Bridging Converts a Noncytotoxic *nor*-Paclitaxel Derivative to a Cytotoxic Analog by Constraining it to the T-Taxol Conformation

Shoubin Tang, Chao Yang, Peggy Brodie, Susan Bane, Rudravajhala Ravindra, Shubhada Sharma, Yi Jiang, James P. Snyder, and David G. I. Kingston

Supporting Data

A. Synthesis

2′-Triisopropylsiloxy-7-triethylsiloxy–bridged-11(15→1)-abeo-paclitaxel (8). Pyridine (0.15 mL) was added to a solution of **7** (18 mg) in anhydrous CH_2Cl_2 (5 mL). The mixture was cooled to -20 °C and thionyl chloride (53 μL) was added. After stirring for 0.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic phase was dried under vacuum and the residue was separated by PTLC on silica gel, developed with 8% EtOAc in hexane, to give compound **8** (8.5 mg, 49%) as a white solid. ^{1}H NMR δ 7.99 (2H, d, *J* = 7.8), 7.70 (2H, d, *J* = 7.0), 7.54 (1H, t, *J* = 7.2), 6.30 (1H, s), 5.88 (1H, t, *J* = 8.0), 5.64 (1H, d, *J* = 8.0), 5.50 (1H, d, *J* = 7.2), 4.94 (1H, d, *J* = 8.4), 4.79 (1H, br, s), 4.65 (1H, d, *J* = 2.0), 4.59 (1H, br, s), 4.43 (1H, t, *J* = 8.4), 4.24 (1H, d, *J* = 8.0), 4.13 (1H, d, *J* = 8.0), 3.47 (1H, d, 7.2), 3.21 (1H, m), 2.82 (1H, m), 2.74 (1H, m), 2.64 (1H, m), 2.60- 2.52 (3H, overlapped), 2.08 (3H, s), 1.96 (1H, m), 1.83 (3H, s), 1.61 (3H, s), 1.58 (3H, s), 1.08-0.82 (30H, overlapped), 0.75-0.64 (6H, overlapped); ^{13}C NMR: δ 201.8, 172.5, 171.3, 169.2, 166.8, 165.7, 145.9, 144.2, 139.5, 138.5, 137.2, 134.3, 133.8, 131.9, 130.5, 130.1, 129.3, 129.0, 128.9, 128.4, 127.2, 127.2, 126.8, 113.5, 84.9, 79.4, 77.9, 75.4, 75.3, 72.9, 71.0, 70.7, 63.5, 57.5, 52.1, 43.7, 38.8, 38.6, 35.4, 33.6,

26.1, 21.0, 20.7, 18.3, 18.1, 17.9, 13.2, 11.6, 9.48, 7.10, 5.5; HRFABMS *m/z* = 1132.5347 $[M+H]^+$, calculated for $C_{64}H_{85}NO_{13}Si_2$ 1132.5438 (Δ = -7.9ppm).

Bridged-11(15→1)-abeo-paclitaxel (4). To a solution of **8** (8.5 mg, 7.5 μmol) in THF (2.5 mL) at 0 °C was added HF/pyridine (0.10 mL, 70 wt%, large excess) and the solution was allowed to warm to room temperature over 1 hour and then stirred overnight. The reaction mixture was quenched with aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by PTLC on silica gel, developed with EtOAc:hexane, 1:3, to yield **4** (5.8 mg, 6.7 μmol, 89%) as a white solid. ^{1}H NMR δ 8.03 (2H, d, *J* = 8.0), 7.70 (2H, d, *J* = 8.0), 7.53 (1H, t, *J* = 7.6), 7.34 (1H, t, *J* = 7.6), 6.30 (1H, s), 5.88 (1H, t, *J* = 8.0), 5.64 (1H, d, *J* = 7.8), 5.50 (1H, d, *J* = 7.8), 4.94 (1H, d, *J* = 8.0), 4.79 (1H, br, s), 4.65 (1H, d, *J* = 1.0), 4.59 (1H, br, s), 4.43 (1H, t, *J* = 8.4), 4.23 (1H, d, *J* = 8.0), 4.14 (1H, d, *J* = 8.0), 3.48 (1H, d, 7.2), 3.24 (1H, m), 2.82 (1H, m), 2.75 (1H, m), 2.64-2.52 (4H, m), 2.08 (3H, s), 1.97-1.91 (2H, m), 1.83 (3H, s), 1.62 (3H, s), 1.58 (3H, s). ^{13}C NMR δ 203.5, 173.5, 172.5, 171.6, 166.9, 165.6, 146.2, 144.9, 140.2, 139.1, 135.8, 134.0, 133.8, 132.1, 130.4, 130.3, 129.4, 129.1, 128.8, 128.7, 127.7, 127.4, 113.7, 85.1, 79.1, 78.8, 75.1, 73.2, 72.7, 71.9, 71.0, 64.1, 57.4, 49.7, 44.1, 39.2, 36.4, 35.4, 33.6, 29.9, 26.8, 20.9, 20.7, 11.9, 8.4. HRFABMS *m/z* = 862.3470 $[M+H]^+$, calculated for $C_{49}H_{52}NO_{13}$ 862.3434 (Δ = 3.1ppm).

Attempted synthesis of compound 6

15(16)-Anhydro-10-deacetyl-7,10,13-tris(triethylsiloxyl)-11(15→1)-abeo-baccatin III (S2).

7,10,13–Tris(triethylsilyl)–10–deacetyl-baccatin III (**S1**)[1] (250 mg) and pyridine (0.25 mL) were dissolved in anhydrous CH_2Cl_2 (10 mL). The mixture was cooled to -20°C, and thionyl chloride (145 μL) was added with stirring. After half an hour, the reaction was quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic phase was dried under vacuum and the residue was separated on silica chromatography with 5% EtOAc in hexane to give compound **S2** as a white solid (117 mg, 48% yield). ^{1}H NMR δ 8.00 (dd, 2H, *J* = 8.0 and 1.5), 7.57 (1H , t, *J* = 8.0), 7.42 (2H, t, *J* = 8.0), 5.57 (1H, d, *J* = 7.6), 5.26 (1H, s), 5.01 (1H, d, *J* = 8.4), 4.90 (1H, d, *J* = 2.0), 4.63 (1H, d, *J* = 2.0), 4.55 (1H, t, *J* = 7.2), 4.47 (1H, dd, *J* = 9.6 and 2.4), 4.24 (1H, d, *J* = 8.0), 4.18 (1H, d, *J* = 8.0), 3.54 (1H, d, *J* = 7.6), 2.58 (1H, ddd, J_1 = 16.0, J_2 = 7.2 and J_3 = 1.6), 2.27 (1H, m), 2.23 (3H, s), 1.94-1.82 (2H, m), 1.79 (3H, s), 1.73 (3H, s), 1.63 (3H, s), 1.00-0.93 (27H, 9CH_3), 0.72-0.57 (18H, 9CH_2); ^{13}C-NMR δ 207.3, 170.2, 165.4, 146.2, 145.9, 137.8, 133.5, 130.0, 129.9, 128.7, 112.1, 84.8, 79.0, 77.0, 76.1, 74.8, 73.9, 72.6, 71.4, 63.5, 56.7, 44.2, 42.8, 38.5, 22.0, 21.3, 11.6, 9.6, 7.23, 7.18, 7.0, 6.11, 5.69, 4.99; HRFABMS *m/z* = 869.4861 $[M+H]^+$ calculated for $C_{47}H_{77}O_9Si_3$ 869.4875 (Δ = -1.7 ppm).

[1] Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guenard, D.; Gueritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631-6637.

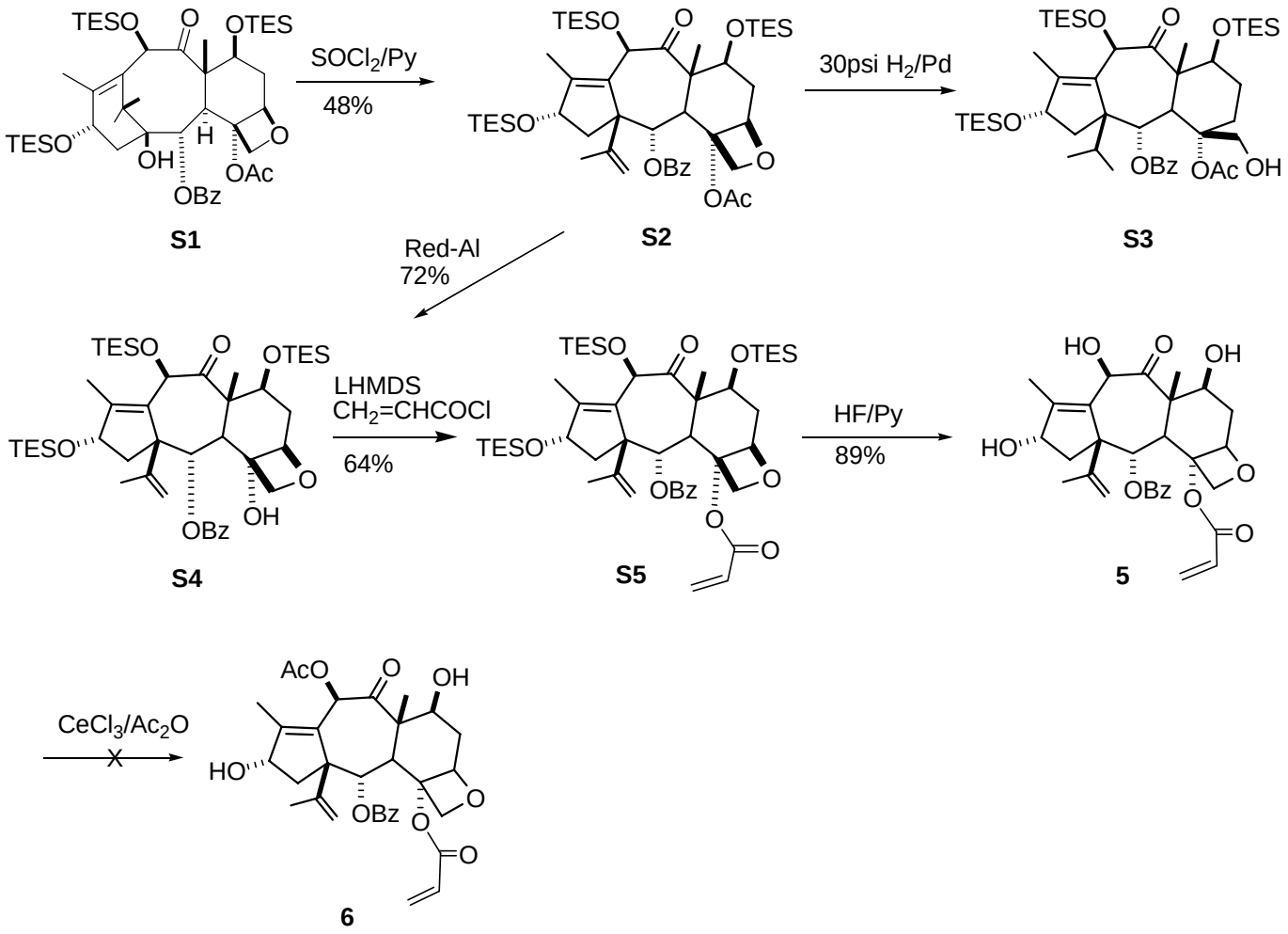

Figure S1. Synthesis of compound **5** and attempted synthesis of compound **6**.

15(16)-Anhydro-10-deacetyl-7,10,13-tris(triethylsiloxyl)-(4,5,15,16)-tetrahydro-11(15→1) abeo-baccatin III (S3). Compound **S2** (50 mg) was dissolved in THF (10 mL) and Pd/C (10% wt, 50 mg) was added. The mixture was then hydrogenated at 30 psi for 12 hrs. After filtration through Celite, the filtrate was evaporated and subjected to PTLC on silica gel developed with 10% EtOAc in hexane to give compound **S3** (23 mg, 46%) as a white solid. ^{1}H NMR δ 7.99 (2H, d, *J* = 7.8), 7.70 (2H, d, *J* = 7.0), 7.54 (1H, t, *J* = 7.2), 5.27 (1H, s), 5.12 (1H, m), 5.03 (1H, d, *J* = 6.8), 4.28 (1H, dd, *J* = 11.2 and 4.4), 3.63 (1H, *J* = 6.8), 3.49 (1H, d, *J* = 10.4), 3.37 (1H, d, *J* = 10.4), 3.21 (1H, br, s), 2.79 (1H, m), 2.35 (1H, m), 2.24 (1H, m), 2.10 (3H, s), 1.96 (1H,

m), 1.91 (3H, s), 1.84-1.73 (2H, overlapped, m), 1.28 (3H, s), 1.00-0.92 (21H, overlapped), 0.79 (3H, d, J = 6.4), 0.70-0.53 (18H, overlapped); ^{13}C NMR δ 208.0, 170.5, 166.5, 142.6, 137.2, 133.1, 130.7, 130.2, 128.5, 77.0, 75.1, 73.6, 72.9, 71.7, 71.2, 66.8, 62.8, 58.3, 44.1, 37.4, 36.0, 33.9, 25.4, 21.3, 19.2, 14.5, 11.9, 7.29, 7.15, 6.63, 5.99, 5.68, 4.14; HRFABMS *m/z* = 873.5115 $[M+H]^+$ calculated for $C_{48}H_{85}O_9Si_3$ 873.5110 (Δ = 0.5 ppm).

15(16)-Anhydro-4,10-di-deacetyl-7,10,13-tris(triethylsiloxy)-11(15→1)-abeo-baccatin III (S4). To a solution of **S2** (70 mg, 0.085 mmol) in anhydrous THF (10 mL) at -20°C, Red-Al (0.18 mL) was added dropwise under nitrogen. The reaction was stirred for 30 min until TLC showed the exhaustion of starting material. After quenching with a few drops of water, 1M sodium potassium tartate (10 mL) was added. The mixture was stirred for 0.5h before it was extracted with EtOAc. The combined organic phase was washed with water and brine, and dried over Na_2SO_4. Column chromatography on silica gel with elution with EtOAc:hexane, 1:4 gave compound **S4** (43 mg, 61%) as a colorless gum. ^{1}H NMR δ 8.03 (dd, 2H, J = 7.6 and 1.2), 7.57 (1H , t, J = 7.6), 7.42 (2H, t, J = 7.6), 5.27 (1H, s), 5.13 (1H, m), 5.03 (1H, d, J = 6.8), 4.28 (1H, dd, J = 11.2 and 4.4), 3.64 (1H, d, J = 6.8), 4.18 (1H, d, J = 8.0), 3.54 (1H, d, J = 7.6), 2.58 (1H, ddd, J_1 = 16.0, J_2 = 7.2 and J_3 = 1.6), 2.27 (1H, m), 2.23 (3H, s), 1.94-1.82 (2H, m), 1.79 (3H, s), 1.73 (3H, s), 1.63 (3H, s), 1.00-0.93 (27H, 9CH_3), 0.72-0.57 (18H, 6CH_2Si); ^{13}C NMR δ 207.0, 165.1, 145.7, 138.3, 133.3, 129.7, 129.6, 128.7, 128.6, 128.5, 127.7, 127.0, 111.7, 87.3, 78.3, 76.7, 73.7, 72.9, 71.7, 63.4, 56.6, 48.7, 42.0, 38.2, 21.0, 11.5, 9.47, 6.97, 6.93, 6.73, 5.72, 5.34, 5.23, 4.70; HRFABMS *m/z* = 827.4736 $[M+H]^+$ calculated for $C_{45}H_{75}O_8Si_3$ 827.4770 (Δ = -4.2 ppm).

15(16)-Anhydro-4,10-di-deacetyl-4-acryloyl-7,10,13-tris(triethylsiloxy)-11(15→1)-*nor*-baccatin III (S5). To a solution of **S4** (41 mg, 0.048 mmol) in THF (2 mL) at -20°C was added LHMDS (40 µl, 2.5 M in THF) and the mixture was stirred for 10 min. Acryloyl chloride (67 µL, 0.1 mmol) was then added. The reaction mixture was stirred for 1 h before being quenched with 2 mL of saturated aqueous NH_4Cl. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine and then dried with Na_2SO_4. The product was purified on preparative TLC (4:6 = EtOAc:hexanes) to give compound **10.12** (25 mg, 0.028 mmol, 63%) as a white solid. ^{1}H NMR δ 8.04 (dd, 2H, *J* = 8.0 and 1.5), 7.64 (1H , t, *J* = 8.0), 7.51 (2H, t, *J* = 8.0), 6.51 (1H, dd, *J* = 17.2 and 1.0), 6.17 (1H, dd, *J* = 17.2 and 10.4), 6.01 (1H, dd, 1H, dd, *J* = 10.4 and 1.0), 5.74 (1H, d, *J* = 8.0), 5.02 (1H, d, *J* = 8.4), 4.79 (1H, d, *J* = 1.2), 4.67 (1H, d, *J* = 1.2), 4.54 (1H, t, *J* = 7.2), 4.37 (1H, d, *J* = 8.0), 4.32 (1H, d, *J* = 8.0), 3.53 (1H, d, *J* = 8.4), 2.55 (1H, ddd, J_1 = 15.2, J_2 = 7.8 and J_3 = 1.6), 2.21 (1H, dd, *J* = 15.2 and 8.0), 2.05 (1H, m), 1.98 (3H, s), 1.91 (1H, m), 1.68 (3H, s), 1.11 (3H, s), 1.01-0.94 (27H, 9CH_3), 0.72-0.58 (18H, 6CH_2Si); ^{13}C-NMR δ 207.3, 165.4, 164.7, 146.9, 145.9, 137.3, 133.5, 131.5, 130.0, 129.9, 129.1, 128.7, 112.1, 84.8, 79.4, 76.9, 75.8, 75.1, 73.9, 72.7, 71.5, 63.4, 56.8, 44.0, 42.9, 38.5, 21.3, 11.7, 9.68, 7.27, 7.22, 7.05, 6.15, 5.69, 5.02. HRFABMS *m/z* = 881.4859 $[M+H]^+$, calculated for $C_{48}H_{77}O_9Si_3$ 881.4875 (Δ = -1.7 ppm)

15(16)-Anhydro-4,10-di-deacetyl-4-acryloyl-11(15→1)-abeo-baccatin III (5). To a solution of **S5** (42 mg, 4.5 µmol) in THF (5 mL) at 0 °C was added pyridine (1 mL) and HF/pyridine (100 µL, 70 wt%, large excess). The solution was allowed to warm up to room temperature in 1 h and stirred overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with water and brine, and dried over

anhydrous Na_2SO_4. Purification by TLC on silica gel developed with EtOAc: hexane, 2:5, gave compound **5** (24 mg, 3.9 μmol, 85%) as a white solid. ^{1}H NMR: δ 8.04 (dd, 2H, *J* = 8.0 and 1.5), 7.64 (1H , t, *J* = 8.0), 7.51 (2H, t, *J* = 8.0), 6.51 (1H, dd, *J* = 17.2 and 1.0), 6.17 (1H, dd, *J* = 17.2 and 10.4), 6.01 (1H, dd, 1H, dd, *J* = 10.4 and 1.0), 5.74 (1H, d, *J* = 8.0), 5.02 (1H, d, *J* = 8.4), 4.79 (1H, d, *J* = 1.2), 4.67 (1H, d, *J* = 1.2), 4.54 (1H, t, *J* = 7.2), 4.37 (1H, d, *J* = 8.0), 4.32 (1H, d, *J* = 8.0), 3.53 (1H, d, *J* = 8.4), 2.55 (1H, ddd, J_1 = 15.2, J_2 = 7.8 and J_3 =1.6), 2.21 (1H, dd, *J* = 15.2 and 8.0), 2.05 (1H, m), 1.98 (3H, s), 1.91 (1H, m), 1.68 (3H, s), 1.11 (3H, s); ^{13}C-NMR δ 207.3, 165.4, 164.7, 146.9, 145.9, 137.3, 133.5, 131.5, 130.0, 129.9, 129.1, 128.7, 112.1, 84.8, 79.4, 76.9, 75.8, 75.1, 73.9, 72.7, 71.5, 63.4, 56.8, 44.0, 42.9, 38.5, 21.3. HRFABMS *m/z* = 539.2297 $[M+H]^+$ calculated for $C_{30}H_{35}O_9$ 539.2281 (Δ = +2.6 ppm).

15(16)-Anhydro-4,10-di-deacetyl-4-acryloyl-11(15→1)-abeo-baccatin III (6). When compound **5** was treated with cerium (III) chloride and acetic anhydride in anhydrous THF,[2] no acetylation was observed over 4 h, even after repeated attempts. A modeling study using the Spartan program indicated that the distance between the oxygen atoms at C-7 and C-9 had increased from 3.194 Å in 10-deacetybaccatin III to 3.427 Å in compound **5**. It thus appears that these two oxygen atoms are too far apart to chelate easily with Ce^{3+} to activate the C-10 hydroxyl group.

[2] These conditions have been used frequently for acetylation on the C-10 position of 10-deacetyl baccatin III analogs, and usually give a rapid and high-yielding reaction.

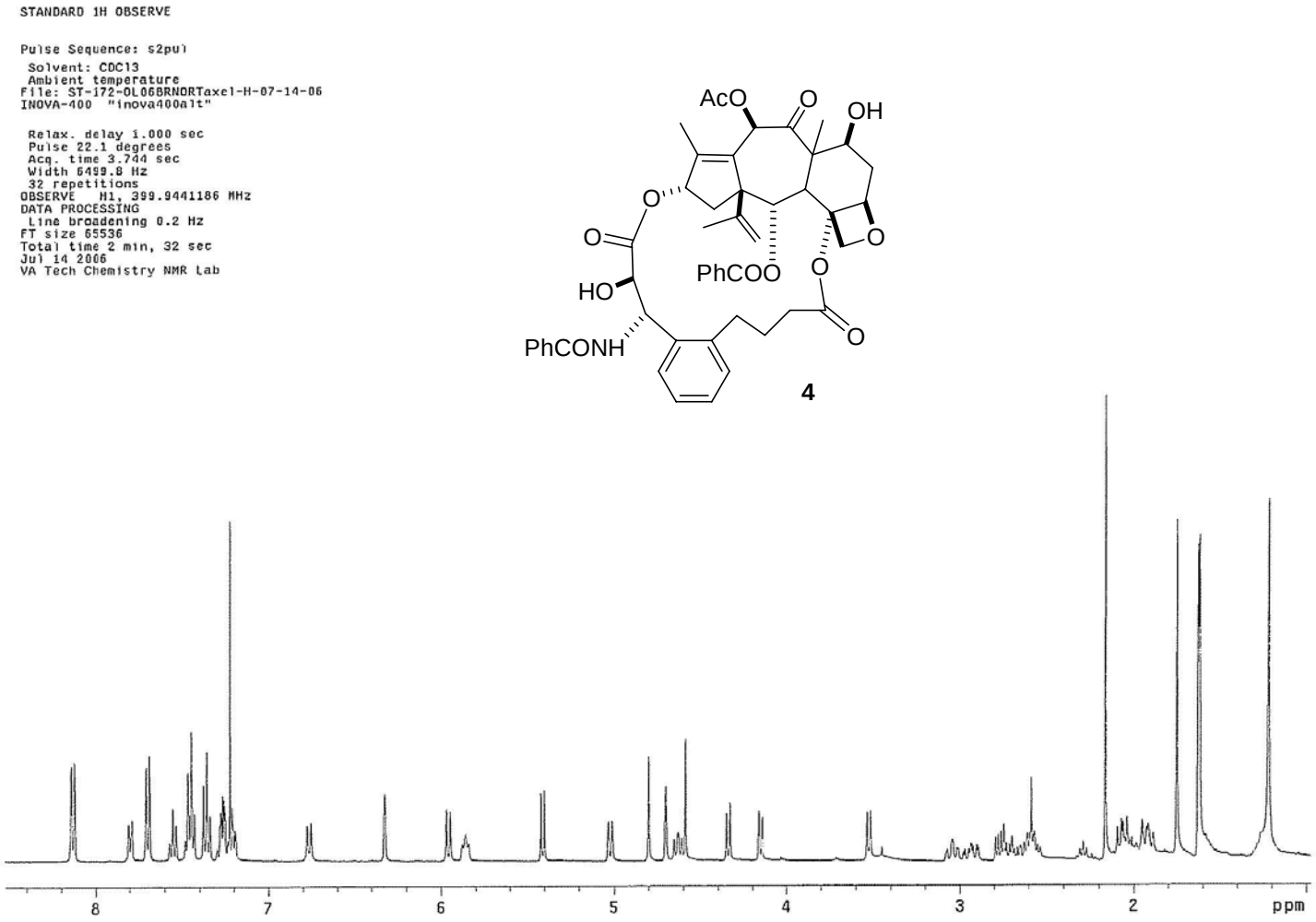

Figure S2. ^{1}H NMR spectrum of bridged A-*nor*-paclitaxel **4**

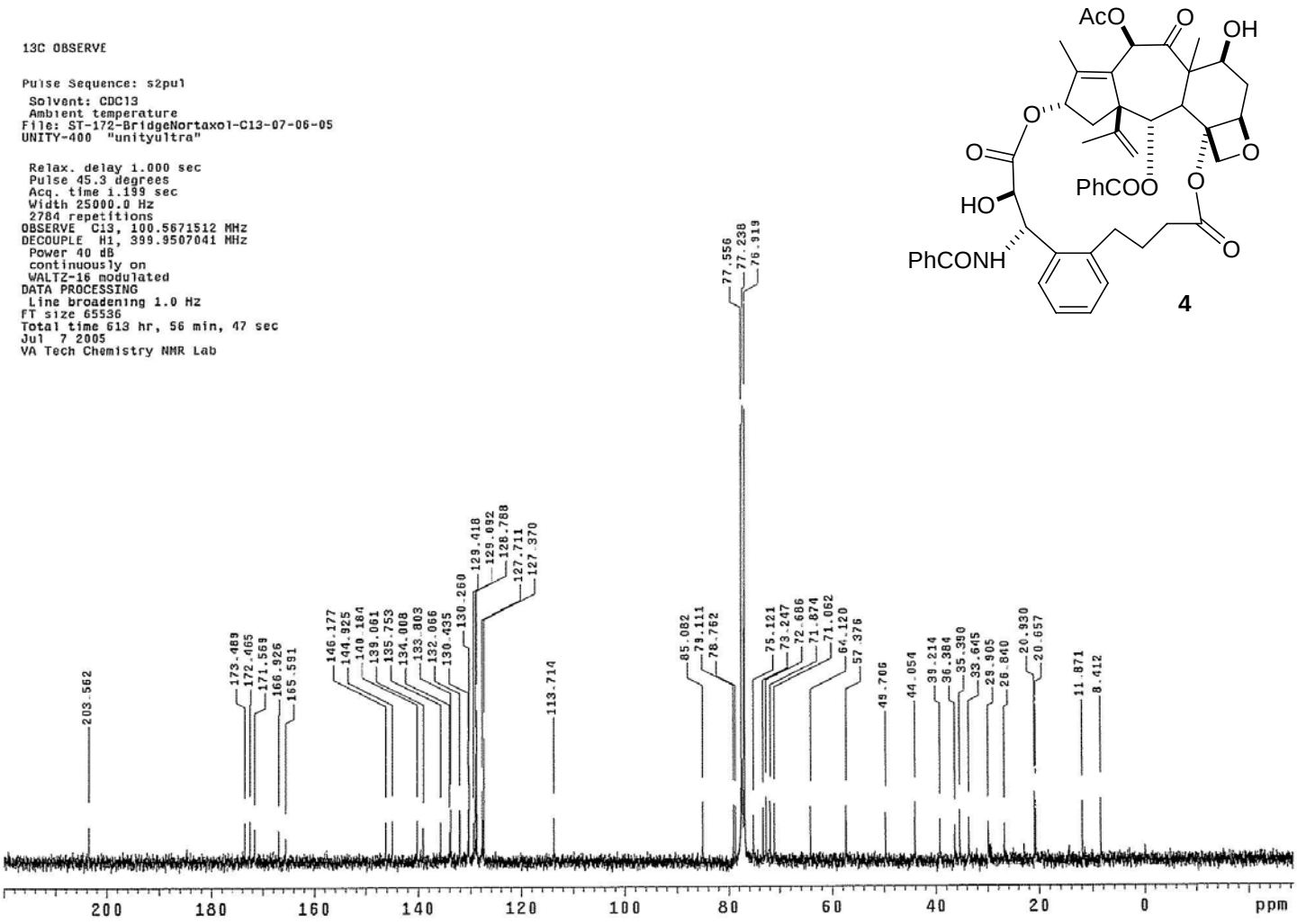

Figure S3. ^{13}C NMR spectrum of bridged A-*nor*-paclitaxel **4**

B. Biochemical Evaluations

Tubulin purification. Tubulin purification and determination of protein concentration was carried out as previously described.[31] Tubulin-GDP, in which the E site GTP is replaced by GDP was prepared according to the method of Seckler *et al*.**[4]** Determination of ED_{50} for tubulin polymerization by compounds was carried out as described in Chatterjee *et al*.[5]

Determination of critical concentration of tubulin. The critical concentrations of tubulin-GTP or tubulin-GDP for **1**, **3** or **4** were determined by the way of assembly experiments performed at 37°C. Protein samples at different concentrations in PME (100 mM PIPES, 1 mM MgSO4, and 2 mM EGTA) buffer were polymerized with equimolar concentrations of **1, 3** or **4**. The extent of assembly in each case was measured in terms of apparent absorption at 350 nm. Critical concentrations were obtained as the *x*-intercepts of plots of apparent absorption at 350 nm (ΔA_{350nm}) vs. tubulin-GDP concentration.

Determination of equilibrium binding constants. Equilibrium binding constants of **1**, **3** and **4** were determined for GMPcPP microtubules. GMPcPP microtubules were prepared by incubating tubulin-GDP with 100 times excess of GMPcPP followed by 20 min incubations in ice and at 37°C respectively. The binding ability of **1**, **3** and **4** to stabilized (GMPcPP) microtubules was assessed by competition between these compounds and fluorescent taxol, N-AB-PT. 5 μM each of GMPCPP-microtubules and N-AB-PT were incubated with varying (0 – 40 μM) concentrations of **1**, **3** or **4** at 37 ºC for 30 min. The fluorescence emission intensity for N-AB-PT

[3] Chatterjee, S. K.; Laffray, J.; Patel, P.; Ravindra, R.; Qin, Y.; Kuehne, M. E.; Bane, S. *Biochemistry* **2002**, *41,* 14010-14018.
[4] Seckler, R.; Wu, G.M; Timasheff, S.N. *J. Biol. Chem.* **1990**, *265*, 7655-7661.
[5] Chatterjee, S. K.; Barron, D. M.; Vos, S.; Bane, S. *Biochemistry* **2001**, *40*, 6964-6970.

bound to microtubules in each sample was measured at 413 nm,[6] using Jobin Yvon spectrofluorimeter. EC_{50} for GMPcPP microtubules was obtained by plotting F/F0 versus log of the concentration of **1**, **3** or **4** where F is the fluorescence of N-AB-PT in the presence of a particular concentration of **1**, **3** or **4** and F0 is the fluorescence intensity of N-AB-PT in the absence of any competitor. The EC_{50} for GMPcPP microtubules was subsequently used to determine the apparent dissociation constant for **1**, **3** or **4** using one site competition relation $K_i = EC_{50}/(1+[\text{N-AB-PT}]/K_d)$, where K_i is the dissociation constant of **1**, **3** or **4**; K_d is the dissociation constant for N-AB-PT with GMPcPP microtubules (15 nM).[6] The inverse of K_i is the apparent equilibrium binding constant K_a.

Determination of cytotoxic activity. The cytotoxicity assay was performed at SUNY as described previously.[7] PC3 cell cultures were grown in Ham's medium supplemented with 10% fetal calf serum, and maintained in a humidified atmosphere of 5% CO_2 in air at 37°C. 5000 cells were plated on to each well of a 96 well plate (Becton Dickinson, Franklin Lakes, NJ, USA). After four days the cells were treated with varying concentrations (2-6600nM) of **1**, **3** or **4** and incubated at 37°C for four days. At the end of incubation, MTT was added and the reaction was terminated after 4 hours by adding 200 μL of isopropanol to each well. The absorbance of MTT dye at 570 nm was recorded using a microplate reader (model # EL 312e, BIOTEK Instruments, Winooski, VT, USA). The IC_{50} of each compound was obtained from the plot of MTT absorbance at 570 nm versus concentration of the compounds. For each compound, IC_{50}

[6] Li, Y.; Edsall, R. Jr.; Jagtap, P. G.; Kingston, D. G. I.; Bane, S. *Biochemistry* **2000**, *39,* 616-623
[7] Chang, M. C.; Uang, B. J.; Wu, H. L.; Lee, J. J.; Hahn, L. J.; Jeng, J. H. *Br. J. Pharmacol.* **2002,** *135*, 619-630.

was determined in two independent experiments. Cytotoxicities in the A2780 assay were determined at VPISU as previously described.[8]

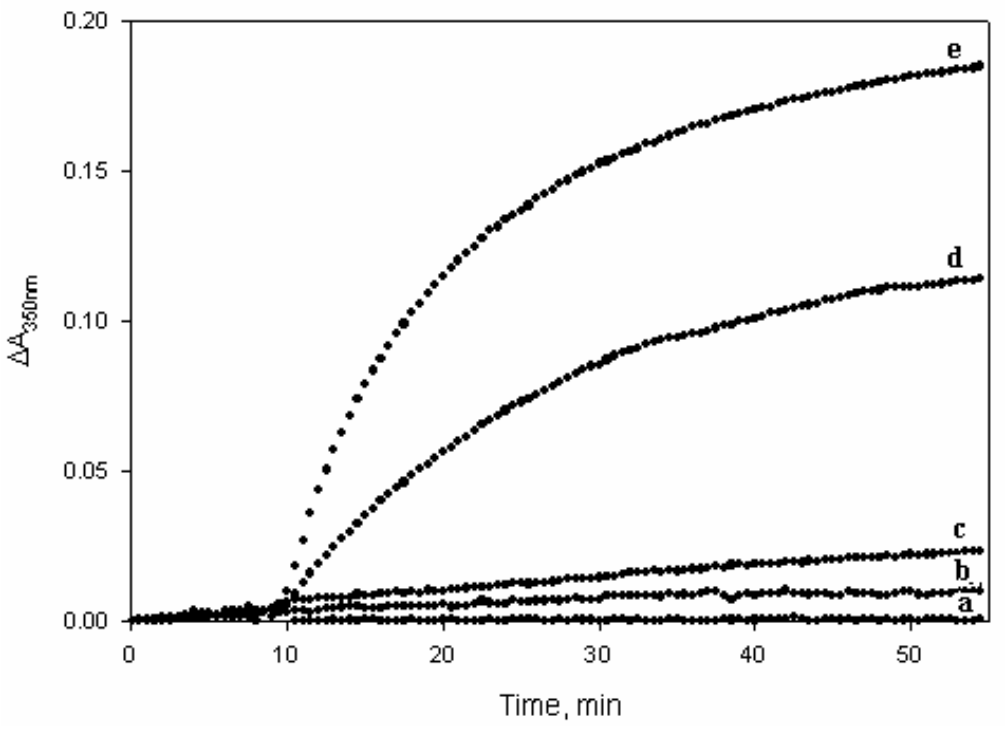

Figure S4. Polymerization of tubulin-GTP by the unbridged A-*nor* analog **3** in the presence of 0.1mM GTP. Tubulin and **3** were used in concentration ratio of 1:1. (a) 1μM, (b) 2μM, (c) 3μM, (d) 4μM and (e) 5μM

[8] Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. *Cancer Res.***1985**, *45*, 2110-2115.

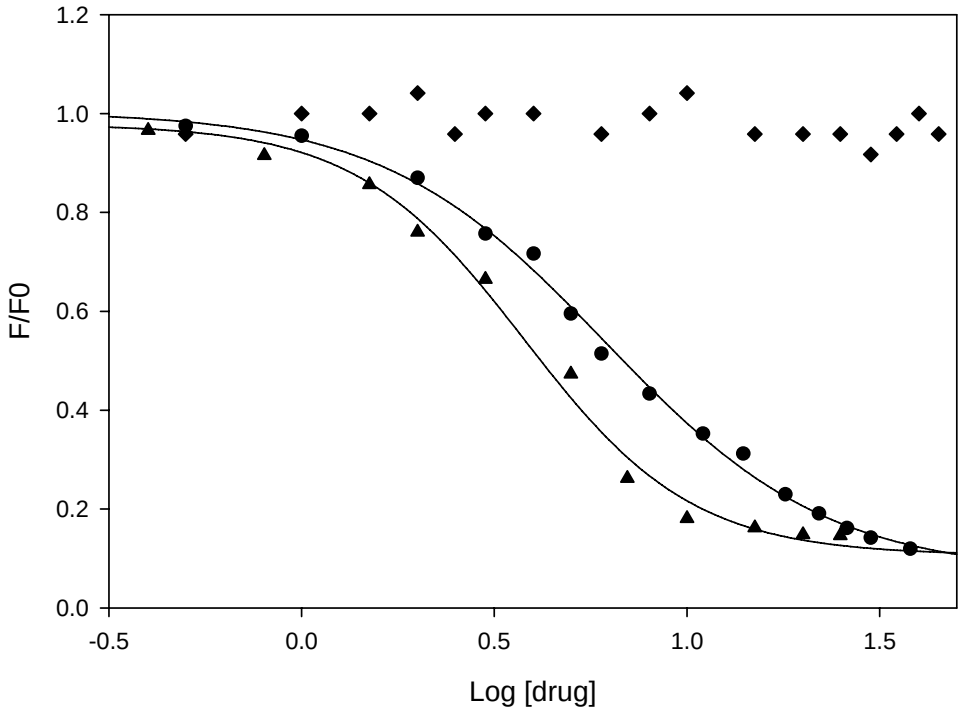

Figure S5. Competition binding curves to GMPcPP microtubules. PTX **1** (●), Unbridged A-*nor* analog **3** (♦) and Bridged A-*nor* analog **4 (▲).**

C. Modeling Studies

Conformational Searching, Conformer Extraction and Binding Site Docking. A 10,000 step MMFF/GBSA/H_2O Monte Carlo MCMM conformational search[9] for A-*nor* PTX **3** and bridged A-*nor* PTX **4** was performed with the Maestro program suite (7 kcal/mol cutoff).[10] This provided 464 and 613 fully-optimized conformations and location of the corresponding global minima 28 and 9 times, respectively. The individual conformational data sets were filtered for T-Taxol conformations by fitting the centroids of the three taxane side chain phenyl rings against those of T-Taxol[11] with the APOLLO software.[12] Bridged **4** delivered 61 T-form minima, while acyclic **3** yielded 75 such conformers. In each case, one of the top two fits provides a very close match to the structure of T-Taxol (Figure 1). In addition, both **3** and **4** were docked into β-tubulin with the Glide protocol[13] using flexible ligand docking and the OPLS force field. Both were found to readily adopt the T-form.

The polar and nonpolar conformers of 3 and 4 were likewise extracted from the conformational searches. Extraction criteria were the same as for T-Taxol, namely fitting the centroids of the three taxane side chain phenyl rings to those of the corresponding taxane conformers described in Refs 9a and 13. For the PTX-NY structure,[14,15] the three centroid constraints were further

---

[9] Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379-4386.
[10] http://www.schrodinger.com/Products/maestro.html
[11] a) Snyder, J. P.; Nevins, N.; Cicero, D. O.; Jansen, J. *J. Am. Chem. Soc.* **2000**, *122*, 724-725; b) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U. S. A.* **2001,** *98***,** 5312-5316.
[12] a) Snyder, J. P.; Rao, S. N.; Koehler, K. F.; Vedani, A.; Pellicciari, R. In *Trends in QSAR and Molecular Modeling92,* Wermuth, C. G., Ed. ESCOM, Leiden, 1993 pp 44-51; b) Jansen, J. M.; Copinga, S.; Gruppen, G.; Molinari, E. J.; Dubocovich, M. L.; Grol, C. J. *Bioorg. Med. Chem.* **1996**, *4*, 1321-1332.
[13] a) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Sander, L. C.; Shenkin, P. S. *J. Med. Chem*. **2004**, *47*, 1739-1749; b) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750-1759; c) Glide, version 4.0, Schrödinger, LLC, New York, NY, 2005.
[14] Geney, R.; Sun, L.; Pera, P.; Bernacki, R. J.; Xia, S.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I. *Chem. & Biol.* **2005**, *12*, 339-348.
[15] a) Johnson, S. A.; Alcaraz, A.; Snyder, J. P. *Org. Lett.* **2005**, *7*, 5549-5552; b) Alcaraz, A. A.; Mehta, A. K.; Johnson, S. A.; Snyder, J. P. *J. Med. Chem*. **2006**, *49*, 2478-2488.

complemented by the location of the hydroxyl oxygen in C2'-OH.[16] Neither the polar nor the PTX-NY conformer types are represented in the conformer database of **4**, although the acyclic set (**3**) contains them. Table S1 provides a breakdown of the conformations found among the 464 and 613 fully-optimized structures for **3** and **4**, respectively.

Rigid Glide docking[13] of the top nonpolar forms of both PTX and **4** into the taxane binding site of tubulin leads to two poses in each case. The first is outside the binding pocket, while the second is a loose-fitting pose in the pocket at odds with the electron crystallographic structure.[17] The source of the latter is the inability of the nonpolar conformer to accommodate His227 which resides between the terminal C-3' CONHPh and C-2 OCOPh phenyl rings in the experimental complex. The situation, along with the predicted reduced activity, is analogous to that observed for a first generation C-4(OAc) – C-3'(*o*-Ph) bridged paclitaxel[18] and serves to eliminate the nonpolar conformation from consideration.

Table S1. Number of different taxoid conformations for four selected conformers found among the optimized datasets for A-*nor*-paclitaxels **3** and **4**.[a]

	Polar	Nonpolar	PTX-NY[14,15]	T-Taxol
Acyclic **3**	72	130	3	75
Bridged **4**	0	286	0	61

[a] The remaining conformations of the 464 and 613 ensembles, respectively, are of other types.

[16] See Figure 2a in Ref. 15.

[17] Nettles, J. H.; Li, H.; Cornett, B.; Krahn, J. M.; Snyder, J. P.; Downing, K. H. The Binding Mode of Epothilone A on ąb-Tubulin by Electron Crystallography. *Science* **2004**, *305*, 866-869.

[18] Metaferia, B. B.; Hoch, J.; Glass, T. E.; Bane, S. L.; Chatterjee, S. K.; Snyder, J. P.; Lakdawala, A.; Cornett, B.; Kingston, D. G. I. *Org. Lett.* **2000**, *3*, 2461-2464.

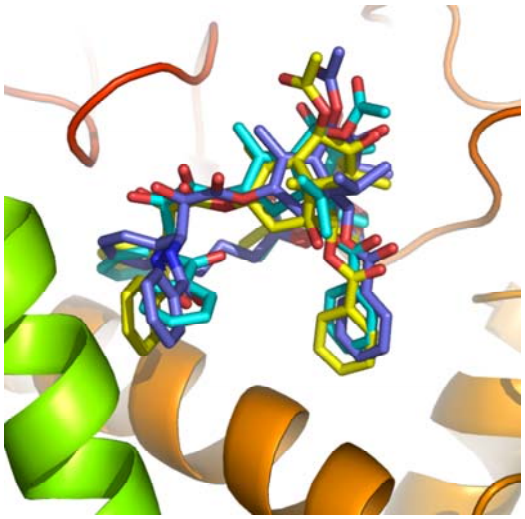

Figure S6. Glide-determined docking poses of acyclic **3** (cyan), bridged **4** (blue) and T-Taxol (yellow) within the tubulin taxane binding site.