Pergamon

Tetrahedron Letters, Vol. 35, No. 37, pp. 6839-6842, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01446-9

Synthesis and Biological Evaluation of 4-Deacetylpaclitaxel

Kurt A. Neidigh, Milind M. Gharpure, John M. Rimoldi and David G. I. Kingston*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

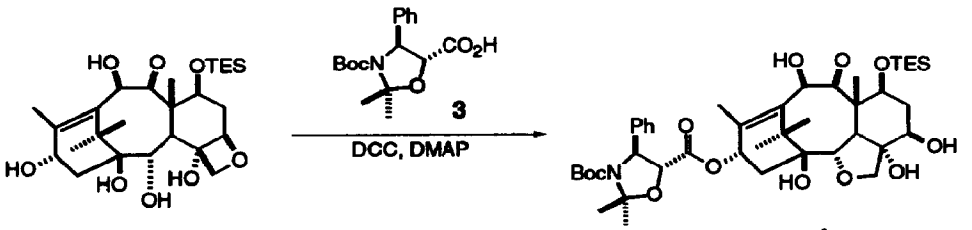
Yuan Qing Jiang and Ernest Hamel*

Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Abstract: 4-Deacetylpaclitaxel, prepared from baccatin III via two synthetic approaches and from paclitaxel via one synthetic approach, has minimal effects on tubulin polymerization and is not cytotoxic to human CA46 Burkitt lymphoma cells.

The anticancer drug paclitaxel (Taxol®) (**1**), a naturally occurring diterpenoid isolated from the bark of the western yew *Taxus brevifolia*, has shown excellent clinical activity against both ovarian cancer and breast cancer, and is currently being developed for general clinical use.[1] Since its discovery by Wall and co-workers in the late 1960s,[2] and the discovery of its unique mode of action by Horwitz in 1979,[3] extensive studies of its chemistry and structure-activity relationships have been reported.[4] From these studies, the important generalizations have emerged that changes in the southern hemisphere[5] of paclitaxel have a much larger effect on its bioactivity than changes in the northern hemisphere. In particular, the oxetane ring appears to be essential to paclitaxel's activity, as is the C-2 benzoate.[4] One of the questions that has so far not been addressed is that of the effect of the C-4 acetate group on the activity of paclitaxel. We have now prepared 4-deacetylpaclitaxel (**9**) by three different routes, and report herein that it is noncytotoxic to human CA46 Burkitt lymphoma cells at concentrations up to 10 μM and has only a minimal stimulatory effect on tubulin polymerization.[6]

Our initial synthetic approach (Scheme 1) began with 4,10-bis(deacetyl)-2-debenzoyl-7-(triethylsilyl)-baccatin III (**2**), obtained from baccatin III as previously described.[7] Previous studies had shown that acetylation

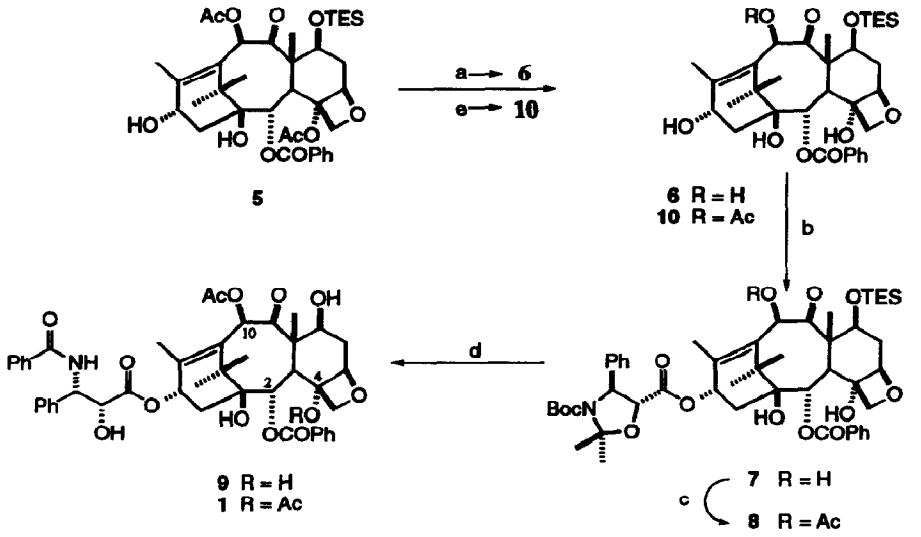


2 **Scheme 1** **4**

of **2** occurred preferentially at C-13,[7] but when reacylation was effected with Commerçon's oxazolidine-protected side chain **3** (prepared from (*S*)-phenylglycine by a modification of published procedures[8]), the only C-13 acylated product observed was the isotaxol derivative **4**. The formation of isomers of baccatin III corresponding to the isotaxol analog **4** has been reported previously.[7,9]

Because of this problem of isomerization when the C-2 position is deacylated, we chose to revise our synthetic approach to utilize 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**6**) as a key intermediate (Scheme 2). This compound has previously been isolated in low yield from the methanolysis of 7-(triethylsilyl)baccatin III (**5**),[7] and the effect of the nature of the base on this selective cleavage reaction was studied. It was found that potassium *tert*-butoxide afforded 4,10-bis(deacetyl)-7-(triethylsilyl)baccatin III (**6**) in the highest yield, 72%. Compound **6** was then coupled with the protected taxol side chain **3** using standard coupling conditions (DCC/DMAP) to give 4,10-bis(deacetyl)-7-triethylsilyl-13-((4*S*,5*R*)-*N*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl-5-oxazolidinyl)baccatin III (**7**) in 90% yield. Acetylation of the C-10 hydroxyl group of compound **7** afforded 4-deacetyl-7-triethylsilyl-13-((4*S*,5*R*)-*N*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl-5-oxazolidinyl)-baccatin III (**8**) in 65% yield, and deprotection of this with 99% formic acid followed by *N*-benzoylation of the resultant free amine gave 4-deacetylpaclitaxel (**9**) in 46% yield from compound **8** and in 19% overall yield from **5**.

A second synthetic approach (Scheme 2) was realized by treatment of 7-(triethylsilyl)baccatin III (**5**) with lithium aluminum hydride,[8a] affording 4-deacetyl-7-(triethylsilyl)baccatin III (**10**) in 10% yield. Compound **10** was coupled to the protected taxol side chain **3** described above, giving 4-deacetyl-7-triethylsilyl-13-((4*S*,5*R*)-*N*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl-5-oxazolidinyl)baccatin III (**8**) in 88% yield and identical to the same compound made by the first approach.



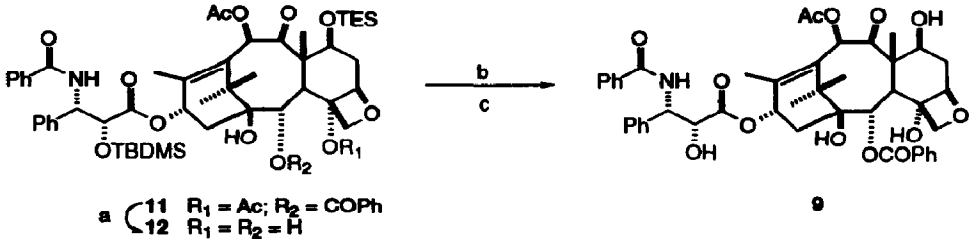
Reagents: (a) KO*t*-Bu, THF; (b) **3**, DCC, DMAP, toluene; (c) Ac_2O, DCC, 4-PP, THF; (d) 99% HCO_2H, then PhCOCl, $NaHCO_3$, EtOAc-H_2O; (e) LAH, THF

Scheme 2

Although both of the preceding approaches did succeed in providing 4-deacetylpaclitaxel, they required several steps and proceeded in unacceptably low yields. We thus sought an approach directly from paclitaxel, which would require the selective hydrolysis of a tertiary acetate in the presence of two other ester groups. Based on our earlier experience with the chemoselective hydrolysis of the 2-benzoate of paclitaxel,[10] we

investigated the use of base hydrolysis in non-hydroxylic solvents, and *found that this method gave a good overall conversion of paclitaxel to its 4-deacetyl analog and is the preferred method for this conversion.*

Treatment of **1** with *tert*-butyldimethylsilyl chloride and imidazole followed by triethylsilyl chloride and imidazole afforded 2'-*tert*-butyldimethylsilyl-7-(triethylsilyl)paclitaxel (**11**) in 92% yield (Scheme 3). Compound **11** was treated with benzyltrimethylammonium methoxide, giving 2'-*tert*-butyldimethylsilyl-2-debenzoyl-4-deacetyl-7-(triethylsilyl)paclitaxel (**12**) in 69% yield. The C-2 hydroxyl group of compound **12** was rebenzoylated to afford 2'-*tert*-butyldimethylsilyl-4-deacetyl-7-(triethylsilyl)paclitaxel in 67% yield, and this compound was then deprotected by treatment with 5% HCl in methanol, affording 4-deacetylpaclitaxel (**9**) in nearly quantitative yield, and in 42% overall yield from paclitaxel.



Reagents: (a) Benzyltrimethylammonium methoxide, CH_2Cl_2; (b) $PhCO_2H$, DCC, DMAP, toluene; (c) 5% HCl in MeOH

Scheme 3

4-Deacetylpaclitaxel (**9**) displayed spectroscopic data fully consistent with the assigned structure. In particular, its ^{1}H-NMR spectrum showed the C-4 acetate peak to be absent, and upfield chemical shifts for the C-3 and C-7 protons and a downfield chemical shift for the C-14 protons were also noted, indicative of the removal of the C-4 acetate. The remaining peaks were similar to those of taxol. FAB MS indicated an (M+H) molecular ion peak at 812 mass units, consistent with the assigned structure.

Table: Effects of Paclitaxel and 4-Deacetylpaclitaxel (9) 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	0.46	0.46
9 (40 μM)	0	0.015	0.60	0.34

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 biological activity of 4-deacetylpaclitaxel(**9**) was determined in a cytotoxicity assay using human CA46 Burkitt lymphoma cells and in a tubulin assembly assay. At concentrations as high as 10 μM, **9** had no inhibitory effect on cell growth, as compared with an IC_{50} value of 30 nM for paclitaxel. In addition **9** was almost inactive in promoting tubulin polymerization and in stabilizing tubulin polymer. Under conditions where assembly occurs without drug at 37 °C but not at 20 °C (Table), paclitaxel gave a feeble reaction even at 0 °C and vigorous assembly at 20 °C. In this system disassembly occurs following assembly when the temperature is reduced to 0 °C in the absence of drug; these observations can be quantitated in terms of maximum assembly and disassembly rates taken from turbidity tracings (Table). No reaction occurred with **9** at 0 °C at concentrations as high as 40 μM, and at 20 °C only a slight reaction was seen at 40 μM. At 37 °C assembly in the presence of 40 μM **9** was slightly faster than in the control. The depolymerization rate was only moderately reduced even at 40 μM **9**, but this effect was reproducible.

In summary, *4-deacetylpaclitaxel is significantly less active than paclitaxel in two different assay systems, suggesting that the 4-acetyl group is necessary for paclitaxel's activity.*

Acknowledgments. Financial support for this work was provided by the National Cancer Institute, grant CA-55131. We thank Mr. K. Harich, Department of Biochemistry, VPI&SU, for obtaining low resolution mass spectrometry data, the Midwest Center for Mass Spectrometry for high resolution mass spectrometry data, and Dr. K. Snader, National Cancer Institute, for a gift of crude paclitaxel-containing fractions from *Taxus brevifolia* which were used as the source of paclitaxel and baccatin III for this work.

References and Notes

1. For a recent review, see Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Sem. Oncol.* **1992**, *19*, 646-662.
2. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325-2327.
3. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665-667.
4. 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. (f) Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222-227.
5. The northern hemisphere encompasses C-7 to C-12, and the southern hemisphere encompasses C-1 to C-5 and C-14: Suffness, M. *Ann. Rep. Med. Chem.* **1993**, *28*, 305-314.
6. Oral presentations of this and similar work have been made both by us and by Georg: Kingston, D. G. I. Studies on the Chemistry of Taxol. *Abstracts of Papers*, 207th National Meeting of the American Chemical Society, San Diego, CA; Abstract MEDI 145; American Chemical Society: Washington DC, 1994; Georg, G. I. Taxol: Semisynthetic and Conformational Studies. *Abstracts of Papers*, 207th National Meeting of the American Chemical Society, San Diego, CA; Abstract MEDI 146; American Chemical Society: Washington DC, 1994.
7. Samaranayake, G.; Neidigh, K. A.; Kingston, D. G. I. *J. Nat. Prod.*, **1993**, *56*, 884-898.
8. (a) Commerçon, A.; Bézard, D.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, *33*, 5185-5188. (b) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1991**, *56*, 6939-6942.
9. (a) Wahl, A.; Guéritte-Voegelein, F.; Guénard, D.; Le Goff, M.-T.; Potier, P. *Tetrahedron* **1992**, *48*, 6965-6974. (b) Farina, V.; Huang, S. *Tetrahedron Lett.* **1992**, *33*, 3979-3982.
10. Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M., Chordia, M.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097-4098.

(*Received in USA* 23 *June* 1994; *revised* 18 *July* 1994; *accepted* 26 *July* 1994)