Synthesis and Antitumor Activity of Novel Mitomycin Derivatives Containing Functional Groups at the C-6-Methyl Position

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A series of C-6-substituted methyl mitomycins was synthesized and evaluated for anticellular and antitumor activities. These novel compounds were prepared by Michael addition of various alcohols or thiols to 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosanes followed by treatment with NH_3 or $MeOH/K_2CO_3$. Most compounds were potent against HeLa S_3, and some of them showed superior activity to that of mitomycin C (MMC) against P388 leukemia and sarcoma 180 in mice. In addition, some compounds exhibited remarkable activity against MMC-resistant P388 in mice. FAB-MS spectra of these mitomycin derivatives showed the elimination of the C-6-methyl substituents from the mitomycin skeletons to form quinonemethides. Interestingly, treatment of 6-demethyl-6-[[(2-pyrimidinyl)thio]methyl]mitomycin C (**12v**) with diethylamine afforded 6-demethyl-6-[(diethylamino)methyl]mitomycin C (**31**) in good yield. These results suggested that the C-6-substituted methyl mitomycins would have different biological character from that of MMC.

Introduction

Mitomycins are well known to be potent antitumor antibiotics, produced by various *Streptomyces* cultures.[1] Among these compounds, mitomycin C (MMC, **1**) has been extensively used in cancer chemotherapy against a variety of solid tumors, but its use is limited by detrimental side effects, such as myelosuppression and gastrointestinal damage. Consequently, about a thousand derivatives intended to have less toxicity and more efficacy have been synthesized by modification mainly at the C-7, N-1a, and C-10 positions.[2] Some of the C-7-substituted mitomycins have been reported to possess superior activity to that of MMC against experimental tumors and are now under clinical investigation.[3] Considering the synthesis of derivatives which have quite a new concept, modification of mitomycins at another position, especially the C-6-methyl position, may be a useful and fascinating strategy to serve desired derivatives for the reasons cited below. The C-6-methyl position is suitable to install *additional* functions because the methyl group does not play an important role in an activation process of mitomycins.[1] Consequently, by the introduction of functional groups into the C-6-methyl position, increasing (decreasing) the lipophilicity[4] or installation of an additional alkylation site[5] should be achieved without affecting the main action of mitomycins. However, these modifications have scarcely been accomplished because of the instability of the mitomycin skeleton under many reaction conditions.[6] During the course of our study of mitomycin chemistry, we reported the novel replacement of hydrogen at the C-6-methyl position by deuterium using 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosane (**7**).[7] Taking the reactivity of **7** as a Michael acceptor into account, nucleophilic introduction of functional groups into the C-6-methyl position should become feasible, and subsequent conversion to the mitomycin skeletons would afford quite novel mitomycin derivatives containing a functional group at the C-6-methyl position. Herein, we describe the synthesis of these C-6-substituted methyl mitomycin derivatives and their antitumor activities.

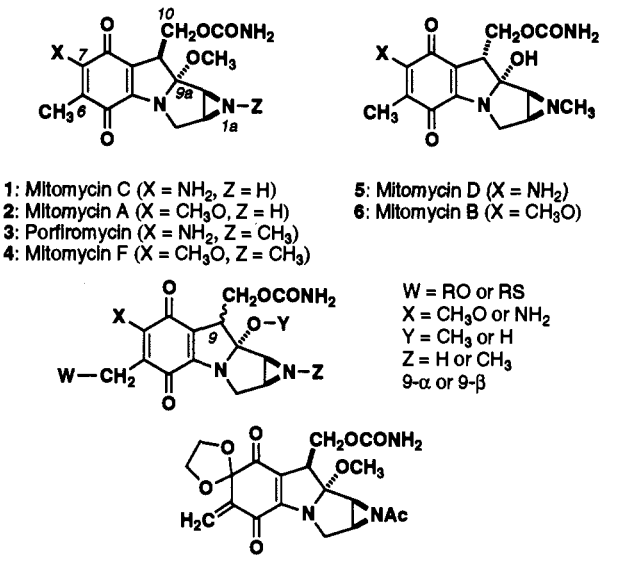

Figure 1. Structure of natural and C-6-substituted methyl mitomycins and 6-methylidene intermediate **7**.

Chemistry

We have already reported the synthesis of 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosane (**7**), a reactive Michael acceptor, by the seleno oxidation of **8** prepared from mitomycin A (MMA, **2**) in three steps.[7] We therefore first tried to react the 6-methylidene intermediate **7** with MeOH for the introduction of a methoxy group into the C-6-methyl position. As mentioned previously,[7] since **7** could not be isolated pure from the reaction mixture

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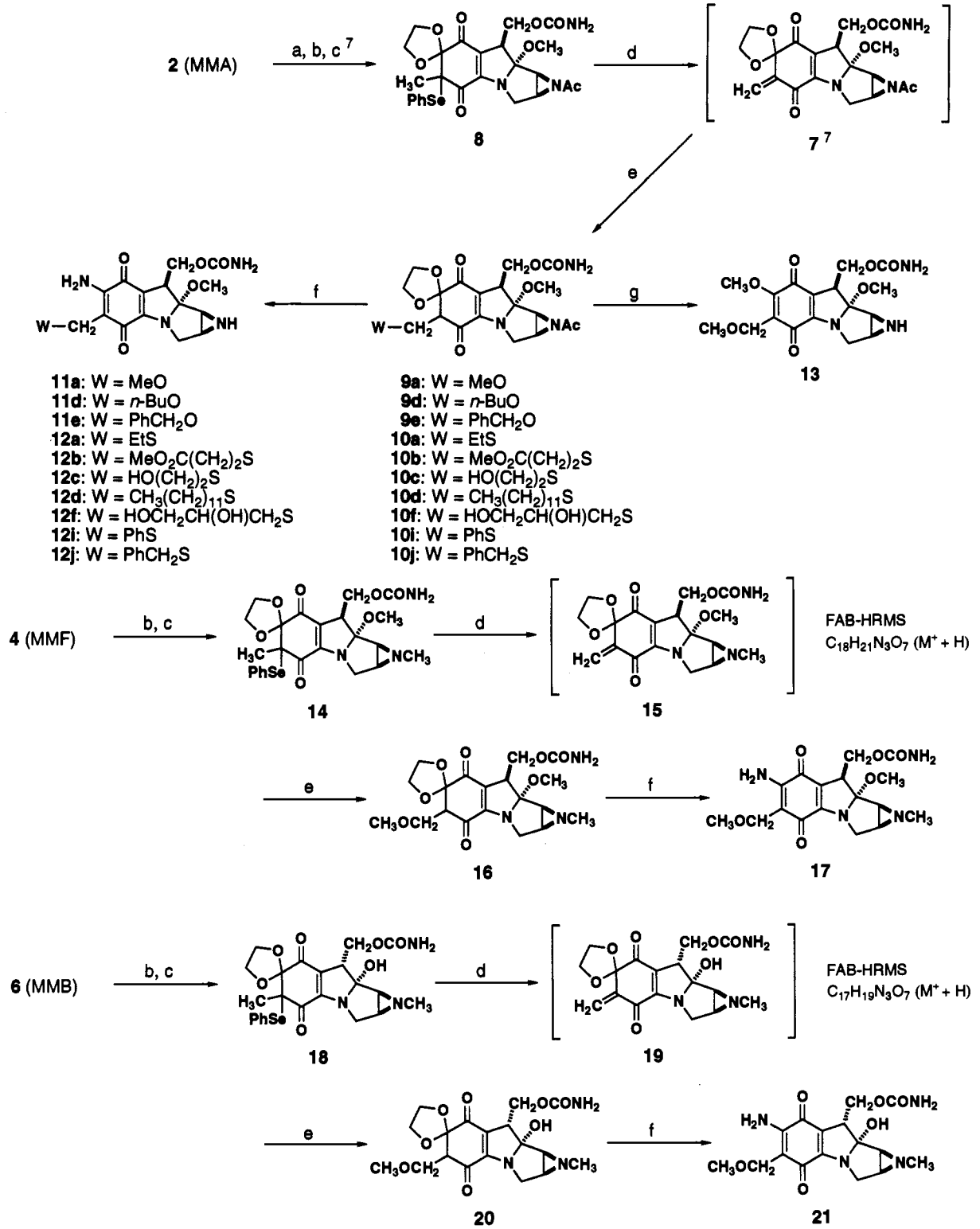
Scheme 1 [a]

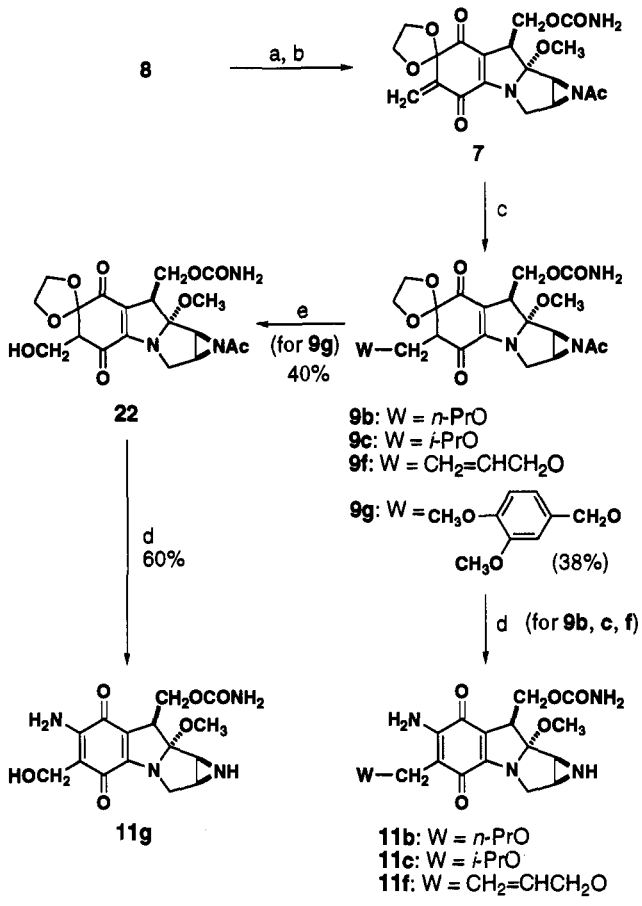


[a] (a) Ac_2O, pyridine, $CHCl_3$; (b) KOH (catalytic), ethylene glycol, THF; (c) PhSeBr, Et_3N, THF; (d) mCPBA, K_2CO_3; (e) ROH or RSH; (f) NH_3, MeOH; (g) MeOH, KOH.

due to the instability on silica gel, the reaction mixture containing **7** was used for further reactions without any treatment (sequential method). 6-Demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(methoxymethyl)mitosane (**9a**) was obtained easily by adding MeOH to the reaction mixture containing **7** at room temperature. A solution of NH_3 in MeOH (method A) was added to the resulting mixture,[7] and after purification, 6-demethyl-6-(methoxymethyl)mitomycin C (**11a**) was obtained in 28% yield based on **8** in three steps. On the other hand, 6-demethyl-6-(methoxymethyl)mitomycin A (**13**) was also prepared from **9a** on treatment with KOH in MeOH (10% yield based on **8**) (Scheme 1).[7]

Similar 6-demethyl-6-(methoxymethyl)mitomycins having various mitomycin skeletons were also prepared to evaluate the antitumor activity. 7,7-(Ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosanes **14** and **18** having mitomycin F (MMF, **4**) and mitomycin B (MMB, **6**) skeletons were prepared by a similar procedure as that described in the synthesis of **8**, *i.e.*, (1) formation of the ethylidene

Scheme 2 [a]

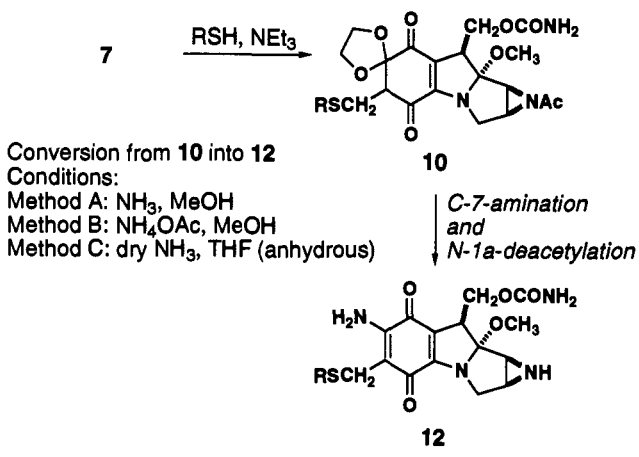


[a] (a) mCPBA, K_2CO_3; (b) isolation; (c) ROH, Triton B (NEt_3 when ROH = *n*-PrOH), $CHCl_3$; (d) NH_3, MeOH; (e) DDQ, H_2O–$CHCl_3$.

acetal at the C-7 position upon treatment with ethylene glycol in the presence of catalytic KOH and (2) introduction of the phenylseleno group into the C-6 position upon treatment with PhSeBr in the presence of NEt_3. The selenoxide fragmentation of **14** and **18** by seleno oxidation using mCPBA in the presence of K_2CO_3 followed by treatment with MeOH afforded mixtures containing **16** and **20**, respectively. They were treated with a solution of NH_3 in MeOH and afforded **17** and **21** in 63% and 20% yields based on **14** and **18**, respectively (sequential method).

For the purpose of introducing other alkoxy groups at the C-6-methyl position, the reaction of the 6-methylidene intermediate **7** with several alcohols was next attempted. The Michael additions of other primary alcohols to **7** were also successful with the same method and afforded the corresponding 6-demethyl-6-(alkoxymethyl)mitomycin C **11** after treatment with NH_3 in MeOH. However, in the case of using secondary alcohols as nucleophiles, the method mentioned above was unsuccessful. In order to obtain the adducts with secondary alcohols, the use of isolated **7**[8] was attempted (improved method). As shown in Scheme 2, isolated **7** was reacted with *i*-PrOH in the presence of catalytic amounts of Triton B, and this reaction successfully afforded the adduct **9c** in 52% yield. This was treated with NH_3 in MeOH to afford 6-demethyl-6-(isopropoxymethyl)mitomycin C (**11c**) in 60% yield. For the introduction of the hydroxy group at the C-6-methyl position, adduct **9g** prepared from **7** and 3,4-dimethoxybenzyl alcohol was oxidized by DDQ[9] to form **22**, which was converted into 6-demethyl-6-(hydroxymethyl)mitomycin C (**11g**).[10] Compound **11g** is the key compound for transformations to the C-6-methyl position intended to control lipophilicity or elimination efficiency since the hydroxy group of **11g** can be modified (*e.g.*, by acylation) rather easily.

Scheme 3



These results are summarized in Table 1.

Furthermore, we tried to introduce various thiols into the C-6-methyl position (Schemes 1 and 3). The adducts **10** were obtained generally in moderate or good yields by the reaction of **7** with slight excesses of thiols without additional bases (for the sequential method in Scheme 1) or in the presence of NEt_3 (for the improved method in Scheme 3). On the contrary, a subsequent conversion step to **12** having the MMC skeleton on treatment with NH_3 in MeOH (method A) often did not afford the desired product **12** in acceptable yield. In the case of the adduct **10** having the thio groups with aromatic heterocycles, the reaction caused the decomposition of substrates and did not afford **12** at all. So, we examined the condition of the C-7-amination/N-1a-deacetylation process using 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-[(phenylthio)methyl]mitosane (**10i**) as a model compound. The complete decomposition of **10** having the thio groups with aromatic heterocycles at the C-6-methyl position suggested that β-elimination had occurred during the basic reaction sequence due to the high elimination efficiency of thiols. To prevent β-elimination, NH_4OAc was used instead of NH_3 to suppress the basicity of the reaction system (method B). By this method, the yield of 6-demethyl-6-[(phenylthio)methyl]mitomycin C (**12i**) was slightly improved (from 29% to 36%). Further improvement was achieved by using anhydrous THF and anhydrous NH_3 gas that were dried over sodium, respectively (method C). Though the reaction rate of this method was very slow because of the poor solubility of NH_3 in anhydrous THF, the product was obtained in good yield (63%). In addition, the formation of the 1a-acetyl derivative of **12i**[11] was observed at the early stage of the reaction, which indicated the rate-determining step of this conversion was the N-1a-deacetylation.

Using the methods described above, various mitomycin derivatives having the thio groups at the C-6-methyl position were prepared. As shown in Table 2, most reactions afforded the corresponding products in reasonable yields.

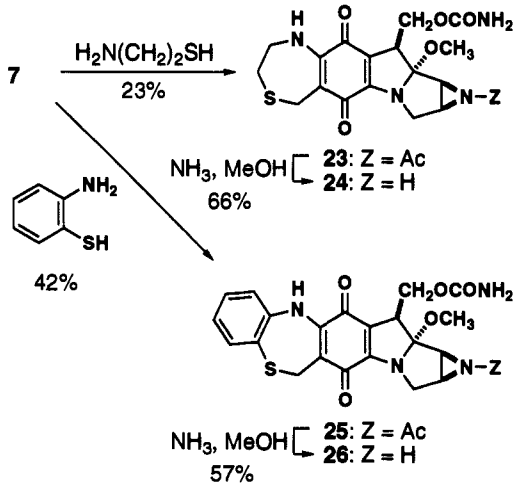
In the case of the reaction with 2-aminoethanethiol or 2-aminothiophenol, compound **7** directly afforded unique cyclization compounds **23** or **25**. N-1a-Deacetylation of **23** and **25** was performed easily by ammonolysis and gave **24** and **26**, respectively.

Table 1. Preparation and Anticellular Activity of 11, 13, 17, and 21

compd	MM skeleton	W	yield (%) 9,[a] 16, and 20	yield (%) product[b]	empirical formula[c]	^{1}H NMR (δ, ppm)[d] C-6 subst	HeLa S_3[e] IC_{50} (μM)
11a	MMC	MeO	*f, g*	28	$C_{16}H_{20}N_4O_6 \cdot 0.3H_2O$	3.31 (s, 3 H), 4.37 (d, J = 12.6 Hz, 1 H), 4.44 (d, J = 12.6 Hz, 1 H)	2.6
13	MMA	MeO	*f, g*	10	$C_{17}H_{21}N_3O_7$	3.37 (s, 3 H), 4.21 (d, J = 10.1 Hz, 1 H), 4.26 (d, J = 10.1 Hz, 1 H)	0.12
17	PFM	MeO	*f, g*	59	$C_{17}H_{22}N_4O_6$	3.17 (s, 3 H), 4.57 (d, J = 11.5 Hz, 1 H), 4.61 (d, J = 11.5 Hz, 1 H)	>100
21	MMD	MeO	*f, g*	20	$C_{16}H_{20}N_4O_6$	3.16 (s, 3 H), 4.50 (d, J = 13.0 Hz, 1 H), 4.54 (d, J = 13.0 Hz, 1 H)	>82
11b	MMC	*n*-PrO	34[h]	18	$C_{18}H_{24}N_4O_6 \cdot 0.8H_2O$	0.80 (t, J = 7.4 Hz, 3 H), 1.51 (m, 2 H), 3.34 (t, J = 6.6 Hz, 2 H), 4.60 (d, J = 11.6 Hz, 1 H), 4.67 (d, J = 11.6 Hz, 1 H)	5.3
11c	MMC	*i*-PrO	52[h]	60	$C_{18}H_{24}N_4O_6$[i]	1.17 (d, J = 6.1 Hz, 3 H), 1,18 (d, J = 6.1 Hz, 3 H), 3.59–3.65 (m, 1 H), 4.40 (d, J = 12.5 Hz, 1 H), 4.49 (d, J = 12.5 Hz, 1 H)	2.5
11d	MMC	*n*-BuO	*f, g*	23	$C_{19}H_{26}N_4O_6$[i]	0.91 (t, J = 7.6 Hz, 3 H), 1.30–1.58 (m, 4 H), 3.41 (t, J = 6.6 Hz, 2 H), 4.40 (d, J = 12.6 Hz, 1 H), 4.47 (d, J = 12.6 Hz, 1 H)	19
11e	MMC	$PhCH_2O$	*f, g*	27	$C_{22}H_{24}N_4O_6$	4.46 (s, 2 H), 4.49 (d, J = 12.6 Hz, 1 H), 4.55 (d, J = 12.6 Hz, 1 H), 7.29–7.37 (m, 5 H)	5.5
11f	MMC	CH_2=$CHCH_2O$	42[h]	41	$C_{18}H_{22}N_4O_6$	3.97 (m, 2 H), 4.64 (d, J = 12.2 Hz, 1 H), 4.71 (d, J = 12.2 Hz, 1 H), 5.09 (ddd, J = 1.7, 3.5, 10.4 Hz, 1 H), 5.30 (ddd, J = 1.7, 3.5, 17.4 Hz, 1 H), 5.95 (m, 1 H)	5.5
11g[h]	MMC	HO	*j*	*j*	$C_{18}H_{24}N_4O_7$[i]	5.00 (d, J = 12.3 Hz, 1 H), 5.05 (d, J = 12.4 Hz, 1 H)	>29
mitomycin C							0.59–1.1
mitomycin A							0.0024

[a] Yield based on 7. [b] Prepared by method A. When 9, 16, and 20 were isolated, the yields were calculated based on them. In other cases, the yields were based on 8, 14, and 18, respectively. [c] Determined by elemental analysis unless otherwise noted. Analytical results were within ±0.40% of theoretical values for C, H, and N. [d] The solvent was $CDCl_3$ (13 and 11a,c–e) or pyridine-d_5 (17, 21, and 11b,f,g). [e] In vitro anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with drugs for 1 h on day 1. The anticellular activity was determined according to the method described previously (see ref 3c). [f] Not isolated. [g] Prepared by the sequential method. [h] Prepared by the improved method. [i] Determined by FAB-HRMS. Analytical results were within ±5 mmu of the theoretical value. [j] See Scheme 2.

Scheme 4



In addition, as previously reported,[8] the Michael adduct of amines was not isolated since the retro-Michael or retro-Mannich reaction had occurred preferentially in the reaction or the purification steps to form 7 or 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosane.

The sulfide moiety in the C-6 substituent of **12e** was cleanly oxidized with mCPBA to afford sulfoxides **27** and **28** and sulfone **29**. Since sulfoxides **27** and **28** were obtained as a diastereomeric mixture bearing the asymmetric center at sulfur, each diastereomer was separated by preparative HPLC.

Biological Activity and Discussion

All C-6-substituted methyl mitomycins were studied in vitro for their anticellular activity against HeLa S_3 cells (Tables 1 and 2). In several C-6-methoxymethyl compounds having various mitomycin skeletons, compounds of the MMC and MMA type (**11a** and **13** in Table 1) showed stronger activity than those of the PFM and MMD type (**17** and **21** in Table 1). None of compounds **11** having various alkoxy groups at the C-6-methyl position showed outstanding anticellular activity. Substituents on the C-6-methyl of **12** also affect largely the anticellular activity. Many compounds having polar functional groups (*e.g.*, OH, NH) in the C-6-methyl substituent did not show sufficient activity (**12c,f,n,x,ak** in Table 2). However, protection of the hydroxy or amino groups in the C-6-methyl substituent resulted in an increase of activity (**12p** vs **12n**, **12y** vs **12x**, and **12aj** vs **12ak** in Table 2). Interestingly, compounds **12o,u** have relatively higher potency, which indicates the importance of the position of the phenyl substituent. A similar relationship was also observed for the activity of **12L** vs **12k** and **12q** vs **12p**. Sulfoxides **27** and **28** and sulfone **29** did not show antitumor activity. Other compounds not mentioned above showed generally significant activity, especially **12b,r,ab,ac,ae,ag** and **26**.

The in vivo activities against several murine tumors of selected compounds are given in Table 3. Most compounds listed in the table were effective in suppressing tumor volume (T/C) against sarcoma 180 murine solid tumor. Among those compounds, **12o** and **26** were shown to have

Table 2. Preparations and Anticellular Activity of 12

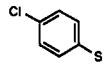
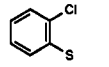
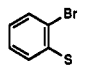
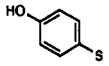
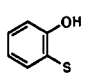
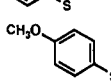
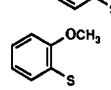
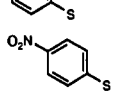
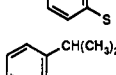
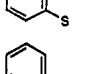
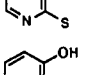
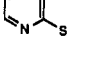
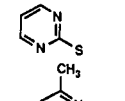
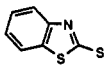
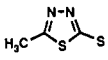
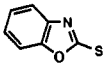
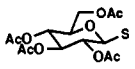
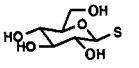
		7 or 8 to 10	10 to 12				
compd	RS	10 yield (%)[a]	method[b]	12 yield (%)	empirical formula[c]	^{1}H NMR (δ, ppm)[d] C-6 subst	HeLa S_3[e] IC_{50} (μM)
12a	EtS	*f, g*	A	29	$C_{17}H_{22}N_4O_5S^h$	1.27 (t, J = 7.4 Hz, 3 H), 2.50 (q, J = 7.4 Hz, 1 H), 3.50 (d, J = 13.6 Hz, 1 H), 3.66 (d, J = 13.6 Hz, 1 H)	2.2
12b	$MeO_2C(CH_2)_2S$	58[g]	A	42	$C_{19}H_{24}N_4O_7S\cdot 0.2H_2O$	2.66–2.76 (m, 4 H), 3.54 (d, J = 13.3 Hz, 1 H), 3.65 (d, J = 13.3 Hz, 1 H), 3.70 (s, 3 H)	0.97
12c	$HO(CH_2)_2S$	60[g]	A	55	$C_{17}H_{22}N_4O_6S^h$	2.87–2.92 (m, 2 H), 3.95 (d, J = 12.9 Hz, 1 H), 4.08 (d, J = 12.9 Hz, 1 H), 4.12 (t, J = 6.2 Hz, 2 H), 6.67 (s, 1 H)	>100
12d	$CH_3(CH_2)_{11}S$	62[g]	A	20	$C_{27}H_{42}N_4O_5S^h$	0.88 (t, J = 6.9 Hz, 3 H), 1.25–1.37 (m, 18 H), 1.59 (m, 2 H), 2.47 (m, 2 H), 3.48 (d, J = 13.4 Hz, 1 H), 3.63 (d, J = 13.4 Hz, 1 H)	5.5
12e	*i*-PrS	85[i]	A	34	$C_{18}H_{24}N_4O_5S$	1.25 (d, J = 6.8 Hz, 3 H), 1.28 (d, J = 6.8 Hz, 3 H), 2.93–3.00 (m, 1 H), 3.82 (d, J = 13.3 Hz, 1 H), 3.98 (d, J = 13.3 Hz, 1 H)	5.4
12f	$HOCH_2CH(OH)CH_2S$	54[g]	A	45	$C_{18}H_{24}N_4O_7S^h$	3.01–3.14 (m, 2 H), 4.04–4.16 (m, 5 H)	>100
12g	*c*-$PrCH_2S$	56[i]	C	44	$C_{19}H_{24}N_4O_5S\cdot 0.4H_2O$	0.18 (m, 2 H), 0.43 (m, 2 H), 1.10 (m, 1 H), 2.52 (dd, J = 7.1, 13.0 Hz, 1 H), 2.60 (dd, J = 7.1, 13.0 Hz, 1 H), 4.04 (d, J = 12.9 Hz, 1 H), 4.57 (d, J = 12.9 Hz, 1 H)	1.1
12h	$CH_2{=}CHCH_2S$	61[i]	C	42	$C_{18}H_{22}N_4O_5S\cdot 0.1CHCl_3$	3.22 (br d, J = 7.3 Hz, 2 H), 3.79 (d, J = 12.9 Hz, 1 H), 3.91 (d, J = 12.9 Hz, 1 H), 5.02 (dd, J = 1.7, 9.4 Hz, 1 H), 5.23 (dd, J = 1.7, 17.1 Hz, 1 H), 5.85–6.05 (m, 1 H)	2.3
12i	PhS	53[g]	A	29	$C_{21}H_{22}N_4O_5S\cdot 0.1CHCl_3$	3.89 (d, J = 12.5 Hz, 1 H), 4.00 (d, J = 12.5 Hz, 1 H), 7.18–7.40 (m, 5 H)	1.0
		71[i]	B	36			
			C	63			
12j	$PhCH_2S$	48[g]	A	44	$C_{22}H_{24}N_4O_5S\cdot 0.5H_2O$	3.47 (d, J = 13.3 Hz, 1 H), 3.52 (d, J = 13.3 Hz, 1 H), 3.68 (d, J = 13.5 Hz, 1 H), 3.71 (d, J = 13.5 Hz, 1 H), 7.21–7.33 (m, 5 H)	2.2
12k		62[g]	D	35	$C_{21}H_{21}ClN_4O_5S^h$	4.22 (br s, 2 H), 7.0–7.6 (m, 4 H)	53
12L		43[i]	C	35	$C_{21}H_{21}ClN_4O_5S^h$	4.26 (d, J = 11.4 Hz, 1 H), 4.34 (d, J = 11.4 Hz, 1 H), 6.97 (dt, J = 1.6, 7.7 Hz, 1 H), 7.05 (dt, J = 1.6, 7.7 Hz, 1 H), 7.27 (dt, J = 1.6, 7.7 Hz, 1 H), 7.31 (dt, J = 1.6, 7.7 Hz, 1 H)	7.3
12m		57[i]	C	40	$C_{21}H_{21}BrN_4O_5S\cdot 0.5CHCl_3$	4.25 (d, J = 11.0 Hz, 1 H), 4.33 (d, J = 11.0 Hz, 1 H), 6.88 (dt, J = 1.3, 7.7 Hz, 1 H), 7.08 (dt, J = 1.3, 7.7 Hz, 1 H), 7.1–7.3 (m, 1 H), 7.50 (dd, J = 1.3, 7.7 Hz, 1 H)	48
12n		68[g]	B	29	$C_{21}H_{22}N_4O_6S^h$	4.25 (d, J = 12.1 Hz, 1 H), 4.35 (d, J = 12.1 Hz, 1 H), 7.0–7.1 (m, 2 H), 7.5–7.6 (m, 2 H)	116
12o		49[i]	C	32	$C_{21}H_{22}N_4O_6S\cdot 0.2H_2O$	4.31 (d, J = 11.6 Hz, 1 H), 4.40 (d, J = 11.6 Hz, 1 H), 6.7–6.8 (m, 1 H), 7.1–7.3 (m, 2 H), 7.5–7.7 (m, 1 H), 12.1 (br s, 1 H)	2.2
12p		63[i]	A	37	$C_{22}H_{24}N_4O_6S^h$	3.61 (s, 3 H), 4.26 (d, J = 12.0 Hz, 1 H), 4.32 (d, J = 12.0 Hz, 1 H), 6.8–6.9 (m, 2 H), 7.5–7.6 (m, 2 H)	14
12q		44[i]	C	15	$C_{22}H_{24}N_4O_6S^h$	3.73 (s, 3 H), 4.23 (d, J = 11.5 Hz, 1 H), 4.32 (d, J = 11.5 Hz, 1 H), 6.8–6.9 (m, 2 H), 7.1–7.2 (m, 1 H), 7.4–7.5 (m, 1 H)	2.3
12r		54[i]	C	4.0	$C_{21}H_{21}N_5O_7S^h$	4.34 (d, J = 11.5 Hz, 1 H), 4.43 (d, J = 11.5 Hz, 1 H), 7.33 (dd, J = 1.9, 7.1 Hz, 2 H), 8.03 (dd, J = 1.9, 7.1 Hz, 2 H)	0.78
12s		55[i]	C	40	$C_{24}H_{28}N_4O_5S\cdot 0.2CHCl_3$	1.18 (d, J = 6.4 Hz, 6 H), 3.50–3.63 (m, 1 H), 4.23 (d, J = 11.7 Hz, 1 H), 4.29 (d, J = 11.7 Hz, 1 H), 7.0–7.4 (m, 4 H)	27
12t		61[i]	B	16	$C_{20}H_{21}N_5O_5S^h$	4.50 (s, 2 H), 6.91 (m, 1 H), 7.20–7.25 (m, 1 H), 7.37 (dt, J = 1.9, 7.8 Hz, 1 H), 8.41 (br d, J = 4.2 Hz, 1 H)	1.8
12u		47[i]	C	30	$C_{20}H_{21}N_5O_6S^h$	5.66 (d, J = 14.5 Hz, 1 H), 5.80 (d, J = 14.5 Hz, 1 H), 6.53 (dd, J = 6.7, 7.7 Hz, 1 H), 7.00 (dd, J = 1.3, 7.7 Hz, 1 H), 7.91 (dd, J = 1.1, 6.7 Hz, 1 H), 9.33 (br s, 1 H)	0.19
12v		68[i]	C	40	$C_{19}H_{20}N_6O_5S^h$	4.53 (s, 2 H), 6.85 (t, J = 4.9 Hz, 1 H), 8.47 (d, J = 4.9 Hz, 2 H)	0.95
12w		69[i]	C	62	$C_{21}H_{24}N_6O_5S\cdot 0.9H_2O$	2.26 (s, 6 H), 4.49 (s, 2 H), 6.52 (s, 1 H)	1.1

Table 2 (Continued)

compd	RS	7 or 8 to 10: 10 yield (%)[a]	10 to 12: method[b]	12 yield (%)	empirical formula[c]	^{1}H NMR (δ, ppm)[d] C-6 subst	HeLa S_3[e] IC_{50} (μM)
12x		28[i]	C	70	$C_{18}H_{20}N_6O_5S^h$	5.18 (d, J = 14.4 Hz, 1 H), 5.30 (d, J = 14.4 Hz, 1 H), 6.70 (d, J = 2.0 Hz, 1 H), 7.14 (d, J = 2.0 Hz, 1 H), 14.0 (br s, 1 H)	92
12y		58[i]	C	51	$C_{19}H_{22}N_6O_5S\cdot 0.2CHCl_3$	3.50 (s, 3 H), 5.10 (d, J = 14.4 Hz, 1 H), 5.21 (d, J = 14.4 Hz, 1 H), 6.81 (d, J = 2.2 Hz, 1 H), 7.04 (d, J = 2.2 Hz, 1 H)	0.76
12z		15[i]	C	28	$C_{17}H_{19}N_7O_5S^h$	5.09 (d, J = 14.5 Hz, 1 H), 5.17 (d, J = 14.5 Hz, 1 H), 8.51 (s, 1 H), 8.8 (br s, 1 H)	6.8
12aa		37[i]	C	70	$C_{18}H_{21}N_7O_5S^h$	3.48 (s, 3 H), 5.46 (s, 2 H), 8.35 (s, 1 H)	2.5
12ab		55[i]	C	25	$C_{17}H_{20}N_8O_5S^h$	3.73 (s, 3 H), 5.40 (d, J = 14.8 Hz, 1 H), 5.46 (d, J = 14.8 Hz, 1 H)	0.12
12ac		53[i]	C	5.0	$C_{22}H_{22}N_8O_5S^h$	5.54 (s, 2 H), 7.3–7.5 (m, 5 H)	0.27
12ad		34[i]	C	32	$C_{19}H_{20}N_4O_5S_2\cdot 0.2CHCl_3$	4.25 (s, 2 H), 6.91 (dd, J = 3.7, 5.4 Hz, 1 H), 7.15–7.25 (m, 1 H), 7.38 (dd, J = 1.2, 5.4 Hz, 1 H)	4.9
12ae		63[i]	C	67	$C_{18}H_{21}N_5O_5S_2^h$	3.04 (t, J = 8.1 Hz, 2 H), 3.96 (t, J = 8.1 Hz, 2 H), 4.81 (d, J = 14.3 Hz, 1 H), 5.13 (d, J = *ca.* 14 Hz, 1 H)	0.82
12af		63[i]	C	63	$C_{18}H_{19}N_5O_5S_2\cdot 0.1CHCl_3$	5.18 (d, J = 14.4 Hz, 1 H), 5.30 (d, J = 14.4 Hz, 1 H), 6.78 (d, J = 4.6 Hz, 1 H), 7.51 (d, J = 4.6 Hz, 1 H)	1.4
12ag		54[i]	C	36	$C_{22}H_{21}N_5O_5S_2^h$	5.59 (d, J = 15.0 Hz, 1 H), 5.71 (d, J = 15.0 Hz, 1 H), 7.15–7.25 (m, 1 H), 7.5–7.6 (m, 2 H), 8.02 (m, 1 H)	0.70
12ah		79[i]	C	49	$C_{18}H_{20}N_6O_5S_2^h$	2.12 (s, 3 H), 5.53 (s, 2 H)	1.1
12ai		68[i]	C	53	$C_{22}H_{21}N_5O_6S^h$	5.28 (br s, 2 H), 7.06–7.17 (m, 2 H), 7.29–7.32 (m, 1 H), 7.81–7.85 (m, 1 H)	5.1
12aj		45[i]	C	60	$C_{29}H_{36}N_4O_{14}S\cdot 1.0H_2O$	1.99 (s, 3 H), 1.99 (s, 3 H), 2.01 (s, 3 H), 2.01 (s, 3 H), 4.02 (d, J = 13.6 Hz, 1 H), 4.13 (ddd, J = 2.5, 4.9, 9.8 Hz, 1 H), 4.22 (d, J = 13.6 Hz, 1 H), 4.40 (dd, J = 2.5, 12.5 Hz, 1 H), 4.58 (dd, J = 4.9, 12.5 Hz, 1 H), 5.23 (d, J = 10.1 Hz, 1 H), 5.52 (t, J = 9.5 Hz, 1 H), 5.58 (t, J = 9.5 Hz, 1 H), 5.74 (t, J = 9.2 Hz, 1 H)	1.1
12ak		*j*	A[j]	79[j]	$C_{21}H_{28}N_4O_{10}S\cdot 2.3H_2O$	3.9–4.2 (m, 6 H), 4.21 (d, J = 13.4 Hz, 1 H), 4.29 (dd, J = 6.8, 11.5 Hz, 1 H), 4.60 (br d, J = 10.1 Hz, 1 H), 5.11 (d, J = 9.2 Hz, 1 H), 6.60 (br s, 1 H), 7.11 (br s, 1 H)	82
24[k]					$C_{17}H_{20}N_4O_5S$	3.08 (br t, J = 5.4 Hz, 2 H), 3.82 (d, J = 16.2 Hz, 1 H), 3.87 (d, J = 16.2 Hz, 1 H), 3.91 (br t, J = 5.4 Hz, 2 H)	1.3
26[k]					$C_{21}H_{20}N_4O_5S^h$	3.84 (d, J = 15.5 Hz, 1 H), 4.19 (d, J = 15.5 Hz, 1 H), 7.03 (dt, J = 1.3, 7.4 Hz, 1 H), 7.26 (dt, J = 1.5, 7.2 Hz, 1 H), 7.36 (dd, J = 1.3, 7.1 Hz, 1 H), 7.48 (dd, J = 1.5, 7.7 Hz, 1 H)	0.31
27[l]	*i*-PrSO				$C_{18}H_{24}N_4O_6S\cdot 0.2CHCl_3$	1.14 (d, J = 6.8 Hz, 3 H), 1.28 (d, J = 6.8 Hz, 3 H),	>10
28[l] (diastereomer of **27**)	*i*-PrSO				$C_{18}H_{24}N_4O_6S\cdot 0.2CHCl_3$	1.14 (d, J = 6.8 Hz, 3 H), 1.28 (d, J = 6.8 Hz, 3 H), 2.83 (septet, J = 6.8 Hz, 1 H), 3.99 (d, J = 13.9 Hz, 1 H), 4.20 (d, J = 13.9 Hz, 1 H)	>10
29[l]	*i*-PrSO$_2$				$C_{18}H_{24}N_4O_7S^h$	1.38 (d, J = 6.8 Hz, 3 H), 1.42 (d, J = 6.8 Hz, 3 H), 3.32 (septet, J = 6.8 Hz, 1 H), 4.43 (d, J = 14.4 Hz, 1 H), 4.58 (d, J = 14.4 Hz, 1 H)	>10
mitomycin C							0.86–2.8

[a] Sequential method: yield based on **8**. Improved method: yield based on **7**. [b] Method A: NH_3, MeOH, room temperature. Method B: NH_4OAc, MeOH, room temperature. Method C: dry NH_3, dry THF, room temperature. Method D: (1) TBSCl, imidazole, DMF, 0 °C, and then (2) NH_3–MeOH, room temperature. [c] Determined by elemental analysis. Analytical results were within ±0.40% of theoretical values for C, H, and N. [d] The solvent was pyridine-d_5 except for **12a,b,d,i,j** and **24** ($CDCl_3$). [e] In vitro anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with drugs for 1 h on day 1. The anticellular activity was determined according to the method described previously (see ref 3c). [f] Not isolated. [g] Prepared by the sequential method. [h] Determined by FAB-HRMS. Analytical results were within ±5 mmu of the theoretical value. [i] Prepared by the improved method. [j] Prepared from **12aj**. [k] See Scheme 4. [l] Prepared by an oxidation of **12e** with mCPBA. See the Experimental Section.

Table 3. Antitumor and Anticellular Activities of Mitomycin Derivatives

		sarcoma 180 (sc–iv)[b]			P388 (ip–ip)[c]			
					ILS max %[g] (OD,[e] mg/kg)			
compd	HeLa S_3[a] IC_{50} (μM)	ED_{50}[d] (mg/kg)	OD[e] (mg/kg)	T/C[f] min.	sensitive to MMC		resistant to MMC	
11c	2.5	15	50	0.38	55	(25)	>97	(25)
12b	0.97		3.1	0.63	69	(3.1)	nt[h]	
12e	5.4	8.4	25	0.27	58	(50)	30	(25)
12g	1.1	13	25	0.29	43	(13)	>115	(1.6)
12h	2.3		14	0.77	nt		33	(6.0)
12i	1.0	6.1	22	0.27	45	(50)	nt	
12o	2.2	22	46	0.14	nt		>67	(46)
12u	0.19	3.7	6.3	0.39	49	(3.1)	26	(3.1)
12v	0.95	8.9	13	0.41	58	(0.78)	>92	(1.6)
12w	1.1		6.3	0.58	50	(3.1)	nt	
12aa	2.5	39	46	0.44	nt		>71	(9.0)
12ad	4.9	29	46	0.35	nt		>56	(46)
12ae	0.82	5.9	6.3	0.45	62	(3.1)	64	(3.1)
12af	1.4	8.9	13	0.40	49	(3.1)	26	(3.1)
12ag	0.70	36	50	0.44	40	(13)	57	(50)
12aj	1.1	24	25	0.45	58	(13)	>143	(13)
26	0.31	35	100	0.16	36	(13)	48	(25)
mitomycin C	0.82–1.4	1.9–3.7	4.0–6.0	0.28–0.46	38–84	(1.0–6.0)	−19–23	(1.0–4.0)

[a] In vitro anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with compounds for 1 h on day 1. The anticellular activity was determined according to the method described previously (see ref 3c). [b] In vivo antitumor activity against sarcoma 180. Sarcoma 180 cells were inoculated sc into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1. [c] In vivo antitumor activity against lymphocytic leukemia P388 in mice. P388 cells were inoculated ip into CDF_1 mice on day 0. Compounds were administered ip on day 1. [d] Dose that gave 50% inhibition of tumor growth calculated from the dose–response curve. [e] Optimal dose. [f] Treated versus control value of tumor volume. Tumor volume was calculated according to the method described previously (see ref 3c). [g] Maximal increase in life span, calculated (T/C − 1) 100, where T and C are mean survival days of treated and control mice, respectively. [h] Not tested.

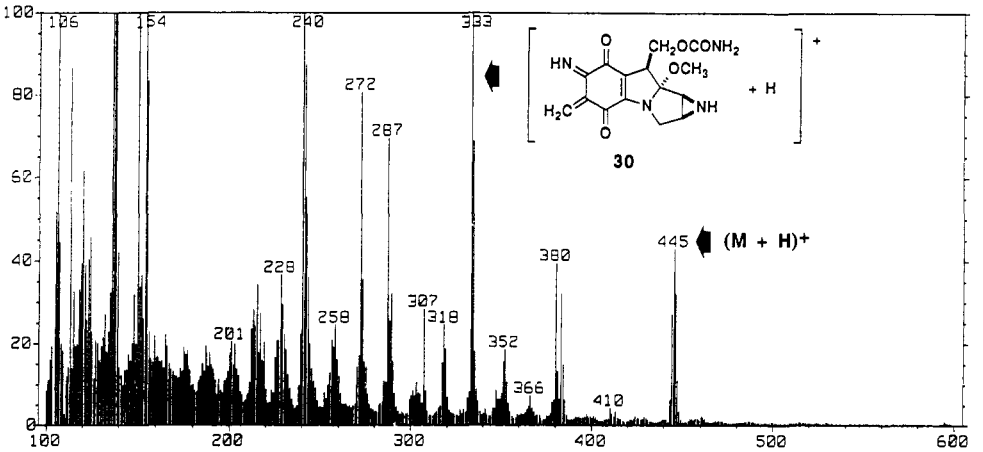


Figure 2. FAB-MS Spectrum of **12v**.

superior activity to that of MMC. However, the optimal dose of those compounds is generally higher than that of MMC. Antitumor activity against P388 murine leukemia is also nearly equal to that of MMC in prolongation of life span (ILS), but the optimal dose is rather variable depending on the C-6-methyl substituent of the derivatives. In general, the compounds having high in vivo activity are potent against in vitro anticellular activity. On the other hand, those having high in vitro activity are not always effective in vivo. The most significant result of these assays is the excellent effectiveness of some derivatives against MMC-resistant P388 murine leukemia (P388/MMC) in ILS. And, surprisingly, even an evidently higher activity against P388/MMC than against P388 (sensitive to MMC) was observed in **11c** and **12g,v,aj**. It is concluded from these results that the C-6-substituted methyl mitomycin derivatives have some different character in antitumor activity from that of MMC. The difference may arise from the nature of the C-6-methyl substituent.

To conjecture the difference of character between such derivatives and MMC, we examined the derivatives from a structural viewpoint. First, from the FAB-MS spectra of C-6-substituted methyl derivatives, the C-6-methyl substituents were found to be eliminated easily from the mitomycin skeleton to form quinonemethide[12] **30** in an ionization process. The fragment peak [*m/z* 333 [(M − RSH) + H]$^+$ or 334 [(M − RSH) + 2H]$^+$] was observed in all compounds except **24** and **26**, and the intensity of most of those was greater than each parent peak. Figure 2 shows the FAB-MS spectrum of the representative derivative **12v**. While the above ionization condition is drastic, the lability compared to the other functional groups, *i.e.*, C-10-carbamoyloxy and C-9a-methoxy which are eliminated in the reductive activation process, is noteworthy. Secondly, we confirmed the ability of the alkylation by nucleophiles at the C-6-methyl position of derivatives using the representative compound **12v**. Diethylamine was used as a nucleophile for its high nucleophilicity and easy determination. As a result, by contact of **12v** with diethylamine at room temperature for 48 h, 6-demethyl-6-[(diethylamino)methyl]mitomycin C (**31**) was obtained in 77% yield, and this demonstrates the effective alkylation at the C-6-methyl position. It is worth

emphasizing that this nucleophilic alkylation occurred without the reductive activation. These findings suggest that the C-6-methyl position of derivatives acts as an alkylation site in addition to the C-1 and C-10 positions, the conventional alkylation sites to DNA. The role of "the third alkylation site" is unclear. However, there is a possibility that the derivatives bind covalently with both DNA and protein through the conventional DNA-alkylation sites and the C-6-methyl position which is positioned away sufficiently from DNA, and such complexes may contribute to characteristic antitumor activity.[13] In view of these results, although limiting, the biological activities characteristic of C-6-substituted methyl mitomycins may result from the newly created alkylation site at the C-6-methyl position.

Conclusions

A series of C-6-substituted methyl mitomycins was prepared, and some of them were effective against several tumors in mice including MMC-resistant P388 leukemia. It was shown that the substituents at the C-6-methyl position play a significant role. Further detailed studies on antitumor spectra and toxicity of some of these derivatives are in progress.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers except for mitomycins and were used without purification. THF was distilled from sodium/benzophenone immediately prior to use. NH_3 gas was also distilled from sodium immediately prior to use. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Bruker AM 400, JEOL JNM-GX270, and JEOL JNM-EX270 instruments. Mass spectral (MS) data were obtained from Hitachi M-80B and JEOL JMS-D300 mass spectrometers. Infrared spectra (IR) were recorded on a Nihon Bunko IR-810 instrument. Elemental analyses were performed by a Perkin-Elmer 2400 C, H, N analyzer. The purity of the samples was checked by chromatographic methods (HPLC and TLC) and careful analysis of NMR spectra. The representative analytical data are listed in Tables 1 and 2.

6-Demethyl-6-(methoxymethyl)mitomycin C (11a). Preparation via the Sequential Method. To a solution of 8[7] (297 mg, 0.516 mmol) in $CHCl_3$ (10 mL) and pyridine (1.0 mL) was added mCPBA (about 70% purity, 200 mg, 0.81 mmol) in one portion at 0 °C. After the solution was stirred for 10 min at room temperature, MeOH (5.0 mL) was added and the mixture was stirred for an additional 5 h. The resulting reaction mixture was poured into a saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator, and the resulting residue was purified by chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent) to afford the adduct **9a** as a yellow paste. To a solution of **9a** in MeOH (5 mL) was added NH_3 in MeOH (6.8 M, 0.5 mL), and the mixture was allowed to stand at room temperature. After 4 h, the resulting mixture was concentrated on a rotary evaporator. The residue was subjected to chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) followed by drying under vacuum to afford **11a** (53 mg, 28% based on 8) as a purple paste.

6-Demethyl-6-(methoxymethyl)mitomycin A (13). Preparation via the Sequential Method. To a solution of 8 (312 mg, 0.542 mmol) in $CHCl_3$ (4 mL) and pyridine (0.5 mL) was added mCPBA (about 70% purity, 274 mg, 1.1 mmol) in one portion at 0 °C. After 1 h, MeOH (2 mL) was added and the reaction mixture was stirred for an additional 3 h. A saturated $NaHCO_3$ aqueous solution was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The resulting residue was subjected to chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) to afford a crude adduct, **9a**. To a solution of **9a** in MeOH (4 mL) was added KOH (1 mg), and the reaction mixture was stirred at room temperature. After 12 h, dry ice and brine were added and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **13** (21 mg, 10% based on 8) as a reddish purple powder.

6-Demethyl-6-(methoxymethyl)porfiromycin (17). Preparation via the Sequential Method. To a stirred solution of **14** (70 mg, 0.13 mmol) in $CHCl_3$ (2.5 mL) and pyridine (0.20 mL) was added mCPBA (about 70% purity, 40 mg, 0.16 mmol) in one portion at 0 °C. After 5 min at 0 °C, MeOH (0.50 mL) was added and stirring of the reaction mixture was continued at room temperature. After 8 h, NH_3 in MeOH (6.8 M, 0.50 mL) was added and the mixture was stirred for an additional 14 h. The volatiles were removed on a rotary evaporator, and the resulting residue was purified by column chromatography (silica gel, 96:4 $CHCl_3$/MeOH as an eluent) to afford the crude product, which was further purified by preparative TLC (silica gel, 9:1 $CHCl_3$/MeOH as a developing solvent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **17** (29 mg, 59%) as a gray powder.

6-Demethyl-6-(methoxymethyl)mitomycin D (21). Preparation via the Sequential Method. To a stirred solution of **18** (368 mg, 0.689 mmol) in $CHCl_3$ (20 mL) and pyridine (1.5 mL) was added mCPBA (about 70% purity, 220 mg, 0.89 mmol) in one portion at room temperature. After 30 min, MeOH (5.0 mL) was added to the reaction mixture. After an additional 15 h, NH_3 in MeOH (6.8 M, 0.50 mL) was added and stirring of the mixture was continued for an additional 3 h. The resulting reaction mixture was poured into a saturated $NaHCO_3$ aqueous solution. The layers were separated, and the aqueous layer was subjected to column chromatography (HP-20, 1:1 water/MeOH as an eluent) to afford a purple fraction. The solvent was removed on a rotary evaporator, and the residue was further purified by column chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent). After drying under vacuum, the desired product **21** (50 mg, 20%) was obtained.

6-Demethyl-6-(isopropoxymethyl)mitomycin C (11c). Preparation via the Improved Method. To a solution of **7** (90 mg, 0.21 mmol) in $CHCl_3$ (3 mL) and *i*-PrOH (4 mL) was added Triton B (1.1% of water solution, 50 μL), and the mixture was stirred at room temperature for 2 days. The reaction was quenched by adding phosphate buffer (pH 4), and the mixture was extracted with $CHCl_3$. The obtained organic extracts were washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. After purification by column chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent), an adduct, **9c** (53 mg, 52% based on **7**), was obtained as a yellow paste. The obtained **9c** (53 mg) was dissolved in MeOH (3 mL) and NH_3 in MeOH (6.8 M, 0.30 mL), and the mixture was stirred at 0 °C. After 16 h, the volatiles were removed on a rotary evaporator and the residue was subjected to chromatography (silica gel, 95:5 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **11c** (26 mg, 60% based on **9c**) which was obtained as a gray powder.

6-Demethyl-6-(hydroxymethyl)mitomycin C (11g). As described in the synthesis of **9c**, compound **7** (207 mg, 0.494 mmol) was treated with 3,4-dimethoxybenzyl alcohol (2.0 mL) and Triton B (1.1% in water, 50 μL) in $CHCl_3$ (15 mL) to afford **9g** (110 mg, 38%). The adduct obtained (34 mg, 0.058 mmol) was dissolved in $CHCl_3$ (10 mL). To the solution were added water (0.3 mL) and DDQ (16 mg, 0.070 mmol), and the resulting mixture was stirred at 0 °C for 6 h. The insoluble materials were filtered off and washed with $CHCl_3$, and the combined organic layer was concentrated on a rotary evaporator. The residue was purified by preparative TLC (silica gel, 95:5 $CHCl_3$/MeOH as a developing solvent) to afford **22** (10 mg, 40%). A similar procedure to that described for the synthesis of **11c** was employed to convert **22** (2.5 mg, 5.7 μmol) into **11g** (1.2 mg, 60%).

6-Demethyl-6-[(isopropylthio)methyl]mitomycin C (12e). Preparation via Method A. To a stirred solution of **7** (230 mg, 0.55 mmol) in $CHCl_3$ (10 mL) was added 2-propanethiol (50 mg, 0.66 mmol). After 1 h at room temperature, the reaction mixture was poured into a saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$. The organic layer was washed with brine,

dried over Na_2SO_4, and concentrated on a rotary evaporator. The resulting residue was purified by column chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent) to afford an adduct, **10e** (231 mg, 85%). A similar procedure to that described for the synthesis of **11c** was employed to convert **10e** (231 mg, 0.467 mmol) into **12e** (65 mg, 34%).

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-[(phenylthio)methyl]mitomycin A (10i). (1) Sequential Method. To a suspension of **8** (292 mg, 0.507 mmol) and powdered K_2CO_3 (149 mg, 1.08 mmol) in $CHCl_3$ (15 mL) was added mCPBA (about 70% purity, 1.49 g, 6.00 mmol) in one portion at 0 °C. The reaction mixture was stirred at room temperature. After 3.5 h, phosphate buffer (0.1 M, pH 7) and aqueous $Na_2S_2O_3$ (0.1 M) were added. The layers were separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with brine and dried over Na_2SO_4 to afford crude **7** as a yellow solution. To this solution (100 mL) were added NEt_3 (100 μL) and thiophenol (60 μL, 0.54 mmol), and the mixture was stirred at room temperature for 3.5 h. The reaction mixture was washed successively with a saturated $NaHCO_3$ aqueous solution and brine, and the volatiles were removed on a rotary evaporator. The resulting residue was purified by column chromatography (silica gel, 97:3 $CHCl_3$/MeOH as an eluent) to afford a brown paste. This paste was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **10i** (142 mg, 53%) as a brown powder.

(2) Improved Method. To a stirred solution of **7** (1.00 g, 2.39 mmol) in CH_2Cl_2 (50 mL) were added NEt_3 (0.50 mL) and thiophenol (0.30 mL, 2.7 mmol). After 1.5 h at room temperature, the reaction mixture was washed successively with phosphate buffer (pH 4) and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The obtained residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford the adduct **10i** (901 mg, 71%) as a brown powder. Compound **10i** was obtained as a mixture of two diastereomers (approximately 1:1) at C-6 in $CDCl_3$: ^1H NMR δ (270 MHz, $CDCl_3$) 2.05 (s, 3/2 H, 1a-acetyl), 2.10 (s, 3/2 H, 1a-acetyl), 3.20 (s, 3/2 H, 9a-OCH_3), 3.22 (s, 3/2 H, 9a-OCH_3), 3.15–3.55 (m, 3 H, 6-CH_2 + 6-H), 3.20–3.25 (m, 1 H, 2-H), 3.41 (dd, J = 1.7, 13.1 Hz, 1/2 H, 3α-H), 3.48 (dd, J = 2.0, 13 Hz, 1/2 H, 3α-H), 3.50 (d, J = 4.5 Hz, 1/2 H, 1-H), 3.51 (d, J = 4.5 Hz, 1/2 H, 1-H), 3.71 (dd, J = 5.0, 8.9 Hz, 1/2 H, 9-H), 3.75 (dd, J = 5.0, 9.4 Hz, 1/2 H, 9-H), 4.0–4.5 (m, 5 H, ethylenedioxy + 10-H_a), 4.01 (d, J = 13.4 Hz, 1/2 H, 3β-H), 4.28 (d, J = 12.9 Hz, 1/2 H, 3β-H), 4.88 (br s, 2 H, 10-$OCONH_2$), 4.93 (dd, J = 5.0, 11.1 Hz, 1/2 H, 10-H_b), 4.95 (dd, J = 5.0, 10.9 Hz, 1/2 H, 10-H_b), 7.11–7.47 (m, 5 H, phenyl). Anal. ($C_{25}H_{27}N_3O_8S$·1.4H_2O) C, H, N.

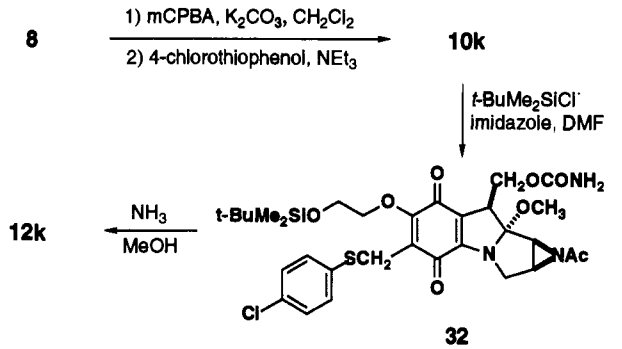
6-Demethyl-6-[(phenylthio)methyl]mitomycin C (12i). (1) Method A: Conversion with NH_3 in MeOH. To a solution of **10i** (110 mg, 0.208 mmol) in MeOH (25 mL) was added NH_3 in MeOH (6.8 M, 0.3 mL), and the mixture was allowed to stand at room temperature. After 11 h, the resulting mixture was concentrated on a rotary evaporator and the residue was subjected to chromatography (silica gel, 9:1 $CHCl_3$/MeOH as an eluent) to afford a purple paste. This paste was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **12i** (27 mg, 29%) as a purple powder.

(2) Method B: Conversion with NH_4OAc in MeOH. To a stirred solution of **10i** (49 mg, 0.093 mmol) in MeOH (10 mL) was added NH_4OAc (150 mg). After 14 h at room temperature, brine was added to the reaction mixture. The mixture was extracted with $CHCl_3$, and the organic layer was dried over Na_2SO_4. The volatiles were removed, and the resulting residue was treated using the foregoing procedure to afford **12i** (15 mg, 36%) as a purple powder.

(3) Method C: Conversion with NH_3 in THF. A solution of **10i** (256 mg, 0.484 mmol) in THF (30 mL) was allowed to stand at room temperature in an atmosphere of dry NH_3 for 1 week. The resulting mixture was concentrated on a rotary evaporator and treated using the foregoing procedure to afford **12i** (136 mg, 63%) as a purple powder: ^1H NMR δ (400 MHz, $CDCl_3$) 0.64 (br s, 1 H, 1a-H), 2.81 (br s, 1 H, 2-H), 2.89 (br s, 1 H, 1-H), 3.19 (s, 3 H, 9a-OCH_3), 3.50 (br d, J = 12.9 Hz, 1 H, 3α-H), 3.60 (dd, J = 4.4, 10.6 Hz, 1 H, 9-H), 3.89 (d, J = 12.5 Hz, 1 H, 6-CH_2), 4.00 (d, J = 12.5 Hz, 1 H, 6-CH_2), 4.21 (d, J = 12.9 Hz, 1 H, 3β-H), 4.51 (br t, 1 H, 10-H_a), 4.68 (dd, J = 4.4, 10.6 Hz, 1 H, 10-H_b), 4.79 (br s, 2 H, 10-$OCONH_2$), 5.85 (br s, 2 H, 7-NH_2), 7.18–7.40 (m, 5 H, phenyl); secondary ion-MS m/z 443 (M^+ + 1); IR (KBr) 3425, 3320, 2886, 1708, 1650, 1600, 1553, 1480, 1439, 1334, 1221, 1165 cm^{-1}. Anal. ($C_{21}H_{22}N_4O_5S$·0.1$CHCl_3$) C, H, N.

6-[[(4-Chlorophenyl)thio]methyl]-6-demethylmitomycin C (12k). Preparation via Method D. A crude solution of **7** in CH_2Cl_2 (50 mL) prepared from **8** (300 mg, 0.523 mmol) was treated in a similar procedure to that described for the synthesis of **10i** (sequential method) with 4-chlorothiophenol (153 mg, 1.06 mmol) to afford **10k** (184 mg, 62%). To the solution of **10k** (114 mg, 0.201 mmol) in DMF (2.5 mL) were added imidazole (50 mg, 0.73 mmol) and *t*-$BuMe_2SiCl$ (82 mg, 0.54 mmol). After being stirred for 105 min at 0 °C, the reaction mixture was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, from 3:1 to 2:1 $CHCl_3$/MeCN as eluents) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **32** (98 mg, 72%) as a reddish purple powder. The desired product **12k** (34 mg, 49%) was prepared by a similar procedure to that described in the synthesis of **12i** (method A) from **32** on a 0.145-mmol scale.

Scheme 5



6-[[(2-Chlorophenyl)thio]methyl]-6-demethylmitomycin C (12L). Preparation via Method C. To a stirred solution of **7** (629 mg, 1.50 mmol) in CH_2Cl_2 (50 mL) were added 2-chlorothiophenol (170 μL, 1.50 mmol) and NEt_3 (150 μL). After 1 h at room temperature, the resulting reaction mixture was washed successively with phosphate buffer (pH 4) and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane to afford an adduct, **10L** (364 mg, 43%), as a red powder. The product **10L** (349 mg, 0.618 mmol) was dissolved in THF (30 mL), and the mixture was allowed to stand under an atmosphere of dry NH_3 at room temperature. After 136 h, the volatiles were removed on a rotary evaporator and the residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent). After trituration with $CHCl_3$–*n*-hexane and drying under vacuum, the desired **12L** (103 mg, 35%) was obtained as a purple powder.

6-Demethyl-6-[[(3-hydroxypyridin-2-yl)thio]methyl]mitomycin C (12u). Preparation via Method C. To a stirred solution of **7** (313 mg, 0.747 mmol) in CH_2Cl_2 (30 mL) were added 3-hydroxy-2-mercaptopyridine (95 mg, 0.747 mmol) and NEt_3 (100 μL). After 30 min at room temperature, the resulting reaction mixture was washed successively with phosphate buffer (pH 4) and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **10u** (190 mg, 47%) as a yellow powder. The obtained **10u** (190 mg, 0.348 mmol) was dissolved in THF (20 mL), and the mixture was allowed to stand under an atmosphere of dry NH_3 at room temperature. After 49 h, the volatiles were removed on a rotary evaporator and the residue was purified by column chromatography (silica gel, from 20:1 to 10:1 $CHCl_3$/MeOH as eluents) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **12u** (48 mg, 30%) as a purple powder.

6-Demethyl-6-[[(2-pyrimidinyl)thio]methyl]mitomycin C (12v). Preparation via Method C. As described in the synthesis

of **12u**, compound **7** (420 mg, 1.00 mmol) was treated with 2-mercaptopyrimidine (111 mg, 0.991 mmol) and NEt_3 (50 μL) in CH_2Cl_2 (40 mL) to afford an adduct, **10v** (362 mg, 68%). A similar procedure to that described for the synthesis of **12u** was employed to convert **10v** (362 mg, 0.681 mmol) into **12v** (121 mg, 40%).

6-Demethyl-6-[[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thio]methyl]mitomycin C (12aj). Preparation via Method C. As described in the synthesis of **12u**, compound **7** (215 mg, 0.513 mmol) was treated with 1-thio-β-D-glucose tetraacetate (195 mg, 0.536 mmol) and NEt_3 (50 μL) in CH_2Cl_2 (20 mL) to afford an adduct, **10aj** (182 mg, 45%). A similar procedure to that described for the synthesis of **12u** was employed to convert **10aj** (182 mg, 0.233 mmol) into **12aj** (98 mg, 60%).

6-Demethyl-6-[(β-D-glucopyranosylthio)methyl]mitomycin C (12ak). Compound **12aj** (25 mg, 0.036 mmol) was dissolved in NH_3 in MeOH (6.1 M, 3.0 mL), and the mixture was allowed to stand at room temperature for 10.5 h. After the volatiles were removed on a rotary evaporator, the obtained residue was purified by a short column chromatography (Bond Elute C-18, from 100:0 to 60:40 water/MeCN as eluents) to obtain a purple aqueous solution. This solution was freeze-dried to afford **12ak** (15 mg, 79%) as a purple amorphous solid.

2-Aminoethanethiol Adduct 24. As described in the synthesis of **10i**, a $CHCl_3$ solution of crude **7** prepared from **8** (320 mg, 0.556 mmol) was treated with 2-aminoethanethiol hydrochloride (127 mg, 1.12 mmol) and pyridine (excess) in THF (10 mL) to afford an adduct, **23** (55 mg, 23%). The adduct **23** (55 mg, 0.13 mmol) was dissolved in MeOH (10 mL) and NH_3 in MeOH (6.8 M, 0.50 mL), and the solution was stirred at 0 °C for 4 h. The volatiles were removed on a rotary evaporator, and the residue was purified by column chromatography (silica gel, 9:1 $CHCl_3$/MeOH as an eluent). After drying under vacuum, the desired product **24** (33 mg, 66%) was obtained.

2-Aminothiophenol Adduct 26. As described in the synthesis of **12L**, compound **7** (299 mg, 0.714 mmol) was treated with 2-aminothiophenol (90 mg, 0.71 mmol) and NEt_3 (100 μL) in CH_2Cl_2 (30 mL) to afford the adduct **25** (146 mg, 42%). The adduct **25** (123 mg, 0.255 mmol) was treated with NH_3 in MeOH as described above and afforded a green paste. This paste was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **26** (76 mg, 57%) as a green powder.

6-Demethyl-6-[(isopropylsulfinyl)methyl]mitomycin C (27 and 28). To a slurry of **12e** (110 mg, 0.270 mmol) and K_2CO_3 (128 mg, 0.926 mmol) in $CHCl_3$ (50 mL) was added a solution of mCPBA (about 80% purity, 130 mg, 0.60 mmol) in $CHCl_3$ (10 mL) over a period of 13 min at 0 °C, and the mixture was stirred for 2 min. The reaction was quenched by adding an aqueous solution of $Na_2S_2O_3$ (0.4 M), and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator to afford a purple paste. This paste was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane to afford the product (73 mg, 64%) as a purple powder. Since the material was found to be a mixture of diastereomers arising from the sulfinyl group at the C-6-methyl position, each diastereomer was isolated by preparative HPLC (ODS, 60:40 water/MeCN as an eluent), affording **27** (22 mg) and **28** (22 mg).

6-Demethyl-6-[(isopropylsulfonyl)methyl]mitomycin C (29). To a slurry of **12e** (75 mg, 0.19 mmol) and K_2CO_3 (102 mg, 0.738 mmol) in $CHCl_3$ (30 mL) was added a solution of mCPBA (about 80% purity, 128 mg, 0.59 mmol) in $CHCl_3$ (10 mL) over a period of 23 min at 0 °C, and the mixture was stirred for 3 min. The reaction was quenched by adding a $Na_2S_2O_3$ aqueous solution (0.4 M), and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator. The paste obtained was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) and further purified by preparative HPLC (ODS, 60:40 water/MeOH as an eluent). MeOH was removed on a rotary evaporator, and the aqueous solution was freeze-dried to afford **29** (16 mg, 20%) as a purple amorphous solid.

6-Demethyl-6-[(diethylamino)methyl]mitomycin C (31). To a solution of **12v** (25 mg, 0.056 mmol) in CH_2Cl_2 (10 mL) was added diethylamine (0.10 mL), and the reaction mixture was allowed to stand at room temperature for 48 h. The volatiles were removed on a rotary evaporator, and the obtained residue was purified by preparative TLC (silica gel, 4:1 $CHCl_3$/MeOH as a developing solvent). After trituration with $CHCl_3$–*n*-hexane and drying under vacuum, the product (17 mg, 77%) was obtained as a reddish purple powder: ^1H NMR (270 MHz, pyridine-d_5) δ 1.25 (t, J = 7.2 Hz, 6 H, ethyl), 2.23 (s, 1 H, 1a-H), 2.80 (dd, J = 1.8, 4.4 Hz, 1 H, 2-H), 3.0–3.2 (m, 5 H, ethyl + 1-H), 3.23 (s, 3 H, 9a-OCH_3), 3.63 (dd, J = 1.8, 12.8 Hz, 1 H, 3α-H), 4.01 (dd, J = 4.3, 11.1 Hz, 1 H, 9-H), 4.17 (s, 2 H, 6-CH_2), 4.52 (d, J = 12.8 Hz, 1 H, 3β-H), 5.01 (br t, J = 11 Hz, 1 H, 10-H_a), 5.34 (dd, J = 4.3, 10.4 Hz, 1 H, 10-H_b), 7.4–7.7 (br, 2 H, 10-$OCONH_2$), 8.8–9.1 (br, 1 H, 7-NH_2), 9.6–10.4 (br, 1 H, 7-NH_2); FAB-MS m/z 406 (M^+ + 1); FAB-HRMS calcd for $C_{19}H_{28}N_5O_5$ (M^+ + H) m/z 406.2091, found 406.2093; IR (KBr) 3430, 3350, 3280, 2970, 2930, 2850, 1720, 1710, 1600, 1570, 1560, 1450, 1340, 1070 cm^{-1}.

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Supplementary Material Available: Complete analytical data (^1H NMR, IR, mass spectrum, elemental analysis) for new compounds except **10i**, **12i**, and **31** and ^1H NMR spectra (65 pages). Ordering information is given on any current masthead page.

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Chem. **1982**, *25*, 730–735. (b) Lin, T.-S.; Antonini, I.; Cosby, L. A.; Sartorelli, A. C. 2,3-Dimethyl-1,4-Naphthoquinones as Bioreductive Alkylating Agents with Cross-Linking Potential. *J. Med. Chem.* **1984**, *27*, 813–815. (c) Driebergen, R. J.; den Hartigh, J.; Holthuis, J. J. M.; Hulshoff, A.; van Oort, W. J.; Postma-Kelder, S. J.; Verboom, W.; Reinhoudt, D. N.; Bos, M.; van der Linden, W. E. Electrochemistry of Potentially Bioreductive Alkylating Quinones. Part 1. Electrochemical Properties of Relatively Simple Quinones, as Model Compounds of Mitomycin- and Aziridinylquinone-Type Antitumor Agents. *Anal. Chim. Acta* **1990**, *233*, 251–268. Addendum of an alkylation site into the mitomycin skeleton was already tried by the synthesis of 7-aziridino-7-deamino mitomycins and 3α-alkoxy mitomycins: (d) Miyamura, S.; Shigeno, N.; Matsui, M.; Wakaki, S.; Uzu, K. The Biological Studies on Mitomycin Derivatives. *J. Antibiot.* **1967**, *20*, 72–76. (e) Kasai, M.; Kono, M.; Shirahata, K. The Derivation of a Novel Mitomycin Skeleton: 3α-Alkoxymitomycin. *J. Antibiot.* **1991**, *44*, 301–308.

(6) The mitomycin skeletons are unstable under acidic, strong basic, and reductive conditions; see: Beijnen, J. H.; Lingeman, H.; Van Munster, H. A.; Underberg, J. M. Mitomycin Antitumor Agents: A Review of Their Physico-Chemical and Analytical Properties and Stability. *J. Pharm. Biomed. Anal.* **1986**, *4*, 275–295. See, also, ref 1. Derivatization at the C-6-methyl position is reported only in one case except our studies; see: Takayanagi, H.; Oshima, J.; Furuhata, K.; Takeda, K.; Ogura, H.; Komiyama, K.; Hata, T. One Step Preparation of 6-Demethyl-6-formylmitomycin C and Cytotoxicity Activity of Its Derivatives. *J. Antibiot.* **1992**, *45*, 1815–1817.

(7) Kanda, Y.; Kasai, M. First Preparation of Mitomycins Specifically Labeled with Deuterium at the C^6-methyl Position. *J. Org. Chem.* **1990**, *55*, 2515–2518.

(8) Arai, H.; Kanda, Y.; Kasai, M. First Synthesis of Mitomycins Completely Labeled at the C-6-Methyl by $^{13}CH_3$ and CD_3. *J. Org. Chem.* **1993**, *58*, 892–897.

(9) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. Specific Removal of *O*-Methoxybenzyl Protection by DDQ Oxidation. *Tetrahedron Lett.* **1982**, *23*, 885–888.

(10) When 6-demethyl-6-[[(3,4-dimethoxybenzyl)oxy]methyl]mitomycin C was oxidized using DDQ, 6-demethyl-6-formylmitomycin C, which was reported in ref 6, was obtained exclusively.

(11) In the case of the conversion of **10r,ac** to **12r,ac**, respectively, it is difficult to suppress the decomposition of the substrate even by use of method C. It seems to be due to the increase of the elimination efficiency by the electron-withdrawing effect (for **10r**) or steric hindrance (for **12ac**).

(12) Molecular formulas of the representative fragment peaks of **12v** were confirmed by FAB-HRMS. *m/z* 445 [$(M + H)^+$]: calculated for $C_{19}H_{21}N_6O_8S$ *m/z* 445.1270, found 445.1281. *m/z* 383 [[(M − $HOCONH_2$ − H) + H]$^+$]: calculated for $C_{18}H_{17}N_5O_3S$ *m/z* 383.1087, found 383.1069. *m/z* 352 [[(M − $HOCONH_2$ − CH_3OH) + H]$^+$]: calculated for $C_{17}H_{14}N_5O_2S$ *m/z* 352.0897, found 352.0882. *m/z* 333 [[(M − RSH) + H]$^+$]: calculated for $C_{15}H_{17}N_4O_5$ *m/z* 333.1243, found 333.1221. *m/z* 272 [[(M − RSH − $HOCONH_2$) + H]$^+$]: calculated for $C_{14}H_{14}N_3O_3$ *m/z* 272.0973, found 272.1004. *m/z* 240 [[(M − RSH − $HOCONH_2$ − CH_3OH) + H]$^+$]: calculated for $C_{13}H_{10}N_3O_2$ *m/z* 240.0757, found 240.0765.

(13) Fujii, N.; Arai, H.; Saito, H.; Kasai, M.; Nakano, H. Induction of Protein-DNA Complexes in HeLa S3 Cells by KW-2149, a New Derivative of Mitomycin C. *Cancer Res.* **1993**, *53*, 4466–4468.