1,3-dioxolan-2-one released from **3**

Protaxel (10 mg) was dissolved in acetonitrile (300 μl) and 2% $NaHCO_3$ (100 μl). After 15 min at room temperature a 250 μl aliquot was removed. For gas chromatography identification of 4-(hydroxymethyl)-1,3-dioxolan-2-one the aliquot was quenched with 0.5N formic acid in methanol (400 μl) to pH 3–5, concentrated under reduced pressure, the residue partitioned between diethyl ether (1 ml), water (100 μl), brine (100 μl) and the aqueous layer sampled. For HPLC identification of **3** and **1** an aliquot (250 μl) from the diethyl ether layer (above) was concentrated under reduced pressure and dissolved in the appropriate injection matrix for sampling. 4-(Hydroxymethyl)-1,3-dioxolan-2-one and **1** were identified by comparison to authentic samples.

Half-life for the reversion of compounds **2–6** to **1** in human serum at 37°C

Solutions of taxoids **2–6** in DMSO (5 mg/ml, 100 μl) were added to human serum (900 μl) and incubated at 37°C. At the desired time points 20 μl aliquots were removed and extracted with ethyl acetate for sampling by HPLC (see method above).

pH dependency of **3** in solution

Solutions of **3** at 40 mg/ml in 50/50 ethanol/Tween with citric acid concentrations of 0, 1, 2 and 5% were prepared, and 0.5 ml aliquots transferred to vials and sealed. These were stored at 22.5°C and removed after 6 months. For analysis, 300 μl of each solution was diluted with 2.7 ml of 5% dextrose in water. Solutions were analyzed for content by HPLC (see method above) and the pH determined.

Stability of **3** in stock solution

A solution of **3** at 40 mg/ml in 50/50 ethanol/Tween with 2% citric acid was prepared, and 0.5 ml aliquots

transferred to vials and sealed. These were stored at −20 and 4°C, and vials removed periodically for analysis. The stability was assessed by HPLC (see method above) after removing 300 μl of each solution and diluting with 2.7 ml of 5% dextrose in water.

Rodent maximum tolerated dose (MTD) studies

A complete report of pharmacology/toxicology studies in mice and rats will be communicated elsewhere. Briefly, toxicity was assessed by determination of acute and chronic MTD, defined as the maximum cumulative dose compatible with unhampered survival. Test subjects were BALB/cAnNIHsd mice, 5–6 weeks old weighing 14–23 g and Sprague Dawley rats, 100–124 g upon arrival. Compound **3** was formulated at 9 mg/ml in a mixture of ethanol, DMSO, Tween 80 and D5W, and filtered through a sterile 0.2 μm filter. Test groups consisted of 10 mice (or rats), five male and five female. The mice or rats were administered either vehicle, as control, or **3** (by i.v. and/or i.p.) at approximately 0.15 ml/min. Mice were observed for signs of toxicity twice daily post injection.

Athymic mice bearing human tumors

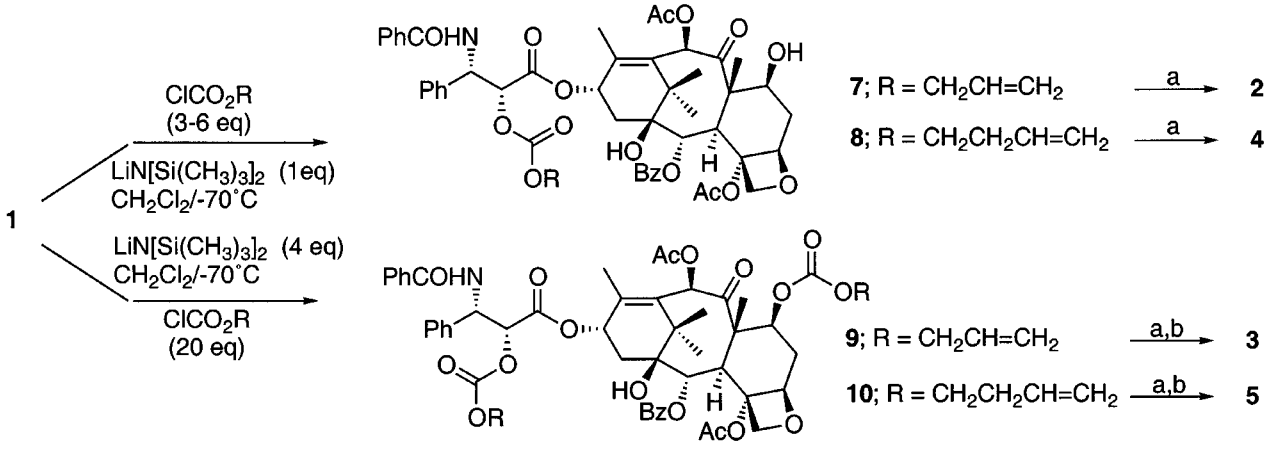
Test subjects were athymic mice, 7 weeks old weighing 25–30 g, injected on day 0 with human cell line xenografts. After measurable tumors were established, mice were treated i.p. with either **3** (formulated at 4 mg/ml in ethanol, Tween 80 and D5W) or paclitaxel (1.2 mg/ml in ethanol, Tween 80 and D5W), a comparator, 4 times at the MTD level every second day. Vehicle was used as a control and the tumor size was obtained every third day.

Results and discussion

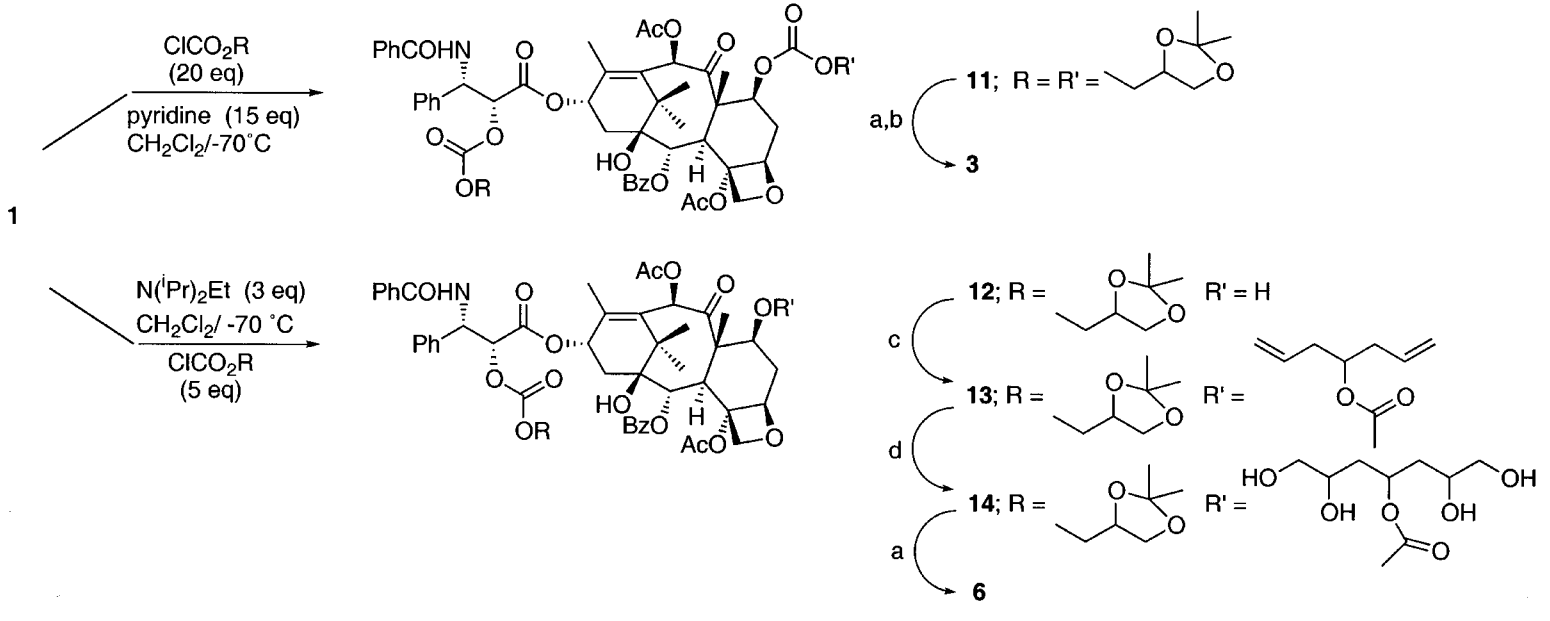
Synthesis of the 1,2-diol substituted 2′-carbonates **2–5** is outlined in Scheme 1. Selective carbonylation at the C-2′ hydroxyl afforded intermediates **7**[17] and **8**, when **1** was combined with the appropriate alkene chloroformate[19] and one equivalent of $LiN[Si(CH_3)_3]_2$ base in CH_2Cl_2 at −70°C. Catalytic oxidation of the intermediates with osmium tetroxide and *tert*-butyl hydroperoxide yielded **2** and **4** after isolation by preparative C-18 chromatography. The disubstituted intermediates **9** and **10** were synthesized under similar conditions. Increasing the equivalencies of chloroformate and base enabled the substitution at both the C-2′ and C-7 position. After oxidation as described above, selective *in situ* cleavage of the C-2′ carbonate by $NaHCO_3$ yielded the C-7 1,2-diol carbonates **3** and **5**.

To avoid the use of toxic osmium tetroxide, compound **3** was alternatively synthesized using the protected 1,2-diol, solketal chloroformate (Scheme 2). The combination of **1** with excess chloroformate and pyridine yielded the intermediate **11**, isolated in high yield by silica gel chromatography using a methylene chloride/acetone mobile phase. Compound **11** was readily converted to **3** by acidification, deprotecting the 1,2-diol functionalities, followed by treatment with dilute base, selectively hydrolyzing the 2′-carbonate. Purification by preparative HPLC gave **3**.

The 2′-carbonate derivative **12** was synthesized in a manner similar to **7** and **8** by limiting the quantities of solketal chloroformate and diisopropylethyl amine.



Scheme 1. Synthesis of paclitaxel derivatives **2–5**. Reagents and conditions: (a) 70% *tert*-butyl hydroperoxide in water (15–40 eq.), 0.16 M OsO_4 (0.2–4 eq.) in *tert*-butanol, 10% formic acid in water, THF, 35°C, 1.5 h. (b) 0.1 M $NaHCO_3$, THF, 0.5-5 h, RT-35°C.



Scheme 2. Synthesis of paclitaxel derivatives **3** and **6** using solketal chloroformate. Reagents and conditions: (a) 1N HCl, 35°C, THF, 3 h. (b) 0.1 M KH_2PO_4 (pH 6.5), THF, 35°C, 2 h. (c) 1-prop-2-enylbut-3-enyl chloroformate (12.5 eq.), $LiN[Si(CH_3)]_2$ (2.5 eq.), THF, −70°C, 2.5 h. (d), 70% *tert*-butyl hydroperoxide in water (2.5 eq.), 0.40 M OsO_4 in *tert*-butanol (4 eq.), water, THF, 35°C, 1 h.

Based on the selective cleavage of the 2′-carbonate in the synthesis of **3**, **12** was a suitable C-2′ protected derivative of **1**. The preparation of **6** (Scheme 2), exemplifies the utility of solketal carbonate as a C-2′ protecting group similar to TES,[20] Troc[21] and Alloc.[18]

All reactions were monitored by C-18 HPLC analysis. Yield, purity and characterization data are listed in Table 1. Substitutions at the 2′ or 7 position were evident by the downfield shift of the C(2′)-H or C(7)-H resonances in the ^{1}H-NMR (Table 1). Aqueous solubility and *in vitro* kinetics (half-life in human serum at 37°C) for reversion of **2–6** to **1** are listed in Table 2.

After preliminary evaluation of compounds **2–6**, taking into account that efficacy of a prodrug would not necessarily be reflected by *in vitro* cell assays, **3** (Protaxel) was selected as a development candidate. Protaxel exhibited the best combination of synthetic efficiency, ease of purification as well as good solubility and stability in delivery vehicles. Protaxel is stable at low pH, and under mild basic conditions (near physiological pH) Protaxel is converted to paclitaxel and the cyclic carbonate 4-(hydroxymethyl)-1,3-dioxolan-2-one (Figure 2). The structure of this intramolecular cyclization bi-product was confirmed by comparison to an authentic sample using gas chromatography. Thus, unlike in previously described carbonate derivatives,[10a] hydrolysis, directly producing glycerol and CO_2, is not the predominant pathway of release of **1** from Protaxel. The large rate difference for release of paclitaxel in human serum between the two C(7) substituted derivatives **3** and **5** (Table 2) also suggests that not simple hydrolysis, but a cyclization mechanism applies to Protaxel. These two compounds, differing by only one methylene group, are sterically similar in the C(7) carbonate region. A comparable rate of hydrolysis should be expected if a similar mechanism is operating. The enthalpic driving force of formation for a five- versus six-membered ring probably accounts for this large $t_{1/2}$ difference.[22]

Lowering the solution pH depresses the reversion rate of **3** to the parent drug **1** (Table 3). Stock solutions of Protaxel formulated at 40 mg/ml in 50/50 ethanol/Tween 80 with 2% citric acid were shown to be stable for at least 1 month at 4°C and for at least 12 months at −20°C. Water content ranging from zero to 1.4% did not affect the stability appreciably. By 10:1 dilution of the stock solution with 5% dextrose or saline containing citric acid as a preservative, the end use solution are obtained with a pH ranging from 4.0 to 4.3. Such solutions remain stable over 24 h at 23°C, can be stored for up to a week at 4°C in a refrigerator and are thus suitable for clinical use.

These studies demonstrate that *in vitro* release of **1** from the prodrug **3** is inversely pH dependent. However, it is probable that upon binding of **3** to bio-macromolecules and *in vivo* extra-vascular distribution a much different rate of release of **1** can be expected. One purpose of constructing a prodrug is

Table 2. Aqueous solubility and half-life (reversion to paclitaxel) in human serum at 37°C of paclitaxel and its derivatives

Paclitaxel and derivatives	Aqueous solubility (mg/ml)[a]	$t_{1/2}$ (min)[b]
1	<0.01	–
2	–	1.5
3	0.05	10
4	–	70
5	0.02	3600
6	–	150

[a]Solubility determined by HPLC.[8e]
[b]$t_{1/2}$ defined as the time when 50% **1** is released from the prodrug as determined by HPLC.

Table 1. Characterization of novel paclitaxel derivatives

Paclitaxel derivative	Yield (%)[a]	HPLC purity (%)[b]	^{1}H-NMR (p.p.m.)[c]			FAB-MS
			C(2′)-H	C(3′)-H	C(7)-H	
1			4.79	5.78	4.39	
2	89	>98	5.44	6.00	4.43	972 $(M+H)^+$
3	88 (83)	>99	4.80	5.80	5.42	1104 $(M+Cs)^+$
4	52	>99	5.44	5.99	4.45	1118 $(M+Cs)^+$
5	75	>95	4.80	5.79	5.44	
6	67	>97	4.79	5.80	5.45	

[a]Isolated yields.
[b]Purity assessed by C-18 chromatography in a 60/40 acetonitrile/5 mM KH_2PO_4 (pH 3.5) mobile phase.
[c]^{1}H-NMR run at 500 MHz ($CDCl_3$), only selected data is shown.

the potential for it to be delivered at a higher tolerated dose, which upon release makes the parent available in higher concentration to target locations. To assess the biological benefits of **3** versus **1**, several *in vivo* parameters were examined.

The MTD for **3** was first assessed by single injection in BALB/cAnNIHsd mice as 315 mg/kg by i.p. and 100–125 mg/kg by i.v. and 130 mg/kg by i.p. and 75–85 mg/kg by i.v., in Sprague Dawley rats. The chronic toxicity of Protaxel versus paclitaxel was shown by repeated i.p. injections in BALB/c mice to be 60–70 and 11–16 mg/kg, respectively. In athymic mice, the chronic MTD of paclitaxel was approximately 14–16 mg/kg/day, as compared to Protaxel at approximately 35–65 mg/kg/day. As a general guideline the prodrug Protaxel is 2.5–3 times better tolerated than

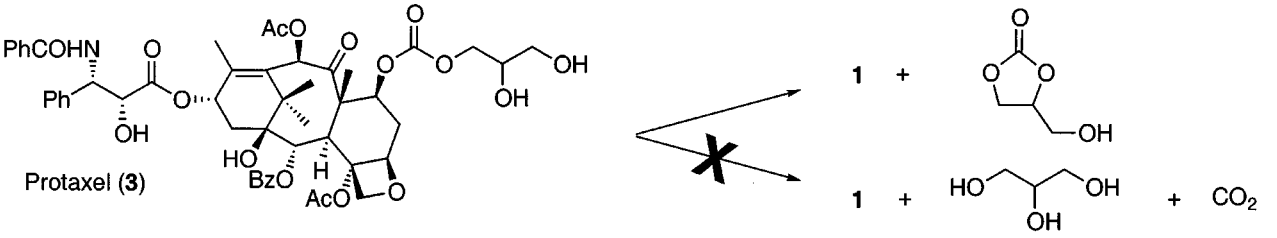


Figure 2. Intramolecular cyclization pathway of paclitaxel (**1**) release from Protaxel (**3**).

Table 3. Stability of Protaxel stock solutions at 22.5°C for 6 months

Citric acid in stock solution (%)	pH of end-use solution[a]	Protaxel (%)[b]	Paclitaxel (%)[b,c]
None	5.87	87.7	10.2
1	4.94	94.7	3.79
2	4.40	98.2	1.35
5	3.72	98.9	0.41

[a]5% Dextrose added.
[b]Purity determined by HPLC.
[c]Other minor impurities omitted.

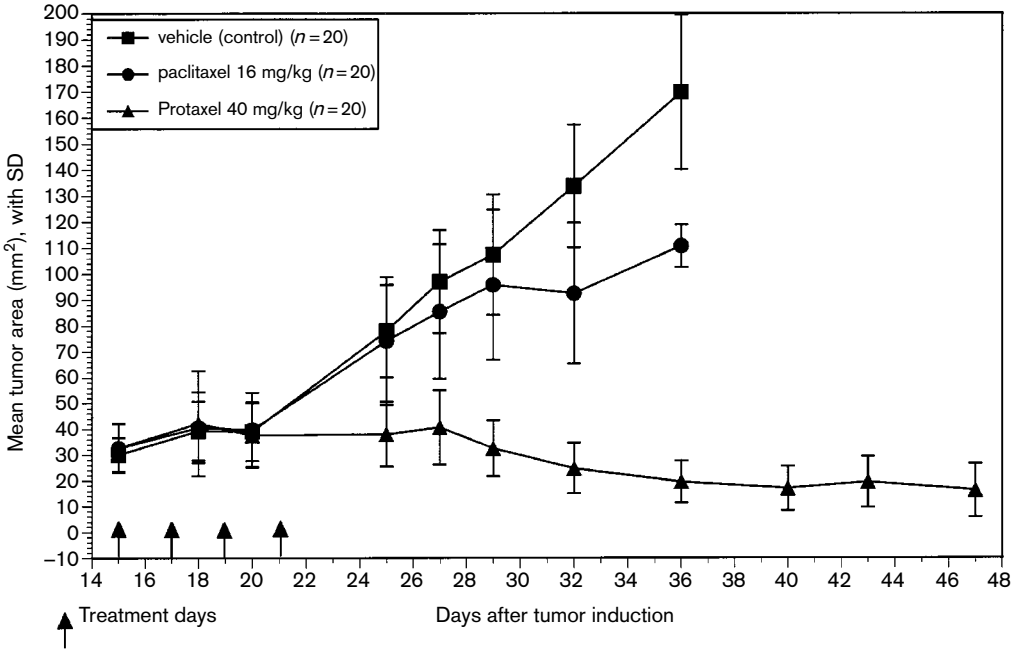


Figure 3. Effects of Protaxel and paclitaxel on the size of PC-3 xenograft in athymic mice.

its parent paclitaxel. From this MTD data, the doses for therapeutic experiments were derived.

Protaxel's therapeutic potential was orientationally assessed in athymic mice bearing human cell line xenografts of prostate (PC-3 and/or DU-145), ovary (OVCAR-3), breast (MDA-MB-468), colon (HT-29), kidney (CAKI-1) and lung (H-460) cancers. By day 36 post-induction, in athymic mice bearing PC-3 tumors, only one out of 10 mice treated with paclitaxel remained alive while 10 out of 10 treated with Protaxel had survived. By day 47 the tumors had regressed to half their original size, shown graphically in Figure 3. The data for all tumor lines surveyed, at 30 days after treatment, is summarized in Figure 4. This cumulative data establishes that the efficacy of Protaxel in several human cancer xenografts including drug-resistant ovarian, breast and prostate surpasses paclitaxel in reducing tumor size. These studies as well as plasma kinetics in rabbits and monkeys will be described in greater detail elsewhere.

Conclusion

A series of analogs of paclitaxel–polyol-carbonates was synthesized with the intention to produce a prodrug. Compound **3**, Protaxel, is stable under mild acidic conditions and gradually releases **1** at rates proportional to increasing pH, by an intramolecular cyclization pathway. Compared to paclitaxel, Protaxel can be formulated in stable clinically applicable solutions, has 2.5–3 times higher systemic tolerance and is more effective than paclitaxel in athymic murine models of certain human cancers.

Acknowledgments

The authors wish to thank Wolfgang Wrasidlo (Department of Pediatric Oncology, Humboldt University, Charité Hospital, Berlin) for his suggestions while consulting on this project with Biophysica.

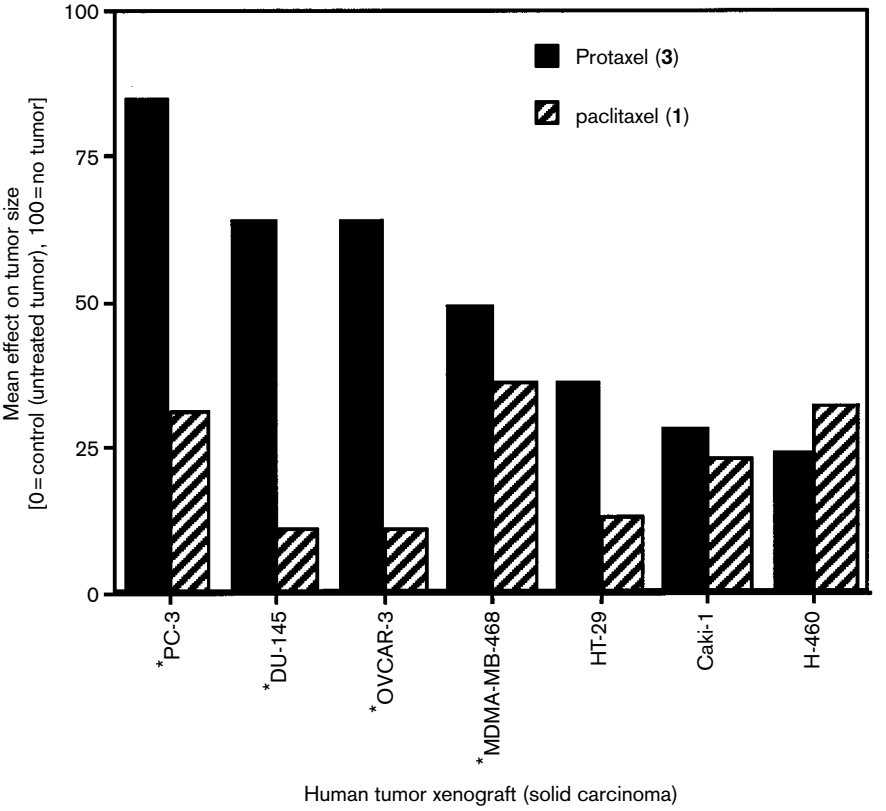


Figure 4. Comparison of Protaxel versus paclitaxel effect on growth of various human tumors in athymic mice. Percent growth inhibition related to control = $100 - [(T_a/T_b/C_a/C_b) \times 100]$; where T_a is the mean tumor area ($n \geqslant 10$) at 30 days following treatment with Protaxel or paclitaxel, T_b is the mean tumor area before treatment, C_a is the mean tumor area of control group and C_b is the mean tumor area of controls before treatment. *Denotes statistically significant difference between groups **3** and **1** ($p<0.05$).

References

1. Spencer CM, Faulds D. Paclitaxel: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer. *Drugs* 1994; **48**: 794–847.
2. Pouvelle B, Farley PJ, Long CA, Taraschi TF. Taxol arrests the development of blood stage *Plasmodium falciparum in vitro* and *Plasmodium chabaudi adami* in malaria infected mice. *J Clin Invest,* 1994; **94**: 413–7.
3. Longanecker DE. Angiotech Initiates First Safety Study for Paclitaxel in Treatment of Multiple Sclerosis. Angiotech Pharmaceuticals, Inc., Vancouver, BC, Canada. Press Release. 30 June 1998.
4. Mross K, Hollaender N, Hauns B, Schumacher M, Maier-Lenz H. The pharmacokinetics of a 1-h paclitaxel infusion. *Cancer Chemother Pharmacol* 2000; **45**: 463–70.
5. Rowinsky EK, Donehower RC. Paclitaxel (Taxol). *N Engl J Med* 1995; **332**: 1004–14.
6. Arbuck SG, Blaylock BA. Taxol: clinical results and current issues in development. In: Suffness M, ed. *Taxol: science and applications.* New York: CRC Press 1995: 379–415
7. (a) Deutsch HM, Glinski JA, Hernandez M, *et al.* Synthesis of congeners and prodrugs. 3. Water soluble prodrugs of Taxol with potent antitumor activity. *J Med Chem* 1989; **32**: 788–92. (b) Paradis R, Page M. New active paclitaxel amino acids derivatives with improved water solubility. *Anticancer Res* 1998; **18**: 2711–26.
8. (a) Zaho Z, Kingston DGI. Modified Taxols, 6. Preparation of water-soluble prodrugs of Taxol. *J Nat Prod* 1991; **54**: 1607–11. (b) Mathew AE, Mejillano MR, Nath JP, Himes RH, Stella VJ. Synthesis and evaluation of some water-soluble prodrugs and derivatives of Taxol with antitumor activity. *J Med Chem* 1992; **35**: 145–51. (c) Bhat L, Liu Y, Victory SF, Himes RH, Georg GI. Synthesis and evaluation of paclitaxel C7 derivatives: solution phase synthesis of combinatorial libraries. *Bioorg Med Chem Lett* 1998; **8**: 3181–6. (d) Yamaguchi T, Harada N, Ozaki K, *et al.* Synthesis of taxoids 5. Synthesis and evaluation of novel water-soluble prodrugs of a 3′-desphenyl-3′-cyclopropyl analogue of docetaxel. *Bioorg Med Chem Lett* 1999; **9**: 1639–44. (e) Damen EWP, Wiegerinck PHG, Braamer L, Sperling D, de Vos D, Scheern HW. Paclitaxel esters of malic acid as prodrugs with improved water solubility. *Bioorg Med Chem* 2000; **8**: 427–32.
9. Nicolaou KC, Riemer C, Kerr MA, Rideout D, Wrasidlo W. Design, synthesis and biological activity of protaxols. *Nature* 1993; **364**: 464–6. (b) Nicolaou KC, Guy RK, Pitsinos EN, Wrasidlo W. A water-soluble prodrug of Taxol with self-assembling properties. *Angew Chem Int Ed Engl* 1994; **33**: 1583–6.
10. (a) Ueda Y, Wong H, Matiskella JD, *et al.* Synthesis and antitumor evaluation of 2′-oxycarbonylpaclitaxels (Paclitaxel-2′-carbonates). *Bioorg Med Chem Lett* 1994;**4**: 1861–4. (b) Vyas DM, Ueda Y, Wong H, *et al.* Phosphatase-activated prodrugs of paclitaxel. In: Georg GI, Chen TT, Ojima, I, Vyas DM. eds. *Taxane anticancer agents: basic science and current status. ACS symp ser 583.* 1995: 124–137. (c) Ueda Y, Matiskella JD, Mikkilineni AB, *et al. Bioorg Med Chem Lett* 1995; **5**: 247–52. (d) Rose WC, Lee FYF, Golick J, Kadow J. Preclinical oral antitumor activity of BMS-185660, a paclitaxel derivative. *Cancer Chemother Pharmacol* 2000; **46**: 246–50
11. (a) Greenwald RB, Pendri A, Bolikal D, Gilbert CW. Highly water soluble taxol derivatives: 2′-polyethyleneglycol esters as potential prodrugs. *Bioorg Med Chem Lett* 1994; **4**: 2465–70. (b) Greenwald RB, Pendri A, Bolikal D. Highly water soluble taxol derivatives: 7-polyethylene glycol carbamates and carbonates. *J Org Chem* 1995; **60**: 331–6. (c) Greenwald RB, Gilber, CW, Pendri A, Conover CD, Xia J, Martinez A. Drug delivery systems: water soluble taxol 2′-poly(ethylene glycol) ester prodrugs—design and *in vivo* effectiveness. *J Med Chem* 1996; **39**: 424–31. (d) Pendri A, Conover CD, Greenwald RB. Antitumor activity of paclitaxel-2′-glycinate conjugated to poly(ethylene glycol): a water-soluble prodrug. *Anticancer Drug Des* 1998; **13**: 387–95.
12. (a) Rodrigues ML, Carter P, Wirth C, Mullins S, Lee A, Blackburn BK. Synthesis and β-lactamase-mediated activation of a cephalosporin–taxol prodrug. *Chem Biol* 1995; **2**: 223–7. (b) Takahashi T, Tsukamoto H, Yamada H. Design and synthesis of a water-soluble taxol analogue: taxol–sialyl conjugate. *Bioorg Med Chem Lett* 1998; **8**: 113–6.
13. Safavy A, Raisch KP, Khazaeli MB, Buchsbaum DJ, Bonner JA. Paclitaxel derivatives for targeted therapy of cancer: toward the development of smart taxanes. *J Med Chem* 1999; **42**: 4919–24.
14. Li C, Yu D-F, Newman RA, *et al*. Complete regression of well-established tumors using a novel water-soluble poly(L-glutamic acid)–paclitaxel conjugate. *Cancer Res* 1998; **58**: 2404–9.
15. (a) Sovak M. Introduction: state of the art and design principles of contrast media. In: Sovak M, ed. *Handbook of experimental pharmacology*. Berlin: Springer-Verlag 1984; **73**: 1–22. (b) Sovak M. Contrast media: a journey almost sentimental. *Invest Rad* 1994; **29**: S4–14.
16. (a) Tannock IF, Rotin D. Regulation of intracellular pH in Tumor cell lines: influence of microenvironmental conditions. *Cancer Res* 1992; **52**: 4441–7. (b) Boyer MJ, Tannock IF. Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res* 1989; **49**: 464–6.
17. (a) Sovak, M. The Harry W. Fischer Lecture Session. Contrast media: a meandering look. *Acad Radiol* 1996; **3**: S241–7. (b) Bressi JC, Douglass III JG, Seligson A, Sovak M. Taxoids. *US Patent 5,801,191,* 1 September, 1998.
18. Compound **7** was first reported by Carboni JM, Farina V, Rao S, Hauck SI, Horwitz SB, Ringel I. Synthesis of a photoaffinity analog of taxol as an approach to identify the taxol binding site on microtubules. *J Med Chem* 1993; **36**: 513–5.
19. (a) Chloroformates were either purchased from Aldrich or synthesized from the corresponding alcohol and triphosgene. (b) Maimaki K, Masunari M, Nakaminami G, Nakagawa M. Total syntheses of carbohydrates. III. DL-Glyceraldehyde and 2-deoxy-DL-*erythro*-pentose. *Bull Chem Soc Jpn* 1972; **45**: 2620–4.
20. Chaudhary AG, Rimoldi JM, Kingston DGI. Modified taxols. 10. Preparation of 7-deoxytaxol, a highly bioactive taxol Derivative, and Interconversion of taxol to 7-epi-Taxol. *J Org Chem* 1993, **58**: 3798–9.
21. Kingston DGI, Samaranayake G, Ivey CA. The chemistry of taxol, a clinically useful anticancer agent. *J Nat Prod* 1990; **53**: 1–12.
22. March J. *Advanced organic chemistry: reactions, mechanisms and structure*. New York: John Wiley 1992: 211–4.

(Received 23 January 2001; accepted 1 February 2001)