Synthesis and Biological Evaluation of Novel C-4 Aziridine-Bearing Paclitaxel (Taxol†) Analogs

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Three novel C-4 aziridine-bearing paclitaxel analogs, **3**–**5**, have been synthesized during the course of our continuing effort at C-4 modification. The key step in the synthesis is the aziridine ring formation at the C-4 position via an intramolecular Mitsunobu reaction. The syntheses and the biological evaluation of these C-4 aziridine-containing derivatives are herein discussed.

The unique structural complexity,[1,2] novel mechanism of action,[3] and clinical importance[4] of the novel antitumor agent paclitaxel (**1**) has rendered it a target of intensive total synthetic[5] and structure–activity relationships (SAR) studies.[6] As a result of extensive SAR examination at the diterpenoid core, we and others have found that minor to major modifications at the C-7,[7] C-9,[8] and C-10[9] positions usually do not dramatically reduce biological activity. In contrast, functional groups at C-2,[10] C-4,[11] and the oxetane ring[12] are essential elements in receptor binding, and thus only minor modifications are tolerated. Very recently, we have disclosed methodology for the derivatization of the C-4 position.[11c] Preliminary biological evaluation of the C-4 cyclopropyl ester analog **2** has shown it to be more potent *in vitro* than paclitaxel.[11c] This promising result has stimulated our interest further in the C-4 modification. In particular, we were interested in replacing the C-4 cyclopropyl group with an isosteric aziridine ring with the intention that the C-4 aziridine ring-bearing analog **3** could potentially function as an alkylating agent[13] at the tubulin binding site. In this vein, we decided to prepare two other aziridine analogs, **4** and **5**, which contain further side chain modifications as shown in Figure 1. The decision to prepare **4** and **5** is based on the literature precedent that the replacement of 3′-phenyl and/or 3′-*N*-benzoyl moiety with 3′-furyl and 3′-*N*-Boc could further enhance the *in vitro* biological activity.[14]

Scheme 1 outlines the synthetic route employed to accomplish the synthesis of a key intermediate (**12**) from 7-TES baccatin (**6**). The C-4 deacetyl baccatin derivative **7** was obtained in high yield from **6**[15] in two steps according to the regioselective C-4 deacetylation method developed in our laboratory.[11c] Deprotonation of **7** with lithium bis(trimethylsilyl)amide in dry tetrahydrofuran and subsequent reaction with *p*-nitrophenyl chloroformate led to the desired C-4 mixed carbonate **8** in modest yield (51%). Standard desilylation (**8** to **9**) and selective C-7 resilylation thus furnished compound **10** in 90% overall yield. Subsequent reaction of **10** with ethanolamine in dry tetrahydrofuran provided the expected carbamate **11** in almost quantitative yield. Conversion of **11** to the aziridine-bearing baccatin **12** was achieved

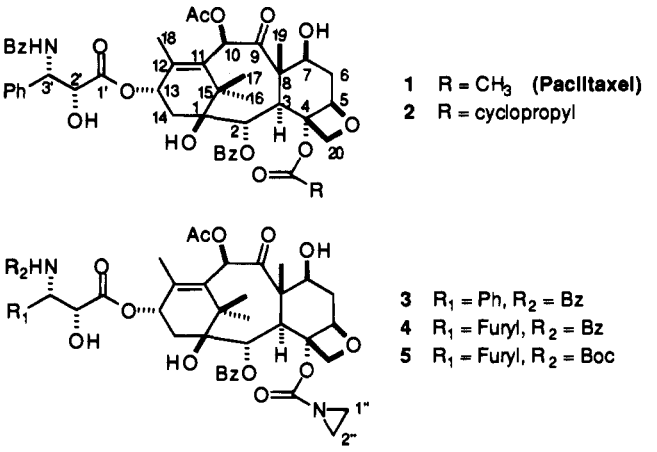

Figure 1.

under standard Mitsunobu conditions[16] in modest yield (see Scheme 1).

Coupling of the aziridino baccatin **12** with three side chain β-lactams (**13a**–**c**)[17] was performed according to the protocol of Holton[18] and provided the desired products **14a**–**c** in high yields. Final desilylation by exposure of **14** to either 48% HF/pyridine or 4 N HCl resulted in the hydrolysis of the C-4 aziridine ring, in addition to the desired desilylation. After some experimentation, it was later found that treatment of **14a**–**c** with tetrabutylammonium flouride in THF provided the desired products **3**–**5** in similar yields, respectively (Scheme 2).

The C-4 aziridine ring-containing derivative **3** was subjected to extensive NMR analysis. The proton (2.46 ppm) and carbon (26.7 ppm) shifts for H-1″ and H-2″ in **3** confirms that *only aziridine, but not oxazoline, is formed in the intramolecular Mitsunobu reaction (**11** to **12**),* although the formation of such oxazoline has been observed under similar conditions.[19] The detailed assignments of proton and carbon spectra of **3** are listed in Table 1. The structures of **4** and **5** are assigned in an analogous manner.

The C-4 aziridine-carrying analogs **3**–**5** were evaluated in a tubulin polymerization assay[20] and an *in vitro* cytotoxicity assay against HCT-116 tumor cell lines.[21] The results of these investigations are summarized in Table 2. Analogs **4** and **5** exhibit slightly better potencies than paclitaxel (**1**) in the tubulin assay, whereas analog **3** possesses 3-fold weaker activity as compared to paclitaxel (**1**) in the same assay. Among the three

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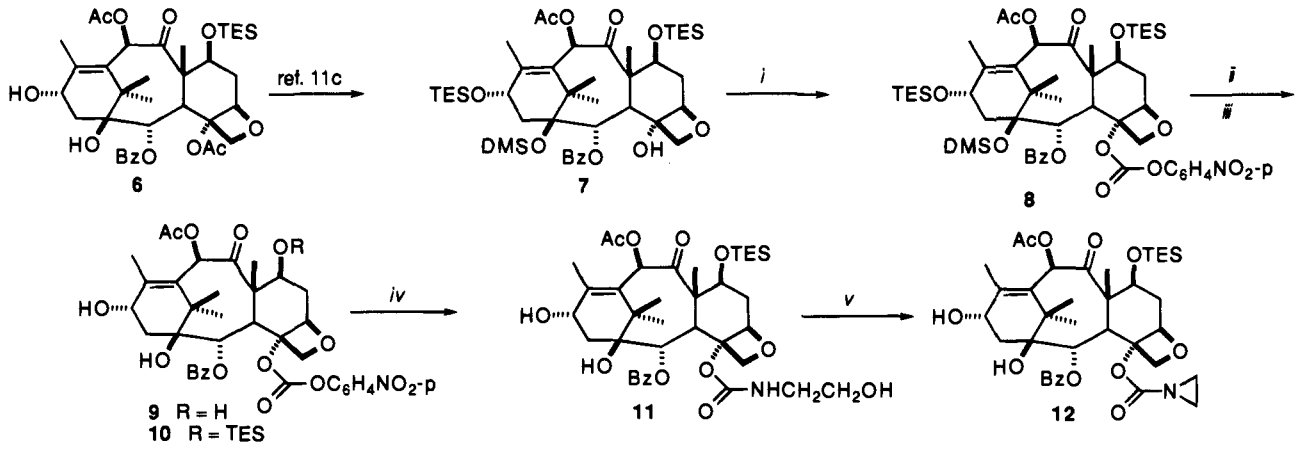
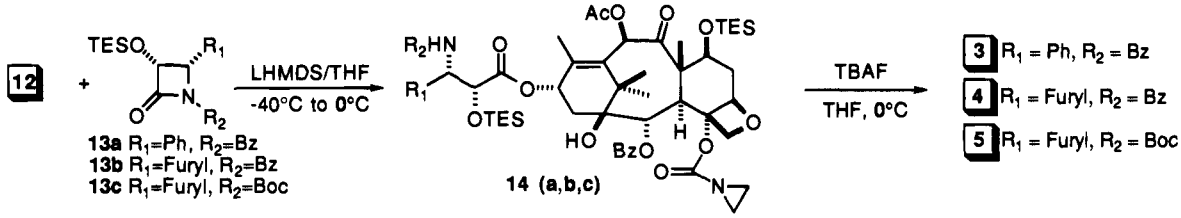
Scheme 1[a]



[a] Reagents and conditions: (i) LHMDS/THF/0 °C, *p*-$NO_2C_6H_4OCOCl$, 51%, plus 25% of recovered **7**; (ii) pyridine/48% HF/CH_3CN/5 °C, 99%; (iii) TESCl/ imidazole/DMF/0 °C, 90%; (iv) ethanolamine/THF/rt, 97%; (v) DEAD/ PPh_3/THF/rt, 49% plus 34% of recovered 11.

Scheme 2[a]



[a] Reagents and conditions: (i) yields for side chain coupling (**14a**) 86%, (**14b**) 69%, (**14c**) 83%; (ii) yields for desilylation (**14a** to **3**) 50% plus 28% of its C-7 epimer, (**14b** to **4**) 52%, (**14c** to **5**) 70%.

Table 1. Diagnostic ^1H and ^{13}C NMR Assignments of **3** ($CDCl_3$, 400 MHz)

no.	proton (ppm)	carbon (ppm)	no.	proton (ppm)	carbon (ppm)
1		78.8	12		142.1
2	5.62 (d, $J = 7.1$)	74.5	13	6.09 (m)	71.2
3	3.84 (d, $J = 7.0$)	45.8	14	2.39 (m)	35.6
4		82.0	15		42.8
5	4.71 (m)	84.5	16	1.18 (s)	26.8
6	2.53, 1.85 (m)	35.4	17	1.11 (s)	21.0
7	4.35 (m)	72.0	18	1.69 (s)	15.1
8		58.4	19	1.66 (s)	9.4
9		203.3	20	4.20 (AB q)	76.0
10	6.24 (s)	75.5	1″	2.46 (m)	26.7
11		138.7	2″	2.46 (m)	26.7

Table 2. Biological Evaluation of Paclitaxel Analogs

no.	chemical modification(s)	tubulin polymerization[a]	IC_{50} (nM) HCT-116
1		1.0	2.4
	3′-furyl	0.85[b]	0.74[b]
	3′-*N*-Boc	0.55[b]	0.55[b]
2	4-cyclopropyl ester	0.3	1.0
3	4-aziridine	2.8	15.6
4	3′-furyl, 4-aziridine	0.7	6.9
5	3′-furyl, 3′-*N*-Boc, 4-aziridine	0.8	2.0

[a] Ratios of analogs relative to paclitaxel ($EC_{0.01}$); ratios < 1 signify analog being more potent. [b] ED_{50}(analog)/ED_{50}(paclitaxel) values (see ref 22 for details).

aziridine analogs tested, the 3′-furyl 3′-*N*-Boc side chain-bearing analog **5** is slightly more cytotoxic than paclitaxel (**1**). On the other hand, analogs **3** and **4** possess 3−6-fold weaker cytotoxicity than paclitaxel (**1**).

In view of the above results, it becomes clear that (i) The *in vitro* biological activities of these C-4 aziridine analogs can be modulated/improved by further side chain modifications, such as replacement of the 3′-phenyl or the 3′-*N*-benzoyl group with a 3′-furyl or 3′-*N*-Boc substituent. Thus, the 3′-furyl/*t*-Boc-carrying analog **5** is more potent than the paclitaxel side chain-bearing analog **3** *in vitro*. This trend parallels Georg's observations in the paclitaxel series as shown in Table 2. This author has shown that both the C-3 furyl and C-3′-*N*-Boc can enhance the *in vitro* potencies of paclitaxel analogs.[14,22] (ii) The fact that replacement of the C-4 cyclopropyl moiety in **2**[11c] with an isosteric aziridine ring in **3** resulted in a 9-fold loss of potency in the tubulin polymerization assay indirectly confirms that the C-4 substituent is indeed involved in intimate binding with tubulin.[11] Thus, a seemingly minor structural change at this position (cyclopropyl vs aziridine) can exert significant impact on the biological activity. Given the fact that the C-4 cyclopropyl group is more sterically demanding than the corresponding aziridine, one can speculate that a C-4 cyclopropyl group presumably fills the binding pocket better than a relatively flat aziridine ring. Further modeling work directed toward probing the exact binding pocket at the C-4 position is ongoing. However, it is also possible that the poor activity of these C-4 aziridine analogs might be attributed, in part, to a covalent interaction with non-tubulin-related proteins. Further efforts aimed at clarification of the intracellular target for these C-4 aziridine analogs is in progress. The full account of this work along with the details of biological evaluation of C-4 paclitaxel analogs[23] will be reported in due time.

Experimental Section

Preparation of Compound 8. To a THF solution (12 mL) of **7** (600 mg, 0.723 mmol) was added at 0 °C LHMDS (0.868 mL, 1 M, 0.868 mmol). After 30 min, *p*-nitrophenyl chloride formate (218.6 mg, 1.085 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h and then the reaction

quenched with NH_4Cl-saturated solution (5 mL). The reaction mixture was extracted with EtOAc (150 mL). The organic phase was washed with water (2 × 15 mL) and brine (15 mL) and dried. The organic layer was filtered and concentrated *in vacuo*. The residue was chromatographed (10% ethyl acetate in hexane) to afford desired **8** (371 mg, 52%), together with 150 mg (25%) of recovered starting material. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.38–8.35 (m, 2H), 8.04–8.01 (m, 2H), 7.69–7.40 (m, 5H), 6.45 (s, 1H), 5.78 (d, J = 7.1 Hz, 1H), 4.98 (m, 2H), 4.56 (m, 1H), 4.40 (dd, J = 6.5 Hz, J' = 10.7 Hz, 1H), 4.27 (AB q, J = 8.5 Hz, 2H), 3.92 (d, J = 7.0 Hz, 1H), 2.42 (m, 2H), 2.17 (s, 6H), 2.15–1.85 (m, 2H), 1.68 (s, 3H), 1.21 (s, 3H), 1.11 (s, 3H), 0.93 (m, 18H), 0.53 (m, 12H), 0.18 (d, J = 1.8 Hz, 3H), −0.29 (d, J = 1.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.4, 169.2, 165.3, 155.4, 150.0, 145.4, 144.4, 133.3, 131.8, 129.9, 128.4, 125.2, 121.8, 84.6, 83.7, 82.0, 75.9, 75.4, 71.8, 68.5, 57.8, 46.8, 44.0, 39.6, 27.4, 22.2, 20.8, 14.4, 10.2, 6.7, 6.6, 5.1, 4.7. Mass calcd for $C_{50}H_{73}NO_{14}Si_3$, (M^+) 995; found, 995.

Preparation of Compound 9. Silylated precusor **8** (851 mg, 0.855 mmol) was dissolved in CH_3CN (17 mL). This solution was treated with pyridine (2.5 mL) followed by 48% HF (7.5 mL) at 0 °C. After 30 min, the reaction mixture was placed at 5 °C overnight. The reaction mixture was then diluted with EtOAc (150 mL) and washed with 1 N HCl (10 mL) followed by water and $NaHCO_3$-saturated solution (3 × 20 mL). The resulting organic layer was dried and concentrated *in vacuo*. The residue was chromatographed (30–50% ethyl acetate in hexane) to afford 600 mg (99%) of **9**. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.29–8.26 (m, 2H), 8.07–8.04 (m, 2H), 7.63–7.42 (m, 5H), 6.28 (s, 1H), 5.63 (d, J = 6.9 Hz, 1H), 5.05 (d, J = 9.3 Hz, 1H), 4.82 (m, 1H), 4.36 (m, 2H), 4.14 (d, J = 8.7 Hz, 1H), 3.94 (d, J = 6.8 Hz, 1H), 2.62–1.06 (m, 19H, incl. s at 2.19, 2.00, 1.63, 3H each, s at 1.07, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.5, 171.3, 166.9, 155.4, 150.5, 146.3, 145.2, 133.7, 131.9, 130.0, 129.0, 128.6, 125.0, 121.9, 83.8, 83.7, 78.7, 76.1, 75.8, 74.4, 72.1, 67.6, 58.2, 46.4, 42.4, 38.9, 35.2, 27.1, 20.7, 20.6, 15.7, 14.0, 9.4. Mass calcd for $C_{36}H_{39}NO_{14}$, (M^+) 709; found, 709.

Preparation of Compound 10. Triol **9** (573 mg, 0.808 mmol) was dissolved in dry DMF (5 mL). To this solution was added imidazole (220 mg, 3.232 mmol) followed by TESCl (0.543 mL, 3.232 mmol) at 0 °C. After 40 min, the reaction mixture was diluted with EtOAc (100 mL) and washed with water (3 × 5 mL) and brine (10 mL). The resulting organic layer was dried and concentrated *in vacuo*. The residue was chromatographed (40% ethyl acetate in hexane) to afford 600 mg (90%) of the desired product **10**. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.26–8.23 (m, 2H), 8.04–8.01 (m, 2H), 7.62–7.39 (m, 5H), 6.42 (s, 1H), 5.62 (d, J = 6.8 Hz, 1H), 5.02 (d, J = 9.1 Hz, 1H), 4.77 (m, 1H), 4.36 (m, 1H), 4.22 (AB q, J = 8.5 Hz, 2H), 3.94 (d, J = 6.7 Hz, 1H), 2.40–0.97 (m, 19H, incl. s at 2.12, 2.11, 1.62, 1.13, 0.97, 3H each), 0.85 (m, 9H), 0.51 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.7, 169.2, 166.9, 155.4, 150.3, 145.1, 143.9, 133.6, 132.8, 130.0, 129.0, 128.5, 125.0, 122.0, 83.7, 83.4, 78.4, 75.8, 75.6, 74.3, 72.0, 67.6, 58.2, 47.4, 42.5, 38.6, 36.9, 26.8, 20.8, 20.0, 15.0, 9.8, 6.6, 5.1. Mass calcd for $C_{42}H_{53}NO_{14}Si$, (M^+) 823; found, 823.

Preparation of Compound 11. To a THF solution (3 mL) of **10** (140 mg, 0.170 mmol) was added ethanolamine (0.031 mL, 0.510 mmol) at room temperature. The reaction mixture was allowed to stir overnight. The solvent was then removed, and the residue was chromatographed (70–80% ethyl acetate in hexane) to afford 122.7 mg (97%) of the desired product 11. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.05–8.02 (m, 2H), 7.60–7.42 (m, 3H), 6.43 (s, 1H), 5.63 (d, J = 6.6 Hz, 1H), 5.49 (m, 1H), 4.96 (d, J = 8.4 Hz, 1H), 4.78 (m, 1H), 4.48–3.04 (m, 10H), 2.56–1.80 (m, 10H, incl. s at 2.20, 2.16, 3H each), 1.65 (s, 3H), 1.18 (s, 3H), 1.02 (s, 3H), 0.90 (m, 9H), 0.56 (m, 6H). HRMS calcd for $C_{38}H_{56}NO_{12}Si$, (MH^+) 746.3572; found, 746.3550.

Preparation of 7-TES-4-aziridine Baccatin (12). Triol 11 (109 mg, 0.146 mmol) was dissolved in dry THF (3 mL). To this solution at room temperature was added DEAD (0.0277 mL, 0.175 mmol) followed by PPh_3. The reaction mixture was stirred at that temperature for 2 h. The solvent was then removed, and the residue was chromatographed (50–80% ethyl acetate in hexane) to afford 52 mg (49%) of the desired aziridine **12**, together with 37 mg (34%) of the recovered starting material. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.13–8.10 (m, 2H), 7.60–7.43 (m, 3H), 6.45 (s, 1H), 5.62 (d, J = 7.0 Hz, 1H), 4.94 (d, J = 7.9 Hz, 1H), 4.76 (dd, J = 6.6 Hz, J' = 10.5 Hz, 1H), 4.24 (AB q, J = 8.4 Hz, 2H), 3.86 (d, J = 7.0 Hz, 1H), 2.76–1.02 (m, 23H, incl. s at 2.48, 2.19, 1.66, 1.18, 1.05, 3H each), 0.90 (m, 9H), 0.55 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 202.0, 169.2, 166.8, 160.8, 145.0, 133.5, 132.0, 130.1, 129.3, 128.4, 83.8, 82.1, 79.1, 75.8, 75.6, 74.7, 72.1, 68.0, 62.1, 58.1, 47.3, 42.7, 39.1, 37.0, 26.2, 25.7, 20.8, 20.5, 14.5, 14.3, 9.9, 6.6, 5.1. HRMS calcd for $C_{38}H_{54}O_{11}NSi$, (MH^+) 728.3466; found, 728.3458.

Preparation of C-4 Aziridine 3 via 14a. A THF solution (2 mL) of **12** (83 mg, 0.114 mmol) was treated at −40 °C with LHMDS (0.148 mL, 1 M, 0.148 mmol) followed by a THF solution (1 mL) of β-lactam **13a** (65.2 mg, 0.171 mmol). The reaction mixture was stirred at 0 °C for 1 h and the reaction quenched with NH_4Cl (1 mL). The reaction mixture was extracted with EtOAc (75 mL) and washed with water and brine. The organic layer was dried and concentrated *in vacuo*. The residue was chromatographed (20–30% ethyl acetate in hexane) to afford 108 mg (86%) of the desired product **14a**. A part of **14a** (92 mg, 0.083 mmol) was dissolved in THF (2.0 mL), and this solution was treated with TBAF (0.415 mL, 1 M, 0.415 mmol) for 1 h. The solvent was removed, and the residue was chromatographed (50–70% ethyl acetate in hexane) to afford 36.4 mg (50%) of the desired C-4 aziridine **3**, together with 20.4 mg (28%) of its C-7 epimer. The physical data of **3** are shown. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.13–8.10 (m, 2H), 7.83–7.76 (m, 2H), 7.64–7.25 (m, 12H), 6.24 (s, 1H), 6.09 (m, 1H), 5.83 (dd, J = 2.3 Hz, J' = 9.2 Hz, 1H), 5.62 (d, J = 7.1 Hz, 1H), 4.72 (m, 2H), 4.36–4.30 (m, 2H), 4.20 (AB q, J = 8.6 Hz, 2H), 3.84 (d, J = 7.0 Hz, 1H), 2.53–0.90 (m, 23H, incl. s at 2.35, 2.23, 1.66, 1.18, 1.12, 3H each). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.3, 171.5, 171.1, 166.6, 166.2, 161.7, 142.1, 138.7, 133.7, 131.7, 131.1, 129.0, 128.5, 128.4, 127.8, 127.1, 126.9, 84.5, 82.0, 78.8, 76.0, 75.5, 74.5, 73.6, 72.0, 71.2, 58.4, 54.3, 45.8, 42.8, 35.6, 26.8, 26.7, 20.9, 20.7, 15.1, 9.4. HRMS calcd for $C_{48}H_{53}N_2O_{14}$, (MH^+) 881.3497; found, 881.3485.

Preparation of C-4 Aziridine 4 via 14b. A THF solution (1.5 mL) of **12** (46 mg, 0.0633 mmol) was treated at −40 °C with LHMDS (0.076 mL, 1 M, 0.076 mmol) followed by β-lactam **13b** (36 mg, 0.095 mmol). The reaction mixture was stirred at 0 °C for 1 h, and then the reaction was quenched with NH_4Cl-saturated solution (1 mL). The reaction mixture was extracted with EtOA and brine. The resulting organic layer was dried and concentrated *in vacuo*. The residue was chromatographed (20–30% ethyl acetate in hexane) to afford 47.8 mg (69%) of the desired **14b**. This material was disolved in THF (1.0 mL) and treated with TBAF (0.193 mL, 1 M, 0.193 mmol) at 0 °C for 1 h. The solvent was then removed, and the residue was chromatographed (50–70% ethyl acetate in hexane) to afford 19.2 mg (52%) of the expected C-4 aziridine **4**, whose physical data are recorded as follows. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.17–7.40 (m, 11H), 7.05 (d, J = 9.4 Hz, 1H), 6.39 (m, 2H), 6.28 (s, 1H), 6.16 (m, 1H), 5.94 (dd, J = 2.8 Hz, J' = 9.2 Hz, 1H), 5.64 (d, J = 7.1 Hz, 1H), 4.74 (m, 2H), 4.39 (m, 1H), 4.21 (AB q, J = 8.6 Hz, 2H), 3.87 (d, J = 7.1 Hz, 1H), 2.57–1.12 (m, 23H, incl. s at 2.37, 2.24, 1.67, 1.20, 1.13, 3H each). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.4, 171.5, 171.1, 166.7, 166.2, 161.8, 151.5, 142.1, 133.6, 133.5, 133.1, 131.8, 130.2, 129.1, 128.5, 127.0, 110.6, 107.9, 84.6, 81.8, 78.9, 76.0, 75.6, 74.6, 72.0, 71.7, 71.5, 58.4, 49.4, 45.8, 42.8, 35.6, 35.4, 26.7, 26.6, 21.1, 20.7, 15.1, 9.4. HRMS calcd for $C_{46}H_{51}N_2O_{15}$, (MH^+) 871.3289; found, 871.3300.

Preparation of C-4 Aziridine Analog 5 via 14c. A THF solution (1.3 mL) of **12** (46.5 mg, 0.064 mmol) was treated at −40 °C with LHMDS (0.083 mL, 1 M, 0.083 mmol) followed by β-lactam **13c** (35.2 mg, 0.096 mmol). The reaction mixture was stirred at 0 °C for 1 h; then the reaction was quenched with NH_4Cl-saturated solution (1 mL). The reaction mixture was extracted with EtOAc (40 mL) and washed with water and brine. The organic layer was dried and concentrated *in vacuo*. The residue was chromatographed (20% ethyl acetate in hexane) to afford 58.3 mg (83%) of desired **14c**. A part of **14c** (47 mg, 0.043 mmol) was dissolved in THF (1mL) and

treated at 0 °C with TBAF (0.215 mL, 1 M, 0.215 mmol) for 1 h. The solvent was then removed, and the residue was chromatographed (60–80% ethyl acetate in hexane) to afford 26 mg (70%) of the desired product **5**. The physical data of **5** are listed as follows. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.16–8.14 (m, 2H), 7.63–7.39 (m, 4H), 6.37–6.19 (m, 4H), 5.63 (d, J = 7.1 Hz, 1H), 5.40 (d, J = 9.6 Hz, 1H), 5.28 (d, J = 10.0 Hz, 1H), 4.72 (m, 2H), 4.40 (m, 1H), 4.19 (AB q, J = 8.5 Hz, 2H), 3.88 (d, J = 7.1 Hz, 1H), 2.56–1.13 (m, 32H, incl. s at 2.35, 2.25, 1.91, 1.22, 1.14, 3H each, 1.34, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.5, 171.8, 171.2, 166.8, 161.7, 154.7, 152.3, 142.4, 141.9, 133.6, 133.5, 132.9, 130.2, 129.0, 128.5, 110.5, 107.2, 84.6, 79.8, 79.0, 76.0, 75.6, 74.6, 72.0, 71.8, 71.5, 58.3, 50.9, 45.7, 42.9, 35.5, 28.1, 26.6, 26.3, 21.3, 20.7, 15.1, 9.4. MASS calcd for $C_{44}H_{55}N_2O_{16}$, (M^+) 866; found, 866.

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Supplementary Material Available: ^{1}H NMR spectral data for compounds **8–12**, **14a–c**, and **3–5** (11 pages). Ordering information is given on any current masthead page.

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