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Synthesis and Biological Evaluation of Paclitaxel Analogs Modified in Ring C

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Abstract: Both 7-deoxy-7α-azidopaclitaxel (**6**) and 7-deoxy-$\Delta^{6,7}$-paclitaxel (**4**) can be prepared from paclitaxel-7-O-triflate (**2b**). Oxidation of 7-deoxy-$\Delta^{6,7}$-paclitaxel with dioxirane yields the epoxide **7**, while oxidation with osmium tetroxide yields 6α-hydroxy-7-epipaclitaxel (**9**), and acylation of this gives the 6α-acyloxy-7-epipaclitaxel derivatives **11a-d**. No compound was as effective at promoting tubulin assembly as paclitaxel, but most stabilized polymer as well as or better than paclitaxel. Compounds **4**, **6**, **7**, **9**, and **11d** differed little from paclitaxel in their cytotoxicity for human Burkitt lymphoma CA46 cells.

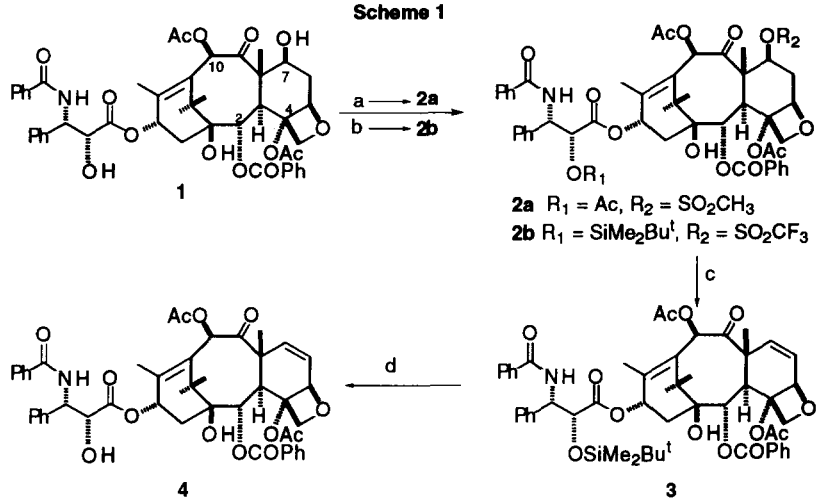
The novel diterpenoid paclitaxel (Taxol®) (**1**), originally isolated[1] from *Taxus brevifolia*, continues to be an exciting target for drug development, both because of its clinical activity[2] and because of its unusual and complex chemistry.[3] Specific transformations of the ring system have included modifications at C-2,[4] at C-4,[5] and at C-7,[6] as well as contraction of rings A and B,[7] oxetane opening,[7,8] and other conversions.[9]

As a part of our continuing studies on the chemistry of paclitaxel, we have had a long-standing interest in transformations of the C-7 hydroxyl group,[6,10] and we now report our studies on transformations of this group leading to the synthesis of 7-deoxy-7α-azidopaclitaxel, 7-deoxy-$\Delta^{6,7}$-paclitaxel, and various analogs derived from the latter compound. The synthesis of 7-deoxy-$\Delta^{6,7}$-paclitaxel has recently and independently been reported by Chen et al.[11] and by Johnson et al.[12]

In our earlier studies on paclitaxel we prepared the 7-mesylate derivative **2a**, but this was inert to treatment with base.[10] Reasoning that the use of a better leaving group at C-7 might lead to reaction at this unreactive neopentyl-type position, we prepared the 7-O-triflate of 2'-(*t*-butyldimethylsilyl)paclitaxel (**2b**). Treatment of **2b** with 1,8-diazabicyclo(5,4,0)undec-7-ene (DBU) at 40°C in dry dichloromethane gave the key intermediate 2'-(*t*-butyldimethylsilyl)-7-deoxy-$\Delta^{6,7}$-paclitaxel (**3**) in 86% yield, together with 14% 7-deoxy-$\Delta^{6,7}$-paclitaxel (**4**). Deprotection of **3** with methanolic HCl yielded the desired 7-deoxy-$\Delta^{6,7}$-paclitaxel (**4**) in 50% yield,[13] together with an oxetane ring-opened product (Scheme 1).

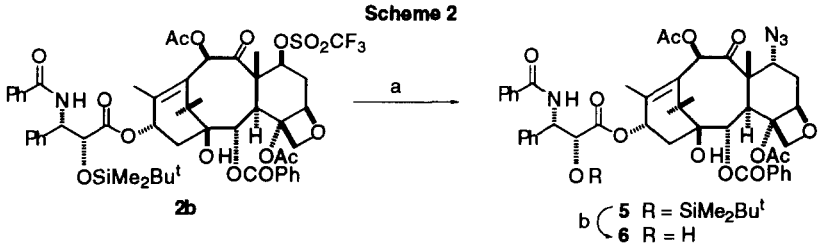
The triflate **2b** could also be converted to a substitution product under appropriate conditions. Reaction of **2b** with NaN_3 in DMF yielded the 7α-azido-7-deoxypaclitaxel derivative **5**, together with lesser amounts of the dehydro derivative **3** and both paclitaxel epimers at C-7 (presumably arising either from traces of water in the solvent or from attack of azide ion on sulfur). Deprotection of **5** with pyridinium hydrofluoride gave 7α-azido-7-deoxypaclitaxel (**6**)[14] (Scheme 2).

Scheme 1



Reagents: (a) Ac_2O/py, then CH_3SO_2Cl/py; (b) Bu^tMe_2SiCl/Imidazole, 60°, 1h, then CF_3SO_2Cl, DMAP, 25°, 2h, 92%; (c) **2b**, DBU, dry CH_2Cl_2, 40°, 4 h; (d) HCl/MeOH, 25°, 1.5 h, 50%.

Scheme 2



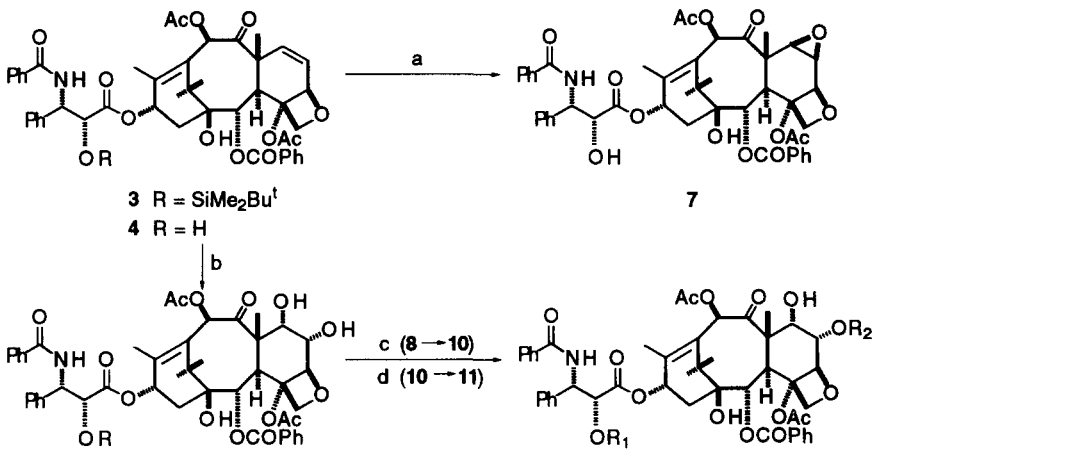
Reagents: (a) NaN_3, DMF, 40°, 24 h, 49%; (b) HF.py, 25°, 1 h, 48%

Further conversions of the dehydropaclitaxel derivative were effected under oxidative conditions (Scheme 3). Oxidation of **4** with mCPBA gave only trace amounts of the epoxide **7**, but oxidation with dimethyldioxirane gave the epoxide **7** in 43% yield. The structure of **7** was confirmed by its spectroscopic data,[15] and its stereochemistry was confirmed by NOE data, which showed that the C-6 and C-7 proton signals were enhanced when the C-3 proton was irradiated, and the signal at C-6 was enhanced when the C-5 proton was irradiated. Attack of dioxirane on **4** thus occurs from the less hindered β face of the ring.

Oxidation of **4** with osmium tetroxide at room temperature yielded 6α-hydroxy-7-*epi*paclitaxel (**9**),[16] and oxidation of protected **3** under the same conditions also proceeded smoothly to give the 2' protected diol **8**. The stereochemistry of hydroxylation was from the face opposite the alkoxy group at C-5, as observed for 2-cyclohexen-1-ol.[17] Acylation of **8** under mild conditions proceeded exclusively on the 6α-hydroxyl group to give the esters **10a** - **10d**, and deprotection with tetrabutylammonium fluoride gave the 6α-acyloxy-7-epipaclitaxel analogs **11a** - **11d** (Scheme 3).

The biological activities of compounds **4**, **6**, **7**, **9**, and **11a** - **11d** were determined in both CA46 cell culture and in a tubulin-assembly assay (Table). None of the analogs was as effective at promoting tubulin

Scheme 3



3 R = $SiMe_2Bu^t$
4 R = H

7

8 R = $SiMe_2Bu^t$
9 R = H

10a R_1 = $SiMe_2Bu^t$, R_2 = CH_3CO
10b R_1 = $SiMe_2Bu^t$, R_2 = C_6H_5CO
10c R_1 = $SiMe_2Bu^t$, R_2 = o-$CH_3C_6H_4CO$
10d R_1 = $SiMe_2Bu^t$, R_2 = cyclo-C_3H_5CO

11a R_1 = H, R_2 = CH_3CO
11b R_1 = H, R_2 = C_6H_5CO
11c R_1 = H, R_2 = o-$CH_3C_6H_4CO$
11d R_1 = H, R_2 = cyclo-C_3H_5CO

Reagents: (a) $(CH_3)_2CO_2$, acetone, 25°, 3 days, 43%; (b) OsO_4, NMO, 25°, 9 h, 71%;
(c) RCOCl, DMAP, CH_2Cl_2, 25°, 4 h, 90-98%; (d) TBAF, THF, 0°, 15 min, 80-95%.

assembly as paclitaxel (e.g. considering the data for 40 μM solutions at 0°C). However, all the analogs with the exception of **9** and **11c** stabilized polymerized tubulin as well as or better than paclitaxel; compounds **11b**, **11c**, and **11d** are particularly interesting in that they have negligible ability to promote tubulin polymerization, but they stabilize the polymer once formed. Compounds **4**, **6**, **7**, **9**, and **11a** differed little from paclitaxel in their cytotoxicity towards human Burkitt lymphoma CA46 cells, while **11b** - **11d** were distinctly less cytotoxic. The apparent divergence of the tubulin and cytotoxicity data is noteworthy but as yet unexplained.

Table: Cytotoxicities and Effects of Analogs on Tubulin Polymerization[a]

Compound	Cytotoxicity IC_{50} values (μM) with CA46 cells	Concentration in tubulin assays	Tubulin Assembly Activity Maximum rate of assembly: ΔA_{350} munit/min			Disassembly Maximum rate: ΔA_{350} munit/min
			0°C	20°C	37°C	
None			0	3.7	220	400
Paclitaxel (**1**)	0.01	10μM	2.9	840	NM	110
Paclitaxel (**1**)		40μM	100	430	NM	70
4	0.03	40μM	2.0	330	NM	60
6	0.01	40μM	0	180	NM	57
7	0.02	40μM	1.7	580	NM	73
9	0.03	40μM	2.1	640	NM	140
11a	0.02	40μM	0	370	NM	70
11b	0.05	40μM	0	6.6	220	47
11c	0.2	40μM	0	2.9	270	120
11d	0.07	40μM	0	7.2	220	45

a. Tubulin data are the average of three determinations; cytotoxicity data are the average of two.

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13. Spectral data for **4** were identical to those previously reported.[12]
14. Spectral data for **6**: ^{1}H-NMR ($CDCl_3$, TMS) δ 8.16 (d, 2H), 7.74 (d, 2H), 7.62 (t, 1H), 7.58-7.30 (m, 5H), 6.99 (d, 1H, J = 9.0, H_{NH}), 6.88 (s, 1H, H_{10}), 6.21 (t, 1H, J = 9.0, H_{13}), 5.83 (q, 1H, J = 9.0, 2.6, $H_{3'}$), 5.73 (d, 1H, J = 7.3, H_2), 5.03 (q, 1H, J = 9.2, 3.9, H_5), 4.82 (q, 1H, J = 4.6, 2.4, $H_{2'}$), 4.45 (d, 1H, J = 8.6, H_{20}), 4.34 (d, 1H, J = 8.6, H_{20}), 3.93 (d, 1H, J = 7.2, H_3), 3.75 (d, 1H, J = 3.0, H_7), 3.46 (d, 1H, J = 4.7, $H_{2'\text{-OH}}$), 2.22-2.62 (m, 4H, H_6, H_{14}), 2.44 (s, 3H, $-CH_3$), 2.20 (s, 3H, $-CH_3$), 1.85 (s, 3H, $-CH_3$), 1.76 (s, 3H, $-CH_3$), 1.23 (s, 3H, $-CH_3$), 1.13 (s, 3H, $-CH_3$).
15. Spectral data for **7**: ^{1}H-NMR ($CDCl_3$, TMS) δ 8.07 (d, 2H), 7.78 (d, 2H), 7.62 (t, 1H) 7.54-7.30 (m, 5H), 7.13 (d, 1H, J = 9.2, H_{NH}), 6.42 (s, 1H, H_{10}), 6.18 (t, 1H, J = 9.2, H_{13}), 5.83 (q, 1H, J = 9.2, 2.6, $H_{3'}$), 5.74 (d, 1H, J = 6.0, H_2), 5.35 (d, 1H, J = 3.0, H_5), 4.80 (q, 1H, J = 2.6, 4.0, $H_{2'}$), 4.48 (d, 1H, J = 8.3, H_{20}), 4.27 (d, 1H, J = 8.3, H_{20}), 4.03 (d, 1H, J = 6.0, H_3), 3.74 (d, 1H, J = 4.0, $H_{2'\text{-OH}}$), 3.26 (q, 1H, J = 3.6, 3.0, H_6), 3.02 (d, 1H, J = 3.6, H_7), 2.42-2.18 (m, 2H, H_{14}), 2.38 (s, 3H, $-CH_3$), 2.22 (s, 3H, $-CH_3$), 1.87 (s, 3H, $-CH_3$), 1.79 (s, 3H, $-CH_3$), 1.22 (s, 3H, $-CH_3$), 1.14 (s, 3H, $-CH_3$).
16. Spectral data for **9**: ^{1}H NMR ($CDCl_3$, TMS) δ 8.14 (d, 2H), 7.71 (d, 2H), 7.60 (t, 1H) 7.54-732 (m, 5H), 7.05 (d, 1H, J = 9.0, H_{NH}), 6.78 (s, 1H, H_{10}), 6.21 (t, 1H, J = 8.7, H_{13}), 5.79 (q, 1H, J = 9.0, 2.4, $H_{3'}$), 5.72 (d, 1H, J = 7.4, H_2), 4.80 (q, 1H, J = 4.7, 2.8, $H_{2'}$), 4.65 (d, 1H, J = 4.9, $H_{7\text{-OH}}$), 4.63 (d, 1H, J = 5.8, H_5), 4.33 (s, 2H, H_{20}), 4.13 (m, 1H, H_6), 3.85 (d, 1H, J = 7.3, H_3), 3.72 (d, 1H, J = 4.7, $H_{2'\text{-OH}}$), 3.65 (q, 1H, J = 4.9, 11.6, H_7), 2.92 (d, 1H, J = 8.2, $H_{6\text{-OH}}$), 2.48 (s, 3H, $-CH_3$), 2.42-2.20 (m, 2H, H_{14}), 2.18 (s, 3H, $-CH_3$), 1.76 (s, 3H, $-CH_3$), 1.62 (s, 3H, $-CH_3$), 1.18 (s, 3H, $-CH_3$), 1.12 (s, 3H, $-CH_3$).
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