Notes

4-Deacetyltaxol and 10-Acetyl-4-deacetyltaxotere: Synthesis and Biological Evaluation

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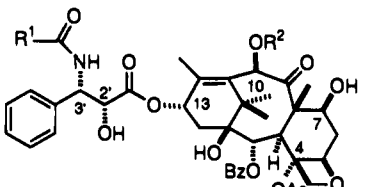
4-Deacetyltaxol and 10-acetyl-4-deacetyltaxotere were synthesized for the first time from 7-(triethylsilyl)-4-deacetylbaccatin III. These analogs were found to be inactive in the microtubule assembly assay.

Taxol (1a) and its potent analog taxotere (**1b**) have proven to be exciting discoveries in the field of cancer chemotherapy (Figure 1). Besides being approved for the treatment of ovarian cancer, taxol also demonstrated encouraging antitumor activity against breast, lung, and head and neck cancer.[1] These promising results have encouraged researchers worldwide to undertake further structure–activity relationship (SAR) studies on taxol.[2–4]

It has been shown that the C-13 phenylisoserine side chain of taxol as well as the diterpene part of the molecule are of importance for biological activity.[5] Much of the early SAR investigations centered on the C-13 phenylisoserine side chain, demonstrating that the 3′-phenyl (or an equivalent group)[6] and the 2′-hydroxyl group are necessary for good cytotoxicity.[7] Deletion of the 3′-amino group from the side chain gave less active analogs.[7] However, replacement of the 3′-*N*-benzoyl group with a variety of other acyl groups was tolerated well.[4] Recent reports have shown the benzoate moiety at C-2 to be essential for biological activity,[8] whereas the acetate group at C-10 has negligible contribution.[9] Similarly, taxol analogs, deoxygenated or modified at C-7 and/or C-10, were found to exhibit essentially identical activity to that of the parent compound.[10–12] 9α-Hydroxytaxol has activity similar to taxol.[13] So far, however, no information is available concerning the role of the acetyl group at C-4 on biological activity. This is probably due to the absence of suitable methodology for the selective hydrolysis of this acetate moiety in the presence of the other ester functionalities in the molecule. In continuation of our ongoing program on SAR studies on taxol, we have recently developed methods for the selective hydrolysis of the esters at C-4 and C-2 of the diterpene baccatin III.[14] This methodology has thus provided a pathway for the synthesis of hitherto unreported 4-hydroxy analogs of taxol and taxotere and to study their biological activity.[15] We are now detailing the observation that the C-4 acetyl group is of crucial importance for the biological activity of taxol and taxotere. The details of these syntheses and the *in vitro* microtubule binding activity of these analogs are reported herein.



1a R^1 = Ph, R^2 = Ac (Taxol)
1b R^1 = *t*-BuO, R^2 = H (Taxotere)

Figure 1. Structures of taxol and taxotere.

Results and Discussion

Our syntheses started with 4-deacetyl-7-(triethylsilyl)baccatin III (**3**), prepared by reaction of 7-(triethylsilyl)baccatin III (**2**) with potassium *tert*-butoxide (1.1 equiv) in THF at −20 °C to 0 °C (Scheme 1).[14] Introduction of the phenyl isoserinate side chain at C-13 was carried out by coupling of **3** with the known precursor **4**[16] (Scheme 2), under standard reaction conditions. Treatment of product **5** with formic acid yielded the amino alcohol **6**, deprotected at C-7, C-2′, and C-3′. This common intermediate **6** was used as such for further *N*-acylation with benzoyl chloride or di-*tert*-butyl dicarbonate, affording the novel 4–deacetyltaxol (**7a**) or 10-acetyl-4-deacetyltaxotere (**7b**) analogs, respectively.

Interestingly, both of the above analogs exhibited very poor activity in the *in vitro* microtubule binding assay (Table 1).[17] A comparison of the conformational analyses of taxol (1)[18] and 4-deacetyltaxol (**7a**)[19] by NMR in DMSO–water revealed that the 4-acetyl group may be responsible for anchoring a hydrophobically clustered conformation in its proper orientation. The observed hydrophobically clustered conformation may be essential for bioactivity. Further work is in progress to introduce and study the effect of other functionalities at the C-4 position.

Experimental Section[17]

4-Deacetyl-7-(triethylsilyl)baccatin III 13-[(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-5–carboxyl-2,2-dimethyl-4-phenyl-1,3-oxazolidine] (5). A mixture of 4-deacetyl-7-(triethylsilyl)baccatin III (325 mg, 0.49 mmol), the oxazolidine carboxylic acid **4** (200 mg, 0.6 mmol), DCC (145 mg, 0.7 mmol), and a catalytic amount of DMAP in dry toluene (12 mL) was stirred under argon at 70 °C for 45 min. The mixture was then cooled to room temperature and filtered. After the residue was washed with CH_2Cl_2 (10 mL), the combined filtrates were concentrated and purified by flash column chromatography

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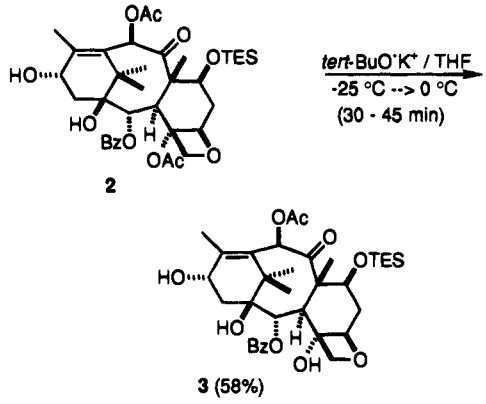
Scheme 1



Scheme 2

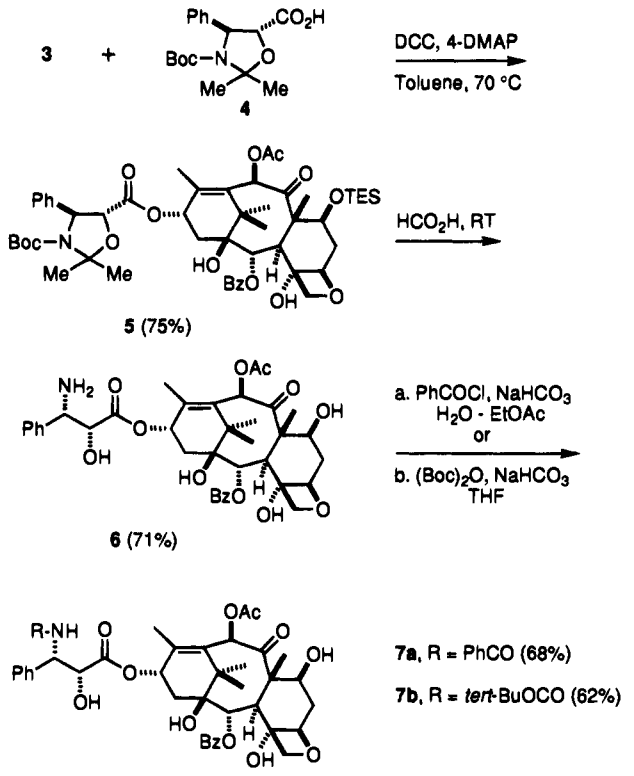


Table 1. Biological Activities of **1a**, **7a**, and **7b**[17]

analog	tubulin assembly	
	ED_{50}[a] (μM)	$ED_{50}/ED_{50\ taxol}$
1a (taxol)	0.93	1
7a	>30	>32
7b	>30	>32

[a] ED_{50} = concentration (μM) which reduces the supernatant protein concentration by 50%.

(SiO_2, hexane/EtOAc = 7/3) to yield the pure product as a white amorphous solid (355 mg, 75%): mp 114–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (q, J = 8 Hz, 6H), 1.12 (t, J = 8 Hz, 9H), 1.27 (s, 3H), 1.34 (br s, 12H), 1.72 (s, 3H), 1.91 (s, 3H), 1.99 (br s, 6H), 2.36 (s, 3H), 2.61 (J = 5 Hz, 1H), 2.80 (m, 2H), 3.30 (d, J = 7 Hz, 1H), 3.35 (br s, 1H), 4.32 (m, 3H), 4.98 (d, J = 5.5 Hz, 1H), 5.07 (br d, J = 8 Hz, 1H), 5.69 (d, J = 6 Hz, 1H), 6.06 (t, J = 8 Hz, 1H), 6.17 (br s, 1H), 6.54 (s, 1H), 7.60–7.93 (m, 8H), 8.28 (d, J = 8 Hz, 2H);^{13}C NMR (75 MHz, DMSO-d_6) δ 5.19, 7.10, 10.23, 20.43, 21.07, 27.51, 28.19, 28.33, 43.52, 48.58, 50.59, 58.38, 63.35, 66.25, 72.83, 72.97, 74.06, 75.49, 76.38, 80.03, 80.74, 88.20, 89.77, 95.96, 106.37, 114.86, 127.92, 128.41, 128.75, 128.82, 128.89, 130.28, 130.62, 133.60, 139.41, 141.34, 143.09, 145.90, 162.61, 165.86, 169.36, 185.30, 203.06; IR (neat) 3400 (br), 1740, 1718, 1700, 1665 cm^{-1}; MS (FAB$^+$) m/e 968 (M + Li); $[\alpha]^{25}_D$ – 96.8° (c = 0.45, $CHCl_3$). Anal. ($C_{52}H_{71}NO_{14}Si$) C, H, N.

4-Deacetyl-*N*-debenzoyltaxol (6). A solution of **5** (350 mg) in 90% formic acid (10 mL) was stirred at room temperature for 6 h. Excess acid was then removed under high vacuum at ambient temperature. The residual solid was then dissolved in $CHCl_3$, washed with dilute $NaHCO_3$ solution and brine, and dried (Na_2SO_4). Solvent evaporation afforded the crude product as a white solid (178 mg, 70%). This product was found to be very polar and was used as such for the next reaction.

4-Deacetyltaxol (7a). To a well-stirred mixture of the amino alcohol **6** (80 mg, 0.11 mmol), ethyl acetate (6 mL), saturated $NaHCO_3$ solution (6 mL), and water (6 mL) was added benzoyl chloride (0.015 mL, 0.13 mmol) dropwise. Stirring was continued for another 15 min. The mixture was then extracted with ethyl acetate (2 × 15 mL). The combined extracts were washed with brine and dried (Na_2SO_4). After the solvent was evaporated, flash column chromatography (CH_2Cl_2/MeOH = 24 /1) yielded the pure product as a white amorphous solid (62 mg, 68%): mp 155–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.13 (s, 3H), 1.79 (s, 3H), 1.87 (s, 3H), 2.08 (m, 1H), 2.24 (s, 3H), 2.58 (m, 2H), 2.78 (dd, J = 6 and 15 Hz, 1H), 3.05 (d, J = 7 Hz, 1H), 3.49 (br s, 1H, exchangeable with D_2O), 4.07 and 4.45 (2d, J = 7.5 Hz, 2H), 4.18 (s, 1H, exchangeable with D_2O), 4.26 (m, 1H), 4.78 (br s, 1H), 4.95 (d, J = 7 Hz, 1H), 5.85 (m, 3H), 6.38 (s, 1H), 6.95–8.04 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.62, 11.96, 20.52, 25.51, 27.07, 35.91, 36.62, 50.31, 54.30, 56.66, 69.06, 70.13, 71.76, 72.43, 72.81, 75.20, 78.68, 81.69, 87.26, 127.13, 127.18, 128.11, 128.60, 128.74, 128.85, 129.09, 129.83, 131.73, 133.29, 134.61, 137.59, 139.17, 145.78, 166.31, 167.53, 169.92, 172.75, 202.52; IR (neat) 3400 (br), 1715 (br), 1650 cm^{-1}; HRMS m/e calcd for $C_{45}H_{50}NO_{13}$ 812.3282 (M + 1), found 812.3278; $[\alpha]^{25}_D$ –39.46° (c = 1.3, $CHCl_3$). Anal. ($C_{45}H_{49}NO_{13}$) C, H, N.

10-Acetyl-4-deacetyltaxotere (7b). To a room temperature solution of the amino alcohol **6** (60 mg, 0.08 mmol) and di-*tert*-butyl dicarbonate (22 mg, 0.1 mmol) in dry THF (5 mL) was added anhydrous $NaHCO_3$, and the mixture was stirred for 4 h. After diluting with ethyl acetate (50 mL) the solution was washed with water and brine, dried (Na_2SO_4), concentrated, and purified by flash column chromatography (SiO_2, CH_2Cl_2/MeOH = 49/1) to yield the product as a white solid (40 mg, 62%): mp 143–146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 3H), 1.19 (s, 3H), 1.31 and 1.47 (2 s, 9H), 1.61 (s, 3H), 1.76 (m, 2H), 2.04 (s, 3H), 2.28 (s, 3H), 2.53 (m, 1H), 2.82 (dd, J = 4 and 15 Hz, 1H), 3.46 (m, 2H), 4.07 (s, 1H), 4.12 (m, 1H), 4.17 and 4.28 (2d, J = 8 Hz, 2H), 4.95 (br d, J = 6 Hz, 1H), 5.24 (br s, 1H), 5.74 (m, 2H), 6.08 (br d, J = 8 Hz, 1H), 6.39 (s, 1H), 7.31–7.61 (m, 8H), 8.18 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.32, 16.69, 19.06, 20.90, 24.91, 25.57, 27.38, 27.58, 28.08, 28.14, 28.28, 33.92, 34.75, 35.91, 42.79, 49.12, 51.29, 53.53, 58.95, 71.37, 72.73, 74.71, 74.80, 76.08, 77.29, 81.01, 83.74, 86.91, 126.74, 127.97, 128.51, 128.55, 128.61, 128.70, 130.19, 133.66, 135.16, 140.91, 152.23, 155.24, 166.91, 167.22, 171.17; IR (neat) 3430 (br), 1710 (br), 1600 cm^{-1}; HRMS m/e calcd for $C_{43}H_{54}NO_{14}$ 808.3544 (M + 1), found 808.3546; $[\alpha]^{20}_D$ –61.6° (c = 0.3, $CHCl_3$). Anal. ($C_{43}H_{53}NO_{14}$) C, H, N.

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