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Synthesis and Biological Evaluation of 4-Deacetoxypaclitaxel

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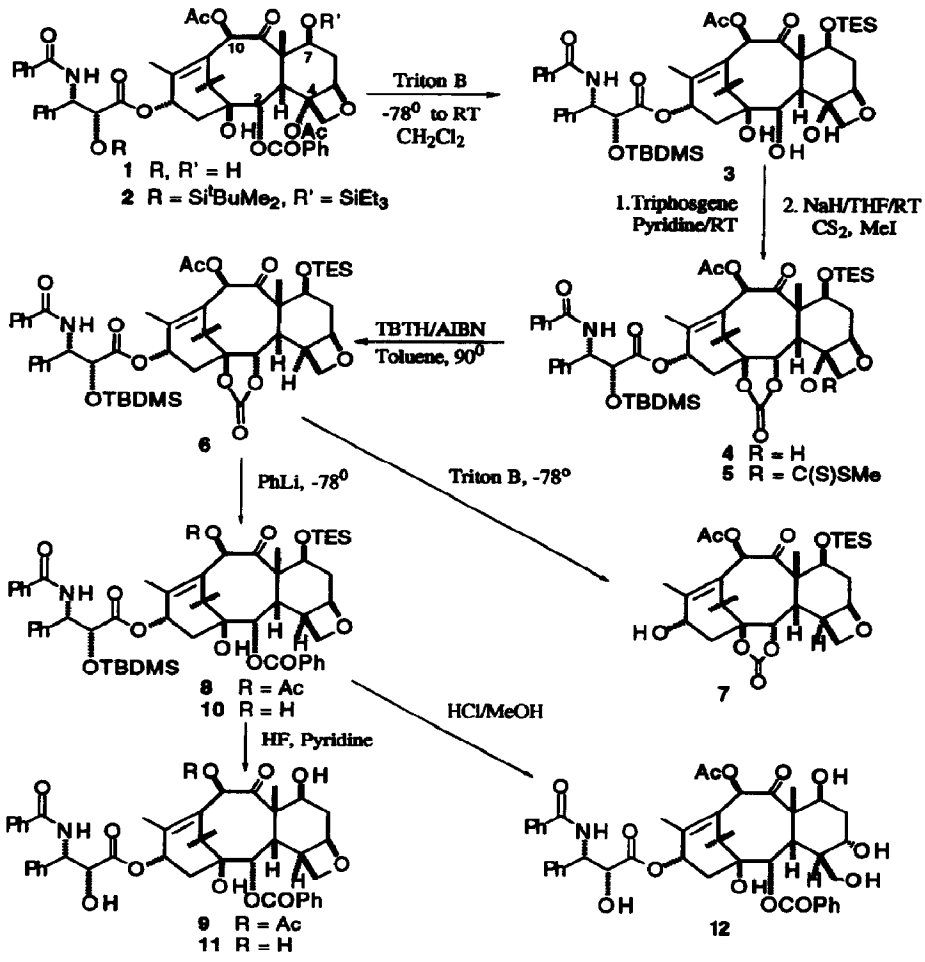
Abstract: 4-Deacetoxypaclitaxel (**9**) has been prepared in seven steps from paclitaxel (Taxol®). It is significantly less active than paclitaxel in tubulin-assembly and cytotoxicity bioassays.

The novel diterpenoid paclitaxel (Taxol®) (**1**), originally isolated[1] from *Taxus brevifolia*, has become established over the last few years as one of the most exciting anticancer drugs in recent history.[2] Its structural complexity and its unique biological activity have combined to catalyze efforts to develop analogs with simpler structures and improved bioactivities, and a number of studies designed to uncover structure-activity relationships have been carried out.[3] From these studies, the important generalizations have emerged that changes in the northern hemisphere[4] of paclitaxel have less effect on its bioactivity than do changes in the southern hemisphere, where relatively small changes can have major effects on activity.[5] In continuing studies on the structure-activity relationships of paclitaxel, we have found that removal of the C-4 acetate group causes a significant loss in tubulin-assembly activity and cytotoxicity.[6]

Although the C-4 acetoxy group appears to be implicated as a requirement for paclitaxel's activity, the simple removal of the acetyl group does not establish this conclusion unambiguously, since the polarity change involved in the conversion of an acetate to an alcohol might also affect the activity. It is also relevant to note that recent evidence indicates that paclitaxel forms a "hydrophobic cluster" between the C-2 benzoate, the C-4 acetate, and the side chain 3' phenyl group in aqueous solvents,[7] and this hydrophobic cluster may be associated with paclitaxel's activity, since an inactive oxetane ring-opened analog[5b] does not show hydrophobic clustering.[8] It is thus possible that the C-4 hydroxyl group could inhibit formation of a hydrophobic cluster, while a C-4 hydrogen would not. For these reasons, it is important to establish the effect of a C-4 deacetoxy transformation on the activity of paclitaxel.

The synthesis of 4-deacetoxypaclitaxel (**9**) is outlined in the Scheme. Conversion of paclitaxel (**1**) to its 2'-(*t*-butyldimethylsilyl)-7-(triethylsilyl) derivative **2** was followed by selective hydrolysis of the C-2 benzoate and C-4 acetate groups with Triton B as previously described[6] to yield the triol **3**. Protection of the 1,2-diol unit as the cyclic carbonate **4** proceeded in 83% yield with triphosgene and pyridine, and set the stage for the key deoxygenation sequence.

Carbonate **4** was converted to its xanthate derivative **5** in 90% yield by treatment with CS_2 and methyl iodide in the presence of NaH. Deoxygenation of **5** by the Barton-McCombie method[9] proceeded smoothly in



the presence of Bu_3SnH and AIBN in toluene at 90 °C to give the 4-deacetoxy derivative **6** in 80% yield. Attempted ring opening of **6** with Triton B gave exclusively the baccatin III derivative **7**, in contrast to the case with the corresponding paclitaxel derivative **2**. Conversion to the paclitaxel analog **8** was thus effected in 60% yield by treatment with PhLi (3 eq.) at -78 °C.[10] If a larger excess of PhLi was used (5 eq.), the 10-deacetyl derivative **10** was formed in addition to the 10-acetyl derivative **8**.

Deprotection of **8** was first attempted with HCl/MeOH, conditions that work perfectly well on silylated paclitaxel derivatives. However, these conditions applied to **8** led surprisingly to the ring opened product **12**, indicating the subtle effects that structural changes have on the reactivity of the functional groups of paclitaxel. In this case, it is apparent that the electron withdrawing effect of the C-4 acetate must stabilize the oxetane ring to a greater extent than the destabilization provided by the anchimeric assistance of the oxygen lone pairs.[5b]

Deprotection was then achieved by treatment of **8** with HF (pyridine, 25 °C) to yield 4-deacetoxypaclitaxel (**9**) in 66% yield. Similar treatment of the 10-deacetyl analog **10** gave 4-deacetoxy-10-deacetylpaclitaxel (**11**).

The spectroscopic data of **9** were fully consistent with its assigned structure.[11] In particular, its ^1H NMR spectrum showed the presence of a C-4 methine proton at 3.84 ppm, overlapped with the signal for the C-7 proton and C-3 methine proton as a triplet centered at 3.32 ppm. The C-5 proton appeared as a multiplet at 5.18 ppm, and the C-20 protons as a doublet at 4.33 ppm. The ^{13}C NMR spectrum of compound **9** showed the presence of the C-4 carbon at 33.5 ppm as compared to 81.1 ppm in paclitaxel.

The biological activities of compounds **9**, **11**, and **12** were determined in a cytotoxicity assay using human CA46 Burkitt lymphoma cells and in a tubulin-assembly assay. The three compounds only marginally inhibited cell growth. Whereas paclitaxel yielded an IC_{50} value of 30 nM with the Burkitt cells, the comparable values were 0.7 μM, 8 μM, and 0.3 μM for compounds **9**, **11**, and **12**, respectively. Compounds **9**, **11**, and **12** were also much less active than paclitaxel both in promoting tubulin polymerization and in stabilizing tubulin polymer to low temperatures: under a reaction condition in which tubulin will not assemble in the absence of an active taxoid,[7] the three new compounds had negligible stimulatory effects. Under conditions where assembly occurs without drug at 37 °C but not at 20 °C (Table), the three compounds gave maximum assembly rates at 20 °C which were between 38 and 940 times smaller than that for paclitaxel at the same concentration, and a higher concentration of drug failed to offset this lack of activity.

Table: Effects of Paclitaxel and Analogs 9, 11, and 12 on Tubulin Polymerization

Compound	Maximum Assembly Rate: ΔA_{350} unit/min			Maximum Depolymerization Rate: ΔA_{350} unit/min
	0 °C	20 °C	37 °C	
None	0	0	0.46	0.56
Paclitaxel (10 μM)	0.010	0.98	NM	0.17
Paclitaxel (40 μM)	0.029	0.95	NM	0.14
9 (10 μM)	0	0.025	NM	0.28
9 (40 μM)	0	0.13	NM	0.28
11 (10 μM)	0	0.001	0.45	0.49
11 (40 μM)	0	0.039	NM	0.30
12(10 μM)	0	0.008	NM	0.48
12 (40 μM)	0	0.023	NM	0.36

Baselines were established with all components (1.0 mg/mL tubulin, 0.6 M monosodium glutamate, 1.0 mM $MgCl_2$, 0.4 mM GTP) in the reaction mixture except drug or dimethyl sulfoxide (the drug solvent). Drug was added, and the reaction followed for 10 min at 0 °C, 15 min at 20 °C, and 15 min at 37 °C. Reaction temperature was then reduced to 0 °C for the depolymerization phase. The experiment was performed in a Gilford model 250 spectrophotometer equipped with an electronic temperature controller; with this device temperature rises at 0.5 °C/min when a higher temperature is set, and cooling from 37 to 0 °C takes about 5 min. Reactions can begin before temperature equilibration is complete, and reaction temperature is thus only nominal. The maximum rate is defined as the maximum interval increase in reading for a reaction. As the cuvette holder has four positions and a dwell time of 5 sec was used, successive readings at each position were approximately 26 sec apart. Averages of duplicate values are presented. NM = Not meaningful, since the extent of reaction at the lower preceding temperature was sufficiently extensive that the maximum rate at the indicated temperature was relatively low.

The reduced activity of compounds 9 and 11 provides definitive proof that an ester substituent at the 4-position of paclitaxel is necessary for its activity, and that the lack of activity of 4-deacetylpaclitaxel[6] is due primarily to its lack of the acetyl group rather than to a polarity change due to the presence of the hydroxyl group. However, the fact that compounds **9** and **11** appear to be slightly more active than 4-deacetylpaclitaxel[6] suggests that polarity changes at C-4 also have an effect on activity. Whether or not these activity changes are associated with the presence or absence of a "hydrophobic cluster" remains to be determined.

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References and Notes

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325-2327.
2. For reviews: (a) Rowinsky, E. K.; Donehower, R. C. *J. Natl. Cancer Inst.*, **1991**, *83*, 1778-1781. (b) Holmes, F. A.; Walters, R. S.; Theriault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *J. Natl. Cancer Inst.*, **1991**, *83*, 1797-1805. (c) Slichenmyer, W. J.; Von Hoff, D. D. *Anti-Cancer Drugs*, **1991**, *2*, 519-530. (d) Rowinsky, E. K.; Donehower, R. C. *Pharmac. Ther.* **1991**, *52*, 35-84. (e) Rowinsky, E. K.; Oretto, N.; Canetta, R. M.; Arbuck, S. G. *Sem. Oncol.* **1992**, *19*, 646-662.
3. For reviews on the chemistry and structure-activity relationships of taxol: (a) Suffness, M.; Cordell, G. A. In *The Alkaloids. Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: New York, 1985; Vol. 25, pp 3-355. (b) Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A. J. Nat. Prod. 1990, 53, 1-12. (c) Blechert, S.; Guénard, D. In *The Alkaloids. Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: San Diego, 1990; Vol. 39, pp 195-238. (d) Kingston, D. G. I. *Pharmac. Ther.* **1991**, *52*, 1-34. (e) Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. *Prog. Chem. Org. Nat. Prod.* **1993**, *61*, 1-188.
4. The northern hemisphere encompasses C-7 to C-12 and the groups attached thereto, and the southern hemisphere encompasses C-1 to C-5 and C-14: Suffness, M. *Ann. Rep. Med. Chem.* **1993**, *28*, 305-314.
5. (a) Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222-227. (b) Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem.*, **1991**, *56*, 5114-5119. (c) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M., Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097-4098. (d) Chen, S.-H.; Wei, J.-M.; Farina, V. *Tetrahedron Lett.* **1993**, *34*, 3205-3206.
6. Neidigh, K. A.; Gharpure, M. M.; Rimoldi, J. M.; Kingston, D. G. I.; Jiang, Y. Q.; Hamel, E. *Tetrahedron Lett.*, preceding paper.
7. Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.*, **1993**, *115*, 11650-11651.
8. Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. NMR Studies of a Hydrophobically Clustered Conformation of Taxol Analogs and its Relationship to Bioactivity. *Abstracts of Papers*, 207th National Meeting of the American Chemical Society, San Diego, CA; Abstract MEDI 099; American Chemical Society: Washington DC, 1994.
9. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin I*, **1975**, 1574-1585.
10. Holton, R. A.; Kim, H.-B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599-1600.
11. Spectroscopic data for compound **9**: FAB mass spectrum, $(M+Na-H)^+$ *m/z* 818.4936. $C_{45}H_{51}NO_{12}Na$ requires 818.3152. ^{1}H-NMR ($CDCl_3$) ∂ 8.11 (2H, d, *J*=7.2, *o*-C_6H_5COO), 7.25-7.53 (13H, m, Ar), 6.94 (1H, d, *J*=9.5, NH), 6.32 (1H, s, H-10), 6.12 (1H, m, H-13), 5.95 (1H, br d, *J*=9.5, H-3'), 5.58 (1H, d, *J*=6.1, H-2), 5.18 (1H, m, H-5), 4.80 (1H, dd, *J*=1, 9.5, H-2'), 4.33 (2H, d, *J*=7.9), 3.84 (2H, m, H-4, H-7), 3.32 (1H, t, *J*=6.1, H-3), 2.62 (2H, dd, *J*=9.5, 16, H-14), 2.50 (2H, m, H-6), 2.25 (3H, s, OAc), 1.95 (3H, s, H_3-18), 1.71 (3H, s, H_3-19), 1.17 (3H, s, H_3-17), 1.16 (3H, s, H_3-16).

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