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Synthesis and Biological Activity of a Novel C4-C6 Bridged Paclitaxel Analog

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Abstract: The novel paclitaxel analog **15** with a bridge between the C-4 and C-6 positions was synthesized and was found to be less active than paclitaxel in *in vitro* tubulin assembly and cytotoxicity assays.

Keywords: taxoids; macrocycles; lactonization; biologically active compounds

The importance of paclitaxel (**1**) as an exciting anticancer agent[2] has stimulated extensive chemical, biological and medicinal research,[3] and a number of research groups have attempted to prepare improved analogs for clinical use. To date, however, the only analog that has been approved is docetaxel (**2**).[4] Since paclitaxel is known to bind to and stabilize polymerized tubulin,[5] an attractive approach to developing an improved analog would be by determining the nature of the microtubule-bound conformation of paclitaxel. The structure of the αβ-tubulin dimer has been determined by electron crystallography at a resolution of 4Å,[6] and this has been built into a 3.7 Å map incorporating a molecule of docetaxel,[7] but the electron density maps were unfortunately not clear enough in the docetaxel/paclitaxel region to definitively determine the microtubule-bound structure of the drug. Various indirect approaches have also provided important information on the nature of the paclitaxel binding site. These include the study of the photoaffinity labeling of tubulin by paclitaxel,[8] the study of the fluorescence of paclitaxel analogs bound to microtubules,[9] and the study of paclitaxel bound to microtubules by NMR methods.[10] On the other hand, conformational analysis of paclitaxel using NMR techniques has also indicated that a "hydrophobic collapse" conformation is predominant in polar solvents.[11] In spite of this impressive body of work, the actual conformation of paclitaxel in the bound state on microtubules remains unknown.

An alternate approach to gleaning information on the nature of the paclitaxel-microtubule interaction is by the synthesis of specific analogs of paclitaxel and the determination of their biological activity. In this way the pharmacophore of paclitaxel can in principle be deduced. The C-4 acetate of paclitaxel has been shown to be

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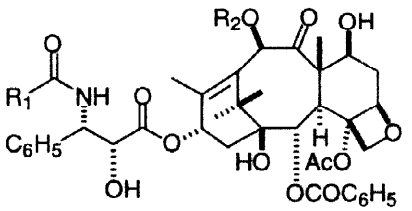
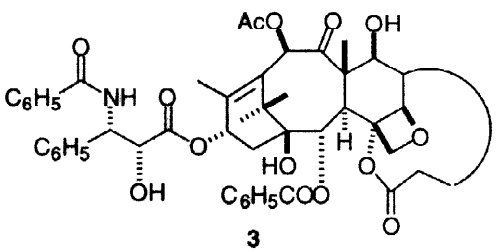
important to its activity, [12] but little is known about the active conformation of the C-4 acetate or about the effect of groups constrained to lie underneath ring C. It was thus our plan to link the C-4 acetyl group to another part of the molecule so that the conformation of the C-4 acyl group would be constrained. The conformation of this constrained compound (**3**) could then be studied through NOESY experiments and modeling. The correlation of its conformation and activity data would be very useful in revealing the interaction of the C-4 acetate and the lower face of the C-ring and microtubules.



1 $R_1 = C_6H_5$, $R_2 = Ac$
2 $R_1 = Me_3CO$, $R_2 = H$



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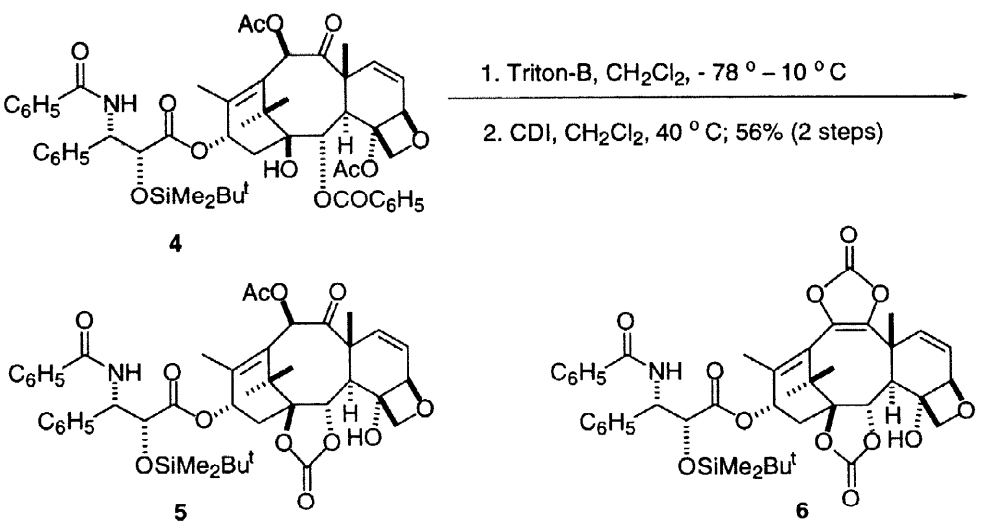
RESULTS AND DISCUSSION

Using a recently developed mild C-4 acylation method under acid/DCC/DMAP conditions, synthetic efforts were made to tether the C-4 acyl group to the C-6 position, which provided a convenient anchor to which to lock the C-4 acyl group. Functionality at C-6 was obtained by dihydroxylation of 7-deoxy-6,7-dehydropaclitaxel[13] (**4**), since modifications at the C-6α hydroxyl group of paclitaxel were known to have only a modest effect on its activity.[13] Because of this any change in the activity of the proposed tethered analog could be attributed primarily to any conformational change at the C-4 acyl group or to the effect of a substituent on the α-face of ring C.

In earlier studies we prepared 6,7-dehydro paclitaxel (**4**) in high yield in three steps.[14] Treatment of compound **4** with Triton-B in methylene chloride sequentially removed the C-2 benzoyl and the C-4 acetyl groups, as previously observed with the protected paclitaxel counterpart.[15] The crude product was subjected directly to reaction with N,N′-carbonyldiimidazole (CDI) without purification to protect the 1,2-diol as its cyclic carbonate **5** (Scheme 1).

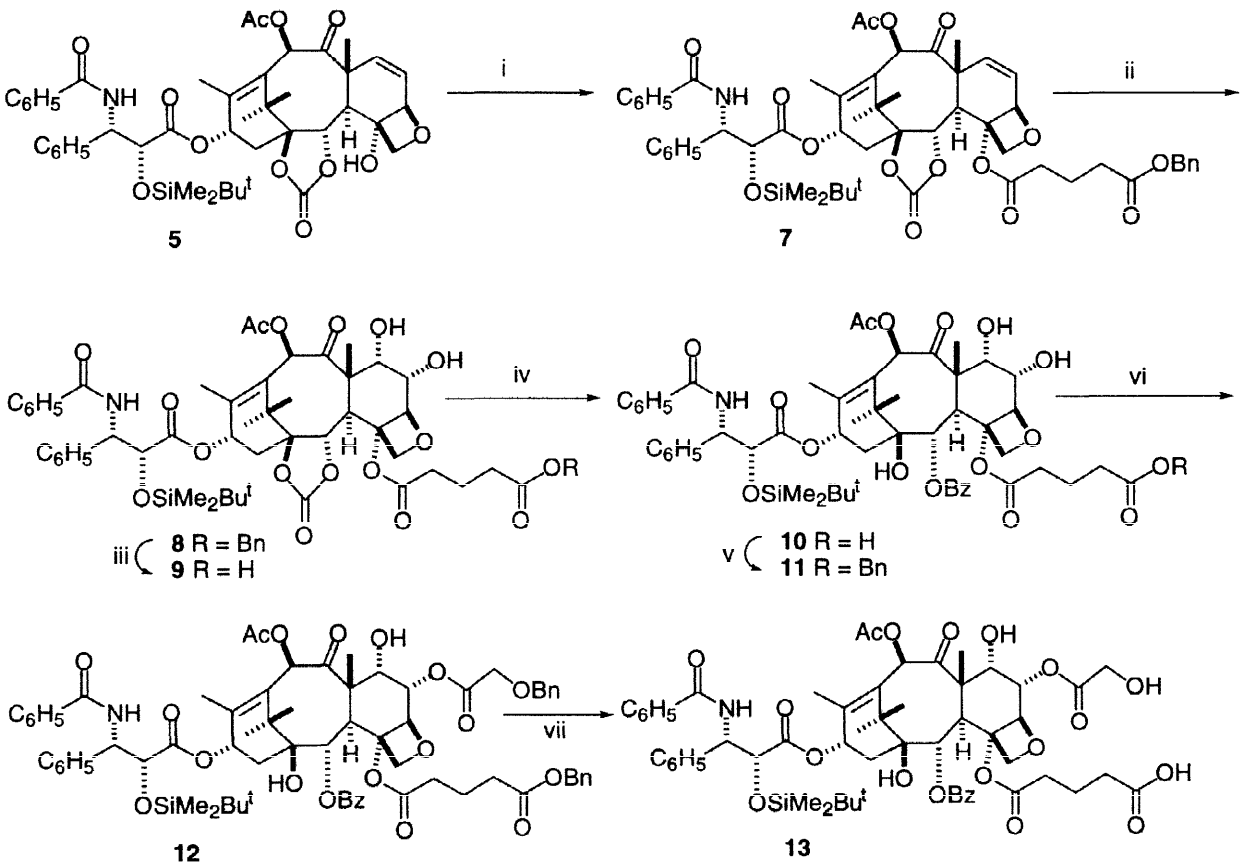


4 **5** **6**

Scheme 1

Interestingly, the dicarbonate **6** was also isolated along with the desired product **5** in a ratio of about 1:2.5. Compound **6** must be formed via a 10-deacetyl intermediate,[16] presumably formed during the Triton-B reaction. Deacetylation of paclitaxel itself has not been observed by us with Triton-B; the increase in reactivity of the C-10 acetate toward hydrolysis may be due to conformational changes caused by the 6,7 olefin in **4** which could decrease steric hindrance at the C-10 position.

Having converted **4** to **5** in two steps in reasonably good yield, C-4 acylation was effected using monobenzyl glutarate under DCC/DMAP conditions, affording the new compound **7** in 86% yield (Scheme 2). The ^{1}H NMR spectrum of **7** showed the presence of a broad singlet for the benzyl protons at 5.08 ppm, along with the methylene protons of the glutaryl moiety at about 2.74-1.78 ppm. Purification of **7** was difficult due to the excess DCC used for the coupling, but flash column chromatography on silica gel provided a mixture containing DCC/DCU that was suitable for the dihydroxylation reaction shown in Scheme 2, in which a more polar product was formed that was easier to purify.



i) $PhCH_2OOC(CH_2)_3COOH$, DCC, DMAP, toluene, 86%; ii) OsO_4, NMO, THF/H_2O, 81%; iii) H_2, Pd/C, EtOAc, 87%; iv) PhLi, THF, -78 °C,78%; v) $PhCH_2OH$, DCC, DMAP, toluene, 94%; vi) $BnOCH_2COCl$, DMAP, CH_2Cl_2, 85%; vii) H_2, Pd/C, EtOAc, 71%.

Scheme 2

The C-4 acylated olefin **7** was treated with OsO_4 and N-methylmorpholine-N-oxide (NMO) in THF/H_2O for 24 h, affording a more polar product in 81% yield. ^{1}H NMR data of the product **8** was typical for the desired transformation in that the signal of the C-6 olefinic proton at 6.15 ppm shifted to 4.22 ppm, and the signal of the C-7 olefinic proton at 6.02 ppm shifted to 3.78 ppm; both of these changes in chemical shift were similar to those observed in the known dihydroxylation of 2′-*O*-*tert*-butyldimethylsilyl-6,7-dehydropaclitaxel.[13] The chemical shift of the C-10 proton also changed from 6.30 ppm in compound **7** to 6.73 ppm in compound **8**, which is characteristic for α–dihydroxylation of 6,7-dehydropaclitaxel derivatives.

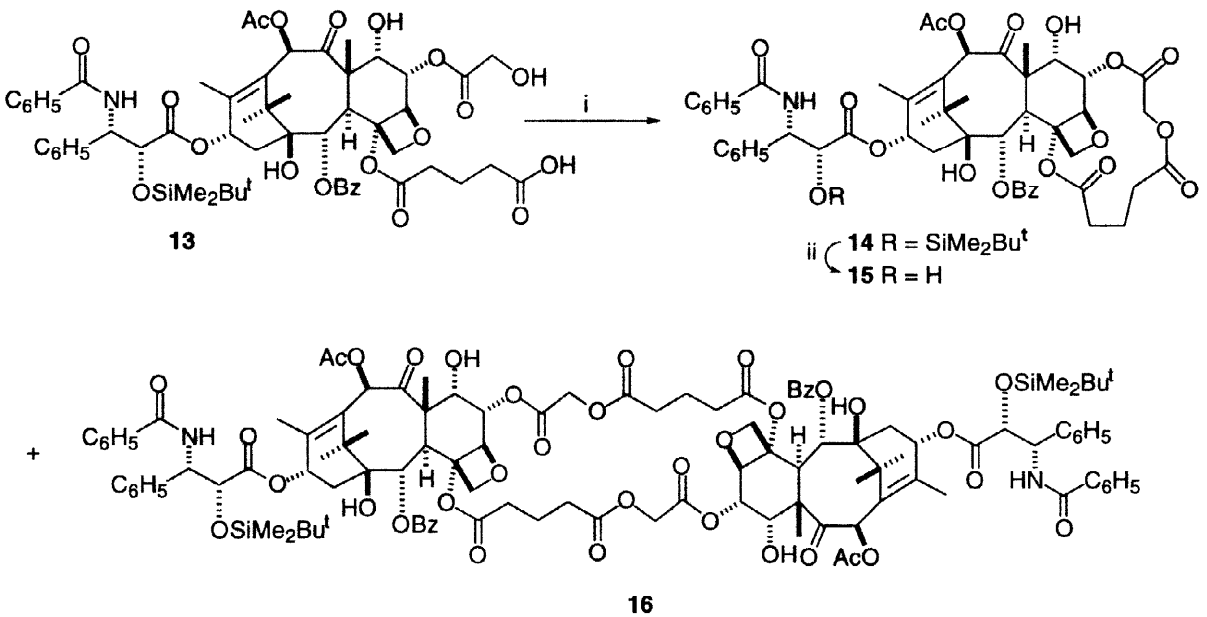
It was envisioned that since the cyclization reaction involving the bridge formation between C-4 and C-6 would be the most difficult step it would be best to leave it to last, and benzoylation at C-2 was therefore the next transformation of choice. Direct benzoylation at C -2 using phenyl lithium (PhLi) failed because of reaction with the C-4 benzyl ester, and an indirect approach using lithium hydroxide to hydrolyze the 1,2 carbonate followed by coupling with benzoic acid would have required protection and deprotection of the more reactive C-6α hydroxyl group. Compound **8** was thus hydrogenated to afford the acid **9** in 87% yield, and reaction of **9** in THF at -78 °C with 10 equivalents of phenyl lithium proceeded regioselectively to give the desired benzoate **10** in good yield. The ^{1}H NMR spectrum of compound **10** showed a characteristic shift of the C-2 proton from 4.63 ppm to 5.74 ppm. The appearance of the two *ortho*-protons at 8.13 ppm also assured the presence of the C-2 benzoyl group. This direct C-2 benzoylation reaction sequence should also be applicable to other analogs of **10** with different lengths of the acid chain at C-4.

A preliminary molecular modeling study using MacSpartan's Sybyl level geometry optimization and energy minimization suggested that the best linker between the C-6α hydroxyl group and the C-4 glutarate would be a 2-3 carbon chain,[17] and 2-hydroxyacetic acid was thus selected as the linker. Direct acylation of the C-6α hydroxyl group of **10** with 2-benzyloxyacetyl chloride and DMAP in methylene chloride gave a low yield of the desired product, so the C-4 acid in compound **10** was protected again as its benzyl ester to give **11** in 94% yield. Reaction of **11** with 2-benzyloxyacetyl chloride proceeded smoothly and cleanly in 85% yield to give the C-6α ester **12** (Scheme 2). In the ^{1}H NMR spectrum of compound **12**, the resonance of the C-6 proton shifted from 4.17 ppm in **11** to 5.39 ppm, as expected for C-6 esterification. The presence of a broad singlet for two protons at 4.62 ppm corresponding to the benzylic protons of the benzyl ether, and a broad singlet at 4.16 ppm for two protons corresponding to the α-protons of the benzyloxyacetate, confirmed the attachment of the linker at the C-6 position. Hydrogenation of compound **12** unmasked both of the benzyl protecting groups and afforded the key intermediate hydroxy acid **13** in 71% yield (Scheme 2).

Among several macrocyclization conditions we tried, only the Mukaiyama conditions using 2-chloro-1-methylpyridinium iodide gave the desired lactone, and then only in low yield. Thus, slow addition of a solution of the hydroxy acid **13** and triethylamine in acetonitrile to a solution of acetonitrile containing 2-chloro-1-methylpyridinium iodide refluxing at 80 °C over 8 h afforded two less polar products, along with starting material and other minor products that were not isolated. One of the major products isolated in 10% yield was identified as the desired lactone **14**, and the other major product isolated in a yield of 12% was tentatively identified as the dilactone **16**. The ^{1}H NMR spectrum of compound **14** was essentially the same as that of **13**, except that the two α-protons of the hydroxyacetyl group at the C-6 position shifted downfield and were split into a pair of doublets. This downfield shift is in accordance with the expected lactonization reaction, as is the observed splitting of the two protons. In compound **13**, the two α-protons of the hydroxyacetyl group at the C-

6 position were almost equivalent due to free rotation, whereas in lactone **14** they experienced different electromagnetic environments because of the rigidity of the lactone ring.

The reason for the low yield of the desired macrolactone is presumably due to the rigidity of the tetracyclic paclitaxel ring system, which forces the two chains at the C-4 and the C-6 positions to be disposed away from each other and makes the desired lactone somewhat strained. This would also explain the formation of the presumed dilactone in greater yield than the monolactone, in spite of the high dilution conditions used.

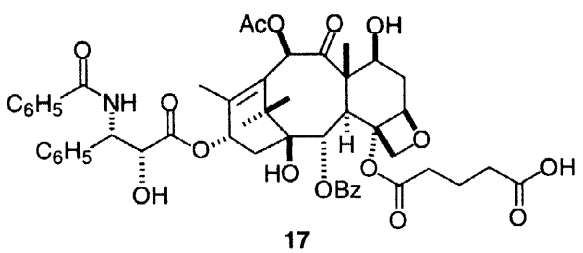


i) 2-Chloro-1-methylpyridinium iodide, Et_3N, CH_3CN, 80 °C, **14** (10%), **16** (12%); ii) HF/pyridine, 64%.

Scheme 3

Compound **14** was deprotected using hydrogen fluoride-pyridine to give the bridged paclitaxel **15**, whose composition was confirmed by ^1H NMR spectroscopy and by FABMS.

The bioactivity of macrolactone **15** was determined in conjunction with that of the 4-glutaryl analog **17**, which was prepared from paclitaxel by standard methods. In a tubulin-assembly assay both compounds **15** and



17 were essentially inactive under our standard conditions.[18] In cytotoxicity assays both **15** and **17** showed less cytotoxicity than paclitaxel. Compound **17** had IC_{50} values of 700 and 900 nM against the human breast carcinoma line MCF-7 and the human prostate carcinoma line PC3, respectively. The macrolactone **15** was still less active, with IC_{50} values of > 5 μM in these cell lines and > 1 μM against the human Burkitt lymphoma line CA-46. Paclitaxel itself gives IC_{50} values of 2-5 nM against these cell lines. Thus, placing a C-4/C-6 bridge

under ring C of paclitaxel does not confer any advantage in terms of activity, and in fact appears to be slightly deleterious, based on the differences in cytotoxicity between compounds **15** and**17**.

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 mesh). Preparative thin layer chromatography (PTLC) plates (silica gel 60 GF) were purchased from Analtech. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Varian Unity 400 spectrometer (operating at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C) or a Bruker WP 360 spectrometer (operating at 360.140 MHz for ^{1}H 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 residual solvent ($CHCl_3$) 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)-2-debenzoyl-4-deacetyl-6,7-dehydropaclitaxel. 2′-*O*-(*tert*-butyldimethylsilyl)-6,7-dehydropaclitaxel[14] (**4**, 723 mg, 0.76 mmol) was dissolved in dry CH_2Cl_2 (18 mL) and cooled to -78 °C for 5 min. To the solution was added benzyltrimethylammonium hydroxide (Triton-B, 30% in MeOH, 0.90 mL, 1.82 mmol). The solution was stirred at -78 °C for 5 min and was allowed to warm up to 15 °C by replacing the acetone-dry ice bath with ethanol. The reaction proceeded at 15 °C for 10 min and was monitored by TLC every 3 min. TLC analysis (EtOAc:hexanes 6:4) indicated that the starting material (Rf~0.7) was converted to a more polar compound (Rf~0.3). The reaction mixture was diluted with ethyl acetate (dry ice cooled) and quenched with dilute HCl (1 N). The organic layer was separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was filtered through a short column (silica gel, EtOAc:hexanes 5:5) to give crude 2′-*O*-(*tert*-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-6,7-dehydropaclitaxel (650 mg), which was subjected to the next reaction without further purification.

2′-*O*-(*tert*-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-paclitaxel (5) and 2′-*O*-(*tert*-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-6,7-dehydro-10-deacetyl-9,10-carbonato-paclitaxel (6). To a solution of crude 2′-*O*-(*tert*-butyldimethylsilyl)-2-debenzoyl-4-deacetyl-6,7-dehydropaclitaxel (650 mg) in dry CH_2Cl_2 (10 mL) was added CDI (2.0 g, 12.3 mmol) and imidazole (catalytic amount) at room temperature. The solution was then stirred at 40 °C for 4 h. The reaction mixture was dilute with EtOAc, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, EtOAc:hexanes 4:6) to afford **5** (253 mg, 40% 2 steps) and **6** (101 mg).

Compound 5: ^{1}H-NMR δ 7.79 (d, J = 7.6, 2H, ArH), 7.56-7.30 (m, 9H, ArH and 3′-NH), 6.34 (s, 1H, H-10), 6.19-6.12 (m, 2H, H-6 and H-7), 5.90 (d, *J* = 8.8, 1H, H-3′), 5.87 (m, 1H, H-13), 5.20 (s, 1H, 4-OH), 5.13 (d, *J* = 7.2, H-5), 4.73 (d, *J* = 8.4, 1H, H-20), 4.67 (d, *J* = 8.4, 1H, H-20), 4.48 (m, 2H, H-2 and H-2′), 3.16 (d, *J* = 5.2, 1H, H-3), 2.90 (m, 1H, H-14), 2.56 (m, 1H, H-14), 2.24 (s, 3H, 10-OAc), 1.98 (s, 3H, 18-CH_3), 1.78 (s, 3H, 19-CH_3), 1.23 (s, 3H, 17-CH_3), 1.17 (s, 3H, 16-CH_3), 0.84 (s, 9H, 2′-OSiMe$_2$^{t}Bu), -0.10 (s, 3H, 2′-OSiMe$_2$^{t}Bu), -0.29 (s, 3H, 2′-OSiMe$_2$^{t}Bu).

Compound 6: ^{1}H-NMR δ 7.80 (d, *J* = 7.6, 2H, ArH), 7.56-7.28 (m, 9H, ArH and 3′-NH), 6.38 (d, *J* = 10.0, 1H, H-7), 6.13 (dd, *J* = 10.0,6.0, 1H, H-6), 5.92 (m, 1H, H-13), 5.79 (d, *J* = 8.4, 1H, H-3′),

5.08 (d, J = 5.6, 1H, H-5), 5.06 (s, 1H, 4-OH), 4.75 (d, J = 4.8, H-2), 4.70 (AB quartet, J = 9.6, 2H, H-20), 4.45 (d, J = 1.6, 1H, H-2′), 3.00 (m, 1H, H-14), 2.59 (m, 1H, H-14), 2.35 (d, J = 4.8, 1H, H-3), 1.82 (d, J = 1.2, 3H, 18-CH_3), 1.75 (s, 3H, 19-CH_3), 1.31 (s, 3H, 17-CH_3), 1.20 (s, 3H, 16-CH_3), 0.83 (s, 9H, 2′-OSiMe$_2$^{t}Bu), -0.11 (s, 3H, 2′-OSiMe$_2$^{t}Bu), -0.32 (s, 3H, 2′-OSiMe$_2$^{t}Bu). ^{13}C-NMR δ 169.9, 167.4, 152.4, 151.7, 151.3, 141.7, 139.2, 138.3, 134.8, 133.4, 132.2, 128.8, 128.6, 128.0, 127.0, 126.2, 125.5, 124.5, 89.6, 84.5, 80.6, 80.3, 75.1, 73.8, 70.1, 55.5, 43.8, 42.5, 40.6, 32.7, 25.5, 23.3, 20.3, 19.9, 18.2, 18.1, -5.6, -6.0.

2′-*O*-(*tert*-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(*O*-benzylglutaryl)-6,7-dehydropaclitaxel (7). To a solution of **5** (82 mg, 0.099mmol) in dry toluene (7.0 mL) was added DCC (600 mg, 2.9 mmol), DMAP (10 mg, catalytic amount), and glutaric acid monobenzyl ester (250 μL, 1.35 mmol). The resulting suspension was stirred at room temperature for 4 days, filtered through a pad of Celite, and rinsed with EtOAc. The filtrate was concentrated and applied directly on preparative TLC (silica gel, 1000 μ, EtOAc:hexanes 4:6) to afford **7** (88 mg, 86%). ^{1}H-NMR δ 7.79 (d, J = 7.6, 2H, ArH), 7.52-7.29 (m, 13H, ArH), 7.04 (d, J = 10.4, 1H, 3′-NH), 6.30 (s, 1H, H-10), 6.15-6.09 (m, 2H, H-6 and H-13), 6.02 (d, J = 10.8, 1H, H-7), 5.65 (dd, J = 10.0,2.4, 1H, H-3′), 5.21 (d, J = 6.4, 1H, H-5), 5.08 (br s, 2H, 4-COOCH$_2$Ph), 4.82 (d, J = 10.0, 1H, H-20), 4.64 (m, 2H, H-2 and H-2′), 4.60 (d, J = 9.6, 1H, H-20), 3.57 (d, J = 6.0, 1H, H-3), 2.74-1.78 (m, 8H, H-14 and 4-OCOCH$_2$CH$_2$CH$_2$COO), 2.20 (s, 3H, 10-OAc), 1.88 (s, 3H, 19-CH_3), 1.84 (s, 3H, 18-CH_3), 1.37 (s, 3H, 17-CH_3), 1.18 (s, 3H, 16-CH_3), 0.81 (s, 9H, 2′-OSiMe$_2$^{t}Bu), -0.05 (s, 3H, 2′-OSiMe$_2$^{t}Bu), -0.23 (s, 3H, 2′-OSiMe$_2$^{t}Bu); HRFABMS *m/z* 996.4539 (MLi-CO_2)$^+$ (calcd for $C_{56}H_{67}O_{13}NSiLi$, 996.4542).

2′-*O*-(*tert*-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-(*O*-benzylglutaryl)-6α-hydroxy-7-*epi*paclitaxel (8). To a solution of **7** (60 mg, 0.058 mmol) in acetone/H_2O (4mL/0.8mL) was added NMO (60 mg, 0.51 mmol) and OsO_4 (catalytic amount). The solution was stirred at room temperature for 40 h, diluted with EtOAc, and washed with saturated Na_2SO_3. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, EtOAc:hexanes 6:4) to give **8** (50 mg, 81%). ^{1}H-NMR δ 7.78 (d, J = 8.4, 2H, ArH), 7.53-7.28 (m, 13H, ArH), 7.04 (d, J = 9.2, 1H, 3′-NH), 6.73 (s, 1H, H-10), 6.18 (t, J = 8.8, 1H, H-13), 5.63 (dd, J = 9.2,2.4, 1H, H-3′), 5.07 (AB quartet, 2H, 4-COOCH$_2$Ph), 4.77 (s, 1H, H-5), 4.75 (d, J = 9.6, 1H, H-20), 4.64 (d, J = 6.0, H-2), 4.62 (d, J = 2.4, 1H, H-2′), 4.56 (d, J = 9.2, 1H, H-20), 4.22, (m, 1H, H-6), 4.17 (d, J = 9.2, 1H, 7-OH), 3.78 (dd, J = 9.2,5.6, 1H, H-7), 3.63 (d, J = 6.4, 1H, H-3), 2.84 (m, 2H, OCOCH$_2$CH$_2$CH$_2$COO and OH), 2.70 (m, 1H, OCOCH$_2$CH$_2$CH$_2$COO), 2.51-1.99 (m, 6H, OCOCH$_2$CH$_2$CH$_2$COO and H-14), 2.18 (s, 3H, 10-OAc), 1.89 (s, 3H, 18-CH_3), 1.63 (s, 3H, 19-CH_3), 1.35 (s, 3H, 17-CH_3), 1.16 (s, 3H, 16-CH_3), 0.81 (s, 9H, 2′-OSiMe$_2$^{t}Bu), -0.04 (s, 3H, 2′-OSiMe$_2$^{t}Bu), -0.23 (s, 3H, 2′-OSiMe$_2$^{t}Bu). ^{13}C-NMR δ 205.2, 173.8, 172.2, 171.6, 169.4, 167.0, 152.4, 144.6, 137.9, 135.6, 134.0, 131.8, 130.5, 128.7, 128.6, 128.3, 128.24, 128.16, 127.0, 126.6, 90.6, 89.8, 82.0, 81.0, 78.4, 75.0, 74.7, 71.7, 70.2, 66.5, 58.8, 55.7, 40.7, 36.3, 34.7, 32.7, 25.5, 24.9, 21.6, 20.7, 20.4, 18.1, 15.9, 14.4, -5.1, -5.6.

2′-*O*-(*tert*-Butyldimethylsilyl)-2-debenzoyl-4-deacetyl-1,2-carbonato-4-glutaryl-6α-hydroxy-7-*epi*paclitaxel (9). A catalytic amount of palladium on activated carbon (5%) was added to a solution of **8** (50 mg, 0.047 mmol) in EtOAc (2 mL). This suspension was stirred under H_2 for 2 h. TLC analysis indicated complete conversion of **8** to a very polar compound. The solid was removed by filtration and the filtrate was concentrated under reduced pressure to afford **9** (41 mg, 87%). ^{1}H-NMR δ 7.80 (d, J = 8.8, 2H, ArH), 7.54-7.21 (m, 8H, ArH and 3′-NH), 6.70 (s, 1H, H-10), 6.26 (t, J = 10.0, 1H, H-13), 5.64 (d, J = 9.2, 1H, H-3′), 4.95 (br s, 1H, H-5), 4.83 (d, J = 10.8, 1H, H-20), 4.63 (d, J = 6.8, 1H, H-2), 4.60 (d, J = 2.0, 1H, H-2′), 4.56 (d, J = 10.4, 1H, H-20), 4.20 (d, J = 6.4, 1H, H-6), 3.75 (d, J = 6.0, 1H, H-7), 3.62 (d, J = 6.8, 1H, H-3), 2.90 (m, 1H, OCOCH$_2$CH$_2$CH$_2$COO), 2.66-2.54 (m, 3H, OCOCH$_2$CH$_2$CH$_2$COO and OH), 2.44-1.96 (m, 5H, OCOCH$_2$CH$_2$CH$_2$COO and H-14), 2.16 (s, 3H, 10-OAc), 1.87 (s, 3H, 18-CH_3),

1.62 (s, 3H, 19-CH_3), 1.34 (s, 3H, 17-CH_3), 1.13 (s, 3H, 16-CH_3), 0.79 (s, 9H, 2′-$OSiMe_2{}^tBu$), -0.08 (s, 3H, 2′-$OSiMe_2{}^tBu$), -0.36 (s, 3H, 2′-$OSiMe_2{}^tBu$).

2′-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-glutaryl-6α-hydroxy-7-*epi*paclitaxel (10) - To a solution of **9** (285 mg, 0.29 mmol) in dry THF (10 mL) at -78 °C was added PhLi (1.8 M in hexanes, 1.95 mL, 3.48 mmol). The solution was stirred at -78 °C for 20 min, diluted with EtOAc, and quenched with dilute HCl (1N). The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with dilute sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, EtOAc:hexanes 7:3) to give **10** (240 mg, 78%). ^{1}H-NMR δ 8.13 (d, *J* = 8.4, 2H, ArH), 7.75 (d, *J* = 7.2, 2H, ArH), 7.57-7.30 (m, 11H, ArH), 7.21 (d, *J* = 8.8, 1H, 3′-NH), 6.81 (s, 1H, H-10), 6.32 (t, *J* = 8.8, 1H, H-13), 5.74 (d, *J* = 7.6, 1H, H-2), 5.67 (d, *J* = 8.8, 1H, H-3′), 4.69-4.65 (m, 3H, H-5, H-2′ and OH), 4.39 (d, *J* = 8.8, 1H, H-20), 4.33 (d, *J* = 8.8, 1H, H-20), 4.17 (m, 1H, H-6), 3.84 (d, *J* = 7.6, 1H, H-3), 3.69 (m, 1H, H-7), 3.15 (m, 1H, $OCOCH_2CH_2CH_2COO$), 2.79 (m, 1H, $OCOCH_2CH_2CH_2COO$), 2.63 (s, 1H, OH), 2.39-1.87 (m, 6H, $OCOCH_2CH_2CH_2COO$ and H-14), 2.20 (s, 3H, 10-OAc), 1.89 (s, 3H, 18-CH_3), 1.64 (s, 3H, 19-CH_3), 1.22 (s, 3H, 17-CH_3), 1.12 (s, 3H, 16-CH_3), 0.77 (s, 9H, 2′-$OSiMe_2{}^tBu$), -0.03 (s, 3H, 2′-$OSiMe_2{}^tBu$), -0.35 (s, 3H, 2′-$OSiMe_2{}^tBu$).

2′-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6α-hydroxy-7-*epi*paclitaxel (11) - To a solution of **10** (140 mg, 0.13 mmol) in dry toluene (1.5 mL) was added DCC (50 mg, 0.24 mmol), DMAP (catalytic amount), and benzyl alcohol (120 μL, 1.2 mmol). The solution was stirred at room temperature for 24 h. TLC analysis indicated complete conversion of **10** to a less polar product. The solution was concentrated under reduced pressure. The resulting syrup was purified on preparative TLC (silica gel, 1000 μM, EtOAc:hexanes 6:4) to afford **11** (143 mg, 94%), which was directly subjected to the next reaction.

2′-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-(*O*-benzylglutaryl)-6α-(2-benzyloxyacetoxy)-7-*epi*paclitaxel (12). To a solution of **11** (86 mg, 0.075 mmol) in CH_2Cl_2 (3.0 mL) was added DMAP (64 mg, 0.75 mmol) and 2-benzyloxyacetyl chloride (60 μL, 0.38 mmol) at 0 °C. The solution was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc, washed with dilute sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ, EtOAc:hexanes 4:6) to afford **12** (82 mg, 85%) which was directly subjected to the next reaction.

2′-*O*-(*tert*-Butyldimethylsilyl)-4-deacetyl-4-glutaryl-6α-(2-hydroxyacetoxy)-7-*epi*paclitaxel (13). A catalytic amount of Pd on activated carbon (5%) was added to a solution of **12** (74 mg, 0.057 mmol) in EtOAc (3 mL). This suspension was stirred under H_2 for 2 h. The solid was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000 μ, EtOAc:hexanes 7:3) to afford **13** (45 mg, 71%). ^{1}H-NMR δ 8.13 (d, *J* = 7.2, 2H, ArH), 7.74 (d, *J* = 7.6, 2H, ArH), 7.58-7.30 (m, 11H, ArH), 7.19 (d, *J* = 8.8, 1H, 3′-NH), 6.77 (s, 1H, H-10), 6.30 (t, *J* = 8.8, 1H, H-13), 5.75 (d, *J* = 7.2, 1H, H-2), 5.69 (d, *J* = 8.8, 1H, H-3′), 5.29 (dd, *J* = 5.2,2.4, 1H, H-6), 4.82 (d, *J* = 2.0, 1H, H-5), 4.81 (d, *J* = 6.4, 1H, OH), 4.66 (d, *J* = 1.6, 1H, H-2′), 4.45 (d, *J* = 8.4, 1H, H-20), 4.37 (d, *J* = 8.8, 1H, H-20), 4.17 (br s, 2H, 6-$OCOCH_2O$), 3.92-3.89 (m, 2H, H-7 and H-3), 3.14 (m, 1H, 4-$COCCH_2CH_2CH_2COO$), 2.80 (m, 1H, 4-$COCCH_2CH_2CH_2COO$), 2.62 (s, 1H, OH), 2.42-2.02 (m, 6H, 4-$COCCH_2CH_2CH_2COO$ and H-14), 2.18 (s, 3H, 10-OAc), 1.87 (s, 3H, 18-CH_3), 1.71 (s, 3H, 19-CH_3), 1.20 (s, 3H, 17-CH_3), 1.12 (s, 3H, 16-CH_3), 0.77 (s, 9H, 2′-$OSiMe_2{}^tBu$), -0.04 (s, 3H, 2′-$OSiMe_2{}^tBu$), -0.32 (s, 3H, 2′-$OSiMe_2{}^tBu$). ^{13}C-NMR δ 205.6, 175.0, 174.1, 171.7, 171.1, 169.5, 167.6, 166.9, 140.5, 137.8, 133.9, 133.6, 132.8, 132.1, 130.2, 129.0, 128.9, 128.8, 128.77, 128.1, 127.1, 126.2, 87.4, 84.0, 79.1, 77.8, 75.3, 75.1, 74.9, 74.8, 70.9, 60.6, 57.7, 55.8, 53.7, 42.7, 39.9, 35.0, 32.6, 29.2, 26.0, 25.5, 21.7, 20.8, 20.3, 18.1, 15.5, 14.8, -5.2, -5.9. HRFABMS *m/z* 1136.4283 $(MNa)^+$ (calcd for $C_{58}H_{71}O_{19}NSiNa$, 1136.4287).

Protected Macrolactone 14. To a continuously stirred solution of 2-chloro-1-methyl-pyridinium iodide (49 mg, 0.19mmol) in acetonitrile (2.5 mL) at 80 °C was added by syringe pump a solution of **13** (29 mg, 0.026 mmol) and triethylamine (37 μL, 0.27 mmol) in acetonitrile (5.0 mL) over a period of 8 h. Stirring was continued for an additional hour at 80 °C and the solution was then concentrated under reduced pressure. The mixture was purified by preparative TLC (silica gel, 1000 μ, EtOAc:hexanes 5:5) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-4-deacetyl-6α-(2′′-hydroxyacetoxy)-7-*epi*paclitaxel 4,2′′-*O*-cycloglutarate (**14**, 2.5 mg, 9%). ^{1}H-NMR δ 8.13 (d, *J* = 7.2, 2H, ArH), 7.70 (d, *J* = 7.2, 2H, ArH), 7.60-7.35 (m, 11H, ArH), 7.05 (d, *J* = 9.2, 1H, 3′-NH), 6.77 (s, 1H, H-10), 6.27 (t, *J* = 8.8, 1H, H-13), 5.74-5.71(m, 2H, H-3′ and H-2), 5.33 (dd, *J* = 5.2,2.4, 1H, H-6), 4.95 (d, *J* = 2.0, 1H, H-5), 4.91 (d, *J* = 16.0, 1H, 6-OCOCH$_2$O), 4.76 (d, *J* = 11.6, 1H, 7-OH), 4.65 (d, *J* = 2.0, 1H, H-2′), 4.40 (d, *J* = 15.2, 1H, 6-OCOCH$_2$O), 4.38 (br s, 2H, H-20), 3.91 (dd, *J* = 11.6,5.2, 1H, H-7), 3.82 (d, *J* = 7.6, 1H, H-3), 3.18 (m, 1H, 4-COCCH$_2$CH$_2$CH$_2$COO), 2.86 (m, 2H, 4-COCCH$_2$CH$_2$CH$_2$COO), 2.51 (m, 2H, 4-COCCH$_2$CH$_2$CH$_2$COO), 2.31 (m, 1H, H-14), 2.19 (s, 3H, 10-OAc), 2.14 (m, 2H, 4-COCCH$_2$CH$_2$CH$_2$COO), 1.89 (s, 3H, 18-CH$_3$), 1.69 (s, 3H, 19-CH$_3$), 1.19 (s, 3H, 17-CH$_3$), 1.12 (s, 3H, 16-CH$_3$), 0.79 (s, 9H, 2′-OSiMe$_2$^{t}Bu), -0.02 (s, 3H, 2′-OSiMe$_2$^{t}Bu), -0.26 (s, 3H, 2′-OSiMe$_2$^{t}Bu).

Macrolactone 15. To a solution of **14** (2.5 mg, 0.0023 mmol) in dry THF (0.5 mL) was added HF-pyridine (70%, 200 μL, excess), and the solution was stirred at room temperature for 4 h. The reaction mixture was diluted with EtOAc and washed with dilute sodium bicarbonate and dilute HCl (1 N). The organic layers were combined and washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500 μ, EtOAc:hexanes 7:3) to afford **15** (1.4 mg, 64%). ^{1}H-NMR δ 8.12 (d, *J* = 7.6, 2H, ArH) 7.77 (d, *J* = 7.2, 2H, ArH), 7.64-7.30 (m, 12H, ArH and 3′-NH), 6.78 (s, 1H, H-10), 6.15 (t, *J* = 8.8, 1H, H-13), 5.81 (dd, *J* = 8.8, 2.8, 1H, H-3′), 5.73 (d, *J* = 7.2, 1H, H-2), 5.32 (dd, *J* = 4.0,4.0, 1H, H-6), 5.12 (d, *J* = 11.6, 1H, 7-OH), 5.02 (m, 1H, H-5), 4.66 (d, *J* = 2.8, 1H, H-2′), 4.47 (d, *J* = 8.8, 1H, H-20), 4.46 (d, *J* = 16.0, 1H, 6-OCOCH$_2$O), 4.39 (d, *J* = 16.0, 1H, 6-OCOCH$_2$O), 4.34 (d, *J* = 8.8, H-20), 4.29 (d, *J* = 4.8, 1H, OH), 3.86 (d, *J* = 7.2, 1H, H-3), 3.74 (dd, *J* = 12.0,4.0, 1H, H-7), 2.84 (t, *J* = 6.8, 2H, 4-COCCH$_2$CH$_2$CH$_2$COO), 2.47-1.86 (m, 6H, H-14, 4-COCCH$_2$CH$_2$CH$_2$COO), 2.09, 1.91, 1.69, 1.19, 1.12. ^{13}C-NMR δ 205.8, 174.3, 172.1, 171.8, 170.3, 167.2, 166.9, 166.6, 141.1, 138.5, 134.05, 133.95, 132.6, 131.7, 130.2, 128.9, 128.8, 128.4, 127.9, 127.4, 127.2, 86.8, 84.4, 79.2, 78.0, 77.2, 76.2, 75.5, 74.9, 72.7, 71.3, 60.7, 57.8, 55.1, 42.5, 40.2, 36.6, 34.6, 32.8, 25.8, 21.5, 20.8, 19.2, 16.7, 14.8. FABMS *m/z* 988 $(M+Li)^+$ (calcd for $C_{52}H_{55}NO_{18}$, 988).

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