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TETRAHEDRON

Synthesis and Biological Evaluation of C-1 and Ring Modified A-*nor*paclitaxels

Haiqing Yuan [1] and David G. I. Kingston*

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

Byron H. Long, Craig A. Fairchild, and Kathy A. Johnston

Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 05843

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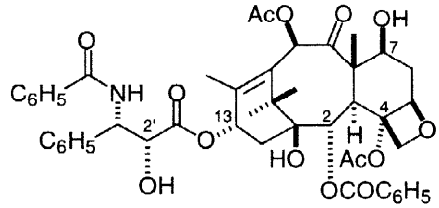
Abstract. 1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel, 1-deisopropenyl-A-*nor*paclitaxel, 1-deisopropenyl-1-acetyl-8,9-oxido-A-*nor*paclitaxel, and A-*nor*-C-*nor*paclitaxel were synthesized. The biological activities of these analogs were determined, and structure-activity relationships for the C-1 position are suggested.

Keywords: taxoids; Baeyer-Villiger reactions; ozonolysis; rearrangements

The novel diterpenoid paclitaxel (**1**) has become one of the most important anticancer agents for the clinical treatment of ovarian and breast cancer,[2] and extensive chemical and SAR studies have been carried out.[3] Among many studies of structural modifications of this complex tetracyclic molecule, analogs prepared by A-ring, B-ring, and C-ring contractions and by oxetane ring manipulations have all appeared in the literature. We[4] and others[5] have reported that paclitaxel undergoes rearrangement under a number of acidic conditions to give the A-ring contracted analog A-*nor*paclitaxel (**2**). Biological studies indicated that A-*nor*paclitaxel was about one third as active as paclitaxel in a tubulin assembly assay but was much less cytotoxic than paclitaxel against Burkitt



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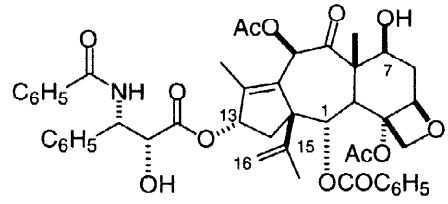

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lymphoma CA 46 cells.[4] In order to extend our knowledge of the SAR of this region, and to explore the difference between the tubulin assembly activity and the cytotoxicity of A-*nor*paclitaxel, we prepared A-*nor*paclitaxel derivatives modified on the C-2 benzoyl group and on the double bond of the C-1 isopropenyl moiety.[6] Interestingly, unlike paclitaxel, where certain modifications of the C-2 benzoyl group usually increase tubulin assembly activity,[7] the same modifications on A-*nor*paclitaxel uniformly decreased tubulin assembly activity slightly. On the other hand, certain modifications at the C-1 isopropenyl moiety enhanced tubulin assembly activity, in some cases to the same level as that of paclitaxel.[6] Geometry and conformation optimized molecular modeling studies using MacSpartan indicated that A-*nor*paclitaxel has an "inverted cup- shape" which is

E:mail: dkingston@vt.edu FAX: (540)231-7702

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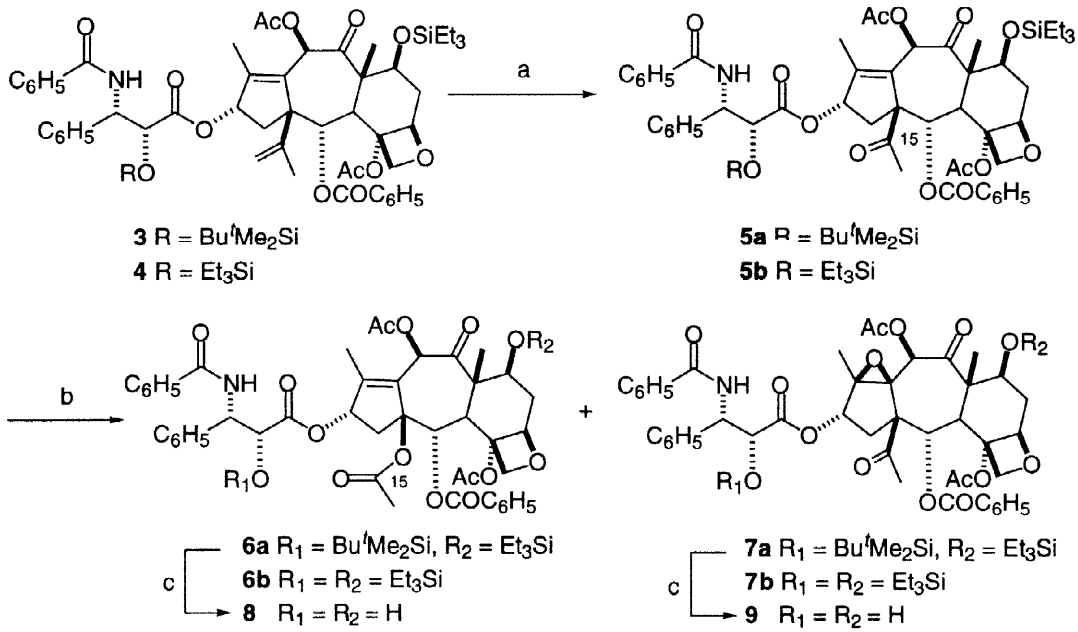
analogous to that of paclitaxel.[8] It thus appeared reasonable to suppose that the spatial volume of the substituent at C-1 may play a role in determining tubulin assembly activity, either by modifying the "hydrophobic collapse" conformation of paclitaxel[9] or by interacting unfavorably with tubulin.

In order to test this assumption, and to obtain more structure-activity relationship (SAR) information for this region, we elected to modify the C-1 substituent and the ring skeleton of A-*nor*paclitaxel; our results are presented below.

RESULTS AND DISCUSSION

Chemistry

Synthesis of 1-deisopropenyl-1-acetoxy-A-norpaclitaxel (8). The key starting material for our investigation was the protected A-*nor*paclitaxel analog **5a**. The related compound **5b** had previously been prepared by ozonolysis of 2',7-di-(*O*-triethylsilyl)-A-*nor*paclitaxel (**4**),[6] and compound **5a** was thus prepared by ozonolysis of 2'-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilyl-A-*nor*paclitaxel (**3**) in methylene chloride followed by reduction with dimethyl sulfide. These conditions, however, gave a mixture of products in which the desired ketone **5a** was only present to the extent of 60-70%. Investigations with different conditions indicated that ozonolysis proceeded cleanly in a methylene chloride/methanol solvent mixture and **5a** was obtained in 91% yield from **3**.



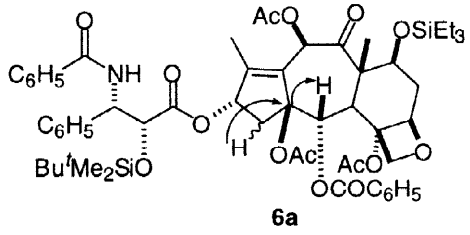
(a) O_3, CH_2Cl_2, CH_3OH, 91%; (b) MCPBA, CH_2Cl_2, **6**, 69%, **7**, 16%; (c) HF, pyridine, **8**, 79%, **9**, 65%

Scheme 1

Initial Baeyer-Villiger oxidation reactions of **5a** using *meta*-chloroperoxybenzoic acid (*m*-CPBA) or trifluoroacetic anhydride and the urea-hydrogen peroxide complex[10] were extremely slow and gave yields of 10% or less; prolonged treatment led to decomposition of the starting material. It was noted, however, that reaction with smaller volumes of solvent usually gave better yields, and reaction of **5a** with *m*-CPBA in a minimum amount of methylene chloride for 24-48 hours afforded the desired compound 2'-*tert*-butyldimethylsilyl-7-triethylsilyl-1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (**6a**) in 69% yield, along with the 11,12-epoxy analog

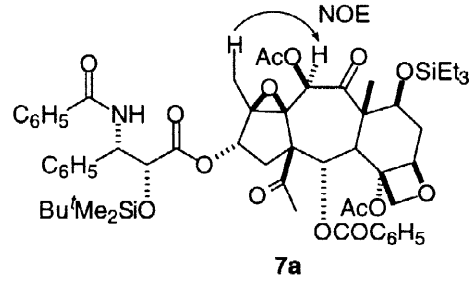
(**7a**) of **5a** in 16% yield. Subsequent deprotection of both products gave 1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (**8**) in 79% yield and 1-deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (**9**) in 65% yield (Scheme 1).

The structure of compound **6a** (and hence of the deprotected derivative **8**) was determined by ^{1}H NMR, ^{13}C NMR, TOCSY, HMQC, HMBC, and NOESY spectroscopy. The key information was the change in the ^{13}C NMR chemical shift of C-15 from 204.5 ppm to 170.1 ppm and of C-1 from 69.8 ppm to 93.6 ppm. HMQC and HMBC established the proton-carbon and carbon-carbon connectivity (Figure 1). The composition of the deprotected product **8** was confirmed by high resolution fast atom bombardment mass spectroscopy (HRFABMS), and its NMR spectra were fully consistent with the assigned structure.



6a

Figure 1

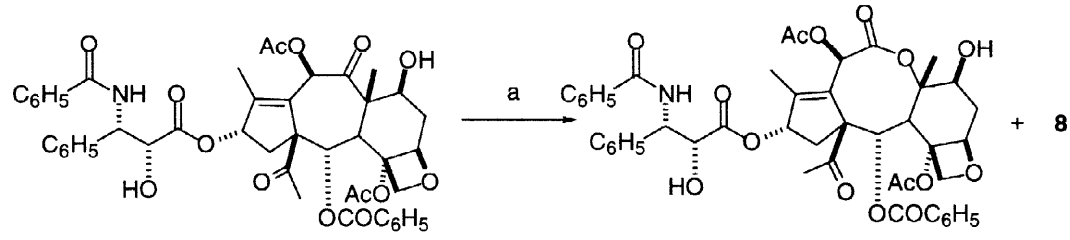


7a

Figure 2

The structure of compound **7a** was determined by ^{1}H NMR, APT, and TOCSY spectroscopy, and its composition was confirmed by HRFABMS. The chemical shifts of the protons at both C-10 and C-13, which were shifted upfield from 6.36 ppm to 5.76 ppm (C-10) and from 5.99 ppm to 5.30 ppm (C-13), were consistent with an oxidation reaction at the C-11,12 double bond. The ^{13}C NMR chemical shifts of both C-11 and C-12 were shifted from the olefinic region (133.3 ppm and 148.0 ppm) to the oxygenated region (two extra carbons observed between 70-84 ppm), confirming this assignment. The stereochemistry of the epoxide was determined by a NOESY experiment on the protected compound **7a**, in which a correlation of the C-18 methyl group and the C-10 proton was observed (Figure 2). The spectroscopic data of the deprotected product **9** were fully consistent with the assigned structure.

Synthesis of 1-deisopropenyl-1-acetyl-8,9-oxido-A-norpaclitaxel. Reaction of the unprotected compound 1-deisopropenyl-1-acetyl-A-*nor*paclitaxel (**10**), rather than the protected compound **5a**, with *m*-CPBA surprisingly gave a second product in addition to the expected product **8**. This second product was characterized as 1-deisopropenyl-1-acetyl-8,9-oxido-A-*nor*paclitaxel (**11**) and it was formed along with **8** in a ratio of about 3.7:1 (Scheme 2). Its ^{13}C NMR spectrum was similar to that of **8** in that both compounds had the same number of



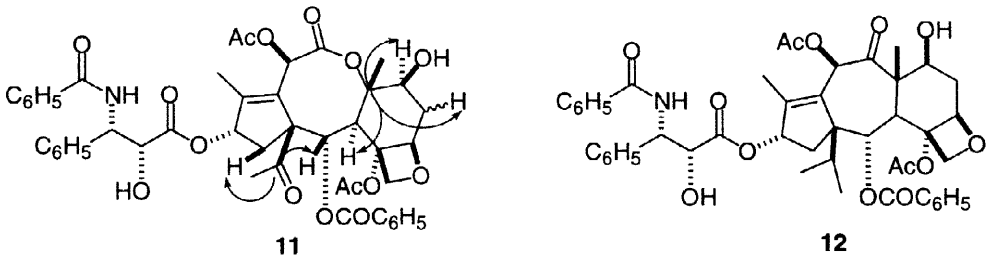
10 **11**

(a) mCPBA, CH_2Cl_2, 24-48 h

Scheme 2

carbonyl, ester carbonyl, quaternary carbon, and oxygenated quaternary carbon signals.

The structure of **11** was assigned unambiguously using a combination of ^{1}H, ^{13}C, DEPT, TOCSY, HMQC, and HMBC spectroscopic techniques, and its composition was confirmed by HRFABMS. Figure 3 shows the key HMBC (2-3 bond) correlations used to assign the resonance at 92.5 ppm to C-8, and thus to establish the location of the inserted oxygen atom.

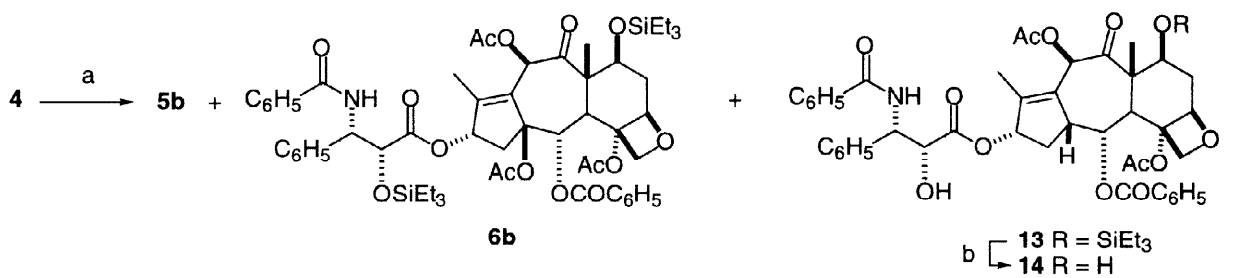


11 **12**

Figure 3

The formation of compound **11** was at first assumed to be due to an anchimeric effect of the free C-2' or C-7 hydroxyl groups. In order to test this assumption, and to prepare analogs of **11**, A-*nor*paclitaxel (**2**) was prepared and hydrogenated to afford 15,16-dihydro-A-*nor*paclitaxel (**12**). When **12** was treated with *m*-CPBA under the same conditions as were used with **10**, no reaction was observed. It thus appeared that the delivery of *m*-CPBA must be assisted by a cooperative effect of the C-15 keto group and the C-7 or the C-2' hydroxyl group.

Synthesis of 1-deisopropenyl-A-norpaclitaxel. As noted earlier, ozonolysis of the double bond of the C-1 isopropenyl group in the protected A-*nor*paclitaxel derivative **3** in methylene chloride followed by reduction with dimethyl sulfide (Me_2S) reproducibly gave a mixture of products among which the major product was found to be the desired keto-compound (**5a**) but only in the moderate yield of 60-70%. The minor products of the ozonolysis reaction of the 2',7-di-*O*-triethylsilyl-A-*nor*paclitaxel **4** in methylene chloride were also examined (Scheme 3). The reaction mixture was found to be unstable and two of its components were converted to two other products.



(a) O_3, CH_2Cl_2, -78 °C; then Me_2S, -78 °C to 25 °C; (b) HF/pyridine.

Scheme 3

These were isolated and identified as 2',7-di-*O*-triethylsilyl-1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (**6b**) and 7-*O*-triethylsilyl-1-deisopropenyl-A-*nor*paclitaxel (**13**) formed in 10% and 5% yields, respectively; the 2'-*O*-triethylsilyl group of **4** was lost in the formation of **13**. Compound **13** was subsequently desilylated to give 1-deisopropenyl-A-*nor*paclitaxel (**14**).

^{1}H NMR spectroscopy showed that the new compound **13** lacked a methyl peak corresponding to the C-17 methyl group and that it had an extra signal for one proton at 2.95 ppm. A TOCSY experiment using a 0.012 ms mixing time (for detection of coupling through 2-3 bonds) showed that the extra proton was coupled to the C-

14α proton at 1.67 ppm and the C-14β proton at 2.42 ppm, as well as to the C-2 proton at 5.60 ppm (Figure 4). ^{13}C NMR spectroscopy indicated the same loss of the C-17 methyl carbon signal at 25.5 ppm, as well as of the C-15 carbonyl carbon signal at 204.4 ppm. The C-1 carbon signal shifted from that of an oxygenated carbon at 69.4 ppm to that of a tertiary carbon at 47.4 ppm. Other signals were very similar to the corresponding signals in the spectrum of **4**.

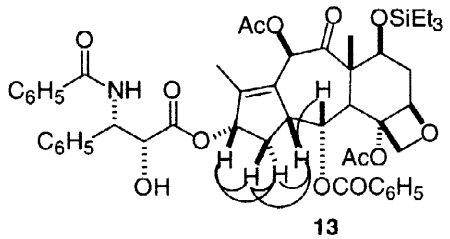


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Figure 4

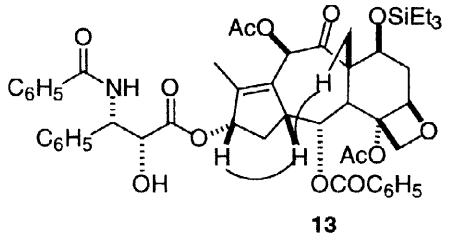

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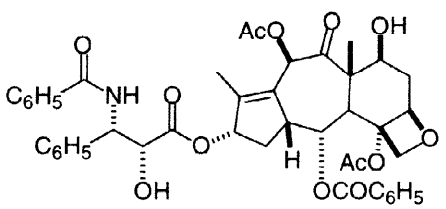
Figure 5

The composition of the new compound was confirmed by HRFABMS. A NOESY experiment using a 0.05 second mixing time and a 5 second delay time established the stereochemistry of the C-1 stereogenic center as shown in Figure 5, based on the observed correlation between the C-1 proton and the C-13 and C-19 protons. Based on this information, the structure of the new compound was assigned as 7-triethylsilyl-1-deisopropenyl-A-*nor*paclitaxel (**13**), and its deprotected analog was thus assigned the structure **14**.

The isolation and identification of compound **14** was significant in three ways. Firstly, this compound is the least sterically encumbered A-*nor*paclitaxel analog possible, and could thus possibly provide us with a sense of the effects of the spatial volume of C-1 substituents on the anticancer activities of these analogs. Secondly, compound **14** would have been very difficult to prepare by standard chemical reactions. Lastly, this compound is a reasonably close A-*nor* analog of the semi-synthetic 1-deoxydocetaxel analog **15**, which showed slightly weaker cytotoxicity than paclitaxel itself.[11] The cytotoxicity of **14** would thus enable a direct comparison to be made of the activity of the A-*nor* series in comparison with the normal series with the same substituent at C-1.



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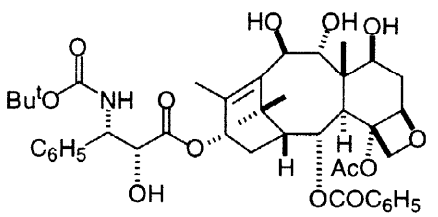
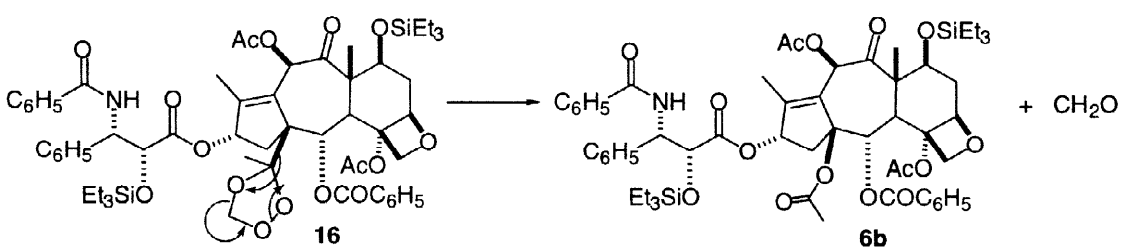


15

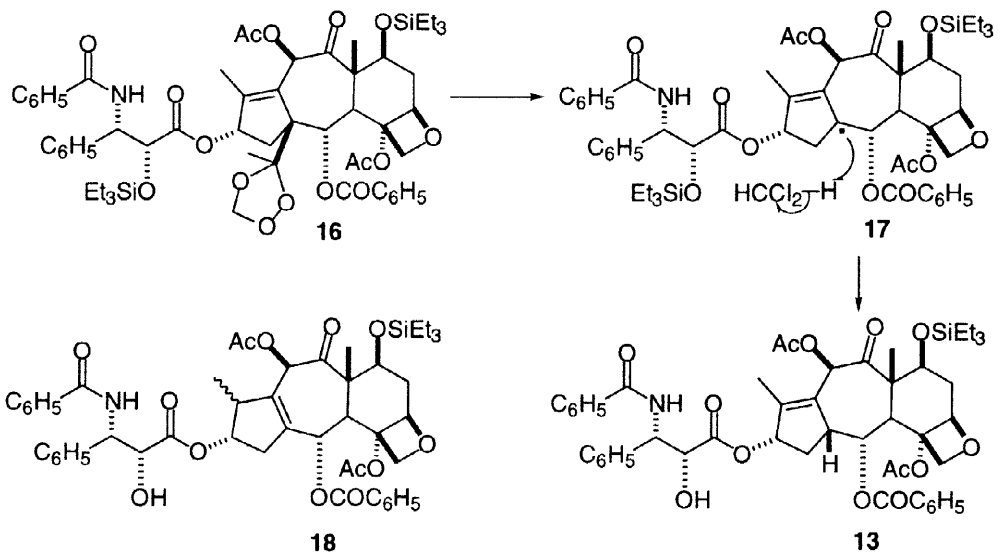
According to the thin layer chromatographic behavior of the reaction mixture following ozonization of **4** in methylene chloride, both compounds **6b** and **13** must be formed via an intermediate secondary ozonide such as **16**. Compound **6b** is presumably formed from the secondary ozonide **16** through the fragmentation shown in Scheme 4.



16 **6b**

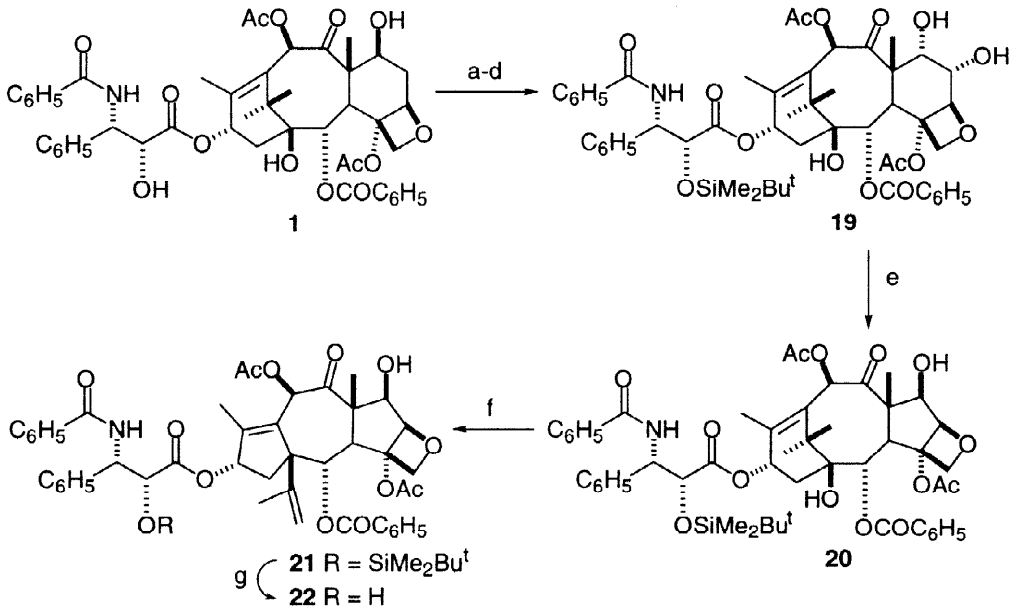
Scheme 4

The mechanism for the formation of **13** is less obvious. However, it could possibly arise from an allylic radical **17** which could be generated from **16** as shown in Scheme 5. Abstraction of a hydrogen atom from solvent (CH_2Cl_2) by **17** could then take place with concomitant loss of the 2'-*O*-triethylsilyl group to give **13**. This process is apparently regioselective, since none of the isomeric product **18** formed from intermediate radical **17** could be detected. This is surprising, because calculations indicated that **13** has a higher strain energy than **18**. The stereoselectivity may be controlled by kinetic factors in that the approach of a hydrogen donor from the less sterically hindered top face would be greatly favored, or alternatively a pathway involving participation of a neighboring group may be involved. It is certainly curious that the reduced analog **13** is isolated with the C-2' protecting group removed, while the co-produced oxidised product **6b** is obtained with the C-2' protecting group intact. Another pathway involving heterolytic cleavage of the C_1-C_{15} bond to give a tertiary allylic anion at C-1 could not be excluded, although it would be expected to be less energetically favorable.



Scheme 5

Synthesis of A-nor-C-norpaclitaxel. Contraction of the C-ring of paclitaxel has been observed in a number of structure modification studies.[12] In particular, C-*nor*paclitaxel was obtained when the carbon-carbon bond between C-6, C-7 of 2'-*O*-*tert*-butyldimethylsilyl-6α-hydroxy-7-*epi*paclitaxel (**19**) was cleaved oxidatively.[13] With the aim of examining the effects of modification of the ring skeleton on the anticancer activity of A-*nor*paclitaxel, we planned a seven-step synthesis of A-*nor*-C-*nor*paclitaxel. Thus, 2'-*O*-*tert*-butyldimethylsilyl-6α-hydroxy-7-epipaclitaxel (**19**) was made by the literature sequence in 75% yield and was treated with lead tetraacetate in the presence of sodium bicarbonate as a buffer to convert it to 2'-*O*-*tert*-butyldimethylsilyl-C-*nor*paclitaxel (**20**) in 50% yield.[14] Compound **20** was then subjected to the A-ring contraction conditions followed by desilylation to give the desired A-*nor*-C-*nor*paclitaxel (**22**) (Scheme 6).



(a)Bu^{t}Me$_2$SiCl, imidazole, DMF, 95%; (b) CF_3SO_2Cl, DMAP, CH_2Cl_2, 98%; (c) DBU, CH_2Cl_2, 40 °C, 95%; (d) OsO_4, NMO, acetone/H_2O, 84%; (e) $Pb(OAc)_4$, $NaHCO_3$, CH_2Cl_2, 0 °C, 50%; (f) $SOCl_2$, pyridine, CH_2Cl_2, 90%; (g) HF/pyridine, THF, 83%.

Scheme 6

Biological Evaluation of Selected A-norpaclitaxel Analogs

The new A-*nor*paclitaxel analogs **8**, **11**, **14**, and **22** were evaluated in a tubulin-assembly assay using microtubular protein (tubulin unresolved from microtubule-associated proteins) at 37 °C and in the HCT 116 cytotoxicity assay. The results are summarized in Table 1, together with the corresponding data for paclitaxel and the 1-deoxydocetaxel analog **15** for comparison.

All of the new A-*nor*paclitaxel analogs except the B-ring lactone **11** were less active than paclitaxel in the tubulin assembly assay, a feature also observed with other A-*nor*paclitaxels prepared previously.[15] In particular, reduction of the size of the group at the C-1 position did not give rise to an increased activity, as had been hoped. Thus compound **14**, with a hydrogen at C-1, was as inactive as compound **8** with an acetoxyl group at this position. The only activity observed (and then only in the tubulin-assembly assay) was for compounds **11** and **22** with modified B and C-rings respectively. It thus appears that the substituent at C-1 is of relatively minor significance in this series, as has also been observed with paclitaxel analogs.[11] On the other hand, the nature of the ring system does make a significant difference to the tubulin-assembly activity, with both a larger B-ring and a smaller C-ring giving compounds with comparable tubulin-assembly activity to paclitaxel.

None of the compounds prepared in this series showed any significant cytotoxicity towards the HCT 116 cell line, suggesting that tubulin-assembly activity is a necessary but not sufficient criterion for cytotoxicity. This is consistent with a recent suggestions that paclitaxel has two sites of action, leading to the induction of two separate apoptotic pathways, and with the finding that paclitaxel binds to human Bcl-2 as a second molecular target.[16]

Table 1. Biological evaluation of A-*nor*paclitaxels

Compounds	Tubulin Assembly Activity (μM)[a]	Cytotoxicity (nM)[b]
paclitaxel	5.8 ± 0.6	1.50
8	>1000	>117
11	5.3 ± 0.8	>117
14	>1000	>125
15[c]	9.1 ± 1.4	3.0
22	9.2 ± 2.0	121.7

[a] $EC_{0.01}$ for polymerization of tubulin.

[b] IC_{50} for cytotoxicity to HCT 116 cell line.

[c] Data from reference 10a.

EXPERIMENTAL SECTION

General Experimental Methods. Unless otherwise noted, all materials were used as received from a commercial supplier without further purification. All anhydrous reactions were performed in oven-dried glassware under argon. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone. Anhydrous toluene was distilled from sodium. Dichloromethane was distilled from calcium hydride. All reactions were monitored by E. Merck analytical thin layer chromatography (TLC) plates (silica gel 60 GF, aluminum back) and analyzed with 254 nm UV light and/or vanillin/sulfuric acid spray. Silica gel for column chromatography was purchased from E. Merck (230-400 mech). Preparative thin layer chromatography (PTLC) plates (silica gel 60 GF) were purchased from Analtech. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Varian Unity 400 spectrometer (operating at 399.951 MHz for ^1H and 100.578 MHz for ^{13}C) or a Bruker WP 360 spectrometer (operating at 360.140 MHz for ^1H and 90.562 MHz for ^{13}C), and were assigned by comparison of chemical shifts and coupling constants with those of related compounds and by appropriate 2D-NMR techniques. All 2D-NMR spectra were obtained on the Varian Unity 400 spectrometer. Chemical shifts were reported as δ-values relative to tetramethylsilane (TMS) as internal reference, and coupling constants were reported in Hertz. Mass spectra (LRFABMS/HRFABMS) were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska.

2′-*O*-(*tert*-Butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (6a) and 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (7a) - A solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-A-*nor*-paclitaxel (**5a**, 250 mg, 0.235 mmol), *meta*-chloroperoxybenzoic acid (57-86%, 175 mg, ~0.7 mmol), and sodium bicarbonate (44 mg, 0.52 mmol) in dichloromethane (5 mL) was stirred at room temperature for 18 hours. The reaction mixture was diluted with EtOAc and washed with saturated sodium sulfite (Na_2SO_3), water, and brine. The organic layer was combined and dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 3:7) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (**6a**, 151 mg, 69%) and 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-triethylsilyl-1-deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (**7a**, 35 mg, 16%). Compound **6a**: amorphous solid; ^1H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.14 (d, J = 7.2, 2H), 7.67 (d, J = 7.2, 2H), 7.56-7.20 (m, 9H), 6.97 (d, J = 8.8, 1H), 6.84 (m, 2H), 6.48 (s, 1H), 5.99 (m, 1H), 5.80 (d, J = 9.6, 1H), 5.17 (d, J = 8.4, 1H), 4.86 (d, J = 9.2, 1H), 4.52 (d, J = 8.8, 1H), 4.43(d, J = 8.4, 1H), 4.43(d, 1H), 4.25 (d, J = 2.0, 1H), 3.21 (d, J = 10.0, 1H), 2.57-2.55 (m, 2H), 2.15 (s, 3H), 2.11 (m,

1H), 1.95 (s, 3H), 1.76 (s, 3H), 1.75 (s, $2CH_3$), 0.94 (t, 9H), 0.78 (s, 9H), 0.58 (q, 6H), -0.14 (s, 3H), -0.31 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 199.1, 171.1, 170.1, 169.5, 169.0, 166.7, 164.6, 147.5, 138.3, 134.3, 133.7, 131.6, 131.6, 130.3, 129.5, 128.6, 128.3, 127.6, 127.0, 126.5, 93.6, 84.4, 79.5, 79.4, 77.2, 75.0, 74.6, 71.9, 71.1, 69.9, 55.7, 54.9, 44.6, 37.6, 37.6, 25.5, 22.0, 21.3, 20.3, 18.2, 12.8, 10.0, 6.9, 5.2, -5.4, -5.8. Compound **7a**: amorphous solid; ^1H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.09 (d, J = 7.2, 2H), 7.69 (d, J = 7.2, 2H), 7.56-7.24 (m, 11H), 7.16 (d, J = 8.8, 1H, NH), 6.16 (d, J = 10.4, 1H), 5.76 (s, 1H), 5.51 (dd, J = 8.4,2.1, 1H), 5.30 (t, J = 8.0, 1H), 5.07 (d, J = 8.8, 1H), 4.60 (dd, J = 10.0,6.8, 1H), 4.44 (d, J = 2.0, 1H), 4.39 (d, J = 8.4, 1H), 4.26 (d, J = 8.4, 1H), 3.95 (d, J = 10.4, 1H), 2.59 (m, 1H), 2.34 (s, 3H), 2.32 (s, 3H), 2.17 (m, 1H), 2.10(s, 3H), 1.89 (m, 1H), 1.74 (m, 1H), 1.64 (s, 3H), 1.19 (s, 3H), 0.91 (t, 9H), 0.73 (s, 9H), 0.55 (q, 6H), -0.30 (s, 3H), -0.45 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 199.1, 171.1, 170.1, 169.5, 169.0, 166.7, 164.6, 147.5, 138.3, 134.3, 133.7, 131.6, 131.6, 130.3, 129.5, 128.6, 128.3, 127.6, 127.0, 126.5, 93.6, 84.4, 79.5, 79.4, 77.2, 75.0, 74.6, 71.9, 71.1, 69.9, 55.7, 54.9, 44.6, 37.6, 37.6, 25.5, 22.0, 21.3, 20.3, 18.2, 12.8, 10.0, 6.9, 5.2, -5.4, -5.8.

1-Deisopropenyl-1-acetoxyl-A-*nor*paclitaxel (8) - A solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-(triethylsilyl)-1-deisopropenyl-1-acetoxyl-A-*nor*paclitaxel (**6a**, 20 mg, 0.019 mmol) in 3% HCl/MeOH (1.0 mL) was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layers were combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, i-PrOH:CH_2Cl_2:hexanes 6:47:47) to afford 1-deisopropenyl-1-acetoxyl-A-*nor*paclitaxel (**8**, amorphous solid, 12.5 mg, 79%). ^1H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.04 (dd, J = 8.0,2.0, 2H), 7.64 (d, J = 7.2, 2H), 7.48-7.25 (m, 9H), 7.02 (dd, J = 8.0,2.0, 2H), 6.62 (d, J = 8.8, 1H), 6.24 (s, 1H), 5.87 (br d, J = 8.4, 1H), 5.76 (d, J = 10.0, 1H), 5.08 (dd, J = 8.8,2.8, 1H), 4.84 (d, J = 9.2, 1H), 4.68 (d, J = 8.8, 1H), 4.46 (d, J = 8.8, 1H), 4.42 (m, 1H), 4.35 (dd, J = 6.0,2.8, 1H), 3.29 (d, J = 6.0, 1H), 3.14 (d, J = 10.4, 1H), 2.60 (m, 1H), 2.57 (m, 1H), 2.39 (d, J = 3.6, 1H), 2.21 (m, 1H), 2.20 (s, 3H), 1.95 (s, 3H), 1.91 (m, 1H), 1.79 (s, 3H), 1.78 (s, 3H), 1.39 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 202.1, 172.9, 171.7, 170.1, 168.8, 166.8, 164.6, 46.4, 137.6, 133.8, 133.7, 131.8, 129.9, 129.5, 129.3, 128.61, 128.60, 128.5, 128.0, 127.2, 126.9, 93.4, 84.7, 81.3, 79.7, 74.4, 72.7, 71.7, 71.4, 70.5, 55.15, 55.12, 44.5, 37.1, 34.7, 22.1, 21.0, 20.5, 13.4, 9.6. HRFABMS *m/z* calcd for $C_{46}H_{47}NO_{15}$ $(M+H)^+$ 854.3024, found 854.3024, error 0.0 ppm.

1-Deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (9) - To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-7-*O*-(triethylsilyl)-1-deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (**7a**, 6.8 mg, 0.0063 mmol) in THF (0.3 mL) was added HF-pyridine (70%, 100 μL) and the solution was stirred at room temperature for 3 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layers were combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500μ, EtOAc:hexanes 6:4) to afford 1-deisopropenyl-1-acetyl-11,12-epoxy-A-*nor*paclitaxel (**9**, amorphous solid, 3.5 mg, 65%). ^1H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.12 (d, J = 7.2, 2H), 7.67-7.24 (m, 13H), 6.67 (d, J = 8.8, 1H), 6.23 (d, J = 11.2, 1H), 5.84 (s, 1H), 5.44 (dd, J = 8.0,2.4, 1H), 5.22 (t, J = 8.0, 1H), 5.05 (d, J = 9.6, 1H), 4.69 (m, 1H), 4.62 (br s, 1H), 4.35 (br s, 2H), 3.83 (d, J = 11.2, 1H), 2.62 (m, 1H), 2.35 (s, 3H), 2.21 (s, 3H), 2.20 (m, 1H), 2.19 (s, 3H), 1.93 (m, 1H), 1.79 (m, 1H), 1.59 (s, 3H), 1.53 (s, 3H). HRFABMS *m/z* calcd for $C_{46}H_{47}NO_{15}$ $(M+H)^+$ 854.3024, found 854.3001, error 2.7 ppm.

1-Deisopropenyl-1-acetyl-8,9-oxido-A-*nor*paclitaxel (11) - A solution of 1-deisopropenyl-1-acetyl-A-*nor*paclitaxel (**10**, 32 mg, 0.038 mmol) and *meta*-chloroperoxybenzoic acid (*m*-CPBA, 70%, 83 mg) in CH_2Cl_2

(0.3 mL) was stirred at room temperature for 48 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layers were combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 7:3) to afford a mixture of two products, which was further separated by preparative TLC (silica gel, 1000μ, MeOH:CH_2Cl_2 5:95) to afford 1-deisopropenyl-1-acetyl-8,9-oxido-A-*nor*paclitaxel (**11**, amorphous solid, 22 mg, 69%) and 1-deisopropenyl-1-acetoxyl-A-*nor*paclitaxel (**8**, amorphous solid, 6.0 mg, 19%). Compound **11**: ^{1}H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 7.91 (d, J = 7.2, 2H), 7.84 (d, J = 7.2, 2H), 7.60-7.32 (m, 11H), 7.21 (d, J = 8.8, 1H), 6.65 (d, J = 6.4, 1H), 6.00 (m, 1H), 5.81 (s, 1H), 5.80 (m, 1H), 4.90 (d, J = 8.8, 1H), 4.75 (dd, J = 2.4,2.4, 1H), 4.43 (d, J = 8.4, 1H), 4.20-4.12 (m, 3H), 2.95 (m, 2H), 2.63 (m, 1H), 2.47 (br s, 1H), 2.26 (s, 6H), 2.07 (s, 3H), 2.02 (s, 3H), 1.94-1.84 (m, 2H), 1.53 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 204.8, 171.9, 171.2, 169.5, 169.3, 166.6, 165.1, 149.2, 138.5, 133.72, 133.67, 132.0, 131.8, 129.7, 129.1, 128.8, 128.71, 128.68, 128.2, 127.1, 127.0, 92.5, 84.3, 82.1, 80.5, 74.5, 74.3, 72.9, 71.3, 69.2, 67.6, 55.0, 48.6, 35.5, 34.8, 25.5, 22.3, 20.2, 14.2, 12.4. HRFABMS *m/z* calcd for $C_{46}H_{47}NO_{15}$ $(M+Na)^+$ 876.2843, found 876.2819, error 2.8 ppm.

Ozonolysis of 2′,7-*O*-bis-triethylsilyl-A-*nor*paclitaxel (4) in methylene chloride - Ozone generated from a micro-ozonizer was carried by oxygen and passed through a solution of 2′,7-bis-*O*-(triethylsilyl)-A-*nor*paclitaxel (**4**, 237 mg, 0.22 mmol) in anhydrous dichloromethane (10 mL) pre-cooled to –78 °C for 12 minutes. The solution was purged with oxygen gas for 30 minutes. Dimethyl sulfide (Me_2S, 1 mL, excess) was then added at –78 °C and the mixture was warmed up to room temperature and stirred for 30 minutes. The solvent was evaporated under reduced pressure and the residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 3:7) to afford 2′,7-bis-*O*-(triethylsilyl)-1-deisopropenyl-1-acetyl-A-*nor*paclitaxel (**5b**, 124 mg, 52%), 2′,7- bis-*O*-(triethylsilyl)-1-deisopropenyl-1-acetoxy-A-*nor*paclitaxel (**6b**, 25 mg, 11%), and 7-*O*-(triethylsilyl)-1-deisopropenyl-A-*nor*paclitaxel (**13**, 11 mg, 5%). Compound **13**: amorphous solid; ^{1}H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 7.99 (dd, J = 8.0,1.6, 2H), 7.65 (dd, J = 8.8,1.6, 2H), 7.47-7.21 (m, 9H), 6.90 (dd, J = 8.0, 1.6, 2H), 6.59 (br s, 1H), 6.51 (d, J = 8.8, 1H), 5.61 (m, 2H), 4.98 (dd, J = 8.8,2.8, 1H), 4.79 (d, J = 8.4, 1H), 4.70 (d, J = 8.4, 1H), 4.48 (m, 2H), 3.98 (s, 1H), 3.16 (br d, J = 3.2, 1H), 3.02 (d, J = 9.6, 1H), 2.95 (m, 1H), 2.54 (m, 1H), 2.41 (m, 1H), 2.17 (s, 3H), 1.84 (m, 1H), 1.73 (s, 3H), 1.66 (m, 1H), 1.59 (s, 3H), 1.33 (s, 3H), 0.92 (t, 9H), 0.56 (q, 6H).

1-Deisopropenyl-A-*nor*paclitaxel (14) - To a solution of 7-*O*-(triethylsilyl)-1-deisopropenyl-A-*nor*paclitaxel (**13**, 10 mg, 0.011 mmol) in dry THF (0.7 mL) was added HF-pyridine (70%, 200 μL) and the solution was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layers were combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford 1-deisopropenyl-A-*nor*paclitaxel (**14**, amorphous solid, 7.5 mg, 86%). ^{1}H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.01 (dd, J = 8.0,1.6, 2H), 7.65 (dd, J = 8.8,1.6, 2H), 7.47-7.19 (m, 9H), 6.90 (dd, J = 8.0, 1.6, 2H), 6.49 (d, J = 8.8, 1H), 6.27 (s, 1H), 5.64 (m, 2H), 5.00 (dd, J = 8.8,2.8, 1H), 4.85 (d, J = 8.8, 1H), 4.70 (d, J = 8.8, 1H), 4.52 (d, J = 8.8, 1H), 4.52 (m, 1H), 4.01 (s, 1H), 3.20 (br s, 1H), 3.08 (d, J = 9.6, 1H), 2.92 (m, 1H), 2.56 (m, 1H), 2.41 (m, 1H), 2.21 (s, 3H), 1.88 (m, 1H), 1.84 (s, 3H), 1.70 (m, 1H), 1.61 (s, 3H), 1.29 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 205.3, 173.1, 171.6, 170.1, 166.8, 165.2, 141.2, 137.8, 133.9, 133.4, 131.7, 130.0, 129.8, 129.6, 128.6, 129.5, 128.4, 127.8, 127.2, 126.9, 84.3, 82.8, 80.0, 74.6, 73.1, 72.5, 70.2, 69.3, 55.2, 54.9, 47.6, 45.5, 34.8, 29.6, 20.8, 20.5, 13.2, 9.4. HRFABMS *m/z* calcd for $C_{44}H_{45}NO_{13}$ $(M+Na)^+$ 818.2789, found 818.2791, error -0.4 ppm.

2′-*O*-(*tert*-Butyldimethylsilyl)-A-*nor*-C-*nor*paclitaxel (21) - To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-C-*nor*paclitaxel (**20**, 36 mg, 0.038 mmol) in dry CH_2Cl_2 (1.5 mL,) was added anhydrous pyridine (72 μL, 0.89 mmol) followed by thionyl chloride ($SOCl_2$) at room temperature. The solution was stirred for 20 minutes. The reaction mixture was then diluted with EtOAc and washed with dilute HCl (1N). The organic layers were combined and washed with dilute sodium bicarbonate, water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 5:5) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-A-*nor*-C-*nor*paclitaxel (**21**, amorphous solid, 20 mg, 57%). ^{1}H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.15 (d, J = 7.6, 2H), 7.82 (d, J = 7.2, 2H), 7.56-7.29 (m, 11H), 7.09 (d, J = 9.2, 1H), 6.74 (d, J = 9.6, 1H), 6.04 (t, J = 8.8, 1H), 5.98 (s, 1H), 5.73 (d, J = 9.2, 1H), 5.19 (d, J = 7.2, 1H), 5.02 (s, 1H), 4.84 (d, J = 10.4, 1H), 4.78 (s, 1H), 4.68 (d, J = 7.2, 1H), 4.56 (d, J = 2.4, 1H), 4.41 (d, J = 10.4, 1H), 3.38 (d, J = 9.6, 1H), 2.30 (m, 1H), 2.19 (m, 1H), 2.13 (s, 3H), 2.07 (s, 3H), 1.98 (s, 3H), 1.80 (s, 3H), 1.57 (s, 3H), 0.77 (s, 9H), -0.11 (s, 3H), -0.33 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 201.8, 170.8, 170.3, 168.8, 166.5, 165.5, 146.3, 143.1, 138.7, 134.6, 134.1, 133.5, 131.6, 130.2, 129.3, 128.7, 128.6, 128.5, 127.7, 127.0, 126.6, 111.2, 87.8, 81.1, 79.7, 78.4, 77.2, 75.6, 73.0, 69.9, 62.8, 58.1, 55.6, 44.9, 39.2, 25.5, 21.5, 20.48, 20.44, 18.2, 11.9, 11.7, -5.6, -6.1.

A-*nor*-C-*nor*paclitaxel (22) - To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-A-*nor*-C-*nor*paclitaxel (**21**, 18 mg, 0.019 mmol) in dry THF (1 mL) was added HF-pyridine (70%, 240 μL) and the solution was stirred at room temperature for 1.5 hours. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate, the organic layers were combined and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford A-*nor*-C-*nor*paclitaxel (**22**, amorphous solid, 12 mg, 76%). ^{1}H NMR ($CDCl_3$, TMS, 399.951 MHz) δ 8.10 (d, J = 8.4, 2H), 7.81 (dd J = 8.0,1.6, 2H), 7.57-7.27 (m, 11H), 7.06 (d, J = 9.6, 1H), 6.99 (s, 1H), 6.74 (d, J = 9.6, 1H), 5.96 (m, 1H), 5.94 (s, 1H), 5.80 (br d, J = 9.2, 1H), 5.14 (d, J = 4.8, 1H), 5.01 (s, 1H), 4.80 (m, 2H), 4.68 (dd, J = 3.6,2.0, 1H), 4.57 (m, 1H), 4.40 (d, J = 8.4, 1H), 3.65 (d, J = 3.6, 1H), 3.35 (d, J = 9.6, 1H), 2.50 (d, J = 6.8, 1H), 2.43 (dd, J = 15.2,8.0, 1H), 2.19 (dd, J = 15.2,6.0, 1H), 2.07 (br s, 6H), 1.95 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H). ^{13}C NMR ($CDCl_3$, TMS, 100.578 MHz) δ 201.7, 172.1, 170.5, 168.8, 166.4, 165.6, 146.1, 142.5, 138.5, 134.9, 134.0, 133.6, 131.8, 130.0, 129.3, 128.8, 128.7, 128.6, 128.0, 127.0, 126.8, 111.3, 87.8, 81.5, 81.3, 78.3, 76.9, 73.9, 73.0, 69.8, 63.3, 58.2, 54.6, 44.9, 39.4, 21.5, 20.45, 20.43, 11.9, 11.7. HRFABMS *m/z* calcd for $C_{47}H_{48}NO_{13}$ $(M+H)^+$ 822.3126, found 822.3129, error -0.4 ppm.

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References

1 Present address: Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

2. For a review of the development and clinical utility of the taxane class of anticancer agents, see Rowinsky, E. K. *Annu. Rev. Med.* **1997**, *48*, 353-374.

3. For reviews of the chemistry and structure-activity relationships of paclitaxel see: (a) Kingston, D. G. I. *Pharmac. Ther.* **1991**, *52*, 1-34. (b) Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222-227. (c) Chen, S.-H.; Farina, V. Paclitaxel Structure-Activity Relationships and Core Skeletal Rearrangements. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; and Vyas, D. M., Eds.; ACS Symposium Series 583, American Chemical Society: Washington, DC, 1994; pp. 247-261. (d) Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. The Medicinal Chemistry of Taxol. In *Taxol: Science and Applications*; Suffness, M.; Ed.; CRC Press, Inc.: Boca Raton, FL, 1995; pp. 317-375. (e) Kingston, D. G. I. Recent Advances in the Chemistry and Structure-Activity Relationships of Paclitaxel. In *Taxane Anticancer Agents: Basic Science and Current Status*; George, G. I.; Chen, T. T.; Ojima, I.; and Vyas, D. M., Eds.; ACS Symposium Series 583, American Chemical Society: Washington, DC, 1994; pp. 203-216. (f) Nicolaou, K. C.; Dai, W.-M.; Guy, R. K.; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15-44. (g) Vyas, D. M.; Kadow, J. F. In *Progress in Medicinal Chemistry*; Ellis, G. P.; Luscombe, D. K., Ed.; Elsevier Science B. V.: Amsterdam, 1995; Vol. 32; pp. 289-337. (h) Guénard, D.; Guéritte-Voegelein, F.; Lavelle, F. *Curr. Pharm. Design* **1995**, *1*, 95-112.
4. Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114-5119.
5. (a) Wahl, A.; Guéritte-Voegelein, G.; Guénard, D. *Tetrahedron* **1992**, 48, 6965-6974. (b) Chen, S. H.; Huang, S.; Wei, J.; Farina, V. *J. Org. Chem.* **1993**, *58*, 4520-4521.
6. Chordia, M. D.; Kingston, D. G. I.; Hamel, E.; Lin, C. M.; Long, B. H.; Fairchild, C. A.; Johnston, K. A.; Rose, W. C. *Bioorg. Med. Chem.* **1997**, *5*, 941-947.
7. Kingston, D. G. I.; Chaudhary, A. G.; Chordia, M. D.; Gharpure, M.; Gunatilaka, A. A. L.; Higgs, P. I.; Rimoldi, J. M.; Samala, L.; Jagtap, P. G.; Giannakakou, P.; Jiang, Y. Q.; Lin, C. M.; Hamel, E.; Long, B. H.; Fairchild, C. A.; Johnston K. A *J. Med. Chem.* **1998**, *41*, 3715-3726.
8. Chordia, M. D.; Kingston, D. G. I. *Tetrahedron* **1997**, *53*, 5699-5710.
9 Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 11650-11651.
10. (a) Cooper, M. S.; Heaney, H.; Newbold, A. J.; and Sanderson, W. R. *Synlett* **1990**, 533-535. (b) Jackson, W. P. *Synlett* **1990**, 536-536.
11. Kingston, D. G. I.; Chordia, M. D.; Jagtap, P. G.; Liang, J.; Shen, Y.-C.; Long, B. H.; Fairchild, C. R.; Johnston, K. A. *J. Org. Chem.*, **1999**, *64*, 1814-1822.
12. Chen, S. H.; Huang, S.; Roth, G. P. *Tetrahedron Lett.* **1995**, *36*, 8933-8936.
13. Liang, X.; Kingston, D. G. I.; Lin, C. M.; Hamel, E. *Tetrahedron Lett.* **1995**, *36*, 2901-2904.
14. Liang, X.; Kingston, D. G. I.; Long, B. H.; Fairchild, C. A.; Johnston, K. A. *Tetrahedron* **1997**, *53*, 3441-3456.
15. Chordia, M. D.; Kingston, D. G. I.; Hamel, E.; Lin, C. M.; Long, B. H.; Fairchild, C. A.; Johnston, K. A.; Rose, W. C. *Bioorg. Med. Chem.* **1997**, *5*, 941-947.
16. Rodi, D. J.; Janes, R. W.; Sanganee, H. J.; Holton, R. A.; Wallace, B. A.; Makowski, L. *J. Mol. Biol.* **1999**, *285*, 197-203.