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Synthesis and Biological Activity of C-6 and C-7 Modified Paclitaxels

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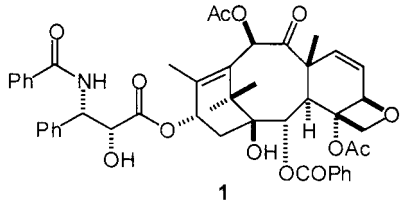
Abstract—Structure modification of paclitaxel at the C-6 and C-7 positions has been achieved using the readily available intermediate 6α-hydroxy-7-*epi*paclitaxel (**2**). While a single diastereomer of the cyclic sulfite of **2** was prepared at lower temperature, both diastereomers were obtained at room temperature. The cyclic sulfate was found to be inert toward nucleophilic attack, which confirms the great steric hindrance of the β-face of the C-6 and C-7 region of paclitaxel. Derivatization at the 6β-position with a nitrogen-containing function was accomplished using the alternate substrate 6α-trifluoromethanesulfonate (**9**), with the 7-*epi* hydroxyl group protected to avoid the formation of C-ring rearranged product. Hydrogenation of the 6β-azide was difficult but could be achieved in moderate yield under high pressure. Similar to other C-6 and C-7 modified analogs reported earlier, these new compounds displayed comparable in vitro cytotoxicity to paclitaxel against the HCT116 human colon cancer cell line and the A2780 human breast cancer cell line.

Introduction

Paclitaxel has established itself as one of the most important cancer chemotherapeutic agents in the past decades through extensive chemical, biological, and medicinal research and development.[1] The chemical synthesis of various paclitaxel analogs has not only provided critical information on the structure–activity relationships of the molecule, but has also rendered access to paclitaxel derivatives with desired chemical and physical properties. Some of these analogs have been utilized in helping to understand the interaction between paclitaxel and its biological target at a molecular level. The results of these studies may be useful in the design and discovery of new generation anticancer drugs that act in a similar way. Structure modification of paclitaxel at the C-6 position has been achieved by our group and others, mainly via the 6,7-olefin (**1**) and the derived 6,7-diol (**2**).[2] A number of analogs have been synthesized, such as 6α-hydroxy-7-deoxy-paclitaxel,[3] C-6 monoesters of 6α-hydroxy-7-*epi*paclitaxel,[2a] and the C-6/C-7 cyclic ester/thioester of 6α-hydroxy-7-*epi*paclitaxel.[2]



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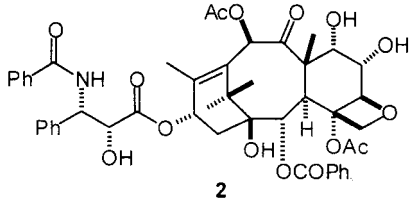

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The extant biological data suggest that modifications of paclitaxel at the C-6/C-7 positions only cause slight changes in its cytotoxicity and tubulin-assembly activity.[2,3] In continuation of our efforts to find paclitaxel derivatives with improved activity and favorable properties, we have made some further studies of analogs at the C-6/C-7 position. In this report, we detail the synthesis and biological evaluation of these analogs.

Results and Discussion

A single diastereomer of 6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (**4**) with respect to the orientation of the

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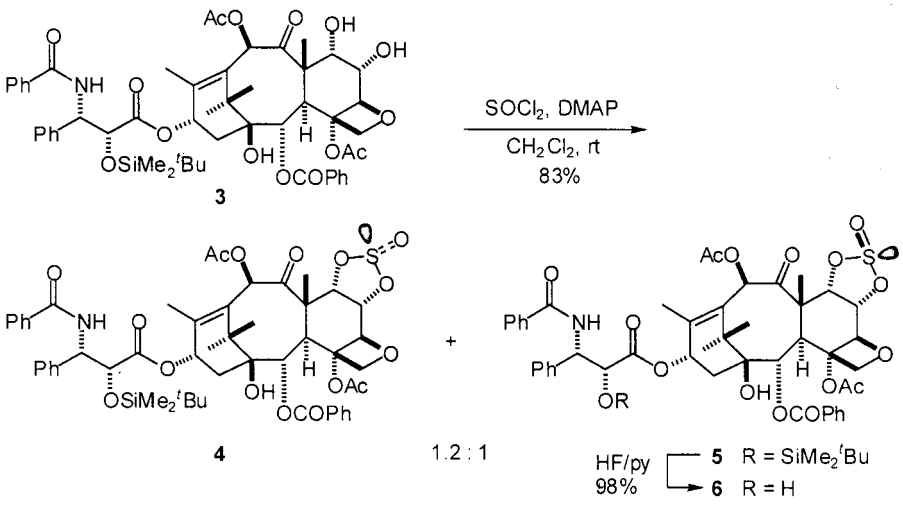


Scheme 1.

lone pair electrons of the sulfur atom was synthesized previously by treating 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel (**3**) with thionyl chloride and 4-dimethylaminopyridine (DMAP) at 0°C.[4] We envisioned that the other diastereomer might exert somewhat different biological properties due to its different local polarity profile, and also that either the cyclic sulfite or the cyclic sulfate would be good precursors to C-6 and/or C-7 paclitaxel derivatives through nucleophilic displacement. The synthesis of **4** was thus revisited under conditions that could give rise to the other diastereomer. Indeed, at room temperature a mixture of the two cyclic sulfites (**4** and **5**) in about a 1.2 : 1 ratio was obtained and was separated by careful multiple elution on preparative TLC (Scheme 1). Deprotection gave the β diastereomer of the cyclic sulfite (**6**).

The two isomers **4** and **5** had similar polarities, and they also shared many common resonances in both ^{1}H and ^{13}C NMR spectra. The significant differences were the protons at the C-5, C-7, C-14α, and C-20β positions. These are listed in Table 1 for comparison (the corresponding data for the cyclic sulfate **7** are also included). Compounds **4** and **7** have very similar proton and carbon signals, and both are considered typical as compared with paclitaxel. The unusual downfield chemical shift of the H-5 and H-14α protons in compound **5** suggested that the lone pair electrons of the sulfur atom in this compound were in close proximity to these protons, which in turn established the orientation of the lone pair electrons as being on the α-face. The opposite orientation (β) was thus assigned to sulfite **4**.

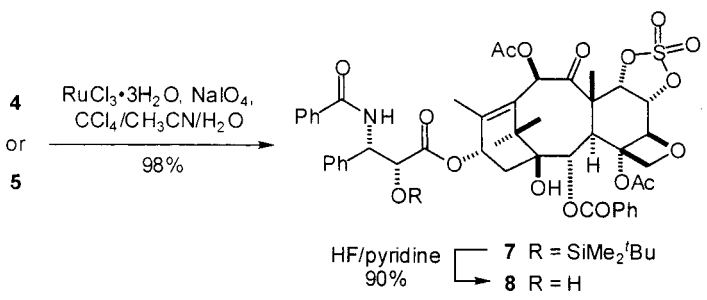
Table 1. Comparison of selected proton chemical shifts (ppm) of compounds **4**, **5** and **7**

Protons	4	5	7
H-5	4.76	5.86	5.03
H-7	4.89	4.39	4.86
H-14α	2.40	3.08	2.47
H-20β	4.35	5.04	4.46

In order to further verify and identify each of the two diastereomers, both sulfites were oxidized to the sulfate with ruthenium trichloride and sodium periodate. As expected, both **4** and **5** were converted to the same 6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (**8**) in excellent yield after deprotection (Scheme 2).

The 1,2 cyclic sulfate has been known as a useful synthon with chemical reactivity similar to an epoxide,[5] and in many cases it is even better than an epoxide for nucleophilic reactions.[6] It was thus expected that nucleophilic substitution at either the C-6 or the C-7 position of **7** would give *trans*-disubstituted paclitaxel analogs.

However, when compound **7** was treated with sodium azide and tetrabutylammonium azide,[7] no detectable reaction was observed. It was reasoned that the stability of the cyclic sulfate **7** toward nucleophilic reaction was due to the steric hindrance of the β face of the molecule caused by the C-19



Scheme 2.

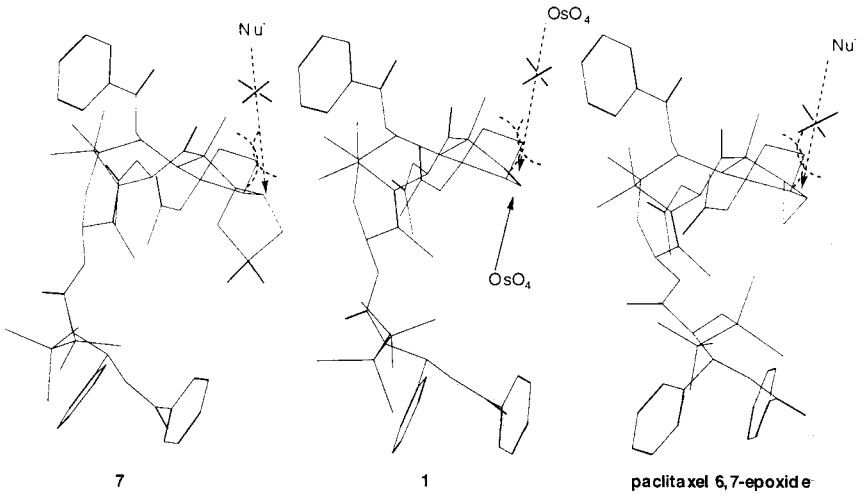
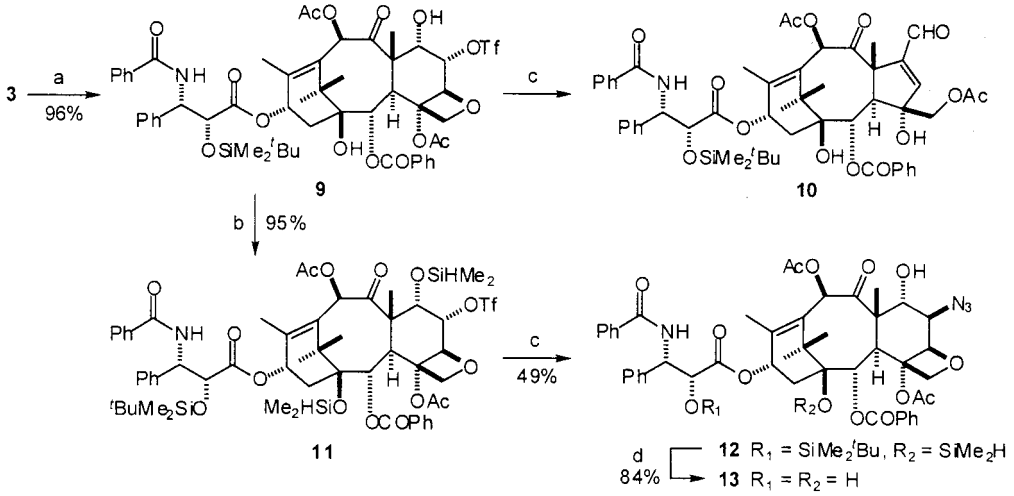


Figure 1. Stereo view of the steric hindrance of the β face of **7** and related compounds.

angular methyl group and the oxetane ring (Fig. 1). This same steric hindrance initially determined the stereochemistry of the dihydroxylation of the 6,7-olefin (**1**), even in the presence of asymmetric induction that favors the other (β) isomer, and uniformly gave the 6α,7α-diol (Fig. 1).[8] This result is also in accordance with the observed stability of paclitaxel 6,7-epoxide. When the epoxide was treated with a variety of reagents with the intention of opening the epoxide ring, only starting material was recovered, and under harsh conditions the epoxide ring remained intact while the oxetane ring was often opened.[9]

Since we were unable to open the cyclic sulfate **7** by nucleophilic substitution, we adopted an alternate approach by converting the 6α hydroxyl group into a good leaving group, which could be substituted directly by nucleophiles. Treatment of 2′-*O*-*tert*-butyldimethylsilyl-6α-hydroxy-7-*epi*paclitaxel (**3**) with trifluoromethanesulfonyl chloride and DMAP furnished the desired 2′-*O*-*tert*-butyldimethylsilyl-6α-trifluoromethanesulfuryl-7-*epi*paclitaxel (**9**) in excellent yield. However, treatment of **9** with sodium azide induced a pinacol-type rearrangement, presumably promoted by the weak base NaN_3, which led to a product with a contracted C-ring (**10**, Scheme 3) instead of the desired substitution product. This competing reaction has also been observed previously with other bases.[2b,2c,4] To avoid this undesired reaction it was necessary to eliminate the retro-aldol pathway by protection of the 7-*epi* hydroxyl group, but this was found to be sterically hindered and stable toward a number of silylation and esterification conditions. Protection was finally achieved with dimethylsilyl chloride to give 2′-*O*-*tert*-butyldimethylsilyl-1,7α-bis-*O*-dimethylsilyl-6α-trifluoromethanesulfuryl-7-*epi*paclitaxel (**11**). In the event, the C-1 hydroxyl group was also protected.[10] Having protected the C-7 hydroxyl group, the azide substitution reaction was effected by treating compound **11** with sodium azide in anhydrous DMF at room temperature for 18 h, to give the desired 2′-*O*-*tert*-butyldimethylsilyl-6β-azido-7-*epi*paclitaxel (**12**) in 49% yield. The C-7 dimethylsilyl protecting group was removed during the reaction,



[a] Conditions: (a) CF_3SO_2Cl, DMAP, CH_2Cl_2; (b) Me_2HSiCl, imidazole, DMF; (c) NaN_3, DMF; (d) HF/pyridine, THF.

Scheme 3.

Table 2. Selected coupling constants of paclitaxel and its known C-6 and C-7 analogs

Compound	H_5–$H_{6\alpha}$	H_5–$H_{6\beta}$	$H_{6\alpha}$–$H_{7\beta}$	$H_{6\beta}$–$H_{7\beta}$
7-*epi*-Paclitaxel[11]	9.0–9.2	3.5–3.7	2.1	5.0
3	–	2.0–2.4	–	4.4–4.8
9	–	2.4	–	4.8
6α-*O*-Esters of **3**[2a]	–	2.5–2.6	–	4.7–4.8
Paclitaxel	9.5–9.6	2.0–2.3	–	–
6α-Hydroxypaclitaxel[9b]	–	0	–	–

which may account for the low yield of this reaction due to the competing rearrangement discussed earlier. Desilylation of **12** afforded **13** in 84% yield.

The stereochemistry at C-6 was determined both by a NOESY experiment and by the determination of coupling constants. The only through-space NOE correlation observed for H-6 of the azide **13** was with 7-*epi* OH, which in turn had correlation with H-3. This suggests an α proton at the C-6 position. To ensure this stereochemistry assignment, the coupling constants of the H-5, H-6, and H-7 protons of **13** were compared with those of C-6 and C-7 modified analogs with known stereochemistry (Table 2). The observed coupling constants of compound **13** (H_5–H_6 8.0–8.4, H_6–H_7 1.6–2.0) also supported an α proton at the C-6 position. The structure of **13** was thus determined to be 6β-azido-7-*epi*paclitaxel. This stereochemistry is what would be expected from an S_N2 reaction.

Having successfully prepared 6β-azido-7-*epi*paclitaxel (**13**), its reduction to the amino analog was the next immediate goal in order to evaluate the effect of an amino group at C-6 on the anticancer activity as well as to afford an analog with improved water solubility in acidic environments. Compound **13** was hydrogenated in methanol catalyzed by 10% palladium on activated carbon under atmospheric pressure. TLC analysis after 1.5 h indicated complete consumption of the starting material and formation of very polar products (R_f~0 in 7:3 EtOAc:hexanes). The major component of the reaction mixture isolated in 40% yield was tentatively identified as 6β-triazeno-7-*epi*paclitaxel (**14**). The ^{1}H NMR spectrum of **14** features an upfield shift of the C-6 proton from 4.12 to 3.0 ppm, in accordance with the reduction of the azide functionality. The HRFABMS revealed, however, that compound **14** had a molecular formula of $C_{47}H_{52}O_{14}N_4$, corresponding to the unusual partially hydrogenated triazeno analog, as in Scheme 4. Although monosubstituted aliphatic triazenes are normally not isolable, being transient intermediates in the hydrogenation of azides, they have been isolated previously in at least one case.[12]

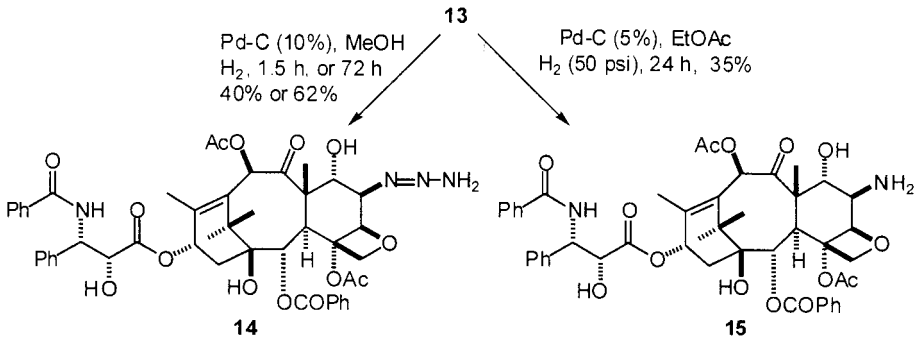
Table 3. Cytotoxicity of C-6/C-7 modified paclitaxels

Compound	IC_{50} (μM)	
	HCT116	A2780
Paclitaxel	0.0021	0.0034
6α-Hydroxy-7-epipaclitaxel	0.0190	0.0112
6,7-*O*,*O*′-cyclosulfite (**6**)		
6α-Hydroxy-7-epipaclitaxel	>0.1073	>0.1073
6,7-*O*,*O*′-cyclosulfate (**8**)		
6β-Azido-7-epipaclitaxel (**13**)	0.00073	0.0021
6β-Amino-7-epipaclitaxel (**15**)	0.0802	0.0460

A stoichiometric amount of Pd–C was then used along with a much longer reaction time, in an effort to completely reduce the azide. However, a complex reaction mixture again resulted after 3 days. The major product, isolated in 62% yield, was identical with **14**, suggesting that the triazene **14** is relatively inert toward hydrogenation. Other paclitaxel azides have proven to be resistant to hydrogenation; thus the 7α-azide could not be hydrogenated at atmospheric pressure,[4] and the only reported hydrogenation of paclitaxel azide in the literature required 48 psi hydrogen for 72 h and gave only 50% yield.[13] A hydrogenation experiment under 50 psi hydrogen pressure was thus conducted and yielded the desired 6β-amino-paclitaxel (**15**) in an unoptimized 35% yield (Scheme 4).

The structure of compound **15** was assigned by ^{1}H, ^{13}C, TOCSY, and HMQC spectra. The 6α proton was found to shift from 4.12 to 3.35 ppm, while the C-6 carbon was shifted from 62.3 to 54.6 ppm. Both changes are consistent with the expected reduction of an azide to an amine. The molecular formula was confirmed by HRFABMS.

Compounds **6**, **8**, **13** and **15** were evaluated in an in vitro cytotoxicity assay against the human colon cancer cell line HCT116 and the breast cancer cell line A2780. The results are summarized in Table 3. Interestingly, while the cyclic sulfite **6** showed modest activity (about tenfold less active than paclitaxel in the HCT116 cell line), the cyclic sulfate **8** was essentially inactive. The aminopaclitaxel **15** was also significantly less active than paclitaxel itself, but the azido analog **13** was 2–3 times more cytotoxic than paclitaxel.



Scheme 4.

Since the amino derivative **15** was not sufficiently active for it to be of interest for drug development, we did not pursue the synthesis of additional analogs in this area.

Experimental

General 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. Anhydrous THF and Et_2O were distilled from sodium/benzophenone. Anhydrous toluene was distilled from sodium. CH_2Cl_2 was distilled from CaH_2. 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. ^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 (TOCSY, HMQC, HMBC, NOESY). All 2D-NMR spectra were obtained on the Varian Unity 400 spectrometer. Chemical shifts were reported as δ-values using residual $CHCl_3$ 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)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (β) (4) and 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (5). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel (3, 40 mg, 0.041 mmol) in dry CH_2Cl_2 (2 mL) was added DMAP (20 mg, 0.16 mmol) and $SOCl_2$ (9 μL, 0.12 mmol) and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was then diluted with EtOAc 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 4:6) to afford 27.8 mg of a mixture of 2′-*O*-(*tert*-butyldimethylsilyl)-6α,7α-*O*-cyclosulfonyl(α)-paclitaxel (**5**) and 2′-*O*-(*tert*-butyldimethylsilyl)-6α,7α-*O*-cyclosulfonyl (β)-paclitaxel (**4**) along with starting material (**3**, 10 mg). The mixture was further separated by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 2.5:7.5, multiple elution) to afford 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (**5**, 9 mg, 37% based on unrecovered staring material) and 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (β) (**4**, 11 mg, 46% based on unrecovered staring material) along with starting material (**3**, 6 mg, decomposed on PTLC plate).

2′-*O*-(*tert*-Butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (5). ^1H NMR δ 8.02 (d, J=7.2 Hz, 2H), 7.75 (d, J=7.2 Hz, 2H), 7.58–7.28 (m, 11H), 7.11 (d, J=9.6 Hz, 1H), 6.79 (s, 1H), 6.33 (t, J=8.8 Hz, 1H), 5.85 (s, 1H), 5.80 (m, 2H), 5.04 (d, J=8.4 Hz, 1H), 4.94 (d, J=6.8 Hz, 1H), 4.65 (d, J=1.6 Hz, 1H), 4.39 (d, J=6.4 Hz, 1H), 4.21 (d, J=8.4 Hz, 1H), 3.80, (d, J=7.2 Hz, 1H), 3.07 (m, 1H), 2.52 (s, 3H), 2.22 (s, 3H), 2.10 (m, 1H), 1.98 (s, 3H), 1.91 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.78 (s, 9H), −0.06 (s, 3H), −0.33 (s, 3H). ^{13}C NMR δ 204.4, 171.2, 169.7, 169.6, 167.2, 166.9, 141.5, 138.3, 134.2, 133.8, 131.9, 131.8, 129.9, 129.1, 128.9, 128.8, 128.7, 127.9, 127.0, 126.3, 88.0, 84.2, 81.7, 80.1, 78.9, 77.2, 75.6, 75.2, 74.6, 70.8, 55.5, 54.2, 42.6, 42.0, 35.8, 25.8, 25.5, 22.9, 21.4, 20.8, 18.1, 15.4, 15.0, −5.3, −5.9. HRFABMS calculated for $C_{53}H_{63}NO_{16}SSi$ $(M+H)^+$ 1030.3716, found 1030.3701, error −1.4 ppm.

6α-Hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (6). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (**5**, 3.2 mg, 0.0031 mmol) in dry THF (100 μL) was added HF-pyridine (70%, 20 μL) 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 sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500μ, EtOAc:hexanes 6:4) to afford 6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (α) (**6**, 2.8 mg, 98%). ^1H NMR δ 7.98 (d, J=8.4 Hz, 2H), 7.78 (dd, J=8.0 Hz, 1.2, 2H), 7.60–7.32 (m, 11H), 7.13 (d, J=9.6 Hz, 1H), 6.72 (s, 1H), 6.17 (t, J=8.0 Hz, 1H), 5.81 (m, 2H), 5.80 (s, 1H), 5.00 (d, J=8.4 Hz, 1H), 4.94 (d, J=6.4 Hz, 1H), 4.78 (m, 1H), 4.36 (d, J=6.8 Hz, 1H), 4.16 (d, J=8.0 Hz, 1H), 3.77 (d, J=6.8 Hz, 1H), 3.43 (d, J=5.2 Hz, 1H), 3.02 (m, 1H), 2.36 (s, 3H), 2.33 (m, 1H), 2.23 (s, 3H), 1.89 (s, 3H), 1.72 (s, 3H), 1.18 (s, 3H), 1.17 (s, 3H). ^{13}C NMR δ 203.6, 172.3, 169.6 (2C), 167.1, 166.7, 140.7, 138.1, 133.9, 133.8, 132.8, 131.9, 129.8, 129.1, 128.9 (2C), 128.7, 128.3, 127.0 (2C), 87.6, 84.2, 81.7, 80.3, 78.4, 77.2, 75.4, 74.1, 73.4, 72.3, 54.8, 54.4, 42.4, 42.0, 35.6, 26.5, 22.6, 20.8, 20.5, 15.7, 14.8. HRFABMS calculated for $C_{47}H_{49}NO_{16}S$ $(M+H)^+$ 916.2851, found 916.2844, error −0.7 ppm.

2′-*O*-(*tert*-Butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (7). *Route A — from **4**:* To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (β) (**4**, 4.6 mg, 0.0045 mmol) in $CCl_4/H_2O/CH_3CN$ (120 μL/240 μL/120 μL) was added sodium periodate ($NaIO_4$, 4.8 mg, 0.022 mmol) and ruthenium trichloride ($RuCl_3 \cdot H_2O$, 1.2 mg, 0.0046 mmol) and the mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered through a pad of silica gel and rinsed with EtOAc (1 mL×2). The filtrate was concentrated under reduced pressure to afford 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfite (**7**, 4.6 mg, 98%).

*Route B—from **5**:* To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*-*O*′-cyclosulfite (α) (**5**, 23 mg, 0.022 mmol) in $CCl_4/H_2O/CH_3CN$

(0.6 mL/1.2 mL/0.6 mL) was added sodium periodate ($NaIO_4$, 24 mg, 0.11 mmol) and ruthenium trichloride ($RuCl_3 \cdot H_2O$, 6 mg, 0.023 mmol) and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was then filtered through a pad of silica gel and rinsed with EtOAc (2 mL×2). The filtrate was concentrated under reduced pressure to afford 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (**7**, 23 mg, 98%). ^{1}H NMR δ 8.15 (dd, *J*=9.2, 1.6 Hz, 2H), 7.73 (dd, *J*=9.2, 1.6 Hz, 2H), 7.62–7.32 (m, 11H), 7.08 (d, *J*=10.0 Hz, 1H), 6.69 (s, 1H), 6.30 (t, *J*=9.6 Hz, 1H), 5.80 (2d, 2H), 5.03 (s, 1H), 5.01 (d, *J*=7.6 Hz, 1H), 4.86 (d, *J*=7.6 Hz, 1H), 4.69 (d, *J*=2.4 Hz, 1H), 4.46 (d, *J*=9.6 Hz, 1H), 4.24 (d, *J*=9.6 Hz, 1H), 4.03 (d, *J*=7.6 Hz, 1H), 2.64 (s, 3H), 2.48 (m, 1H), 2.21 (s, 3H), 2.13 (m, 1H), 1.99 (br s, 3H), 1.90 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 0.78 (s, 9H), −0.04 (s, 3H), −0.32 (s, 3H).

6α-Hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (8). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (**7**, 4.6 mg, 0.0044 mmol) in dry THF (250 μL) was added HF-pyridine (70%, 40 μL) 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 sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 500μ, EtOAc:hexanes 6:4) to afford 6α-hydroxy-7-*epi*paclitaxel 6,7-*O*,*O*′-cyclosulfate (**8**, 3.7 mg, 90%). ^{1}H NMR δ 8.13 (d, *J*=7.2 Hz, 2H), 7.74 (d, *J*=7.6 Hz, 2H), 7.64–7.35 (m, 11H), 7.01 (d, *J*=9.2 Hz, 1H), 6.65 (s, 1H), 6.22 (t, *J*=8.8 Hz, 1H), 5.82 (2d, 2H), 4.99 (d, *J*=7.2 Hz, 1H), 4.98 (s, 1H), 4.84 (br s, 1H), 4.83 (d, *J*=7.2 Hz, 1H), 4.43 (d, *J*=8.0 Hz, 1H), 4.21 (d, *J*=8.0 Hz, 1H), 4.03 (d, *J*=7.2 Hz, 1H), 3.59 (br d, *J*=4.4 Hz, 1H), 2.45 (s, 3H), 2.44 (m, 1H), 2.33 (m, 1H), 2.21 (s, 3H), 1.88 (s, 3H), 1.86 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H). ^{13}C NMR δ 202.1, 172.7, 169.5, 169.1, 167.1, 166.9, 141.5, 138.0, 134.0, 132.0, 131.9, 130.2, 129.0, 128.9, 128.7, 128.3, 127.0, 84.8, 82.5, 80.3, 80.0, 78.3, 77.3, 74.1, 73.1, 71.8, 55.2, 54.7, 42.5, 39.2, 35.9, 25.8, 22.3, 21.1, 20.6, 15.1, 13.5. HRFABMS calculated for $C_{47}H_{49}NO_{17}S$ $(M+H)^+$ 932.2800, found 932.2813, error 1.4 ppm.

2′-*O*-(*tert*-Butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*epi*paclitaxel (9). 2′-*O*-(*tert*-Butyldimethylsilyl)-6α-hydroxy-7-*epi*paclitaxel (**3**, 170 mg) was dissolved in dry CH_2Cl_2 (2 mL) and treated with 4-dimethylaminopyridine (102 mg, 5 eq.) and trifluoromethanesulfonyl chloride (47 μL, 2 eq.) at 0°C with stirring for 1 h. The reaction mixture was then diluted with EtOAc (4.0 mL) and the precipitate filtered off on Celite. The resulting solution was evaporated and the residue purified by preparative TLC (silica gel, 1:1 EtOAc:hexanes) to furnish 2′-*O*-(*tert*-butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*epi*paclitaxel (**9**, 176 mg, 96%). ^{1}H NMR δ 8.15 (d, 2H), 7.70 (d, 2H), 7.62 (t, 1H), 7.64–7.26 (m, 10H), 7.07 (d, *J*=9.2 Hz, 1H), 6.77 (s, 1H), 6.29 (t, *J*=8.6 Hz, 1H), 5.80 (dd, *J*=8.8, 2.4 Hz, 1H), 5.73 (d, *J*=7.3 Hz, 1H), 5.28 (dd, *J*=4.6, 2.7 Hz, 1H), 4.93 (d, *J*=2.8 Hz, 1H), 4.90 (d, *J*=12.0 Hz 1H) 4.67 (d, *J*=2.3 Hz, 1H), 4.47 (d, *J*=8.9 Hz, 1H), 4.38 (d, *J*=8.9 Hz, 1H), 3.97 (d, *J*=7.3 Hz, 1H), 3.92 (dd, *J*=4.6, 12.0 Hz, 1H), 2.71 (s, 3H), 2.40 (dd, *J*=9.6, 15.2 Hz, 1H), 2.20 (s, 3H), 2.11 (dd, *J*=9.6, 15.2 Hz, 1H), 1.91 (s, 3H), 1.68 (s, 3H), 1.20 (s, 3H), 1.13 (s, 3H), 0.78 (s, 9H), −0.05 (s, 3H), −0.30 (s, 3H). HRFABMS calculated for $C_{53}H_{63}NO_{14}SiNa$ $(M+Na\text{-}CF_3SO_3H)^+$ 988.3915, found 988.3913, error −0.2 ppm.

1-*O*-Dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*O*-dimethylsilyl-7-*epi*paclitaxel (11). To a solution of 2′-*O*-(*tert*-butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*epi*paclitaxel (**9**, 68 mg, 0.060 mmol) in dry CH_2Cl_2 (3 mL) was added imidazole (80 mg, 1.2 mmol) and chlorodimethylsilane (DMSCl, 35 μL, 0.31 mmol) and the solution was stirred at room temperature for 1.75 h. The reaction mixture was then directly subjected to column chromatography (silica gel, EtOAc:hexanes 3:7) to afford 1-*O*-dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*O*-dimethylsilyl-7-*epi*paclitaxel (**11**, 71 mg, 95%). ^{1}H NMR δ 8.16 (d, *J*=8.4 Hz, 2H), 7.75 (m, 2H), 7.61–7.32 (m, 11H), 7.05 (d, *J*=9.2 Hz, 1H), 6.39 (s, 1H), 6.36 (t, *J*=8.4 Hz, 1H), 5.91 (dd, *J*=9.2 Hz, 2.0, 1H), 5.64 (d, *J*=7.2 Hz, 1H), 5.33 (dd, *J*=6.4 Hz, 2.4, 1H), 5.02 (d, *J*=6.4 Hz, 1H), 4.84 (m, 1H), 4.71 (d, *J*=2.4 Hz, 1H), 4.65 (d, *J*=8.4 Hz, 1H), 4.32 (m, 1H), 4.19 (2d, 2H), 4.04 (d, *J*=2.0 Hz, 1H), 2.69 (s, 3H), 2.50 (m, 1H), 2.35 (m, 1H), 2.19 (s, 3H), 1.91 (br s, 3H), 1.69 (s, 3H), 1.15 (s, 3H), 1.07 (s, 3H), 0.78 (s, 9H), 0.36 (d, *J*=2.8 Hz, 3H), 0.33 (d, *J*=2.8 Hz, 3H), −0.04 (s, 3H), −0.18 (d, *J*=2.8 Hz, 1H), −0.30 (s, 3H), −0.47 (d, *J*=2.8 Hz, 3H).

1-*O*-Dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6β-azido-7-*epi*paclitaxel (12). To a solution of 1-*O*-dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6α-trifluoromethanesulfuryl-7-*O*-dimethylsilyl-7-*epi*paclitaxel (**11**, 71 mg, 0.057 mmol) in DMF (1.5 mL) was added sodium azide (NaN_3, 100 mg, 1.5 mmol) and the solution was stirred at room temperature for 18 h. The reaction mixture was then diluted with EtOAc 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 4:6) to afford 1-*O*-dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6β-azido-7-*epi*paclitaxel (**12**, 25 mg, 49%, based on unrecovered starting material), the starting material (**11**, 11 mg) along with other products that were not fully characterized. ^{1}H NMR δ 8.18 (d, *J*=8.0 Hz, 2H), 7.72 (m, 2H), 7.60–7.31 (m, 11H), 7.03 (d, *J*=9.6 Hz, 1H), 6.79 (s, 1H), 6.32 (t, *J*=8.8 Hz, 1H), 5.87 (dd, *J*=9.6, 2.4 Hz, 1H), 5.78 (d, *J*=7.6 Hz, 1H), 5.05 (d, *J*=8.0 Hz, 1H), 4.68 (d, *J*=2.4 Hz, 1H), 4.57 (d, *J*=11.2 Hz, 1H), 4.36 (s, 2H), 4.26 (m, 1H), 4.13 (d, *J*=9.2 Hz, 1H), 3.86 (d, *J*=7.2 Hz, 1H), 3.68 (dd, *J*=10.8, 1.2 Hz, 1H), 2.72 (s, 3H), 2.31 (m, 2H), 2.19 (s, 3H), 2.04 (s, 3H), 1.88 (s, 3H), 1.79 (s, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 0.76 (s, 9H), −0.05 (s, 3H), −0.15 (d, *J*=2.4 Hz, 1H), −0.31 (s, 3H), −0.49 (d, *J*=2.8 Hz, 3H). ^{13}C NMR δ 206.0, 172.6, 170.9, 169.5, 166.8, 165.6, 139.5, 138.0, 133.9, 133.4, 132.8, 131.7, 130.4, 129.9, 128.75, 128.69, 128.6, 127.9, 127.1, 126.4, 82.7, 81.9, 80.7, 78.6, 77.8, 75.7, 75.4, 70.5, 62.1, 57.5, 55.4, 43.5, 39.9, 34.8, 26.8, 25.5, 22.7, 22.1, 20.9, 18.2, 14.8, 14.7, 0.3, −0.4, −5.3, −6.0. FT-IR 2104.5 cm^{-1} (strong).

6β-Azido-7-*epi*paclitaxel (13). To a solution of 1-*O*-dimethylsilyl-2′-*O*-(*tert*-butyldimethylsilyl)-6β-azido-7-*epi* paclitaxel (**12**, 25 mg, 0.023 mmol) in dry THF (0.5 mL) was added HF-pyridine (70%, 100 μL) and the solution was stirred at room temperature for 3 h. The reaction mixture was then 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 sodium sulfate, and concentrated under reduced pressure. The crude product was purified by preparative TLC (silica gel, 1000μ, EtOAc:hexanes 6:4) to afford 6β-azido-7-*epi*paclitaxel (**13**, 17.6 mg, 84%). ^{1}H NMR δ (8.16 (d, *J*=7.6 Hz, 2H), 7.71 (m, 2H), 7.62–7.35 (m, 11H), 7.05 (d, *J*=8.8 Hz, 1H), 6.77 (s, 1H), 6.20 (t, *J*=8.8 Hz, 1H), 5.80 (dd, *J*=8.8, 2.0 Hz, 1H), 5.76 (d, *J*=7.6 Hz, 1H), 5.03 (d, *J*=8.0 Hz, 1H), 4.79 (m, 1H), 4.49 (d, *J*=10.8 Hz, 1H), 4.42 (d, *J*=8.8 Hz, 1H), 4.27 (d, *J*=8.8 Hz, 1H), 4.12 (m, 1H), 3.86 (d, *J*=7.2 Hz, 1H), 3.68 (br s, 1H), 3.66 (m, 1H), 2.50 (s, 3H), 2.35 (m, 1H), 2.26 (m, 1H), 2.18 (s, 3H), 1.77 (s, 3H), 1.76 (s, 3H), 1.17 (s, 3H), 1.13 (s, 3H). ^{13}C NMR δ 205.6, 172.6, 172.4, 169.5, 167.1, 167.07, 139.6, 137.9, 133.8, 133.6, 133.3, 132.0, 130.2, 129.1, 129.0, 128.8, 128.7, 128.3, 127.0, 126.9, 82.6, 80.5, 79.0, 78.4, 78.1, 77.2, 75.0, 73.2, 72.0, 62.3, 57.7, 54.9, 42.6, 40.1, 36.0, 25.8, 22.3, 21.1, 20.8, 14.76, 14.73. HRFABMS calculated for $C_{47}H_{50}N_4O_{14}$ $(M+H)^+$ 895.3402, found 895.3401, error −0.1 ppm.

Hydrogenation of 6β-azido-7-*epi*paclitaxel (13). To a solution of 6β-azido-7-*epi*paclitaxel (**13**, 10 mg, 0.011 mmol) in MeOH (1 mL) was added a catalytic amount of palladium on activated carbon (10%). Hydrogen gas was bubbled through a needle into the suspension for 1.5 h. TLC analysis showed complete disappearance of the starting material and formation of a very polar spot. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500μ, MeOH:CH_2Cl_2 8:92) to give a complex reaction mixture. The major component (4.0 mg, 40%) was isolated and identified as 6β-triazeno-7-*epi*paclitaxel (**14**).

6β-Triazeno-7-*epi*paclitaxel (14). To a solution of 6β-azido-7-*epi*paclitaxel (**13**, 7.8 mg, 0.0088 mmol) in MeOH (2 mL) was added excess amount of palladium on activated carbon (10%). This suspension was stirred under hydrogen atmosphere for 72 h. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 500μ, MeOH:EtOAc 5:95) to afford 6β-triazeno-7-*epi*paclitaxel (**14**, 4.8 mg, 62%). ^{1}H NMR δ 8.12 (dd, *J*=8.0 Hz, 1.2, 2H), 7.77 (dd, *J*=8.0, 1.2 Hz, 2H), 7.62–7.34 (m, 11H), 7.17 (d, *J*=9.6 Hz, 1H), 6.80 (s, 1H), 6.15 (t, *J*=8.4 Hz, 1H), 5.83 (dd, *J*=8.8, 2.4 Hz, 1H), 5.80 (d, *J*=6.0 Hz, 1H), 5.15 (d, *J*=3.2 Hz, 1H), 4.80 (d, *J*=2.4 Hz, 1H), 4.39 (d, *J*=8.0 Hz, 1H), 4.19 (d, *J*=7.6 Hz, 1H), 4.03 (d, *J*=6.0 Hz, 1H), 3.82 (d, *J*=6.4 Hz, 1H), 3.80 (m, 1H), 3.61 (br s, 1H), 3.01 (m, 1H), 2.41 (s, 3H), 2.33 (m, 1H), 2.23 (m, 1H), 2.21 (s, 3H), 1.91 (s, 3H), 1.75 (s, 3H), 1.20 (s, 3H), 1.18 (s, 3H). ^{13}C NMR δ 203.8, 171.9, 170.9, 169.4, 167.0, 166.8, 139.3, 138.1, 133.9, 133.8, 133.7, 131.9, 130.1, 129.1, 128.9, 128.7, 128.2, 127.02, 127.05, 82.5, 80.8, 79.3, 78.3, 77.2, 75.3, 73.8, 73.4, 71.8, 69.1, 57.8, 54.7, 42.8, 42.6, 40.5, 35.7, 25.8, 22.5, 20.9, 20.8, 17.5, 14.7. LRFABMS calculated for $C_{47}H_{52}N_4O_{14}$ $(M+H)^+$ 897.8, found 897.4; $(M+Na)^+$ 919.3, found 919.4; $(M+Li)^+$ 903.4, found 903.4.

6β-Amino-7-*epi*paclitaxel (15). To a solution of 6β-azido-7-*epi*paclitaxel (**13**, 34 mg, 0.038 mmol) in EtOAc (10 mL) was added catalytic amount of palladium on activated carbon (5%). This suspension was stirred under 50 psi hydrogen atmosphere for 24 h. The suspension was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, 1000μ, MeOH:CH_2Cl_2 8:92) to furnish 6β-amino-7-*epi*paclitaxel (**15**, 12 mg, 35%). ^{1}H NMR δ 8.17 (dd, *J*=7.2, 1.6 Hz, 2H), 7.71 (dd, *J*=6.8, 1.6 Hz, 2H), 7.62–7.34 (m, 11H), 7.11 (d, *J*=9.2 Hz, 1H), 6.81 (s, 1H), 6.19 (t, *J*=8.8 Hz, 1H), 5.79 (dd, *J*=8.8, 2.4 Hz, 1H), 5.75 (d, *J*=7.6 Hz, 1H), 4.90 (d, *J*=8.4 Hz, 1H), 4.78 (d, *J*=2.4 Hz, 1H), 4.39 (d, *J*=8.0 Hz, 1H), 4.17 (d, *J*=8.4 Hz, 1H), 3.85 (d, *J*=7.6 Hz, 1H), 3.52 (br s, 1H), 3.35 (d, *J*=8.0 Hz, 1H), 2.47 (s, 3H), 2.36 (m, 1H), 2.23 (m, 1H), 2.1–2.2 (br, 3H), 2.17 (s, 3H), 1.75 (s, 3H), 1.73 (s, 3H), 1.16 (s, 3H), 1.13 (s, 3H). ^{13}C NMR δ 206.5, 172.6, 172.1, 169.5, 167.2, 167.1, 139.4, 138.0, 133.7, 133.6, 133.2, 131.9, 130.2, 129.3, 129.0, 128.8, 128.6, 128.3, 127.1, 126.9, 83.5, 83.0, 80.7, 79.1, 78.0, 76.2, 75.2, 73.2, 72.0, 58.0, 54.9, 54.6, 42.5, 40.2, 36.0, 25.8, 22.3, 21.1, 20.9, 14.8, 14.5. HRFABMS calculated for $C_{47}H_{52}N_2O_{14}$ $(M+H)^+$ 869.3497, found 869.3504, error 0.8 ppm.

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