Reaction of Glycosyl Halides with 7-Hydroxy-9a-methoxymitosane Sodium Salt

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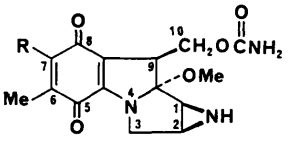
The reaction of 7-*O*-hydroxy-9a-methoxymitosane sodium salt with glycosyl halide derivatives gave the corresponding 7-*O*-glycosyl-9a-methoxymitosanes in reasonable yields. A major product, 7-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-9a-methoxymitosane, was isolated from the reaction mixture of 7-*O*-hydroxy-9a-methoxymitosane sodium salt with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide, in addition to two minor by-products, bissaccharide derivatives of mitomycin. The structures of glycosyl derivatives of mitomycin were elucidated by analysis of the nuclear magnetic resonance spectra. Field desorption mass spectrometry was also successfully used for the confirmation of these structures. The cytocidal, antibacterial, and antitumor activities of 7-*O*-glycosyl-9a-methoxymitosanes were also examined.

Keywords mitomycin C; mitomycin A; 7-*O*-hydroxy-9a-methoxymitosane sodium salt; glycosyl halide; Williamson reaction; antitumor activity

Since mitomycin A and B were first discovered in 1956 by Hata *et al.*,[1] many studies have been carried out on the mitomycins, including the well-known antitumor antibiotic, mitomycin C.[2]

The mitomycins are acknowledged as excellent antitumor antibiotics, but bone marrow damage is one of the major complications of treatment with mitomycin C.[3] The relatively highly toxic nature of the compounds has prompted prior syntheses of numerous mitomycin derivatives and analogues in an attempt to secure compounds having equal or enhanced antitumor activity but lesser toxicity than the naturally occurring mitomycins. As a part of the program on the syntheses of glycoconjugates of mitomycins, we now report the reaction of 7-hydroxy-9a-methoxymitosane sodium salt with glycosyl halides, including monosaccharides and a disaccharide. The structures of the glycosylation products were elucidated on the basis of the field desorption (FD) mass spectra (MS) and the nuclear magnetic resonance (NMR) spectra. Some of the biological activities of those derivatives were also investigated.



1 (mitomycin A): R=OMe
2 (mitomycin C): R=NH_2

Chart 1

Several methods are available for preparing mitomycin A (**1**) and its analogues,[4-7] namely 7-alkoxymitosanes from mitomycin C (**2**), but it is very difficult to synthesize 7-*O*-glycosyl-9a-methoxymitosanes by those methods. We have developed a facile method for the synthesis of 7-*O*-glycosyl-9a-methoxymitosanes by the treatment of 7-*O*-hydroxy-9a-methoxymitosane sodium salt (**3**) with glycosyl halides under Williamson reaction conditions. This procedure gave a satisfactory yield of 7-*O*-glycosyl-9a-methoxymitosanes.

The starting material **3** was prepared from **2** as described by Matsui *et al.*[8] and Kinoshita *et al.*[9] The amino group on C-7 of **2** was hydrolyzed with 0.1 N NaOH to give crude **3** as a bluish fine powder, which was submitted to the next glycosylation step without purification because of its instability.

Condensation of **3** and 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide (**4**) gave a mixture of one major product and many minor by-products, as demonstrated by thin layer chromatography (TLC). The reaction mixture was separated by column chromatography on silica gel to give 7-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-9a-

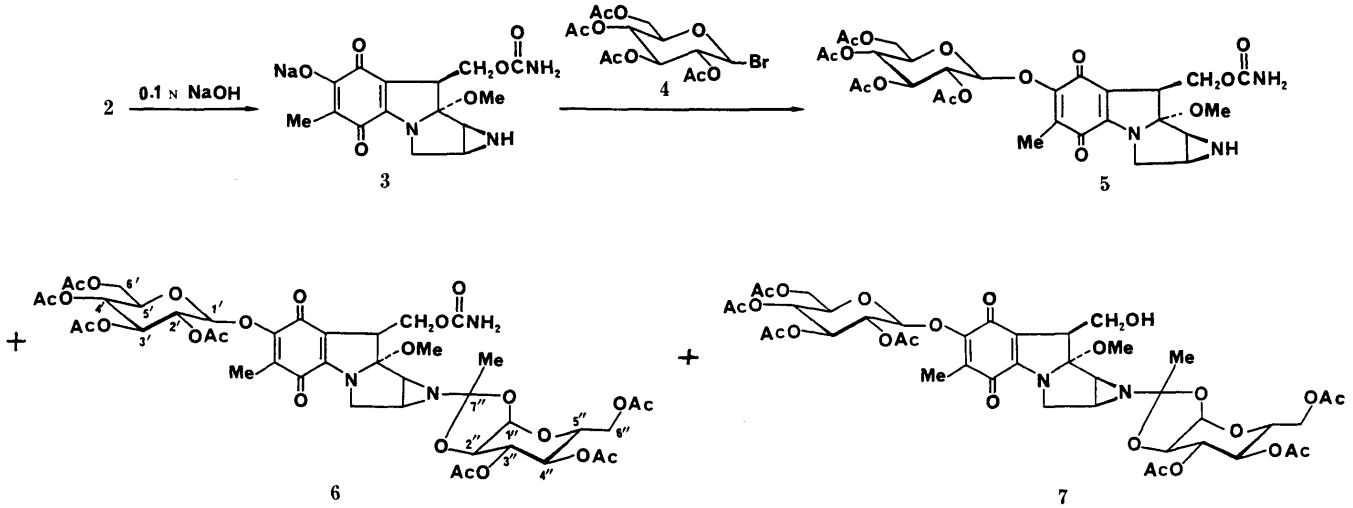


Chart 2

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methoxymitosane (**5**) in 42% yield, 3'',4'',6''-tri-*O*-acetyl-1'',2''-*O*-(7''-(1-*N*-(7-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-9a-methoxymitosanyl))ethylidyne)-α-D-glucopyranose (**6**) in 1% yield and 3'',4'',6''-tri-*O*-acetyl-1'',2''-*O*-(7''-(1-*N*-(7-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-10-decarbamoyl-10-hydroxy-9a-methoxymitosanyl))ethylidyne)-α-D-glucopyranose (**7**) in 0.5% yield.

The molecular formulae of **5**, **6**, and **7** were determined to be $C_{29}H_{35}N_3O_{15}$, $C_{43}H_{53}N_3O_{24}$, and $C_{42}H_{52}N_2O_{23}$, respectively, from the FD-MS, ^{1}H- and ^{13}C-NMR spectra. Their structures were elucidated on the basis of the ^{1}H- and ^{13}C-NMR spectra (Tables I, II, and III). Proton assignments for these compounds were made by two-dimensional (2-D) ^{1}H–^{1}H correlation spectroscopy (COSY), and carbon chemical shifts were assigned by distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{13}C COSY, and long-range selective proton decoupling (LSPD) techniques.

Consistent patterns were noted in the ^{1}H- and ^{13}C-NMR spectra for **5**, suggesting the presence of 9a-methoxymitosane and a 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose moiety. The ^{1}H- and ^{13}C-NMR spectra of **5** showed an anomeric proton and carbon signal at δ 5.52 and δ 99.7, respectively. The coupling constant of $J_{1',2'}=8.0$ Hz in the ^{1}H-NMR of **5** indicated that the glycosidic bond has the β-configuration. Moreover, the ultraviolet (UV) spectrum of **5** was similar to that typical of **1**[11,12] as shown in Fig. 1.

On the other hand, when **5** was treated with ammonia in methanol for 30 min, **2** was produced as a major product. Moreover, when **5** was treated with sodium methoxide in methanol for 20 min, **1** was produced as a major product. These products were identified by comparing their spectral (^{1}H-NMR spectra) and chromatographic (TLC) properties with those of authentic samples of **1** and **2**. The structure of **5** was elucidated on the basis of these results.

For further investigation, **5** was treated with approximately 1.1—1.2 equivalent of **4** in *N*,*N*-dimethylformamide (DMF) for 18 h, and the reaction products were isolated by preparative TLC to give **6**. In the ^{13}C-NMR spectra of **6**, two anomeric carbon signals of C-1' and C-1'' were observed at δ 96.8 and δ 99.6, indicating β and α configuration, respectively. When the ^{13}C-NMR spectra of **5** were compared with those of **6**, the signals due to the aglycone moiety of **6** corresponded with those of **5** except for the signals at C-1 and C-2, which were different due to *N*-alkylation. The ^{13}C-NMR spectra of **6** showed the C-7'' signal of the ortho ester group at δ 116.8. Moreover, the ^{1}H- and ^{13}C-NMR spectra of **6** revealed signals due to a methyl group on the ortho ester group at δ 1.68 and δ 25.1, respectively. Compound **6** was obtained as a single isomer but its stereochemistry at the new asymmetric center at C-

Table I. ^{1}H-NMR Data for the Mitosane Moiety in **5**, **6**, and **7**

Hydrogen number	5	6	7
1-H	2.92 br s	2.84 d (5.2)	2.77 d (5.5)
2-H	2.83 br s	2.65 d (1.8, 5.2)	2.67 dd (2.0, 5.5)
NH	2.09 s	—	—
3-H	3.48 dd (4.5, 12.6)	3.41 dd (1.8, 13.0)	3.44 dd (2.0, 13.0)
3-H'	4.02 d (12.6)	3.99 d (13.0)	3.99 d (13.0)
6-Me	1.87 s	1.85 s	1.88 s
9-H	3.62 dd (4.5, 10.5)	3.59 dd (4.5, 10.5)	3.37 t (7.0)
10-H	4.55 t (10.5)	4.22 t (10.5)	4.14 m
10-H'	4.70 dd (4.5, 10.5)	4.92 dd (4.5, 10.5)	
9a-OMe	3.20 s	3.13 s	3.16 s
NH_2	4.80 s	4.15 s	

Table II. ^{13}C-NMR Data for **5**, **6**, and **7**

Carbon number	Mitosane moiety			Carbon number	Glucose moiety		
	5	6	7		5	6	7
1	36.9	41.0	40.6	1'	99.7	99.6	99.9
2	32.9	37.1	37.6	2'	71.5	71.5	71.5
3	49.8	49.5	49.5	3'	72.0	72.5	72.5
5a	128.6	128.9	128.6	4'	68.2	68.3	68.2
5	182.3	182.3	182.3	5'	72.5	71.9	72.0
6	151.5	150.7	150.8	6'	61.5	61.9	61.5
6-Me	7.8	8.7	8.8	1''	—	96.8	97.3
7	152.7	152.8	152.8	2''	—	75.1	75.2
8	177.3	177.1	178.3	3''	—	70.8	70.8
8a	114.5	114.1	116.5	4''	—	67.5	67.5
9	43.2	42.5	46.7	5''	—	68.1	68.2
9a	106.3	106.2	106.5	6''	—	61.5	61.4
9a-OMe	49.7	48.9	49.1	7''	—	116.8	117.1
10	62.5	62.8	62.7	7''-Me	—	25.1	25.2
10-$OCONH_2$	156.4	156.2	—	OAc[a]			

a) –OCOMe; **5**: 20.5×2, 20.7, 20.8, **6**: 20.6×2, 20.7×4, 20.8, **7**: 20.6×3, 20.7×2, 20.8×2. –OCOMe; **5**: 169.6, 169.7, 169.9, 171.0, **6**: 169.5×2, 169.6, 169.7, 170.0, 170.6, 170.7, **7**: 169.3, 169.5, 169.7, 170.1, 170.6×2.

Table III. ^{1}H-NMR Data for the Glucosyl Moiety in **5**, **6**, and **7**

Hydrogen number	5	6	7	Hydrogen number	6	7
1'-H	5.52 d (8.0)	5.57 d (7.5)	5.53 d (7.5)	1''-H	5.69 d (4.6)	5.80 d (5.0)
2'-H	5.18 dd (8.0, 9.0)	5.21 dd (7.5, 9.0)	5.21 dd (9.0, 7.5)	2''-H	4.20 t (4.5)	4.25 dd (4.0, 5.0)
3'-H	5.27 t	5.26 t	5.28 t	3''-H	5.10 t	5.13 t
4'-H	5.13 t (10.0)	5.12 dd (10.0, 9.0)	5.15 dd (10.0, 9.0)	4''-H	4.85 dd (4.5, 9.5)	4.86 dd (4.5, 9.5)
5'-H	3.72 ddd (2.5, 4.0, 10.0)	3.77 m	3.76 ddd (2.5, 4.0, 10.0)	5''-H	3.80 m	3.98 m
6'-H	4.13 dd (2.5, 12.0)	4.11—	4.15 dd (2.0, 12.0)	6''-H	4.03—	3.56 dd (11.5, 2.5)
6'-H'	4.21 dd (4.0, 12.0)	4.26 m	4.28 dd (4.0, 12.0)	6''-H'	4.21 m	4.16 dd (2.0, 11.5)
OAc[a]				7''-Me	1.68 s	1.70 s

a) **5**: 2.02, 2.03, 2.06, 2.08. **6**: 2.00, 2.02, 2.07×2, 2.08. **7**: 2.03, 2.04, 2.05, 2.09×2, 2.10.

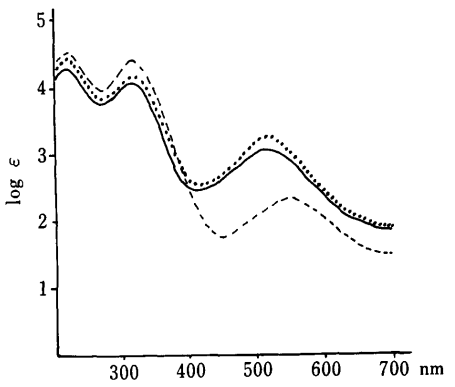

Fig. 1. UV Spectra of **1**, **2**, and **5** (in MeOH)
1; ——, **2**; – – –, **3**; ------.

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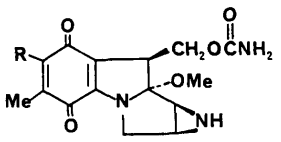

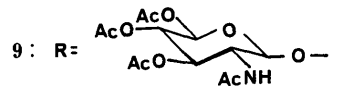

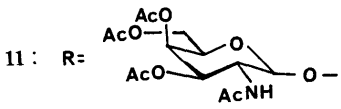

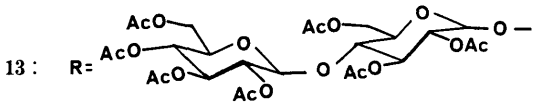

Chart 3

TABLE IV. Growth Inhibitory Concentrations of **2**, **5**, **9**, **11**, and **13** against HeLa Cells and *E. coli*

Compound number	Cytocidal activity[a]	Inhibitory zone (mm)[b]
2	0.024	37.3
5	1.56	8.9
9	1.56	11.4
11	1.56	11.1
13	1.56	9.7

a) Minimum inhibitory concentration (μg/ml). *b*) Paper disc (6 mm, thin) was infiltrated with antibiotic solution at a concentration of 10 μg/ml.

TABLE V. Antitumor Activity of **2**, **5**, **9**, **11**, and **13** against Sarcoma 180

Total dose (mg/kg/d)	Percent increase in life span				
	2	**5**	**9**	**11**	**13**
160	—	Toxic	85	419	307
40	Toxic	248	211	107	196
10	107	70	48	63	19
2.5	78	48	0	4	0
0.78	19	4	0	0	0

7″ could not be determined from the NMR spectra. On the basis of these results, the structure of **6** was elucidated.

The structure of **7** was established by comparing its ^{13}C-NMR spectrum with that of **6**. One of the major differences is that **7** lacks a signal due to a carbamoyl group at δ 156.2. When **2** was hydrolyzed with 0.1 N NaOH, it seemed that 10-decarbamoyl-7,10-dihydroxy-9a-methoxymitosane sodium salt[13] was produced as a minor by-product. It can therefore be presumed that **7** was produced by the reaction of the minor by-product with **4**.

In the same manner, treatment of **3** in DMF with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl chloride (**8**), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-galactopyranosyl chloride (**10**), or 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-D-lactosyl bromide (**13**), gave the corresponding 7-*O*-(β-D-glycosyl)-9a-methoxymitosane, **9**, **11**, or **12**, respectively, in 10—15% yield as a major product.

These 7-*O*-glycosyl-9a-methoxymitosanes were examined for cytocidal, anti-*E. coli*, and antitumor activities. Cytocidal activities against HeLa cells and anti-*E. coli* activities of these derivatives are shown in Table IV. All the compounds showed lower anti-HeLa and antibacterial activities than those of **2**. The antitumor activities of these derivatives against sarcoma 180 in mice are shown in Table V. All compounds showed higher antitumor activities than that of **2**. Though detailed experiments on the antitumor activity are required, it seems that these derivatives are more potent than **2** under the present experimental conditions.

Experimental

Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. TLC was performed on Silica gel GF-254 (Merck) plates. FD-MS, UV spectra, and infrared (IR) spectra were measured with a JEOL JMA-3100, Hitachi 340, and JASCO IR-A2 instrument, respectively. The NMR spectra were measured in chloroform-*d* ($CDCl_3$) or pyridine-d_5 (C_5D_5N) with tetramethylsilane (TMS) as an internal standard, with a Varian VXR-300 spectrometer. Column chromatography was conducted on Silica gel 60 (70—230 mesh, Merck).

7-*O*-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-9a-methoxymitosane (5), 3″,4″,6″-Tri-*O*-acetyl-1″,2″-*O*-(7″-(1-*N*-(7-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-9a-methoxymitosanyl))ethylidyne)-α-D-glucopyranose (6), and 3″,4″,6″-Tri-*O*-acetyl-1″,2″-*O*-(7″-(1-*N*-(7-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-10-decarbamoyl-10-hydroxy-9a-methoxymitosanyl))ethylidyne)-α-D-glucopyranose (7) A 0.1 N NaOH solution (1.5 ml) was added to a solution of **2** (20 mg, 0.06 mmol) in methanol (1 ml) under an argon atmosphere. The mixture was stirred for 40 h at room temperature and the progress of the reaction was monitored by TLC with chloroform–methanol (10 : 1). Then methanol in the reaction mixture was removed by using an evaporator under reduced pressure at 20 °C and water was removed by lyophilization. The residue was dried over P_2O_5 to give a bluish compound (**3**), which was used for the following reaction without purification. It was dissolved in dry DMF (2 ml), then **4** (62 mg, 0.15 mmol) and Ag_2CO_3 (50 mg) were added to the stirred solution with exclusion of moisture, stirring was continued for 18 h at room temperature. The reaction mixture was diluted with chloroform (15 ml) and the precipitates were removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with chloroform–methanol (50 : 1) to give four fractions. The first fraction gave **7** (0.3 mg, yield 0.5%). The second fraction gave **6** (0.6 mg, yield 1%). The third fraction yielded an intractable mixture (2 mg). The last fraction gave the desired compound (**5**) as a reddish purple amorphous powder (16.9 mg, yield 42%). **5**: FD-MS *m/z*: 666 ($M^+ + 1$). *Anal.* Calcd for $C_{29}H_{35}N_3O_{15}$: C, 52.32; H, 5.30; N, 6.31. Found: C, 52.32; H, 5.34; N, 6.32. UV λ_{max}^{MeOH} nm (log ε): 215 (4.45), 305 (4.30), 513 (3.22). IR ν_{max}^{KBr} cm^{-1}: 2950, 1750, 1630, 1590. NMR data are given in Tables I—III.

6: FD-MS *m/z*: 1018 ($M^+ + Na$), 996 ($M^+ + 1$). UV λ_{max}^{MeOH} nm (log ε): 215 (4.15), 236 sh (3.97), 305 (3.85), 518 (3.09). IR ν_{max}^{KBr} cm^{-1}: 1750, 1670, 1625, 1590. NMR data are given in Tables I—III.

7: FD-MS *m/z*: 975 ($M^+ + Na$), 953 ($M^+ + 1$). UV λ_{max}^{MeOH} nm (log ε): 215 (4.16), 285 (3.82), 305 (3.78), 518 (2.98). IR ν_{max}^{KBr} cm^{-1}: 1750, 1625. NMR data are given in Tables I—III.

Synthesis of 6 from 5 Compound **4** (4.9 mg, 0.012 mmol), Ag_2CO_3 (10 mg) and molecular sieves 4A (50 mg) were added to a solution of **5** (6.7 mg, 0.01 mmol) in dry DMF (0.5 ml) under an argon atmosphere. The reaction mixture was stirred for 16 h at room temperature and subjected to preparative TLC with chloroform–methanol (10 : 1). The reddish purple band (*Rf* 0.60) was scraped off and extracted with chloroform–ethanol. Removal of the solvent gave **6** (4.5 mg, yield 45%), as a reddish purple amorphous powder, showing chromatographic (TLC) behavior and spectral (IR and ^1H-NMR) properties identical with those of **6** described before.

Synthesis of 2 from 5 A solution of **5** (3.4 mg, 0.005 mmol) in methanol (2 ml) was treated with 2 N NH_4OH (0.2 ml). The reaction mixture was stirred for 30 min at room temperature and subjected to preparative TLC (neutral alumina) with acetone–benzene (5 : 1) to give **2** (1.4 mg, yield 80%). This product was crystallized from pyridine as dark bluish purple needles. It was identified by direct comparison of its ^1H-NMR spectrum with that of an authentic sample of **2**.

Synthesis of 1 from 5 A solution of **5** (6.7 mg, 0.01 mmol) in dry methanol (5 ml) was treated with 0.1% sodium methoxide solution in methanol (10 mg). The reaction mixture was stirred for 20 min at room

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temperature and subjected to preparative TLC with chloroform–methanol (10:1) to give **1** (2.1 mg, yield 60%) as a dark reddish purple solid. The structure of this product was identified by direct comparison of its ^{1}H-NMR spectrum with that of an authentic sample of **1**.

7-*O*-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranosyl)-9a-methoxymitosane (9) Compound **8** (55 mg, 0.15 mmol) and Ag_2CO_3 (50 mg) were added to a solution of **3** (obtained from the hydrolysis of **2** (20 mg)) in DMF (2 ml) under an argon atmosphere. The reaction mixture was stirred for 15 h at room temperature and diluted with chloroform (15 ml), and the precipitates were removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residue was purified by preparative TLC with chloroform–methanol (10:1) to give **9** (6.0 mg, yield 15%) as a reddish purple amorphous powder. FD-MS *m/z*: 665 ($M^+ + 1$). UV λ_{max}^{MeOH} nm (log ε): 215 (4.11), 308 (3.92), 510 (2.86). IR ν_{max}^{KBr} cm^{-1}: 1760, 1670, 1590. ^{1}H-NMR ($CDCl_3$): mitosane moiety; 1.98 (3H, s, 6-Me), 2.88 (1H, br s, 2-H), 2.95 (1H, br s, 1-H), 3.22 (3H, s, 9a-OMe), 3.50 (1H, br s, J=13.0 Hz, 3-H), 3.63 (1H, dd, J=4.5, 10.5 Hz, 9-H), 4.04 (1H, d, J=13.0 Hz, 3-H), 4.52 (1H, t, J=10.5 Hz, 10-H), 4.73 (1H, dd, J=4.5, 10.5 Hz, 10-H′), 4.89 (2H, br s, –NH), *N*-acetylglucosamine moiety; 1.90 (3H, s, OAc), 2.01 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 3.63 (1H, m, 5-H), 4.12 (1H, dd, J=3.0, 12.0 Hz, 6-H), 4.19 (1H, dd, J=12.0, 4.5 Hz, 6-H′), 4.32 (1H, dt, J=8.5, 10.0 Hz, 2-H), 5.30 (1H, dd, J=10.0, 8.5 Hz, 3-H), 5.14 (1H, t, J=10.0 Hz, 4-H), 5.26 (1H, d, J=10.0 Hz, 1-H), 6.68 (1H, d, J=8.5 Hz, NH).

7-*O*-(2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-galactopyranosyl)-9a-methoxymitosane (11) Compound **10** (55 mg, 0.15 ml) and molecular sieves 4A (50 mg) were added to a solution of 3 (obtained from the hydrolysis of **2** (20 mg, 0.06 mmol)) in dry DMF (2 ml) under an argon atmosphere. The reaction mixture was stirred for 10 h at room temperature and diluted with chloroform (15 ml), and the precipitates were removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residue was purified by preparative TLC with chloroform–methanol (10:1) to give **11** (4.4 mg, yield 11%) as a reddish purple amorphous powder.

FD-MS *m/z*: 665 ($M^+ + 1$). UV λ_{max}^{MeOH} nm (log ε): 214 (4.20), 306 (3.82), 510 (2.78). IR ν_{max}^{KBr} cm^{-1}: 1760, 1670, 1590. ^{1}H-NMR ($CDCl_3$): mitomycin moiety; 1.91 (3H, s, 6-Me), 2.86 (1H, br s, 2-H), 2.95 (1H, br s, 1-H), 3.22 (3H, s, 9a-OMe), 3.50 (1H, br d, J=13.0 Hz, 3-H), 3.63 (1H, dd, J=4.5, 10.5 Hz, 9-H), 4.05 (1H, d, J=13.0 Hz, 3′-H), 4.52 (1H, t, J=10.5 Hz, 10-H), 4.74 (1H, dd, J=4.5, 10.5 Hz, 10-H′), 4.84 (1H, br s, –NH), *N*-acetylgalactosamine moiety; 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.04 (3H, s, OAc), 2.19 (3H, s, OAc), 3.85 (1H, br t, J=6.5 Hz, 5-H), 4.10 (1H, m, 6-H), 4.52 (1H, dt, J=11.0, 8.5 Hz, 2-H), 4.96 (1H, dd, J=3.5, 11.0 Hz, 3-H), 5.01 (1H, d, J=8.5 Hz, 1-H), 5.34 (1H, br d, J=3.5 Hz, 4-H), 6.53 (1H, br d, J=8.5 Hz, NH).

7-*O*-(Hepta-*O*-acetyl-β-D-lactosyl)-9a-methoxymitosane (13) Compound **12** (210 mg, 0.3 mmol) and molecular sieves 4A (100 mg) were added to a solution of **3** (obtained from the hydrolysis of **2** (40 mg, 0.12 ml)) in dry DMF (3 ml) under an argon atmosphere. The reaction mixture was stirred for 18 h at room temperature then diluted with chloroform (20 ml) and the precipitates were removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The residue was purified by preparative TLC on silica gel (20×20 cm, 0.25 mm thick) with chloroform–methanol (20:1). The reddish purple band (*Rf* 0.38) was scraped off and extracted with chloroform–ethanol. Removal of the solvent gave **13** (28 mg, yield 10%) as a reddish purple amorphous powder. FD-MS *m/z*: 954 ($M^+ + 1$). *Anal.* Calcd for $C_{41}H_{51}N_3O_{23}$: C, 51.62; H, 5.38: N, 4.40. Found: C, 50.65; H, 5.64; N, 4.48. UV λ_{max}^{MeOH} nm (log ε): 215 (4.15), 306 (3.72), 512 (2.82). IR ν_{max}^{KBr} cm^{-1}: 1755, 1670, 1590. ^{1}H-NMR ($CDCl_3$): mitosane moiety; 1.89 (3H, s, 6-Me), 2.84 (1H, br s, 2-H), 2.92 (1H, br s, 1-H), 3.20 (3H, s, 9a-OMe), 3.48 (1H, br d, J=12.5 Hz, 3-H), 3.62 (1H, dd, J=4.5, 10.5 Hz, 9-H), 4.02 (1H, d, J=12.5 Hz, 3-H′), 4.56 (1H, t, J=10.5 Hz, 10-H), 4.72 (1H, dd, J=4.5, 10.5 Hz, 10-H′); lactose moiety: 1.98 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 2.09 (3H, s, OAc), 2.12 (3H, s, OAc), 5.80 (1H, d, J=8.0 Hz, 1″-H), 5.53 (1H, d, J=7.8 Hz, 1′-H).

Cytocidal and Antibacterial Activities of 2, 5, 9, 11, and 13 HeLa cells were maintained in monolayers in Eagle's minimum essential medium supplemented with 10% bovine serum and kanamycin (100 μg/ml) at 37 °C. To determine the cytocidal activities of 7-*O*-glycosyl-9a-methoxymitosanes (**2**, **5**, **9**, **11**, and **13**), 0.2 ml of cell suspension (4×10^4/ml) was placed in a tissue culture microplate (Falcon, 96-well) and incubated for 24 h at 37 °C in a 5% CO_2–95% air atmosphere. Then 5 μl of medium containing a different concentration of derivative for testing was added to each well, and the plate was reincubated for 72 h. The cells were fixed with methanol, and stained with Giemsa solution. The growth rate and morphological change were examined under a light microscope.

Anti-*E. coli* activity was determined by the paper disc method using nutrient agar plates.

Antitumor Activity of 2, 5, 9, 11, and 13 Sarcoma 180 cells (1×10/mouse) were inoculated i.p. into ICR mice on day 0. Mice were given various doses of 7-*O*-glycosyl-9a-methoxymitosanes on days 1, 5, and 9. Antitumor activity was evaluated in terms of the increase in life span (ILS): $(T/C-1)\times 100\%$ where "T" is the median survival in days (MSD) of the treated group and "C" is the MSD of the control group.

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