Highly Water Soluble Taxol Derivatives: 7-Polyethylene Glycol Carbamates and Carbonates

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The first examples of highly water soluble taxol derivatives (0.1 mmol/mL) were prepared by the attachment of polyethylene glycol (molecular weight 2–5 kD) at the 7-position of taxol via a urethane or carbonate linkage. When lower molecular weight polyethylene glycols (350 and 750) were used, the solubilities were considerably lower (1.87×10^{-3} mmol/mL) but still substantially greater than taxol itself. Additional 7-substituted taxol derivatives were also prepared by utilizing small molecules consisting of sugars and ionic and multifunctional compounds. However, most of these derivatives had solubilities, calculated from HPLC retention times, that did not differ significantly from taxol itself. The use of methoxyacetate as a protecting group during these syntheses is discussed.

Early difficulties in obtaining sufficient quantities of taxol for clinical studies to assess antitumor activity in ovarian, breast, and lung cancer have been overcome, but the associated problem of poor aqueous solubility still remains. Modification of both the 3′ and 10 position has been achieved by French researchers[1] to yield a 10-deacetyl *tert*-butyl carbamate derivative (Taxotere) which produced a slightly more water soluble taxol analogue. Taxotere has also been reported to possess antitumor activity comparable to or greater than that of taxol itself.

Considering the scope of interest manifested in taxol, few modifications of the 7 position and subsequent oncolytic activities have been reported. Most accounts to date have been concerned with esterifications of the hindered secondary alcohol moiety at the 7 position which could improve solubility while maintaining cytotoxic activity.[2-4,8-10]

Polyethyleneglycol (PEG) is known to enhance water solubility[5a] and reduce immunogenicity of high molecular weight protein adducts[5b-d] and therefore seemed like an excellent candidate for conjugation to taxol. In this study we report the synthesis of PEG[6] conjugated taxol linked via carbamate and carbonate functionalities and the effect on solubility of the molecule by varying the molecular weight of the attached polymer. The chemistry developed for this purpose was also used to obtain nonpolymeric taxoids,[7] and an HPLC methodology was employed which was useful in predicting water solubilities of these derivatives.

Results

The 7-OH functionality of taxol can be modified by several different routes to provide the first examples of 7-carbamate derivatives of taxol. Further, the intermediates in this scheme can also be utilized to obtain carbonates not easily accessible by other means. The most direct route to accomplish this conversion employs condensation of substituted isocyanates with 2′-*O*-acetyltaxol (**2a**) (Figure 1). The use of dibutyltin dilaurate in stoichiometric amounts instead of catalytic amounts[11] was necessary in order to obtain reasonable yields of product. No epimerization or rearrangement of the taxane structure was detected, although the use of other electrophilic reagents has been implicated in these types of undesired conversions.[12,13]

Limitations of this procedure due to a lack of commercial sources of key isocyanates and occasional unexplained variation in yield prompted us to explore routes of greater utility, and which would not threaten the integrity of the taxane ring system. The first of these was conversion of 2′-*O*-acetyltaxol to the 7-chloroformate derivative **6a** using the phosgene equivalent bis(trichloromethyl) carbonate (triphosgene) and either *N*,*N*-diisopropylethylamine (DIEA) or pyridine (Figure 2). In practice it was found that approximately 6 equiv of triphosgene and 9 equiv of base were required to give yields of only 60–70% of **6a**.[14] Chloroformates are generally water-reactive: therefore, no attempts were

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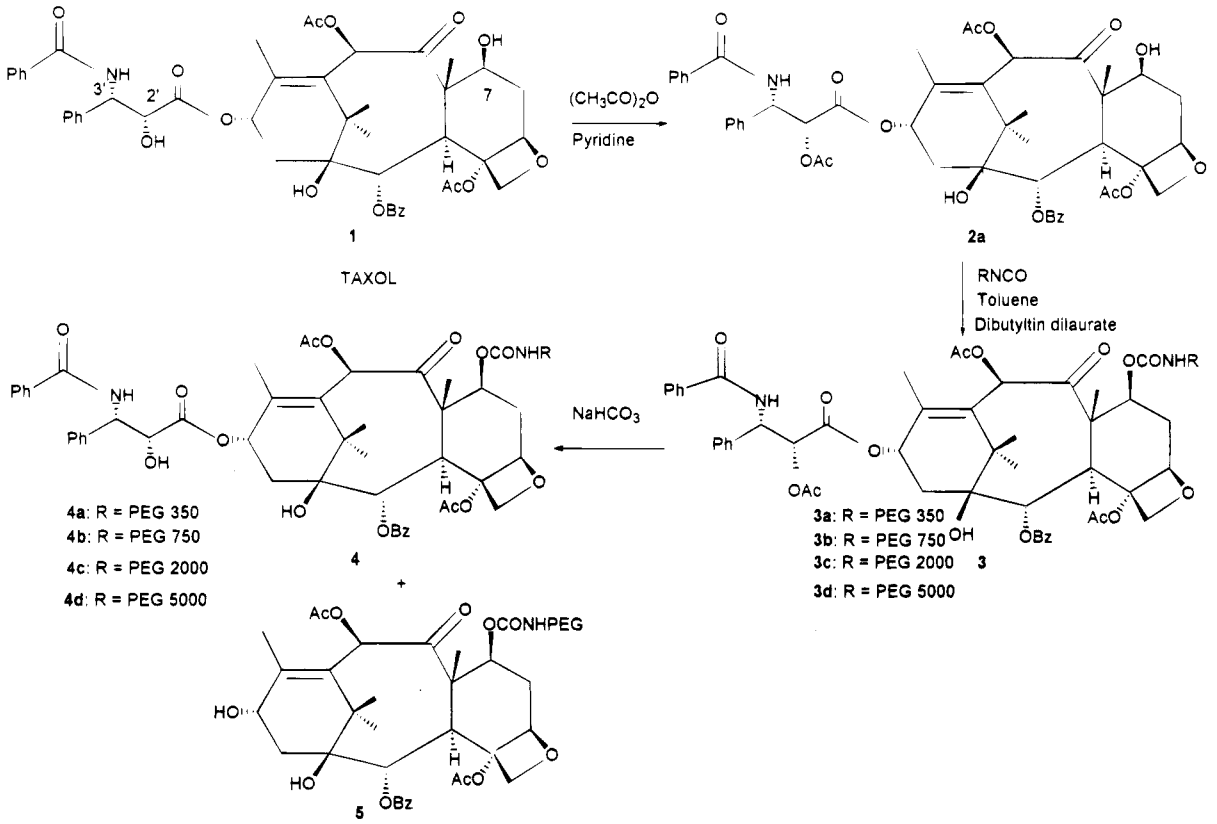

Figure 1.

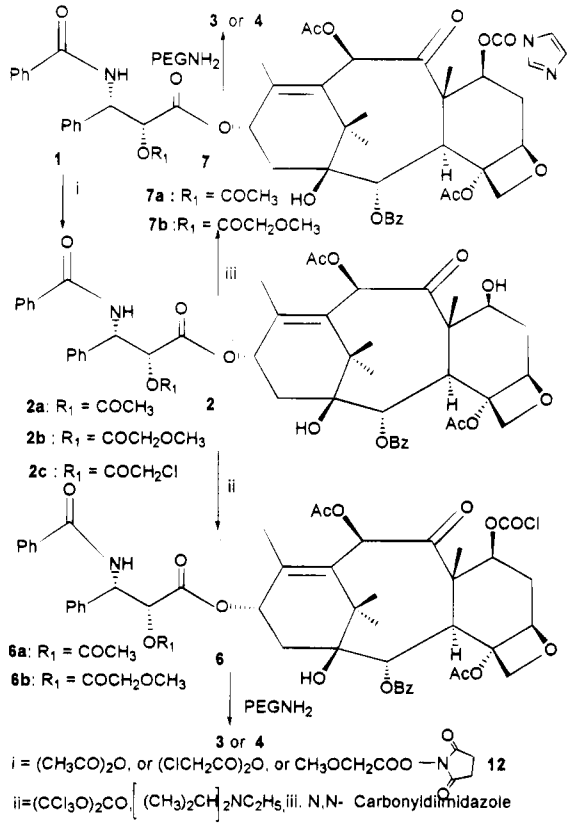

Figure 2.

made to isolate **6a**, which was reacted directly with excess amine to afford the 7-carbamate derivative.

As an alternative to employing triphosgene, it was found that **2a** reacted smoothly with an excess of *N,N*-carbonyldiimidazole to give high yields of the easily isolated and relatively stable carbonylimidazole derivative **7a**. Compound **7a** did not react readily with amines in chloroform solution, but in 2-propanol the 2′-*O*-acetyl carbamates were produced in high yield. Hydrolysis of the 2′-*O*-acetate group with $NaHCO_3$ was slow and was accompanied by partial hydrolysis of the C-13 side chain ester as reported[15] (Figure 1). Employing a nucleophilic amine in methanol–water was found to give some aminolysis of the 2′-*O*-acetyl group, but yields were unacceptably low. To circumvent these difficulties, a more labile protecting group was employed at the 2′ position. Thus 2′-*O*-(chloroacetyl)taxol (**2c**) was prepared according to Samaranayake,[16] but was found to be unstable toward water, hydrolyzing rapidly back to taxol. Methoxyacetate esters are reported to hydrolyze 20 times as fast as acetates.[17] A comparison of pK_a values[18] for substituted carboxylic acids shows methoxyacetic acid with a value of 3.48, approximately midway between chloracetic (2.81) and acetic (4.76) acid. Therefore methoxyacetate (MAc) esters should exhibit suitable stability in polar solvents while reacting rapidly with nucleophiles. This was indeed found to be the case, and 2′-*O*-(methoxyacetyl)taxol (2′-MAc-taxol; **2b**) was substantially more stable in aqueous alcohol solutions. Deprotection of **2b** was performed with a variety of amines, used in excess, to produce taxol without rupture of the C-13 side chain. Functionalization of 2′MAc-taxol with *N,N*-carbonyldi-

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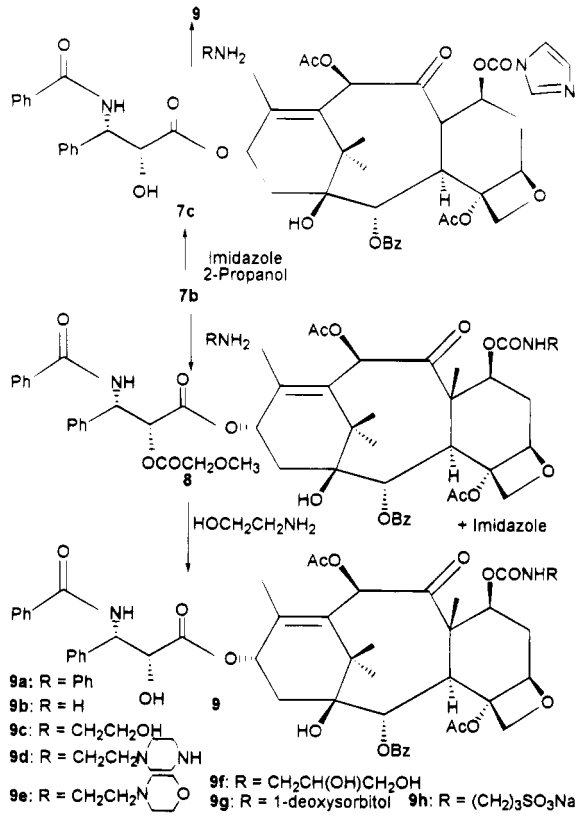


Figure 3.

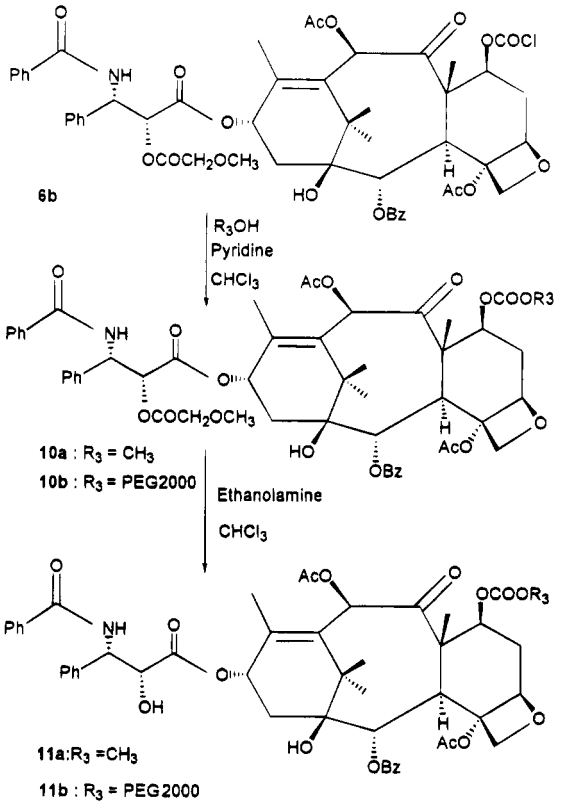


Figure 4.

Table 1. NMR Chemical Shifts for Taxol Derivatives[a] (ppm)

compound	2′-H	3′-H	7-H	other peaks[b]
1	4.79	5.78	4.42	
2b	5.60	6.05	4.45	4.25 (d, 2H), 3.55 (s, 3H)
7b	5.63	6.04	5.69	4.25 (d, 2H), 3.55 (s, 3H)
7c	4.81	5.82	5.65	
11a	4.80	5.78	5.45	3.8 (s, 3H)
9a[c]	4.80	5.68	5.50	7–7.7 (m, 5H)
9b	4.80	5.78	5.42	3.5 (br s, 2H)
9c	4.80	5.78	5.42	3.5 (m, 2H), 3.0 (br m, 2H)
9d	4.79	5.81	5.45	3.28 (m, 2H), 2.87 (m, 4H), 2.46 (m, 6H)
9e	4.79	5.80	5.44	3.70 (br m, 4H), 3.29 (br m, 6H), 2.47 (br m, 2H)
9f[b]	4.73	5.60	5.30	3.85–3.95 (m), 3.63 (br s), 3.43 (m)
9g	*d*	*d*	5.35	
9h[e]	4.76	5.62	5.47	3.8 (m, 2H), 2.97 (m, 2H), 3.08 (m, 2H)

[a] The OH and NH peaks are not listed. [b] Only significant peaks are provided. [c] **9a** was prepared as depicted in Figure 1. [d] The ^{1}H NMR spectrum of **9g** in $CDCl_3$ was very poorly resolved. [e] CD_3OD was used as solvent.

imidazole gave **7b** (Figure 2) which was then utilized in carbamate synthesis (Figure 3, R groups listed in Table 3).

It appears that substitution of the imidazole group of **7b** by amine occurs first, producing the 2′-MAc-7-carbamate derivative (**8**), which in turn can react with either the free imidazole produced or the excess of amine present to give the final product. In fact, the reaction of **7b** with 1 equiv of imidazole cleaved the MAc protecting group and provided an example of compound **7c** which was isolated and characterized by NMR (Table 1).

2-Propanol was generally the most satisfactory solvent for carrying out these reactions with no competing solvolysis occurring. In those cases where reactants were insoluble in 2-propanol, DMSO could be substituted. Utilization of the 2′-MAc group was found to be advantageous for most syntheses requiring the 2′ position to be protected. Not only was MAc easily removed by the addition of excess primary amine (generally ethanolamine was used) without affecting other esters on the taxol circumference, but by employing *N*-hydroxysuccinimidyl methoxyacetate, MAc-NHS (**12**), in the reaction with taxol only the 2′-ester was observed to form. On the other hand, less discriminating acylating agents such as methoxyacetyl chloride reacted with taxol to give 10–20% of 2′,7-diMAc-taxol and consequently lower yields of the desired product.

For the preparation of 7-carbonate derivatives of taxol, it was necessary to employ the more reactive 2′-MAc-7-chloroformate intermediate **6b** with the appropriate alcohol in dry chloroform in the presence of pyridine (Figure 4). After the formation of carbonate was complete as confirmed by HPLC, addition of an excess of ethanolamine cleaved the MAc group to yield the desired 2′-OH group. Anomalously, methanol exhibited enhanced nucleophilic character and simply dissolution of **7b** for 18 h in methanol with 2 equivalents of ethanolamine led to the deblocked methyl carbonate derivative **11a**.

^{1}H NMR spectra were routinely used to follow reactions and also for characterization of the various taxol derivatives. ^{1}H NMR spectral assignments for the taxol derivatives were made by comparison of their spectral characteristics with those of taxol and other taxol analogues reported in the literature.[2,24] For example, on acetylation of the 2′-OH the chemical shift of the 2′-H changes from 4.71 to 5.51 ppm and that of 3′-H from 5.72 to 5.95 ppm.[2] Similar changes were observed on acetylation of the 2′-OH in our studies. Methoxyacetylation of the 2′-OH resulted in a chemical shift of 5.60 ppm for 2′-H and 6.05 ppm for 3′-H. Similarly, in 7-*O*-acetyltaxol

Table 2. Estimated Aqueous Solubility of PEG-Taxol Derivatives[a]

entry	taxol-7-O-CO-X	X	estimated aqueous solubility mg/mL	estimated aqueous solubility nM	HPLC t_R (min)
1	**4a**	NH PEG 350	≤1[b]	≤8.1×10^5	15.1
2	**4b**	NH PEG 750	30–60 (est)[b]	$1.9–3.8 \times 10^7$ (est)	15.2
3	**4c**	NH PEG 2000	≥666	≥2.2×10^8	15.3
4	**4d**	NH PEG 5000	≥666	≥1×10^8	15.2
5	**11b**	O PEG 2000	≥666	≥2.2×10^8	16.1

[a] Measured at ambient temperature (27 °C). [b] Slightly turbid solution obtained; no sedimentation occurred on centrifugation.

a chemical shift of 5.54 ppm (d of d) is reported for the 7-H.[2] We observed a chemical shift of 5.45 ppm in taxol 7-carbonates and 5.30 to 5.50 in taxol 7-carbamates for the 7-H. The changes in the spectral characteristics of other protons in the molecule were considerably smaller and similar to those reported for 7-*O*-acetyltaxol. The multiplicities and coupling constants of the peaks did not change as a result of the above mentioned substitution. In some cases the NMR spectra in $CDCl_3$ solvent were very poorly resolved. In those cases better resolved spectra could be obtained in deuterated methanol. Table 1 lists important spectral characteristics and also any additional peaks that appear due to the protons of the substituent of these derivatives that are significantly different from those of taxol.

Discussion

A series of PEG substituted taxols (Table 2) were prepared by the methods described. The solubilities of the 350 and 750 molecular weight derivatives were obtained by sonicating 100 mg of the compound in 1 mL of water, centrifuging, lyophilizing the supernatant, and weighing the residue. The solubilities of the 2 and 5 kD PEG derivatives were estimated by adding water in small portions to 100 mg of substance, until dissolution occurred. About 100 μL of water gave a clear but extremely viscous solution. An additional 50 μL of water gave a flowable liquid and this total volume was used for calculations. On the basis of the value of 150 μL, the solubility of the PEG compounds were calculated to be minimally 666 mg/mL (1.2×10^8 nM). Taxol solubility has been reported as 3×10^2 nM,[9,19] so on a molar basis it can be seen that entries 3–5 in Table 2 are approximately 30000 times more soluble than taxol.

A series of nonpolymeric 7-carbamate and carbonate derivatives, some containing hydrophilic groups, was also prepared in the hope that greater water solubility would be achieved. None of these derivatives appeared to possess solubility characteristics significantly different from the parent with the exception of **9h** (Table 3). Substitution of 2′- and/or 7-hydroxy groups systematically affected the retention times on reverse phase HPLC analysis. As would be expected, substitution by nonpolar groups increased the retention times. Since 7-OH is in the hydrophilic region of the molecule,[20] modification of 7-OH made the molecule more nonpolar compared to modification of the 2′-OH.[2] Interestingly, when the 7-OH was functionalized with ethanolamine (entry 7), leaving a single hydroxyl group, the retention time was almost identical to that of taxol. When the substituents were more polar than a single hydroxy group, the retention times were decreased (e.g. 1-amino-1-deoxysorbitol or 3-aminopropanesulfonic acid, sodium salt). The retention times of the taxol derivatives were used in estimating water solubilities since correlations between retention time (t_R) and reverse phase-high pressure liquid chromatography have been developed to estimate water solubilities of highly insoluble organic compounds.[21] A modification of the equation developed by Swann and coworkers[22] (eq 1) was employed for the taxol series presented in Table 3,

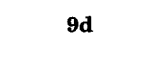
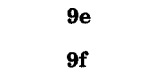
$$\ln \text{WS (mg/mL)} = -K \ln t_R - 0.01 (\text{mp} - 25\ ^\circ\text{C}) + 18.328 \quad \text{(eq 1)}$$

where WS = water solubility, t_R = retention time (min), mp = melting point, K = a constant whose value was determined to be 8.55 for current HPLC conditions,[23] and taxol solubility taken as 0.01 mg/mL.

The actual solubility of a pure sample of **9h** was determined in order to verify the predictive value of the equation and was done as follows. To 2 mg of **9h** in a sample tube was added 300 μL of deionized water and the tube was sonicated for 15 min. The resulting suspension was filtered using a 0.45 μm syringe filter. A known weight of the filtrate was lyophilized and the weight of residue was measured. This gave a solubility of 7.24 mg/mL. The melting point of **9h** was found to be 195 °C dec, and using this value in equation 1 a solubility of 7.43 mg/mL was calculated for **9h** which is in very close agreement to the actual solubility observed.

However, for most compounds the melting point factor did not change the result to any significant degree and eq 1 was simplified to eq 2 where K was determined to be 9.29.

Table 3. Calculated Aqueous Solubilities, Yields, and FAB-MS Data for Various Taxol Derivatives

entry	taxol-7-OC(O)-X	X	WS (mg/mL)[a]	yield (%)[b]	$(M + H)^+$	$(M + Na)^+$
1	**9a**	NHPh	0.000025	60	973.6	
2	**7b**	(2′-MAc) imidazolyl	0.000059	90	1020.3	
3	**7c**	imidazolyl	0.000179	35	948.2	
4	**11a**	OCH_3	0.00042	50		934.3
5	**2b**	(2′-MAc) no 7-*O*-acyl group	0.0048	88–95	926.3	
6	**9b**	NH_2	0.0093	60–70		919.4
7	**9c**	$NHCH_2CH_2OH$	0.0099	55–60		963.3
8	**9d**		0.010	50–55	1010.5	
9	**9e**		0.0105	60–70	1010.3	1032.5
10	**9f**	$NHCH_2CH(OH)CH_2OH$	0.0195	40–50	971.4	993.412
11	**9g**	NH-1-deoxysorbitol	0.054	55	1062.0	1084.3
12	**9h**	$NH(CH_2)_3SO_3Na$	11.43	50	1041.4	1063.3

[a] Taxol solubility was taken as 0.01 mg/mL. [b] No attempts were made to optimize yields.

$$\ln \text{WS (mg/mL)} = -K \ln t_R + 18.328 \quad \text{(eq 2)}$$

Note that in the case of **9h**, eq 2 gives a solubility of 11.43 mg/mL which is a 37% difference from the observed value. An advantage of this procedure is that only microgram quantities of compound need be employed to provide a reasonable and rapid estimation of taxoid solubility. Surprisingly, using 0.1 N HCl with piperazine derivative **9d** did not dissolve as much as 1 mg/mL of material. A summary of this solubility data is presented in Table 3. Interestingly, an amphiphilic PEG substituent in the 7-position of taxol produced a shift to longer retention times *regardless of the molecular weight of the PEG chain*. The t_Rs were also clustered in a narrow range and are listed in Table 2.

Summary

The use of PEG derivatives of MW ≥ 2000 to modify the 7-position of taxol has been shown to produce highly water soluble derivatives that still maintain a cytotoxic profile.[7] In vitro testing of these compounds employing a P388 cell line gave IC_{50} values which were in the low micromolar range as compared to taxol itself which is active at nanomolar quantites. Activation of the 7-hydroxy moiety of taxol with reactive acylating agents was accomplished with such reagents as triphosgene and *N,N*-carbonyldiimidazole provided that the 2′-hydroxy functionality was protected. The balance between facile removal of the 2′-protecting group and stability during various reactions was met by employing a methoxyacetate ester.

Experimental Section

General Methods. Unless stated otherwise, all reagents and solvents were used without further purification. Analytical HPLC's were performed using a C_8-reverse phase column (Beckman-ultrasphere) under isocratic conditions with a 75:25 mixture (v/v) of methanol–water as the mobile phase. For preparative HPLC, a 47 mm × 300 mm C_8 cartridge column (Waters PrepPack) under a radial pressure of 600 psi was employed. In both cases the peak elutions were monitored at 227 nm. NMR spectra were obtained using a 270 MHz spectrometer. Deuterated chloroform was used as the solvent unless otherwise specified. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and FAB-MS analyses were done at the Mass Spectrometry Facility of Yale Medical School, New Haven, CT. 2′-*O*-Acetyltaxol was prepared by the reaction of taxol with acetic anhydride/pyridine using known procedures.[2] Better yields were obtained when 1.5 equiv of acetic anhydride was used (instead of 5 equiv). PEG 2000 and 5000 were obtained from NOF America (New York, NY), and PEG 350 and 750 from Aldrich Chemical Co. (Milwaukee, WI). All PEG compounds were dried under vacuum or by azeotropic distillation from toluene prior to use.

***N*-Hydroxysuccinimidyl Methoxyacetate (MAc-NHS, 12).** To a suspension of 2.53 g (0.022 mmol) of *N*-hydroxysuccinimide and 2.17 g (0.02 mmol) methoxyacetyl chloride in 10 mL of methylene chloride was added 2.84 g (0.022 mmol) of diisopropylethylamine in 15 mL of methylene chloride over a period of 30 min. After stirring at room temperature for 4 h the reaction mixture was washed with water, dried over sodium sulfate, and evaporated to dryness. The crude product was recrystallized from 1:1 ethyl acetate–hexane to give colorless needles (yield 2.4 g, 64%): mp 59–60 °C; IR 1837, 1784, 1735 cm^{-1}; FAB-MS $(M + H)^+$ 188.055.

2′-*O*-(Methoxyacetyl) taxol (2b). A solution of 100 mg (0.12 mmol) of taxol, 60 mg (0.32 mmol) of MAc-NHS, and 38 mg (0.30 mmol) of diisopropylethylamine in 3 mL of dry dichloromethane was refluxed for 3 h under nitrogen, followed by stirring at room temperature for 18 h. The reaction was quenched by adding 8 μL (0.2 mmol) of methanol. After stirring for 15 min, the reaction mixture was washed with 2 × 3 mL portions of 0.1 N HCl. The dichloromethane layer was separated and dried over magnesium sulfate. The solvent was then removed by distillation *in vacuo* at room temperature, to yield 90 mg (82% yield) of product. Purity, determined by HPLC analysis, was greater than 95%. The product was characterized by NMR and FAB-MS $(M + H)^+$ 926.3. The ^1H NMR spectrum of the product was similar to that of taxol except for the following major differences: δ 5.60 (d, 1H), 6.04 (d, 1H).

2′-*O*-(Methoxyacetyl)-7-*O*-(imidazolylcarbonyl)taxol (7b). In a 25 mL round bottomed flask were placed **2b** (102 mg, 0.11 mmol), *N,N*-carbonyldiimidazole (53 mg, 0.33 mmol), and methylene chloride (5 mL). The resulting clear solution was stirred at room temperature under a nitrogen atmosphere for 5 h. The reaction mixture was diluted with 5 mL of methylene chloride, washed with 2 × 5 mL of water, dried over anhyd magnesium sulfate, and evaporated to dryness. The crude product thus obtained (105 mg, 94%) was used without further purification for the preparation of 7-substituted taxol derivatives. The FAB-MS exhibited an $(M + H)^+$ peak at 1020.3. The ^1H NMR spectrum was similar to that of **2b** except for the following major changes: δ 5.69 (d of d, 1H), 5.02 (d, 1H).

7-O-(Imidazolylcarbonyl)taxol (7c). A solution of 20 mg (0.02 mmol) of **7b** and 2.67 mg (0.039 mmol) of imidazole in 1 mL of anhydrous 2-propanol was stirred at reflux temperature for 48 h. The solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane (2 mL) and washed with 2 × 3 mL of 0.1 N hydrochloric acid, followed by 1 × 2 mL of water. The dichloromethane was separated and dried over anhydrous magnesium sulfate. The solvent was removed by distillation *in vacuo* at room temperature to yield a solid residue (15 mg) which was purified by prep HPLC to give 10 mg (54%) of the product. FAB-MS $(M + H)^+$ 948.3.

Taxol 7-Carbamates. Method 1 (employing isocyanates). PEG-NH_2. The preparation of this derivative was accomplished by the two step procedure of Buckmann.[25]

(A) PEG-Cl. Methoxypolyethylene glycol (PEG-OH) of mw 5000 (50 g, 10 mmol) was placed in a 1 L round-bottomed flask and warmed to 80 °C under vacuum (0.1 Torr) for 6 h to remove traces of water. To the dried PEG-OH was added 9.5 g (80 mmol) of thionyl chloride and the reaction mixture was refluxed for 16 h. The unreacted thionyl chloride was removed by distillation and the product was purified by recrystallization from 2-propanol to yield 48 g (97.6%) of colorless crystalline PEG-Cl.

(B) PEG-NH_2. PEG-Cl, mol wt 5000, (40 g, 8.0 mmol) was dissolved in 40 mL of deionized water and to this resulting solution was added 80 mL of ammonia solution (28–30%). The resulting clear solution was heated undisturbed at 60 °C for 4 days in a pressure vessel. Sodium carbonate (25 g) was added to the reaction mixture which was then extracted with methylene chloride (3 × 200 mL). The methylene chloride solution was dried over magnesium sulfate and then evaporated to dryness. Recrystallization from 2-propanol gave 42 g (84%) of the PEG-NH_2. Similar procedures were employed

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to obtain PEG-NH_2 of mw 2000 (yield 85%), mw 750 (yield 65%) and mw 350 (yield 60%).

(C) 2′-*O*-Acetyltaxol 7-PEG Carbamates (3c and 3d). PEG-NH_2, mol wt 5000 (7.5 g, 1.5 mmol), was dried under vacuum (25 °C, 0.1 Torr, 3 h) and then dissolved in 20 mL of chloroform that had been distilled from P_2O_5. To this solution was added, with stirring, 164 mg (0.55 mmol) of triphosgene followed by 400 mg (4.0 mmol) of triethylamine. The reaction mixture was refluxed for 2 h at which time a strong band at 2262 cm^{-1} was observed indicating the presence of PEG-NCO. To the reaction mixture were then added 0.44 g (0.5 mmol) of 2′-*O*-acetyltaxol (**2a**) and 0.55 g (0.87 mmol) of dibutyltin dilaurate and the reaction was heated at 50 °C for 48 h. After evaporation of the reaction mixture to dryness, the product was recrystallized from 2-propanol and further purified by preparative HPLC to give 1.91 g of **3d** (65%). The product was characterized by ^1H and ^{13}C NMR.

Using similar procedures the corresponding PEG-2000 derivative **3c** was also obtained.

(D) Taxol 7-Phenylcarbamate (9a). In a 10 mL round-bottomed flask were placed 17.5 mg (0.019 mmol) of **2b**, 4.5 mg (0.038 mmol) of phenyl isocyanate, 12 mg (0.019 mmol) of dibutyltin dilaurate, and 2 mL of dry methylene chloride. The reaction mixture was refluxed for 18 h. To the cooled reaction mixture was added 5 mL of hexane and the precipitated white solid was isolated by centrifugation. The ^1H NMR spectrum of the product showed the expected multiplet for the 7-H at 5.56 ppm. To the crude product was added 1 mL of methanol and 10 μL of ethanolamine, and the solution was stirred at room temperature for 15 min. The reaction mixture was diluted with 5 mL of methylene chloride, washed with water, and after the usual workup was purified by preparative HPLC to give 9.8 mg. of **9a** (53%).

Method 2. The following general procedure was used for the preparation of taxol 7-carbamates from **7b**. 2-Propanol was used as the solvent and in those cases where the amine was insoluble, DMSO was employed. Hydrolysis of **7b** to taxol was usually insignificant in most cases. The product was purified by silica gel chromatography or by preparative HPLC.

Taxol 7-(2-Hydroxyethyl)carbamate (9c). In a 5 mL sample tube were placed 26 mg (0.025 mmol) of **7b**, 1 mL of anhydrous 2-propanol, and 7.3 mg (0.12 mmol) of ethanolamine. The reaction mixture was stirred at 40 °C for 12 h, diluted with 5 mL of methylene chloride, extracted with 2 mL of 0.1 M HCl, and washed twice with 2 mL of water. The organic layer was dried over magnesium sulfate, evaporated to dryness, and dried under high vacuum to obtain 15 mg (58%) of (9c). The ^1H NMR, δ 5.40 (m, 1H), 5.04 (m, 2H), 4.80 (d, 1H), 3.75 (m, 2H), 3.5 (m, 2H) (other spectral characteristics were similar to those of 7-*O*-acetyltaxol[2]) and FAB-MS $(M + Na)^+$ 963.3 were in agreement with the structure. The following compounds were also prepared using this procedure: **9b**, **9d**–**9f**, **9g** (DMSO), **9h** (DMSO).

Taxol 7-PEG_{2000} Carbamate 4c. A solution of 7b (14.4 mg, 0.015 mmol) and PEG_{2000}-NH_2 (29 mg, 0.015 mmol) in anhydrous 2-propanol was refluxed for 21 h. Ethanolamine (0.44 μL, 0.007 mmol) was added to the reaction mixture and reflux was continued for another 21 h. The reaction mixture was cooled to room temperature and the separated solid was filtered and washed with ether to give 30 mg of crude product. This was purified by preparative HPLC to give 20 mg (50%) of pure 4c. The ^1H NMR showed the expected shift of the 2′-H doublet from 5.60 to 4.80 ppm.

Method 3. 2′-*O*-Acetyltaxol 7-PEG_{5000} Carbamate 3d. In a 25 mL three-necked round bottomed flask equipped with a reflux condenser, a magnetic stirrer, and a guard tube containing NaOH pellets were placed 25 mg (0.028 mmol) of 2-*O*-acetyltaxol (**2a**) and 5 mL of anhydrous methylene chloride. To this solution were added triphosgene (17 mg, 0.057 mmol) and pyridine (20 mg, 0.22 mmol), and stirring was continued for 30 min. Dry nitrogen was bubbled through the reaction mixture until dryness. To this reaction mixture was added PEG-NH_2 (mw 5000, 280 mg, 0.056 mmol) in 5 mL of methylene chloride and the solution stirred for 15 min. After the usual workup the product was purified by preparative HPLC using a reverse phase column (C_8) to give 65 mg (31%) of 3d. The ^1H NMR, ^{13}C NMR, and HPLC of the pure product were found to be identical to those of 3d obtained by other methods.

Taxol 7-PEG_{2000} Carbonate 11b. 2′-MAc-taxol (**2b**, 25 mg, 0.027 mmol) was converted to the corresponding chloroformate (**6b**) using procedures similar to the one described in the synthesis of **3d**. To the chloroformate thus obtained was added 162 mg (0.081 mmol) of PEG-OH (mol wt 2000) and 7 mg (0.09 mmol) of pyridine in 5 mL of methylene chloride. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with 5 mL of methylene chloride and washed with three 5 mL portions of water. The organic layer was dried over $MgSO_4$ and concentrated to 2 mL. To this solution was added 8.3 mg (0.14 mmol) of ethanolamine and the mixture was stirred at room temperature for 5 h. After removal of solvent by evaporation under reduced pressure the crude **11b** was recrystallized from 10 mL of 2-propanol. The product was additionally purified by preparative HPLC to yield 59 mg (37%). The ^1H NMR showed the following peaks: δ 5.54 (m,1H), 4.81 (d, 1H), in addition to the characteristic resonances of PEG and taxol.

Preparation of Taxol 7-(Methyl carbonate) 11a. To 25 mg (0.025 mmol) of **7b** in 1 mL of anhydrous methanol was added 3.4 mg (0.056 mmol) of ethanolamine, and the solution was stirred at room temperature for 24 h. The reaction mixture was evaporated to dryness and dissolved in 5 mL of methylene chloride, and the solution was washed successively with 2 × 2 mL portions of 0.1 M HCl and 2 mL of water, dried over anhydrous $MgSO_4$, concentrated, and dried under vacuum to leave 11 mg (50%). The product was characterized by ^1H NMR.

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Supplementary Material Available: Figure 5 showing the solubility of PEG taxoids and copies of selected ^1H and ^{13}C NMR spectra for compounds **2b**, **3c**, **4c**, **7b,c**, **9a**–**h**, **11a,b**, **12** (20 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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