Synthesis of *N*-Substituted 10-Des(carbamoyloxy)-10-azidomitomycins

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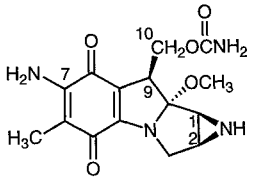
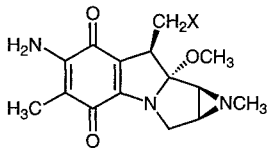
A general method for the synthesis of *N*-substituted 10-des(carbamoyloxy)-10-azidomitomycins **5** has been developed. These compounds are expected to be rapidly converted to the corresponding C-10 isothiocyanate derivatives allowing mitomycins to couple to biomolecules. The key synthetic intermediate was 10-des(carbamoyloxy)-10-azidomitomycin C **(12)**. Compound **12** was prepared by decarbamoylation of mitomycin C **(1)** followed by aziridine protection with Fmoc-chloride and activation of the C-10 site with methanesulfonyl chloride. Deprotection of the Fmoc unit with morpholine and treatment with NaN_3 gave **12**.

Mitomycin C **(1)** is an important anticancer agent whose clinical properties derive from its sequence-selective modification of DNA.[1] In vivo reductive activation of **1** leads to mono- and dialkylated DNA adducts. The reaction proceeds selectively at the 2-amino site in deoxyguanosine (G*) residues and preferentially occurs with 5′CG* sequences.[2]



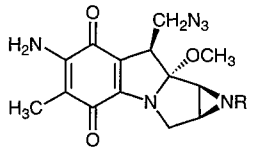
1

Efforts have been made to extend the therapeutic value of mitomycin C by linking it to select biomolecules.[3–6] Recently, we showed that 10-des(carbamoyloxy)-10-isothiocyanatoporfiromycin **(2)** can be efficiently coupled to phosphorothioate-containing oligodeoxynucleotides possessing a hexylamino spacer at the 5′ terminus to give mitomycin-DNA conjugates.[6] Preliminary molecular modeling experiments predicted that attaching the oligodeoxynucleotide to the C-10 site of the drug leads to minimal double helical distortion upon base pairing with the complementary strand, thus permitting guanine modification of a distal CG* site.[7] In our synthetic procedure, 10-des(carbamoyloxy)-10-azidoporfiromycin **(3)** served as a late-stage, stable intermediate suitable for rapid conversion to the C-10 isothiocyanate derivative **2**.[6] Catalytic reduction of **3** in pyridine gave **4** upon oxidative workup; **4** was then treated with di(2-pyridyl) thionocarbonate to yield **2**.



2 X = NCS
3 X = N_3
4 X = NH_2

The potential value of this technique is not restricted to 5′CG* DNA sequences. We demonstrated that placement of electron-withdrawing substituents (SO_2Me, C(O)SMe, CO_2Me) at the *N*(1a)-aziridine site in **1** changes the preferred site of mitomycin alkylation from 5′CG* sequences to 5′AG* and 5′TG* sequences.[8] In this paper, we report on a general method for the syntheses of *N*-substituted 10-des(carbamoyloxy)-10-azidomitomycins **5**.

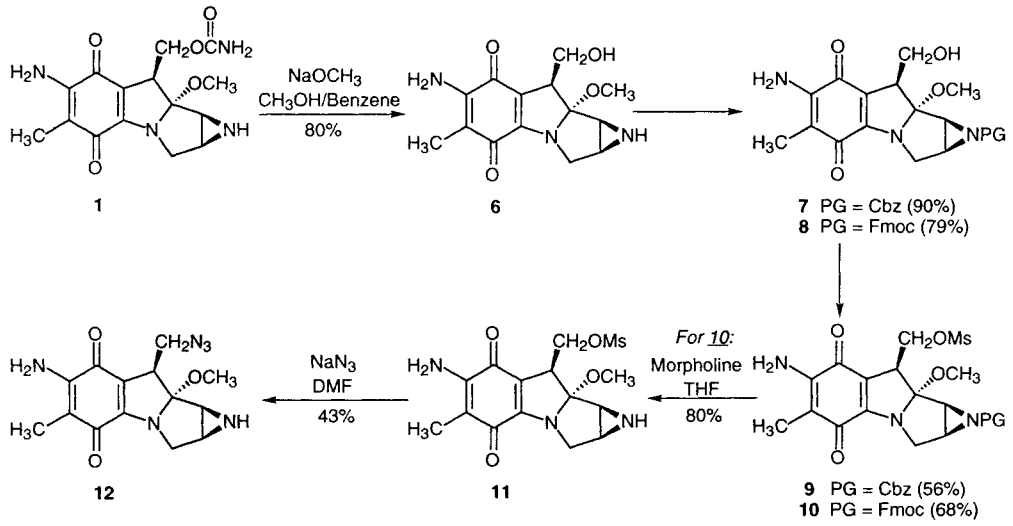


5

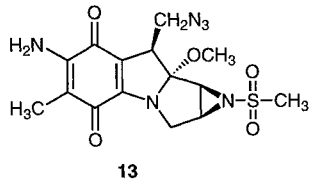
Our strategy required 10-des(carbamoyloxy)-10-azidomitomycin C **(12)** which would permit us to introduce a wide range of substituents at the *N*(1a)-aziridine site. We envisioned a five-step sequence for the synthesis of **12** (Scheme 1). Treatment of mitomycin C **(1)** with NaOMe gives 10-decarbamoylmitomycin C[9,10] **(6)**, which can then be selectively protected at the *N*(1a)-aziridine site. Activation of the C-10 position by mesylation followed by removal of the protecting group would allow the introduction of the C-10 azido group to give **12**. Key to the success of this method was the selection of the aziridine *N*(1a) protecting group. The instability of mitomycin C to moderate acid and base conditions and to reductive conditions in protic solvents restricted our choices.

We elected first to protect the *N*(1a)-aziridine nitrogen with the Cbz (carbobenzyloxy) group. Decarbamoylation of mitomycin C followed by treatment with Cbz-chloride and triethylamine gave **7** in a 72% overall yield. Activation of the C-10 site in **7** with methanesulfonyl chloride in pyridine provided **9** (56% yield). When we attempted to remove the Cbz group with PtO_2 and H_2 in anhydrous pyridine we were unsuccessful but did recover the starting material. This finding led us to prepare the Fmoc (9-fluorenylmethoxycarbonyl)-protected mitomycin **10** in place of the Cbz derivative **9**. Treatment of **10** with excess morpholine in THF at 0 °C led to the smooth removal of the Fmoc group[11] and the generation of **11** (80% yield). Displacement of the C-10 mesylate group in **11** with NaN_3 (DMF, 70 °C, 3 h) gave **12** in a 43% yield.

The usefulness of **12** for the synthesis of *N*-substituted 10-des(carbamoyloxy)-10-azidomitomycins **5** was demonstrated by its conversion to the *N*-methanesulfonyl derivative **13** with methanesulfonyl chloride.

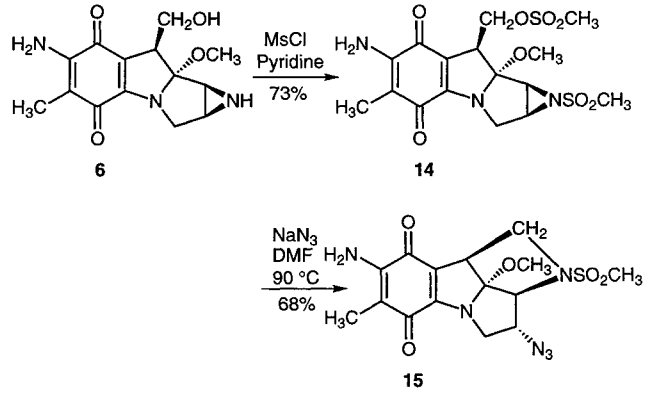


Scheme 1



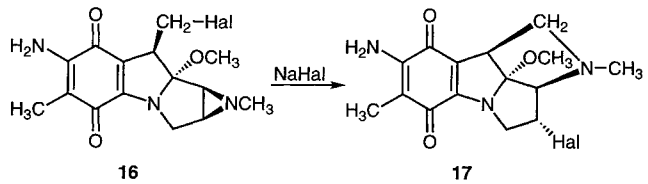
13

The availability of 10-decarbamoylmitomycin C (**6**) prompted us to explore an alternative procedure for **13** (Scheme 2). Addition of excess methanesulfonyl chloride to **6** in pyridine gave the *N*-methanesulfonyl mesylate **14**. Treatment of **14** with NaN_3 (90 °C) did not give **13** but rather rearranged **15** (68 % yield).



Scheme 2

This transformation is similar to the conversion of C-10 halogen 10-des(carbamoyloxy)porfiromycin **16** to **17** upon treatment with halide ions (Scheme 3).[10]



Scheme 3

The spectroscopic properties of the C-10 substituted mitomycins were consistent with the proposed structures. The Table lists key ^1H NMR data for **1** and **7–14**. Two distinctive patterns were observed. First, placement of an electron-withdrawing substituent at the aziridine *N*(1a)-site (**7–10**, **13**, **14**) resulted in a downfield shift ($\Delta\delta = 0.27–1.12$) in the C-1 and C-2 methine protons compared with **1**, **11**, and **12**. Second, substitution of the carbamoyloxy group in mitomycin C led to distinctive shifts for the diastereotopic C-10 methylene protons. We observed that these protons were more shielded than **1** when the C-10 substituent was a hydroxy group (**7**, **8**) and an azido moiety (**12**, **13**), and less shielded than **1** when the C-10 substituent was a methanesulfonyloxy unit (**9–11**, **14**).

In summary, a general procedure for the synthesis of *N*-substituted 10-des(carbamoyloxy)-10-azidomitomycins **5** has been developed. Use of this method is expected to permit the synthesis of substituted mitomycin conjugates through the intermediacy of the corresponding C-10 isothiocyanate derivative.

^1H NMR and ^{13}C NMR spectra were recorded on either a Nicolet NT-300 or a General Electric QE-300 spectrometer. Chemical shifts are expressed in parts per million relative to the solvent employed, and coupling constants (*J* values) are given in Hertz. MS data were obtained on a Finnegan TSQ-70 triple quadrupole mass spectrometer under positive CI conditions by Dr. Mehdi Moini at the University of Texas at Austin. Melting points were determined with Fisher-Johns melting point apparatus and are uncorrected. UV-vis absorption spectra were run on a Hitachi Model 100–80 spectrometer. FT-IR spectra were run on Mattson Galaxy Series FT-IR 5000 and Genesis infrared spectrophotometers, and absorption values are expressed in wavenumbers (cm^{-1}). pH measurements were determined with either a Radiometer pHM 26 or a pHM 84 research meter equipped with a Radiometer GK 2320C combination glass electrode, which was standardized against aqueous buffer solutions. HPLC analyses were conducted with the following Waters Associates Units: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The peak areas in the HPLC were determined with Waters Associates 740 and 745 and Hewlett Packard 3392A integrators. The products were eluted from a C18 μBondapak (stainless steel) column (3.9 × 300 mm) using the following linear gradient condition: 90 % A (0.025 M triethylammonium acetate, pH 7.0) and 10 % B (MeCN)

Table. Key ^{1}H NMR Data for Substituted Mitomycins

Product	^{1}H NMR ($CDCl_3$/TMS, 300.1 MHz) δ, J (Hz)				
	1-H	2-H	9-H	10-*H*H′	10-H*H*′
1[a]	2.90 (d, 4.5)	2.78 (dd, 2.0, 4.5)	3.52 (dd, 4.3, 10.9)	4.20 (dd, 10.6, 10.9)	4.61 (dd, 4.3, 10.6)
7	3.36 (d, 4.8)	3.29 (dd, 1.8, 4.8)	3.47–3.51 (m)	3.89–3.97 (m)	4.09 (dd, 10.9, 14.6)
8	3.38–3.57 (m)	3.05 (br s)	3.66–3.73 (m)[b]	3.66–3.73 (m)[b]	3.93 (br t, 8.8)[b]
9[c]	3.90 (d, 4.8)	3.54–3.65 (m)	4.13 (dd, 4.2, 10.9)	–[d]	5.60 (dd, 4.2, 9.9)
10	3.39 (d, 4.8)	3.13 (dd, 1.8, 4.8)	3.78 (dd, 4.5, 11.1)	4.35–4.53 (m)	5.04 (dd, 4.5, 9.9)
11	3.04–3.06 (m)	2.86 (dd, 2.4, 4.8)	3.67 (t, 7.2)	4.73 (d, 7.2)	
12	3.02 (br d, 4.8)	2.83 (br d, 4.8)	3.44 (dd, 3.9, 11.1)	3.65 (dd, 11.1, 12.0)	4.16 (dd, 3.9, 12.0)
13	3.83 (d, 5.4)	3.46 (dd, 1.8, 5.4)	3.50–3.64 (m)	3.50–3.64 (m)	4.22 (dd, 3.9, 12.0)
14	3.78 (d, 5.1)	3.46 (dd, 2.4, 5.1)	3.77 (dd, 4.8, 9.8)	4.37 (dd, 9.8, 10.2)	5.07 (dd, 4.8, 10.2)

[a] The solvent used was CD_3OD.
[b] These assignments are tentative and may be reversed.
[c] The solvent used was pyridine-d_5.
[d] The signal is believed to be beneath the water peak.

isocratic for 5 min, then from 90% A and 10% B to 45% A and 55% B in 30 min.

H_2O used for the reactions was HPLC grade. Mitomycin C was purchased from Janssen Chimica (Spectrum Chemical Mfg. Corp., New Brunswick, N.J.). THF was distilled from Na metal and benzophenone, and pyridine was distilled from KOH. All other solvents were the best commercial grade available and used without further purification. Analytical and preparative TLC (PTLC) were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521), and silica gel GF preparative uniplates (20 × 20 cm; Analtech No. 02013).

N(1a)-Cbz-10-decarbamoylmitomycin C (7):
To a THF solution (4 mL) of **6**[10] (6.2 mg, 0.02 mmol) and Et_3N (10 μL, 0.07 mmol) was added benzyl chloroformate (Cbz-chloride) (20 μL, 0.14 mmol). The mixture was stirred for 2 h at r.t. under Ar. The mixture was concentrated by a stream of Ar and the residue was purified by PTLC (5% MeOH/$CHCl_3$) to yield **7** as a dark brown solid (8.0 mg, 90%); HPLC t_R 31.9 min; R_f 0.44 (5% MeOH/$CHCl_3$).

UV/Vis (MeOH): λ_{max} = 350 nm.

^{1}H NMR ($CDCl_3$): δ = 1.75 (s, 3 H, 6-CH_3), 3.17 (s, 3 H, 9a-OCH_3), 3.29 (dd, 1 H, J = 1.8, 4.8 Hz, 2-H), 3.36 (d, 1 H, J = 4.8 Hz, 1-H), 3.47 (dd, 1 H, J = 1.8, 13.5 Hz, 3-H_α), 3.47–3.51 (m, 1 H, 9-H), 3.89–3.97 (m, 1 H, 10-*H*H′), 4.09 (dd, 1 H, J = 10.9, 14.6 Hz, 10-H*H*′), 4.39 (d, 1 H, J = 13.5 Hz, 3-H_β), 5.06 (d, 1 H, J = 16.0 Hz, OC*H*H′Ph), 5.11 (d, 1 H, J = 16.0 Hz, OCH*H*′Ph), 5.20 (br s, 2 H, 7-NH_2), 7.33 (br s, 5 H, C_6H_5). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.9, 40.6, 43.0, 45.2, 48.8, 49.7, 61.7, 68.9, 105.0, 105.3, 113.8, 126.9, 128.5, 128.7, 134.9, 147.1, 154.6, 160.9, 178.3, 178.8.

MS (+CI): m/z (rel. intensity) = 427 $[M + 2, 43]^+$, 426 $[M + 1, 100]^+$.

HRMS (+ CI): m/z = 426.167 39 $(M + 1)^+$ (calcd for $C_{22}H_{24}N_3O_6$, 426.166 51).

N(1a)-Cbz-10-decarbamoyl-10-(methanesulfonyl)mitomycin C (9):
To an anhyd pyridine solution (2 mL) of **7** (5.9 mg, 13.9 μmol) was added $MeSO_2Cl$ (5 μL, 65 μmol) and the mixture was stirred at r.t. under Ar (1 h). The solution was concentrated under a stream of Ar and the residue was purified by PTLC using 5% MeOH/$CHCl_3$ as the eluent to yield **9** as a dark brown solid (3.9 mg, 56%); HPLC t_R 38.0 min; R_f 0.68 (5% MeOH/$CHCl_3$).

UV/Vis (MeOH): λ_{max} = 356 nm.

^{1}H NMR (pyridine-d_5): δ = 2.00 (s, 3 H, 6-CH_3), 3.17 (s, 3 H, SO_2CH_3), 3.34 (s, 3 H, 9a-OCH_3), 3.54–3.65 (m, 2 H, 2-H, 3-H_α), 3.90 (d, 1 H, J = 4.8 Hz, 1-H), 4.13 (dd, 1 H, J = 4.2, 10.9 Hz, 9-H), 4.75 (d, 1 H, J = 13.5 Hz, 3-H_β), 5.22 (s, 2 H, OCH_2Ph), 5.60 (dd, 1 H, J = 4.2, 9.9 Hz, 10-H*H*′), 7.24–7.55 (m, 5 H, C_6H_5). The C-10 *H*H′ signal is believed to be beneath the water peak in pyridine, the assignments were consistent with the COSY spectrum.

^{13}C NMR (pyridine-d_5): δ = 8.8, 37.5, 40.8, 43.7, 43.9, 49.5, 49.8, 67.6, 68.9, 104.9, 105.2, 109.7, 128.8, 128.9, 129.0, 132.0, 148.8, 155.7, 161.3, 177.3, 177.7.

MS (+CI): m/z (rel. intensity) = 505 $[M + 2, 21]^+$, 504 $[M + 1, 69]^+$.

HRMS (+CI): m/z = 504.144 06 $(M + 1)^+$ (calcd for $C_{23}H_{25}N_3O_8S$, 504.144 06).

N(1a)-Fmoc-10-decarbamoylmitomycin C (8):
To an ice-cooled THF solution (4 mL) of **6** (3.1 mg, 0.01 mmol) and Et_3N (20 μL, 0.14 mmol) was added 9-fluorenylmethyl chloroformate (Fmoc-chloride; 4.9 mg, 0.02 mmol). The ice-bath was removed, and the mixture was stirred at r.t. under Ar (4 h). The THF was removed in vacuo, and the residue was purified by PTLC (10% MeOH/$CHCl_3$) to afford **8** (4.3 mg, 79%); HPLC t_R 36.4 min; R_f 0.58 (10% MeOH/$CHCl_3$).

UV/Vis (MeOH): λ_{max} = 356 nm.

^{1}H NMR ($CDCl_3$): δ = 1.75 (s, 3 H, 6-CH_3), 3.05 (br s, 1 H, 2-H), 3.14 (s, 3 H, 9a-OCH_3), 3.38–3.57 (m, 2 H, 1-H, 3-H_α), 3.66–3.73 (m, 2 H, 9-H, 10-*H*H′), 3.93 (br t, 1 H, J = 8.8 Hz, 10-H*H*′), 4.17 (t, 1 H, J = 5.6 Hz, Fmoc-CH), 4.36 (d, 1 H, J = 13.3 Hz, 3-H_β), 4.47–4.60 (m, 2 H, Fmoc-CH_2), 5.20 (br, 2 H, 7-NH_2), 7.26–7.76 (m, 8 ArH). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.8, 40.2, 42.9, 45.2, 47.1, 48.7, 49.7, 61.2, 68.0, 105.0, 105.4, 113.7, 120.1, 124.7, 127.2, 128.0, 141.3, 143.1, 147.0, 154.6, 160.9, 176.7, 178.4.

MS (+CI): m/z (rel. intensity) = 515 $[M+2, 23]^+$, 514 $[M+1, 100]^+$.

HRMS (+CI): m/z = 514.197 33 $(M+1)^+$ (calcd for $C_{29}H_{28}N_3O_6$, 514.197 81).

N(1a)-Fmoc-10-decarbamoyl-10-(methanesulfonyl)mitomycin C (10):
A pyridine solution (0.5 mL) of **8** (4.1 mg, 0.01 mmol) was stirred at 0°C under Ar. $MeSO_2Cl$ (10 μL, 0.12 mmol) was added and allowed to stir at r.t. (5 h). The mixture was diluted with $CHCl_3$ (10 mL), and the insoluble salts were filtered. The filtrate was concentrated in vacuo and purified by PTLC (3% MeOH/$CHCl_3$) to give **10** as a dark brown solid (3.2 mg, 68%); HPLC t_R 38.2 min; R_f 0.48 (3% MeOH/$CHCl_3$).

UV/Vis (MeOH): λ_{max} = 356 nm.

^{1}H NMR ($CDCl_3$): δ = 1.77 (s, 3 H, 6-CH_3), 3.04 (s, 3 H, SO_2CH_3), 3.13 (dd, 1 H, J = 1.8, 4.8 Hz, 2-H), 3.20 (s, 3 H, 9a-OCH_3), 3.39 (d, 1 H, J = 4.8 Hz, 1-H), 3.44 (dd, 1 H, J = 1.8, 13.5 Hz, 3-H_α), 3.78 (dd, 1 H, J = 4.5, 11.1 Hz, 9-H), 4.21 (t, 1 H, J = 6.6 Hz, Fmoc-CH), 4.35–4.53 (m, 4 H, Fmoc-CH_2, 3-H_β, 10-*H*H′), 5.04 (dd, 1 H,

$J = 4.5$, 9.9 Hz, 10-H*H'*), 5.25 (br, 2H, 7-NH_2), 7.26–7.75 (m, 8 ArH). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.9, 37.5, 39.8, 42.2, 43.0, 46.9, 48.7, 50.0, 66.6, 68.6, 105.5, 105.7, 109.4, 120.1, 124.9, 127.2, 127.9, 141.3, 143.2, 146.8, 154.9, 160.5, 176.0, 178.2.

MS (+CI): *m/z* (rel. intensity) = 592 $[M+1, 100]^+$, 591 $[M, 44]^+$.

HRMS (+CI): *m/z* = 592.174 90 $(M+1)^+$ (calcd for $C_{30}H_{30}N_3O_8S$, 592.175 36).

10-Decarbamoyl-10-(methanesulfonyl)mitomycin C (11):

To a THF solution (1 mL) of **10** (3.2 mg, 5.5 μmol) was added carefully a THF solution (0.2 mL) of morpholine (10 μL, 114 μmol) at 0 °C. The mixture was allowed to stir under Ar (1 h). The solvent was removed under reduced pressure, and the residue was redissolved in a minimum amount of 5% MeOH/$CHCl_3$ and purified by PTLC (5% MeOH/$CHCl_3$); dark brown solid (1.6 mg, 80%); HPLC t_R 22.5 min; R_f 0.48 (10% MeOH/$CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.74 (s, 3H, 6-CH_3), 2.86 (dd, 1H, J = 2.4, 4.8 Hz, 2-H), 3.04–3.06 (m, 4H, 1-H, SO_2CH_3), 3.20 (s, 3H, 9a-OCH_3), 3.52 (dd, 1H, J = 2.4, 12.9 Hz, 3-H_α), 3.67 (t, 1H, J = 7.2 Hz, 9-H), 4.19 (d, 1H, J = 12.9 Hz, 3-H_β), 4.73 (d, 2H, J = 7.2 Hz, 10-H_2), 5.25 (br, 2H, 7-NH_2). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.9, 33.0, 36.1, 37.4, 43.4, 49.9, 50.0, 66.6, 105.0, 105.4, 108.6, 147.2, 155.4, 175.6, 178.3.

MS (+CI): *m/z* (rel. intensity) = 369 $[M, 100]^+$, 367 $[M-2, 88]^+$.

HRMS (+CI): *m/z* = 370.106 59 $(M+1)^+$ (calcd for $C_{15}H_{20}N_3O_6S$, 307.10728).

10-Des(carbamoyloxy)-10-azidomitomycin C (12):

A mixture of **11** (2.5 mg, 8.0 μmol) and NaN_3 (4 mg, 0.06 mmol) in DMF (1 mL) was heated at 70 °C (3 h) under Ar. The yellow-orange mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by PTLC (5% MeOH/$CHCl_3$) to yield **12** as a dark brown solid (1.1 mg, 43%); HPLC t_R 25.6 min; R_f 0.58 (10% MeOH/$CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.74 (s, 3H, 6-CH_3), 2.83 (br d, 1H, J = 4.8 Hz, 2-H), 3.02 (br d, 1H, J = 4.8 Hz, 1-H), 3.20 (s, 3H, 9a-OCH_3), 3.44 (dd, 1H, J = 3.9, 11.1 Hz, 9-H), 3.53 (dd, 1H, J = 2.4, 13.2 Hz, 3-H_α), 3.65 (dd, 1H, J = 11.1, 12.0 Hz, 10-*H*H'), 4.16 (dd, 1H, J = 3.9, 12.0 Hz, 10-H*H'*), 4.21 (d, 1H, J = 13.2 Hz, 3-H_β), 5.20 (br, 2H, 7-NH_2). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.9, 36.5, 43.2, 49.3, 49.8, 49.9, the remaining signals were not detected.

MS (+CI): *m/z* (rel. intensity) = 317 $[M+1, 100]^+$, 316 $[M, 71]^+$, 315 $[M-1, 46]^+$.

HRMS (+CI): *m/z* = 316.128 30 $(M)^+$ (calcd for $C_{14}H_{16}N_6O_3$, 316.128 39).

***N*(1a)-Methanesulfonyl-10-des(carbamoyloxy)-10-azidomitomycin C (13):**

To an anhyd pyridine solution (0.5 mL) of **12** (1.2 mg, 3.9 μmol), was added $MeSO_2Cl$ (5 μL, 64.6 mmol). The mixture was stirred under Ar (30 min) and concentrated by a stream of N_2. The residue was purified by PTLC (5% MeOH/$CHCl_3$) to obtain **13** as a dark brown solid (0.9 mg, 60%); HPLC t_R 29.0 min; R_f 0.67 (5% MeOH/$CHCl_3$).

IR (KBr): ν = 2106, 1649, 1559 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 1.76 (s, 3H, 6-CH_3), 3.05 (s, 3H, SO_2CH_3), 3.21 (s, 3H, 9a-OCH_3), 3.46 (dd, 1H, J = 1.8, 5.4 Hz, 2-H), 3.50–3.64 (m, 3H, 3-H_α, 9-H, 10-*H*H'), 3.83 (d, 1H, J = 5.4 Hz, 1-H), 4.22 (dd, 1H, J = 3.9, 12.0 Hz, 10-H*H'*), 4.42 (d, 1H, J = 13.8 Hz, 3-H_β), 5.15–5.23 (br, 2H, 7-NH_2).

HRMS (+CI): *m/z* = 395.113 41 $(M+1)^+$ (calcd for $C_{15}H_{19}N_6O_5S$, 395.113 77).

***N*(1a)-Methanesulfonyl-10-decarbamoyl-10-(methanesulfonyl)mitomycin C (14):**

To an anhyd pyridine solution (2 mL) of **6** (15.4 mg, 0.05 mmol) cooled in an ice-bath was added $MeSO_2Cl$ (15 μL, 0.19 mmol). The ice-bath was removed, and the mixture was stirred at r.t. under Ar (1 h). The solution was concentrated by a stream of Ar, and the residue was purified by PTLC using 5% MeOH/$CHCl_3$ as the eluent to yield **14** as a dark brown solid (17.3 mg, 73%); HPLC t_R 27.9 min; R_f 0.36 (5% MeOH/$CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.76 (s, 3H, 6-CH_3), 3.04 (s, 3H, SO_2CH_3), 3.09 (s, 3H, OSO_2CH_3), 3.21 (s, 3H, 9a-OCH_3), 3.46 (dd, 1H, J = 2.4, 5.1 Hz, 2-H), 3.59 (dd, 1H, J = 2.4, 13.8 Hz, 3-H_α), 3.77 (dd, 1H, J = 4.8, 9.8 Hz, 9-H), 3.78 (d, 1H, J = 5.1 Hz, 1-H), 4.37 (dd, 1H, J = 9.8, 10.2 Hz, 10-*H*H'), 4.47 (d, 1H, J = 13.8 Hz, 3-H_β), 5.07 (dd, 1H, J = 4.8, 10.2 Hz, 10-H*H'*), 5.35 (br, 2H, 7 NH_2). The assignments were consistent with the COSY spectrum.

^{13}C NMR ($CDCl_3$): δ = 7.9, 37.7, 40.0, 42.7, 42.8, 43.6, 48.5, 50.0, 66.0, 105.5, 105.7, 109.5, 143.9, 154.0, 176.0, 178.1.

HRMS (+CI): *m/z* = 448.08432 $(M+1)^+$ (calcd for $C_{16}H_{22}N_3O_8S_2$, 448.08483).

[2a*R*-(2aα,3α,9bα,9cα)]-8-Amino-3-azido-2,2a,3,4,9b,9c-hexahydro-9c-methoxy-7-methyl-2-methylsulfonyl-1*H*-benzo[*b*]pyrrolo[2,3,4-*gh*]pyrrolizine-6,9-dione (15):

A mixture of **14** (17.2 mg, 0.04 mmol) and NaN_3 (13.5 mg, 0.21 mmol) in DMF (1.5 mL) was heated at 90 °C for 1 h. The mixture was diluted with $CHCl_3$ (10 mL) and filtered through a fritted funnel. The filtrate was concentrated in vacuo, and the residue was purified by PTLC using 5% MeOH/$CHCl_3$ as the eluent to yield **15** as a dark brown solid (10.3 mg, 68%); HPLC t_R 30.4 min; R_f 0.63 (5% MeOH/$CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.79 (s, 3H, 6-CH_3), 2.91 (s, 3H, SO_2CH_3), 3.24 (s, 3H, 9a-OCH_3), 3.40 (dd, 1H, J = 7.8, 13.1 Hz, 3-H_α), 3.54–3.65 (m, 2H, 9-H, 10-*H*H'), 3.77 (d, 1H, J = 2.2 Hz, 1-H), 3.83 (d, 1H, J = 9.7 Hz, 10-H*H'*), 4.49 (dt, 1H, J = 2.2, 7.8 Hz, 2-H), 4.76 (dd, 1H, J = 7.8, 13.1 Hz, 3-H_β), 5.25 (br, 2H, 7-NH_2).

^{13}C NMR ($CDCl_3$): δ = 7.9, 33.8, 40.2, 51.5, 54.8, 56.3, 66.2, 75.5, 106.5, 114.7, 116.9, 146.3, 154.0, 177.0, 178.5.

MS (+CI): *m/z* (rel. intensity) = 396 $[M+2, 82]^+$, 363 $[M-31]^+$.

HRMS (+CI): *m/z* = 395.114 63 $(M+1)^+$ (calcd for $C_{15}H_{19}N_6O_5S$, 395.113 77).

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