multiwell dishes. After 24 h of culture, compounds **9** and **10** were added to the culture dishes according to the protocol described previously.[32] Estradiol was also added to evaluate its extent of antagonism of growth inhibition of compounds **9** and **10**. Final concentrations were as follows: estradiol, 10^{-8} M; compounds **9** and **10**, 10^{-8}, 10^{-7}, and 10^{-6} M. After 5 days of culture, the monolayer was fixed with 90% ethanol and colored with hematoxylin.[32] The intensity of the coloration giving a measure of the number of cells was determined with a multiscan spectrophotometer at 540 nm (Flow Laboratories Inc.).

Acknowledgment. This work was funded by grants from the Cancer Research Campaign and Medical Research Council (to R.M.) and from the Caisse Générale d'Epargne et de Retraite de Belgique (to G.L.). We thank Dr. M. Jarman for suggesting the method used to distinguish the enantiomers of **19**.

Registry No. (±)-**7**, 109640-20-2; (±)-**8**, 109640-21-3; (1*R*,2*S*)-**8a**, 109717-23-9; (1*S*,2*R*)-**8b**, 109717-24-0; (±)-**9**, 109669-15-0; (±)-**10**, 109669-16-1; (*E*)-**11**, 103628-15-5; (*Z*)-**12**, 103628-14-4; (±)-**13**, 109640-22-4; (±)-**14**, 109640-23-5; (*R*)-(−)-**15a**, 938-79-4; (*R*)-(−)-**15a**·(*R*)-(+)-PhCH(Me)NH_2, 109640-25-7; (±)-**15b**, 7782-29-8; (*S*)-(+)-**15b**, 4286-15-1; (*S*)-(+)-**15b**·(*S*)-(−)-PhCH(Me)NH_2, 13491-02-6; (*R*)-(−)-**16a**, 109640-26-8; (*S*)-(+)-**16b**, 109640-27-9; (1*S*,2*R*)-(+)-**17a**, 109640-28-0; (1*R*,2*S*)-(−)-**17b**, 109640-29-1; (1*R*,2*S*)-(−)-**18a**, 109640-30-4; (1*S*,2*S*)-**18a**, 109640-31-5; (1*S*,2*R*)-**18b**, 109640-32-6; (1*R*,2*R*)-**18b**, 109640-33-7; (1*R*,2*S*[)-(+)-**19a**, 109640-36-0; (1*S*,2*R*)-(−)-**19b**, 109640-37-1; **20a**, 109640-38-2; (1*R*,2*S*)-EtCH(Ph)CH(Ph)C_6H_4OH-*p*, 109640-34-8; (1*S*,2*S*)-EtCH(Pg)CH(Ph)C_6H_4OH-*p*, 109640-35-9; (*Z*)-tamoxifen, 10540-29-1; (*E*)-tanoxifen, 13002-65-8; (*R**,*R**)-(±)-1-(4-(2-dimethylaminoethoxy)phenyl)-1-(4-(2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy)phenyl)-2-phenylbutane, 109640-24-6.

(32) Madeddu, L.; Roy, F.; Leclercq, G. *Anticancer Res.* **1986**, *6*, 11.

Synthesis and Antineoplastic Activity of 1a-Formyl and 1a-Thioformyl Derivatives of Mitomycin C and 2-Methylaziridine

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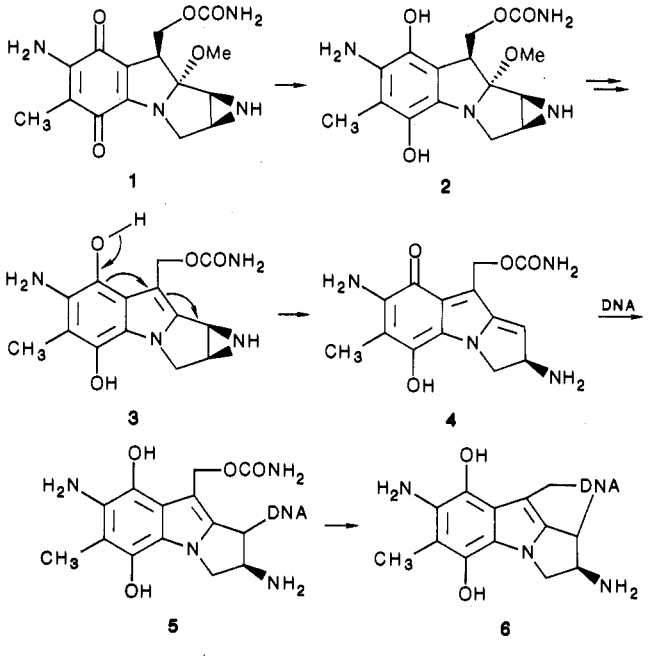
A select number of 1-formyl- and 1-thioformyl-2-methylaziridine derivatives and the corresponding 1a-substituted mitomycin C analogues were synthesized and tested for antineoplastic activity by using an in vivo test with murine P388 leukemia. Select compounds were also tested in vivo with murine melanoma B16. Several of the mitomycin C derivatives displayed activity and some of the mitomycin C analogues were comparable in activity to the parent compound.

Mitomycin C (**1**) is a clinically used anticancer drug.[1] It acts as a prodrug that requires initial reductive activation in a cancer growth.[2] The antitumor agent is believed to express its action by mono- and dialkylating (cross-linking) DNA.[2] It has been suggested that the reaction with the genetic material involves quinone methide **4**.[2] This species then undergoes attack by DNA at carbon 1 to give the monoalkylated intermediate **5** followed by an intramolecular attack at carbon 10 to give the bisalkylated product **6** as shown in Scheme I.[2]

The clinical use of mitomycin C is limited by its toxicity.[3] An explanation for the toxicity involves the aziridine ring.[4] Aziridines undergo acid-catalyzed ring opening.[5] Mitomycin C is known to undergo ring opening in acid media to generate a reactive carbon 1 cationic species.[6] A comparable intermediate might form in vivo before reaching a cancer cell and serve as a potent electrophile for other biomolecules, thus being toxic. la-Acyl- and la-sulfonylmitomycin C derivatives have been shown to be less toxic than the parent compound **1**.[7] The presence of electron-withdrawing groups at the la-position is expected to decrease the basicity of the aziridine nitrogen atom, and thereby diminish the possibility of an acid-catalyzed ring

Scheme I



(1) Crooke, S. T. In *Mitomycin C. Current Status and New Developments*; Carter, S. K., Crooke, S. T., Eds.; Academic: New York, 1979; pp 1–2.
(2) Moore, H. W.; Czerniak, R. L. *Med. Res. Rev.* **1981**, *1*, 249–280.
(3) Bradner, W. T. In *Mitomycin C. Current Status and New Developments*; Carter, S. K., Crooke, S. T., Eds.; Academic: New York, 1979; p 33.
(4) Remers, W. A. In *Mitomycin C. Current Status and New Developments*; Carter, S. K., Crooke, S. T., Eds.; Academic: New York, 1979; p 28.
(5) Dermer, O. C.; Ham, G. E. *Ethylenimine and Other Aziridines*; Academic: New York, 1969; p 107.
(6) McClelland, R. A.; Lam, K. *J. Am. Chem. Soc.* **1985**, *107*, 5182–5186.
(7) Masanao, M.; Yamamoto, I.; Wakakai, S.; Soeda, Y. Jp Patent 13760, 1965; *Chem. Abstr.* **1965**, *63*, 11508*g*. Kyowa Fermentation Industry Co., Ltd. Neth. Patent 6412941, 1966; *Chem. Abstr.* **1966**, *65*, 13712*h*. Kyowa Fermentation Industry Co., Ltd. Fr. Patent 1437402, 1966; *Chem. Abstr.* **1966**, *65*, 20128*e*.

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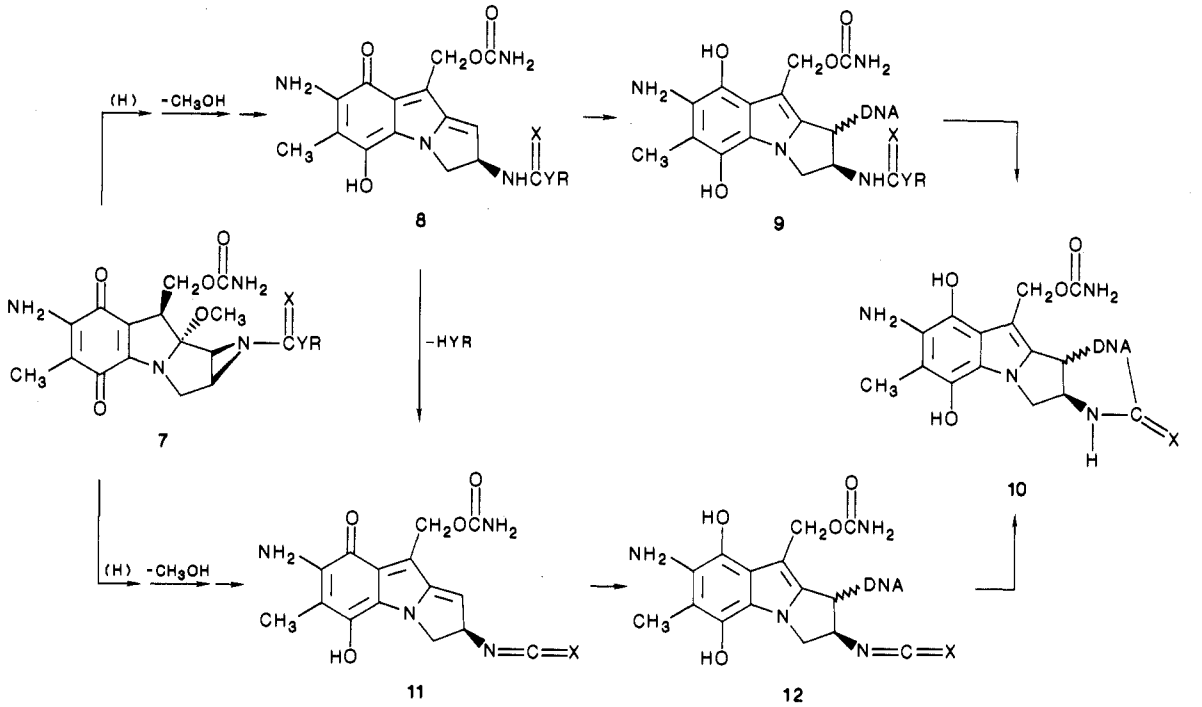
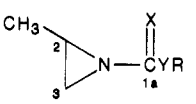
Scheme II[a]



[a] X = O, S; Y = O, NH, S; R = Ph, CH_3.

Table I. Characteristic Physical and Spectral Data for Aziridines 15–21 and 23



							^{1}H NMR[b]			^{13}C NMR[b]		
no.	X	Y	R	% yield	mp or bp, °C	IR[a]	2	3α	3β	1a	2	3
15[c]	O	O	Ph	63	*d*	1745	2.45–2.75 (m)	2.46 (d, 5.8)	2.07 (d, 3.7)	161.18	34.12	32.65
16[c]	O	O	Me	85	107 (160 Torr)	1730	2.36–2.63 (m)	2.31 (d, 5.7)	1.93 (d, 3.7)	163.82	33.55	32.36
17[c]	O	NH	Ph	95	62.5–65.0	1715	2.36–2.63 (m)	2.36–2.63 (m)	1.91 (d, 3.7)	162.77	34.79	33.04
18[e]	O	NH	Me	80	45 (0.03 Torr)[f]	1680	2.25–2.50 (m)	2.25–2.50 (m)	1.80 (d, 3.8)	165.79	33.79	32.56
19[c]	O	S	Ph	89	*d*	1700	2.47–2.63 (m)	2.47 (d, 6.1)	2.02 (d, 3.6)	179.36	35.80	34.01
20[c]	O	S	Me	63	70 (20 Torr)	1685	2.37–2.75 (m)	2.37–2.75 (m)	2.05 (d, 3.4)	181.93	35.31	33.75
21[c]	S	O	Ph	80	*d*	1160, 1185	2.79–2.93 (m)	2.72 (d, 6.2)	2.40 (d, 4.4)	203.15	39.51	37.69
22[g]	S	O	Me									
23[c]	S	NH	Ph	39	73.0–74.5	1180	2.54–2.62 (m)	2.54–2.62 (m)	2.21 (d, 4.0)	195.34	40.33	37.92
24[g]	S	NH	Me									
25[g]	S	S	Ph									
26[g]	S	S	Me									

[a] Value is in cm^{-1} and was obtained in CCl_4. [b] Value in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant in hertz when appropriate. [c] Satisfactory C, H, N analysis was secured for this compound. [d] Obtained as a nonvolatile oil. [e] Satisfactory high-resolution mass spectral analysis obtained for this adduct. [f] Oven temperature and pressure of Kugelrohr distillation. [g] Compound not isolated; see text.

opening of 1 prior to reaching the cancer growth. Unfortunately, these derivatives were also less potent than 1.[8] Thus, substituents on the aziridine nitrogen of mitomycin C that decrease the toxicity of the drug candidate while still maintaining a high degree of potency are desirable.

By use of the appropriate substituent on the 1a-position of mitomycin C, a third potential alkylation site may be revealed. The mechanism by which this could occur is illustrated in Scheme II for mitomycin C analogues **7**. The unravelling of the aziridine ring is expected to lead to either a carbamate-type derivative **8** or directly to the isocyanate-type analogue **11**. Both pathways provide an additional potential alkylation site. Ring opening should be facilitated in these cases since the aziridine is considered to be "activated".[9] The presence of three different DNA binding sites may prove beneficial. The additional alkylation site is four atoms away from the next nearest binding site. This 1,4 relationship has been found to be important for the interstrand cross-linking of DNA by dimesylates[10] and diepoxides.[11] Suggestions have been made that the cross-linked adduct for mitomycin C and DNA leads to the cytotoxic event.[12]

(8) Usubuchi, I.; Sorbajima, Y.; Hongo, T.; Kawaguchi, T.; Sugawara, M.; Matsui, M.; Wakaki, S.; Uzu, K. *Gann* **1967**, *58*, 307–313.

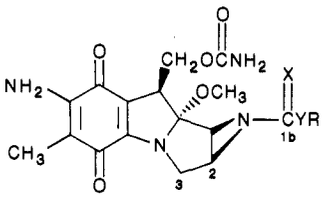
(9) Reference 5, pp 205–206.

(10) Remers, W. A. In *Antineoplastic Agents*; Remers, W. A., Ed.; Wiley: New York, 1984; p 106.

(11) Lawley, P. D. In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; Vol. 1, p 460.

(12) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355–362.

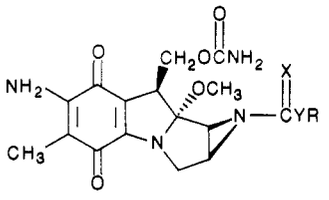
Table II. Characteristic Physical and Spectral Data for Mitomycin C Derivatives **30–36** and **38–41**



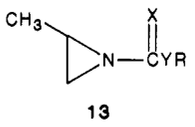
							^{1}H NMR[c]		^{13}C NMR[c]		
no.	X	Y	R	% yield	mp,[a] °C	IR[b]	1	2	1b	1	2
30	O	O	Ph	89	232–236	1750[d]	3.85 (d, 4.7)[e]	3.67–3.72 (m)	159.96[e]	43.19	41.34
31	O	O	Me	88	118–122	1735[d]	3.42 (d, 4.5)[f]	3.30 (dd, 4.5, 1.9)	161.48[f]	42.08	39.92
32	O	NH	Ph	92	200–202	1710[g]	4.02–4.07 (m)[h]	3.48–3.60 (m)	160.96[h]	43.41	42.80
33	O	NH	Me	86	144–150	1680[g]	3.40 (d, 4.8)[i]	3.21 (dd, 4.8, 1.7)	163.63[i]	43.48	41.66
34	O	S	Ph	95	106–108	1700[d]	3.38–3.47 (m)[f]	3.65–3.71 (m)	178.18[f]	42.81	42.17
35[j]	O	S	Me	94	110–115	1680[d]	3.63 (d, 4.5)[f]	3.39 (d, 4.5)	179.94[f]	42.06	41.87
36	S	O	Ph	85	150–152	1150, 1185[d]	3.79 (d, 4.5)[f]	3.58–3.63 (m)	201.06[f]	42.04	42.04
37[k]	S	O	Me								
38	S	NH	Ph	73	210	1150[g]	3.90 (d, 4.2)	3.20 (br s)	193.0[l]	47.35	42.5[l]
39	S	NH	Me	55	170–172	1165[d]	3.71 (d, 4.7)[i]	3.38 (dd, 4.7, 1.6)	195.05[i]	43.33	42.27
40	S	S	Ph	88	135	1065[d]	3.95 (d, 4.5)[f]	3.28 (dd, 4.5, 0.9)	216.52[f]	48.34	41.98
41	S	S	Me	95	135	1055[d]	3.83 (d, 4.6)[f]	3.48 (d, 4.6)	218.13[f]	47.94	42.01

[a] All the adducts decomposed at the specified temperatures. [b] Values reported are in cm^{-1}. [c] Value in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coulpling constant in hertz when appropriate. [d] Spectrum obtained in $CHCl_3$. [e] Spectrum obtained in acetone-d_6. [f] Spectrum obtained in $CDCl_3$. [g] Spectrum obtained in KBr. [h] Spectrum taken in pyridine-d_5. [i] Spectrum taken in CD_3CN. [j] Satisfactory high-resolution mass spectral analysis obtained for this adduct. [k] Compound **37** not isolated; see text. [l] A low-intensity signal was obtained for this resonance.

In this paper, a novel approach for the development of new potent mitomycin C derivatives **7**, as well as the corresponding 1-methylaziridine analogues **13**, is described. The appropriate physical and spectral data as well as the biological data are also presented.



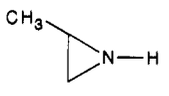
7



13

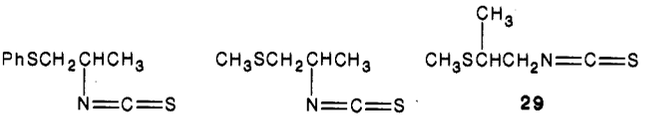
Chemistry

By considering the different combinations of X, Y, and R suggested in Scheme II there arises 12 different mitomycin C derivatives. As a model to explore the derivatization reactions for the aziridine ring in mitomycin C, 2-methylaziridine (**14**) was chosen due to its ready availability. The model derivatives were prepared by



14

using the appropriate chloroformate in the presence of Et_3N (**15**, **16**, **19**, **20**), isocyanate (**17**, **18**), chlorothionoformate in the presence of Et_3N (**21**), or isothiocyanate (**23**). The yields ranged from 39% (**23**) to 95% (**17**) with an average of 80% (Table I). Compound **22** was not detected in the reaction between **14** and methyl chlorothionoformate. A myraid of compounds were formed as determined by TLC. The inability to isolate **22** has been attributed by us to the instability of the chlorothionoformate[13] under the reaction conditions. Evidence (^{1}H NMR) has been secured that aziridine **24** was produced from the reaction of **14** with methyl isothiocyanate. However, after immediate purification by column chromatography, the clear oil obtained rapidly converted to an opaque white viscous oil either in solution (hexane, EtOAc, Et_2O, or CH_2Cl_2) or in neat phase (0–25 °C). ^{1}H NMR spectroscopy of the opaque oil revealed a number of new unidentified products and TLC indicated at least 50% decomposition to a polymeric material. A comparable result has been observed with 1-(aziridine)thiocarbonyl chlorides which isomerize at room temperature to 2-chloroalkyl isothiocyanates and unidentified polymers.[14] The reaction between **14** and phenyl chlorodithioformate did not lead to any detectable amounts of the desired aziridine **25** but rather to the isothiocyanate **27**. This type of rearrangement has been reported for **19**,[15] but it is apparently facile enough for **25** under a variety of reaction conditions that it proceeds in situ. The rearrangement also appears to have occurred in the reaction of **14** with methyl chlorodithioformate. Both IR and ^{1}H NMR spectroscopy indicate that **28** and **29** were produced along with several other adducts. A comparable observation has been seen in the rearrangements of the 1-(aziridine)thiocarbonyl chlorides.[14]



27 **28** **29**

Characteristic physical and spectral data for aziridines **15–21** and **23** are presented in Table I. The infrared spectra display the diagnostic *N*-acyl absorptions and the decrease in the magnitude of the carbonyl stretch (X = O) as the Y group changes from oxygen to nitrogen to sulfur is expected.[16] In the ^{1}H NMR spectra for **15–21**

(13) Barany, G.; Schroll, A. L.; Mott, A. W.; Halsrud, D. A. *J. Org. Chem.* **1983**, *48*, 4750–4761.

(14) Tomalia, D. A. *J. Heterocycl. Chem.* **1966**, *3*, 384–386.

(15) Tomalia, D. A.; Sheetz, D. P.; Ham, G. E. *J. Org. Chem.* **1970**, *35*, 47–52.

(16) Pretsch, E.; Seibl, J.; Simon, W. *Strukuraufklarung organisher Verbindugen mit spektroskopischen Methoden*; Springer-Verlag: Heidelberg, 1981.

and **23** the resonances for the 2, 3α, and 3β protons were downfield from the corresponding resonances in the parent aziridine **14**. A similar deshielding effect was noted for the carbons 2 and 3 signals in the ^{13}C NMR spectra. In the carbonyl series (X = O) the average downfield shift for protons 2, 3α, and 3β was 0.65 ppm. A larger deshielding effect (0.90 ppm) was noted for the thiocarbonyl adducts (X = S). The ^{13}C NMR absorptions for the la carbons were consistent with expected values.[16]

The mitomycin C derivatives **7** were prepared similarly to the model analogues **13** (Table II). In general, mitomycin C (**1**) was less reactive than **14** toward acylation, necessitating the use of higher reaction temperatures in these transformations. For example, phenyl isothiocyanate reacted with mitomycin C (**1**) in a refluxing DME solution while with **14** at 0 °C in Et_2O. The lower reactivity of mitomycin C (**1**) vs. **14** is probably due, in part, to the adverse steric effects encountered in the transition state for the substitution process and the reduced basicity of the aziridine nitrogen in **1** vs. **14**.[17] Mitomycin C derivative **37** was not formed. Once again, this has been attributed to the instability of the chlorothionoformate. It is interesting to note that the mitomycin C derivatives **39–41** were synthesized in good yield (80% average) by the same method that had been employed for the attempted preparation of the corresponding aziridines **24–26**.

Key physical and spectral data for the mitomycin C derivatives are listed in Table II. The infrared spectral data recorded for the acyl group in compounds **30–36** and **38–41** were in agreement with the absorbances observed for aziridines **15–21** and **23**. The trends observed in the ^1H and ^{13}C NMR spectra of the mitomycin C adducts **7** paralleled those noted in the simple aziridine compounds **13**.

Biological Activity

The 2-methylaziridine derivatives were tested in a number of in vitro assays[18] (see supplementary material available paragraph). None of the compounds exhibited significant activity and as a result few were selected for in vivo testing[19] (supplementary material). Moreover, under in vivo conditions, the 2-methylaziridine adducts **13** displayed significantly weaker antineoplastic activity than the corresponding mitomycin C derivatives.

The data for the in vivo tests with mitomycin C derivatives **30–36** and **38–41** against P388 leukemia as well as the results of the tests of select compounds with B16 melanoma are in Table III. In contrast to the simpler aziridines, several of the mitomycin C derivatives exhibited pronounced biological activity. The results of the tests for the mitomycin derivatives must be discussed first with respect to their efficacy and then with respect to their potency. In this series, compounds **35**, **36**, and **40** displayed the highest efficacy (optimum T/C %) in the P388 test when compared to the concurrently run mitomycin C controls. Of the remaining mitomycin C derivatives, **30**, **31**, and **34** exhibited moderate activity, while **32**, **33**, **38**, **39**, and 41 did not display significant activity. We note that within the series of compounds where X is oxygen, activity generally increased as the basicity of the YR group[20] decreased. This trend does not appear for the compounds where X is sulfur. Compounds **30** (T/C % = 131, 1.6 mg/kg per dose), **31** (T/C % = 125, 3.2 mg/kg per dose), and **34** (T/C % = 150, 1.6 mg/kg per dose) have good potency.

Three adducts (**30**, **31**, and **34**) were chosen for evaluation with B16 melanoma (Table III). Their efficacy was comparable to that of mitomycin C, and **34** (T/C % = 144, 1.6 mg/kg per dose) displayed significant activity at low doses.

Table III. In Vivo Test Results for Mitomycin C Derivatives **30–36** and **38–41**[a]

	P388 screen[b]		B16 screen[c]	
no.	maximum[d] % T/C	MED[e]	maximum[d] % T/C	MED[f]
30	194 (25.6) [275 (4.8)]	1.6 [0.2]	165 (3.2) [147 (2.0)]	3.2 [2.0]
31	194 (25.6) [275 (4.8)]	3.2 [0.2]	165 (6.4) [147 (2.0)]	6.4 [2.0]
32	116 (25.6) [142 (4.8)]			
33	121 (25.6) [142 (4.8)]			
34	200 (12.8) [275 (4.8)]	1.6 [0.2]	156 (3.2) [147 (2.0)]	1.6 [2.0]
35	132 (12.8) [142 (4.8)]	12.8 [3.2]		
36	162 (51.2) [152 (3.2)]	51.2 [1.6]		
38	127 (25.6) [200 (4.8)]	25.6 [1.6]		
39	95 (3.2) [152 (3.2)]			
40	167 (51.2) [172 (4.8)]	12.8 [0.4]		
41	122 (51.2) [>333 (3.2)]			

[a] Tests conducted at Bristol-Myers Laboratories. [b] P388 lymphocytic leukemia screen: the tumor was ip as was the treatment. [c] Subcutaneous B16 melanoma test: the tumor was sc and the treatment administered iv. [d] The values reported are the median survival time for the test substrate at the optimal dose listed in parentheses. The corresponding values for the concurrently run mitomycin C samples appear in the brackets. % T/C = (median survival time of drug treated/median survival time of control) × 100. [e] The values reported are the minimum effective dose (% T/C ≥ 125) in mg/kg per dose observed for the substrate tested, followed by the corresponding values for mitomycin C in brackets. MED = minimum effective dose. [f] The values reported are the minimum effective dose (% T/C ≥ 140) in mg/kg per dose observed for the substrates tested, followed by the corresponding values for mitomycin C in brackets. MED = minimum effective dose.

Conclusions

Select 1a-formyl- and 1a-thioformylmitomycin C derivatives **7** have been found to have comparable efficacies to mitomycin C (**1**) in both P388 and B16 tests. Moreover, the potencies of several of these compounds were comparable to that observed for mitomycin C in the subcutaneous B16 melanoma test. These results are in contrast to the low activity reported for the previously prepared mitomycin C derivatives with carbonyl or electron-withdrawing groups on the 1a-position.[8] The precise factors (protection of the aziridine toward acid-catalyzed hydrolysis, activation of the aziridine for ring opening, or unmasking a new alkylation site) that contribute to the enhanced activity of these adducts have not been ascertained. Evaluation of the toxicity of select compounds is under review.

(17) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Uzu, K. *J. Med. Chem.* **1964**, *8*, 1–10.
(18) For in vitro testing procedures, see: Catino, J. J.; Francher, D. M.; Edinger, K. J.; Stringfellow, D. A. *Cancer Chemother. Pharmacol.* **1985**, *15*, 240–243.
(19) For in vivo testing procedures, see: Bradner, W. T.; Rose, W. C.; Schurig, J. E.; Florczyk, A. P.; Huftalen, J. B.; Catino, J. J. *Cancer Res.* **1985**, *45*, 6475–6481.
(20) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; Harper and Row: New York, 1976; pp 149–150.

Experimental Section

General Procedures. All reagents and solvents were purified and dried according to the prescribed methods[21] unless otherwise stated. Specifically, Et_2O was distilled under an atmosphere of N_2 from Na/benzophenone, DME from $LiAlH_4$, 1,4-dioxane from Na/benzophenone, and Et_3N from CaH_2. All reactions were carried out in flame-dried glassware under an atmosphere of N_2 that had been dried by passage through a column of NaOH and Drierite. Concentration refers to rotary evaporation under reduced pressure.

Thin-layer chromatography was performed with 250-μm plates from Analtech Co. coated with silica gel GHLF. The reported R_f values are not standardized. Flash column chromatography used EM Super-60 silica gel supplied by E. Merck Co. and was 230–400-mesh size. Gravity column chromatography was done with EM 60 silica gel supplied by E. Merck and was 70–230-mesh size. The eluting solvents were of commercial grade and are reported as volume/volume. Melting points were obtained on either a Thomas-Hoover capillary or a Fisher-Johns melting point apparatus and are corrected unless otherwise stated.

IR spectra were obtained on a Perkin-Elmer 1330 spectrophotometer in the indicated solvent. The positions of the absorption bands are expressed in cm^{-1}. ^1H NMR data were determined on a Varian FT-80A or Nicolet NT300 spectrometer. ^{13}C NMR data were obtained on a Nicolet NT300 spectrometer. The NMR data were taken in the indicated solvent and are relative to the internal tetramethylsilane standard as values in parts per million. Coupling constants (J, hertz) are apparent and are reported as the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, complex multiplet; br, broad. The mass spectrometry studies were conducted by Dr. John Chinn at the Department of Chemistry, University of Texas at Austin. Low-resolution mass spectral data were obtained on a Bell and Howell 21-491 mass spectrometer. CH_4 was used as the ionizing gas for all spectra obtained under CI conditions. High-resolution mass spectral data were obtained on a CEC21-110B double-focusing magnetic-sector spectrometer. Of the 11 mitomycin C derivatives prepared, only compound **35** gave a discernible parent ion in the high-resolution mass spectrum.

Microanalyses were obtained from Spang Microanalysis Laboratory, Eagle Harbor, MI.

General Procedure for 2-Methylaziridine Derivatives Using a Chloroformate or Chlorothionoformate. To a solution of 310 μL (250 mg, 4.38 mmol) of **14** (Fluka Corp., 98%, stabilized with NaOH) and 609 μL (443 mg, 4.38 mmol) of Et_3N in Et_2O (45 mL) was added 1.1 equiv of the chloroformate or chlorothionoformate unless otherwise indicated. This was then heated to just below reflux (about 32 °C) for 1 h. The reaction mixture was then filtered through a pad of Celite to remove most of the Et_3NHCl, concentrated, and purified by the indicated method.

Phenyl 2-Methyl-1-aziridinecarboxylate (15). Using 604 μL (754 mg, 4.82 mmol) of phenyl chloroformate (Aldrich Co., 97%) gave 730 mg of a colorless oil, which was purified by gravity column chromatography (98/2 $CHCl_3$/MeOH) to give 450 mg (63%) of **15** as a colorless oil: R_f 0.73 (98/2 $CHCl_3$/MeOH); IR (CCl_4) 3070, 1745, 1600, 1495, 1305, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38–6.88 (m, 5), 2.75–2.45 (m, 1), 2.46 (d, J = 5.8, 1), 2.07 (d, J = 3.7, 1), 1.36 (d, J = 5.4, 3); ^{13}C NMR ($CDCl_3$) 161.18, 150.90, 129.09 (2 C), 125.39, 121.04 (2 C), 34.12, 32.65, 16.98 ppm; MS, m/e (relative intensity) (EI) 177 (M^+, 4), 121 (17), 94 (100), 84 (11), 77 (16), 65 (19), 56 (66); MS, m/e (relative intensity) (CI) 178 (M + 1, 100), 135 (18), 123 (9), 95 (66), 84 (90). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

Methyl 2-Methyl-1-aziridinecarboxylate (16). Making use of 1.5 equiv (508 μL, 621 mg, 6.57 mmol) of methyl chloroformate gave 457 mg of an orange liquid. This was distilled to give 429 mg (85%) of **16** as a colorless liquid: bp 107 °C (160 Torr); IR (CCl_4) 1730, 1440, 1305, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.71 (s, 3), 2.63–2.36 (m, 1), 2.31 (d, J = 5.7, 1), 1.93 (d, J = 3.7, 1), 1.29 (d, J = 5.3, 3); ^{13}C NMR ($CDCl_3$) 163.82, 53.21, 33.55, 32.36, 17.22 ppm; MS, m/e (relative intensity) (EI) 115 (M^+, 4), 100 (3), 84 (7), 59 (9), 56 (100); MS, m/e (relative intensity) (CI) 116 (M + 1, 100), 84 (16), 72 (27). Anal. ($C_5H_9NO_2$) C, H, N.

S-Phenyl 2-Methyl-1-aziridinecarbothioate (19). Employing 662 μL (851 mg, 4.82 mmol) of phenyl chlorothioloformate (Alfa Co.) gave 907 mg of an orange liquid. This was purified by flash column chromatography (9/1 hexane/EtOAc) to give 757 mg (89%) of **19** as a light yellow liquid (lit.[22] mp 28–29 °C): R_f 0.60 (7/3 hexane/EtOAc); IR (CCl_4) 3070, 1700, 1415, 1270, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50–7.18 (m, 5), 2.63–2.47 (m, 1), 2.47 (d, J = 6.1, 1), 2.02 (d, J = 3.6, 1), 1.24 (d, J = 5.4, 3); ^{13}C NMR ($CDCl_3$) 179.36, 134.70, 129.20 (2 C), 129.03 (2 C), 128.05, 35.80, 34.01, 17.32 ppm; MS, m/e (relative intensity) (EI) 193 (M^+, 27), 137 (100), 123 (31), 109 (30), 56 (34); MS, m/e (relative intensity) (CI) 194 (M + 1, 100), 151 (39), 111 (37), 84 (42). Anal. ($C_{10}H_{11}NOS$) C, H, N.

S-Methyl 2-Methyl-1-aziridinecarbothioate (20). Using 417 μL (533 mg, 4.82 mmol) of methyl chlorothioloformate (Alfa) gave 500 mg of a light yellow liquid. This was distilled to yield 363 mg (63%) of **20** as a colorless liquid, bp 70 °C (20 Torr), which is a potent *lachrymator*: R_f 0.52 (7/3 hexane/EtOAc); IR (CCl_4) 1685, 1410, 1270, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.75–2.37 (m, 2), 2.32 (s, 3), 2.05 (d, J = 3.4, 1), 1.32 (d, J = 5.3, 3); ^{13}C NMR ($CDCl_3$) 181.93, 35.31, 33.75, 17.52, 12.83 ppm; MS, m/e (relative intensity) (EI) 131 (M^+, 4), 84 (33), 75 (34), 56 (100), 41 (38); MS; m/e (relative intensity) (CI) 132 (89), 102 (100), 89 (97). Anal. (C_5H_9NOS) C, H, N.

O-Phenyl 2-Methyl-1-aziridinecarbothioate (21). Employing 666 μL (832 mg, 4.82 mmol) of phenyl thionochloroformate (Aldrich, 97%) gave 806 mg of a light yellow oil which was purified by flash column chromatography (9/1 hexane/EtOAc) to give 677 mg (80%) of **21** as a light yellow oil: R_f 0.52 (7/3 hexane/EtOAc); IR (CCl_4) 3010, 2935, 1605, 1350, 1200, 1185, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (d of d, J = 7.5, J' = 7.5, 2), 7.26 (t, J = 7.5, 1), 7.07 (d, J = 7.5, 2), 2.93–2.79 (m, 1), 2.72 (d, J = 6.2, 1), 2.40 (d, J = 4.4, 1), 1.44 (d, J = 5.5, 3); ^{13}C NMR ($CDCl_3$) 203.15, 154.05, 129.47, 126.34, 122.16, 39.51, 37.69, 17.12 ppm; MS, m/e (relative intensity) (EI) 193 (M^+, 10), 137 (26), 121 (32), 109 (40), 94 (64), 84 (98), 77 (100); MS, m/e (relative intensity) (CI) 194 (M + 1, 30), 157 (43), 135 (100), 100 (59). Anal. ($C_{10}H_{11}NOS$) C, H, N.

2-Methyl-N-phenyl-1-aziridinecarboxamide (17). To 310 μL (250 mg, 4.38 mmol) of **14** (Fluka, 98%, stabilized with NaOH) in Et_2O (45 mL) was added 524 μL (574 mg, 4.82 mmol) of phenyl isocyanate. The solution was then heated to just below reflux (about 32 °C) for 1 h. The reaction solution was then concentrated to give 830 mg of a colorless oil which was purified by flash column chromatography (65/35 hexane/EtOAc) to give 740 mg (95%) of **17** as a white amorphous solid: mp 62.5–65.0 °C (uncorrected); R_f 0.26 (7/3 hexane/EtOAc); IR (CCl_4) 3310, 3040, 1715, 1610, 1590, 1515, 1440, 1315 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (br s, 1), 7.45–6.80 (m, 5), 2.63–2.36 (m, 2), 1.91 (d, J = 3.7, 1), 1.26 (d, J = 5.1, 3); ^{13}C NMR ($CDCl_3$) 162.77, 138.32, 128.87 (2 C), 123.57, 119.23 (2 C), 34.79, 33.04, 17.41 ppm; MS, m/e (relative intensity) (EI) 176 (M^+, 54), 161 (10), 147 (22), 134 (42), 119 (88), 106 (30), 93 (100), 84 (15), 77 (40), 65 (35), 56 (57); MS, m/e (relative intensity) (CI) 177 (M + 1, 100). Anal. ($C_{10}H_{12}N_2O$), C, H, N.

N,2-Dimethyl-1-aziridinecarboxamide (18). To 310 μL (250 mg, 4.38 mmol) of **14** (Fluka, 98%, stabilized with NaOH) in Et_2O (45 mL) was added 390 μL (380 mg, 6.57 mmol) of methyl isocyanate (Aldrich). The solution was then heated to just below reflux (about 32 °C) for 1 h. The solution was then concentrated to give 550 mg of a colorless oil. This was purified by Kugelrohr distillation (oven temperature 45 °C, 0.030 Torr) to give 400 mg (80%) of **18** as a colorless oil: R_f 0.35 (97.5/2.5 $CHCl_3$/MeOH); IR (CCl_4) 3310, 1680, 1510, 1295 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.32 (br s, 1), 2.78 (d, J = 4.9, 3), 2.50–2.25 (m, 2), 1.80 (d, J = 3.8, 1), 1.25 (d, J = 5.2, 3); ^{13}C NMR ($CDCl_3$) 165.79, 33.75, 32.56, 27.16, 17.48 ppm; MS, m/e (relative intensity) (EI) 114 (M^+, 3), 84 (9), 70 (4), 57 (100), 42 (42); MS, m/e (relative intensity) (CI) 115 (M + 1, 100); high-resolution MS calcd for $C_5H_{10}N_2O$ m/e 114.0793, found 114.0795.

2-Methyl-N-phenyl-1-aziridinecarbothioamide (23). To 310 μL (250 mg, 4.38 mmol) of **14** (Fluka, 98%, stabilized with

(21) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: New York, 1980.

(22) Pretsch, E.; Seibl, J.; Simon, W. *Strukuraufklarung organisher Verbindugen mit spektroskopischen Methoden*; Springer-Verlag: Heidelberg, 1981.

NaOH) in 5.7 mL of Et_2O at 0 °C was added 524 μL (592 mg, 4.38 mmol) of phenyl isothiocyanate. The reaction mixture was stirred 90 min at 0 °C during which time a white precipitate formed. The precipitate was collected by vacuum filtration to give 466 mg of a white powder. This was triturated twice with very cold Et_2O (2 × 2.8 mL) to give 324 mg (39%) of **23** as an analytically pure white powder: mp 73.0–74.5 °C; R_f 0.38 (7/3 hexane/EtOAc); IR (CCl_4) 3400, 3200, 2990, 1605, 1535, 1500, 1415, 1380, 1180, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.26 (br s, 1), 7.35–7.20 (m, 5), 2.62–2.54 (m, 2), 2.21 (d, J = 4.0, 1), 1.10 (d, J = 6.0, 3); ^{13}C NMR ($CDCl_3$) 195.34, 136.05, 129.17, 126.18, 123.62, 40.33, 37.92, 17.02 ppm; MS, m/e (relative intensity) (EI) 192 (M^+, 60), 177 (82), 118 (100), 104 (42), 77 (76); MS, m/e (relative intensity) (CI) 193 (M + 1, 100), 164 (16), 136 (53), 103 (18). Anal. ($C_{10}H_{12}N_2S$) C, H, N.

2-Isothiocyanato-1-(phenylthio)propane (27). To a solution of 340 μL (450 mg, 2.4 mmol) of phenyl chlorodithioformate[23] in 3.6 mL of Et_2O cooled in an ice/water bath was added 330 μL (240 mg, 2.4 mmol) of Et_3N in Et_2O (1.2 mL) over 10 min. After the addition was complete, the reaction mixture was left to stir 20 min. During this time, a small amount of a white precipitate formed. Aziridine 14 (170 μL, 140 mg, 2.4 mmol) was now added over 10 min. A voluminous white precipitate formed immediately. The cold bath was removed once the addition was complete and the reaction mixture left to stir for 30 min. The mixture was then filtered through a pad of Celite and concentrated to leave 410 mg of a bright orange liquid. This was purified by flash column chromatography (92.5/7.5 hexane/EtOAc) to give 260 mg (52%) of **27** as an orange liquid: R_f 0.63 (7/3 hexane/EtOAc); IR (CCl_4) 3075, 2060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53–7.20 (m, 5), 3.86–3.80 (m, 1), 3.14–2.97 (m, 2), 1.39 (d, J = 6.5, 3); ^{13}C NMR ($CDCl_3$) 134.52, 130.83 (3 C), 129.56 (2 C), 127.21, 53.30, 41.63, 21.01 ppm; MS, m/e (relative intensity) (EI) 209 (M^+, 21), 123 (100), 109 (33), 77 (20), 65 (23); MS, m/e (relative intensity) (CI) 151 (100). Anal. ($C_{10}H_{11}NS_2$) C, H, N.

General Procedure for the Preparation of 7-Amino-1a-formyl(or thioformyl)-9a-methoxymitosanes Using Chloroformates or Thionochloroformates. To a solution of 100 mg (299 μmol) of mitomycin C and 41.6 μL (30.3 mg, 299 μmol) of Et_3N in DME (50 mL) was added 1.1 equiv of the chloroformate or chlorothionoformate. This was then heated to 45 °C (1 h), vacuum filtered through a pad of Celite to remove most of the Et_3NHCl, concentrated, and purified by flash column chromatography.

7-Amino-9a-methoxy-1a-(phenoxycarbonyl)mitosane (30). Using 41.3 μL (51.5 mg, 329 μmol) of phenyl chloroformate gave 186 mg of a purple solid, which after purification (97.5/2.5 $CHCl_3$/MeOH) gave 122 mg (89%) of **30** as an amorphous purple solid: mp 232–236 °C dec; R_f 0.46 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3380, 1750, 1575, 1355 cm^{-1}; ^1H NMR ($(CD_3)_2CO$) δ 7.39–7.17 (m, 5), 6.38 (br s, 2), 5.85 (br s, 2), 4.99 (d of d, J = 10.9, J' = 4.7, 1), 4.49 (d, J = 13.5, 1), 4.24 (d of d, J = 10.9, J' = 10.9, 1), 3.85 (d, J = 4.7, 1), 3.72–3.67 (m, 2), 3.55 (d of d, J = 13.5, J' = 1.6, 1), 3.27 (s, 3), 1.79 (s, 3); ^{13}C NMR ($(CD_3)_2CO$) 178.64, 177.08, 159.96, 157.33, 155.28, 152.10, 148.89, 129.98 (2 C), 126.47, 122.25 (2 C), 111.37, 106.70, 104.92, 62.06, 49.95, 49.52, 45.01, 43.19, 41.34, 8.15 ppm; MS, m/e (relative intensity) (EI) 422 (0.1), 411 (0.3), 362 (0.2), 94 (100), 66 (71), 44 (70); MS, m/e (relative intensity) (CI) 455 (M + 1, 44), 422 (7), 412 (4), 362 (100), 95 (56).

7-Amino-9a-methoxy-1a-(methoxycarbonyl)mitosane (31). Employing 1.5 equiv (34.7 μL, 42.4 mg, 449 μmol) of methyl chloroformate gave 140 mg of a purple solid. Purification (95/5 $CHCl_3$/MeOH) yielded 103 mg (88%) of **31** as an amorphous purple solid: mp 118–122 °C dec; R_f 0.41 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3480, 1735, 1570, 1445, 1350, 1280 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.24 (br s, 2), 4.84 (d of d, J = 9.6, J' = 4.8, 1), 4.75 (br s, 2), 4.44 (d, J = 13.2, 1), 4.30 (d of d, J = 9.6, J' = 9.6, 1), 3.70 (s, 3), 3.66 (d of d, J = 9.6, J' = 4.8, 1), 3.51 (d of d, J = 13.2, J' = 1.9, 1), 3.42 (d, J = 4.5, 1), 3.30 (d of d, J = 4.5, J' = 1.9, 1), 3.20 (s, 3), 1.77 (s, 3); ^{13}C NMR ($CDCl_3$) 178.22, 176.05, 161.48, 156.90, 154.56, 147.51, 110.36, 105.41, 104.04, 62.07, 53.94, 49.77, 48.76, 43.40, 42.08, 39.92, 7.93 ppm; MS, m/e (relative intensity) (EI) 360 (3), 349 (5), 300 (39), 269 (100), 241 (58), 91 (87), 43 (57); MS, m/e (relative intensity) (CI) 393 (M + 1, 18), 360 (6), 332 (30), 300 (100).

7-Amino-9a-methoxy-1a-[(phenylthio)carbonyl]mitosane (34). Making use of 45.2 μL (58.1 mg, 329 μmol) of phenyl chlorothioloformate (Alfa) gave 200 mg of a purple solid. Purification (95/5 $CHCl_3$/MeOH) yielded 135 mg (95%) of **34** as an amorphous purple solid: mp 106–108 °C dec; R_f 0.38 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3375, 1740, 1700, 1655, 1575, 1450, 1350, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45–7.28 (m, 5), 5.55 (br s, 2), 5.10 (br s, 2), 4.94 (d of d, J = 10.5, J' = 4.7, 1), 4.21 (d, J = 13.6, 1), 4.12 (d of d, J = 10.5, J' = 10.5, 1), 3.18 (s, 3), 3.71–3.65 (m, 2), 3.47–3.38 (m, 2), 1.78 (s, 3); ^{13}C NMR ($CDCl_3$) 178.27, 178.18, 176.10, 156.78, 154.30, 147.57, 134.95, 129.90, 129.39, 127.13, 110.56, 105.59, 105.04, 61.97, 49.81, 48.59, 44.51, 42.81, 42.17, 7.98 ppm; MS, m/e (relative intensity) (EI) 427 (0.1), 317 (3), 218 (23), 110 (100), 84 (52), 77 (38), 66 (62); MS, m/e (relative intensity) (CI) 471 (M + 1, 4), 219 (30), 111 (100).

7-Amino-9a-methoxy-1a-[(methylthio)carbonyl]mitosane (35). Using 28.5 μL (36.4 mg, 329 μmol) of methyl chlorothioloformate (Alfa) furnished 184 mg of a purple solid, which after purification (95/5 $CHCl_3$/MeOH) gave 115 mg (94%) of **35** as an amorphous purple solid: mp 110–115 °C dec; R_f 0.52 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3390, 1740, 1680, 1660, 1610, 1575, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.75 (br s, 2), 5.39 (br s, 2), 4.92 (d of d, J = 10.5, J' = 4.8, 1), 4.48 (d, J = 12.9, 1), 4.13 (d of d, J = 10.5, J' = 10.5, 1), 3.67 (d of d, J = 10.5, J' = 4.8, 1), 3.63 (d, J = 4.5, 1), 3.54 (d, J = 12.9, 1), 3.39 (d, J = 4.5, 1), 3.20 (s, 3), 2.29 (s, 3), 1.78 (s, 3); ^{13}C NMR ($CDCl_3$) 179.95, 178.05, 175.99, 156.91, 154.31, 147.74, 110.23, 105.48, 104.85, 61.84, 49.69, 48.73, 44.13, 42.06, 41.87, 12.97, 7.90 ppm; MS, m/e (relative intensity) (EI) 376 (0.4), 365 (2), 47 (100); MS, m/e (relative intensity) (CI) 409 (M + 1, 71), 366 (59), 348 (26), 316 (100); high-resolution mass spectrum calcd for $C_{17}H_{20}N_4O_6S$ m/e 408.1103, found 408.1114.

7-Amino-9a-methoxy-1a-(phenoxythiocarbonyl)mitosane (36). Employing 45.5 μL (56.8 mg, 329 μmol) of phenyl thionochloroformate (Aldrich, 99%) produced 170 mg of a purple solid. Purification (95/5 $CHCl_3$/MeOH) left 120 mg (85%) of **36** as an amorphous purple solid: mp 150–152 °C dec; R_f 0.46 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 1730, 1605, 1565, 1335, 1185, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40–7.01 (m, 5), 5.27 (br s, 2), 4.93 (d of d, J = 10.5, J' = 4.7), 4.68 (br s, 2), 4.60 (d, J = 13.9, 1), 4.36 (d of d, J = 10.5, J' = 10.5, 1), 3.79 (d, J = 4.5, 1), 3.73 (d of d, J = 10.5, J' = 4.7, 1), 3.63–3.59 (m, 2), 3.24 (s, 3), 1.79 (s, 3); ^{13}C NMR ($CDCl_3$) 201.06, 178.43, 176.08, 156.40, 154.04, 153.73, 147.30, 129.55, 126.67, 121.96, 110.58, 105.84, 105.32, 62.17, 49.89, 48.72, 45.99, 42.04 (2 C), 8.05 ppm.

7-Amino-9a-methoxy-1a-[(phenylthio)thiocarbonyl]mitosane (40). To a solution of 100 mg (299 μmol) of **1** and 41.6 μL (30.3 mg, 299 μmol) of Et_3N in 50 mL of DME was added 46.6 μL (62.1 mg, 329 μmol) of phenyl chlorodithioformate.[23] The reaction solution was heated to 70 °C (3 h) and then vacuum filtered through a pad of Celite. The solution was then concentrated to give 190 mg of a purple solid which was purified by flash column chromatography to furnish 127 mg (88%) of **40** as an amorphous purple solid: mp 135 °C dec; R_f 0.47 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3500, 3420, 3365, 3000, 1730, 1650, 1605, 1565, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50–7.29 (m, 5), 5.62 (br s, 2), 5.15 (br s, 2), 4.91 (d of d, J = 10.4, J' = 4.6, 1), 4.08 (d of d, J = 10.4, J' = 10.4, 1), 3.95 (d, J = 4.5, 1), 3.65 (d of d, J = 10.4, J' = 4.6, 1), 3.37 (d, J = 13.7, 1), 3.28 (d of d, J = 4.5, J' = 0.9, 1), 3.20 (d, J = 13.7, 1), 3.16 (s, 3), 1.84 (s, 3); ^{13}C NMR ($CDCl_3$) 216.52, 177.70, 176.11, 156.81, 154.09, 147.46, 134.76, 130.65 (2 C), 129.85 (3 C), 110.59, 106.14, 104.91, 62.05, 49.73, 49.61, 49.17, 48.34, 41.98, 8.02 ppm; MS, m/e (relative intensity) (EI) 218 (100), 110 (96).

7-Amino-9a-methoxy-1a-[(methylthio)thiocarbonyl]mitosane (41). To a solution of 100 mg (299 μmol) of **1** and 41.6 μL (30.3 mg, 299 μmol) of Et_3N in DME (50 mL) was added 32.5 μL (41.7 mg, 329 μmol) of methyl chlorodithioformate.[23] The reaction solution was heated to just below reflux (ca. 80 °C) for 1 h and then vacuum filtered through a pad of Celite. It was then concentrated to leave 190 mg of a purple solid which was purified by flash column chromatography (93/7 $CHCl_3$/MeOH) to give 120 mg (95%) of **41** as an amorphous purple solid: mp 135 °C dec; R_f 0.44 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3510, 3420, 3370,

(23) Bogeman, M.; Petersen, S.; Schultz, O.-E.; Soll, H. In *Methoden der Organischen Chemie (Houben-Weyl)*; Muller, E., Ed.; Georg Theime Verlag: Stuttgart, 1955; Vol. 9, p 808.

1735, 1660, 1610, 1575, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.56 (br s, 2), 5.15 (br s, 2), 4.89 (d of d, J = 10.3, J' = 4.6, 1), 4.59 (d, J = 13.5, 1), 4.18 (d of d, J = 10.3, J' = 10.3, 1), 3.83 (d, 4.6, 1), 3.66 (d of d, J = 10.3, J' = 4.6, 1), 3.55 (d of d, J = 13.5, J' = 1.5, 1), 3.48 (d, J = 4.6, 1), 3.19 (s, 3), 2.54 (s, 3), 1.74 (s, 3); ^{13}C NMR ($CDCl_3$) 218.13, 178.23, 176.03, 156.86, 154.00, 147.63, 110.42, 106.33, 105.10, 62.09, 49.76, 49.47, 49.05, 47.94, 42.01, 19.72, 7.96 ppm; MS, m/e (relative intensity) (EI) 424 (M^+, 100); MS, m/e (relative intensity) (CI) 425 (M + 1, 28).

7-Amino-9a-methoxy-1a-(phenylcarbamoyl)mitosane (32). To a solution of 100 mg (299 μmol) of 1 in DME (50 mL) was added 35.8 μL (39.2 mg, 329 μmol) of phenyl isocyanate. The reaction solution was heated to 45 °C (1 h) and then concentrated to give 152 mg of a purple solid which was purified by flash column chromatography (92.5/7.5 $CHCl_3$/MeOH) to furnish 125 mg (92%) of **32** as an amorphous purple solid: mp 200–202 °C dec; R_f 0.49 (9/1 $CHCl_3$/MeOH); IR (KBr) 3320, 1710, 1605, 1550, 1450, 1345, 1100 cm^{-1}; ^1H NMR (C_5D_5N) δ 7.93 (d, J = 7.7, 2), 7.29 (d of d, J = 7.7, J' = 7.7, 2), 7.06 (t, 7.7, 1), 5.53 (d of d, J = 11.0, J' = 4.7, 1), 4.80 (d of d, J = 11.0, J' = 11.0, 1), 4.48 (d, J = 13.2, 1), 4.07–4.02 (m, 2), 3.60–3.48 (m, 2), 3.17 (s, 3), 2.05 (s, 3). The N-H protons were not detected. ^{13}C NMR (C_5D_5N) 178.00, 176.93, 160.96, 158.21, 155.17, 140.28, 129.18, 119.88, 111.07, 106.70, 104.33, 62.11, 50.00, 49.61, 43.94, 43.41, 42.80, 8.83 ppm. Signals associated with the solvent may have obscured two peaks. MS, m/e (relative intensity) (EI) 119 (80), 93 (100), 66 (47); MS, m/e (relative intensity) (CI) 120 (100), 94 (32).

7-Amino-9a-methoxy-1a-(methylcarbamoyl)mitosane (33). To a solution of 100 mg (299 μmol) of 1 in DME (50 mL) was added 88.2 μL (85.3 mg, 1500 μmol) of methyl isocyanate (Aldrich). The solution was then heated to 45 °C (1 h) and concentrated to give 137 mg of a purple solid. Purification by flash column chromatography (9/1 $CHCl_3$/MeOH) gave 100 mg (86%) of **33** as an amorphous purple solid: mp 144–150 °C dec; R_f 0.41 (9/1 $CHCl_3$/MeOH); IR (KBr) 3365, 1715, 1680, 1600, 1545, 1445, 1345, 1285 cm^{-1}; ^1H NMR (CD_3CN) δ 6.25 (br q, J = 4.7, 1), 5.90 (br s, 2), 5.50 (br s, 2), 4.81 (d of d, J = 10.4, J' = 4.6, 1), 4.29 (d, J = 13.3, 1), 4.06 (d of d, J = 10.4, J' = 10.4, 1), 3.56 (d of d, J = 10.4, J' = 4.6, 1), 3.44 (d of d, J = 13.3, J' = 1.7, 1), 3.40 (d, J = 4.8, 1), 3.21 (d of d, J = 4.8, J' = 1.7, 1), 3.17 (s, 3), 2.63 (d, J = 4.7, 3), 1.70 (s, 3); ^{13}C NMR (CD_3CN) 178.94, 176.92, 163.63, 157.71, 155.65, 149.14, 111.23, 106.97, 105.07, 62.30, 50.26, 50.23, 43.77, 43.48, 41.66, 27.47, 8.38 ppm; MS, m/e (relative intensity) (CI) 392 (M + 1, 33), 349 (58), 335 (50), 299 (100), 292 (89), 242 (50).

7-Amino-9a-methoxy-1a-(phenylthiocarbamoyl)mitosane (38). To a solution of 50 mg (150 μmol) of 1 in DME (50 mL) was added 19.7 μL (22.2 mg, 165 μmol) of phenyl isothiocyanate. The solution was then heated to reflux (90 min). Another 19.7 μL of phenyl isothiocyanate was then added and refluxing continued (5 h). The solution was then concentrated to leave 70 mg of an amorphous purple solid which was purified by flash column chromatography to give 51 mg (73%) of **38** as a purple solid: mp 210 °C dec; R_f 0.42 (9/1 $CHCl_3$/MeOH); IR (KBr) 3420, 3310, 1715, 1650, 1600, 1550, 1150 cm^{-1}; ^1H NMR (CD_3CN) δ 7.31–7.23 (m, 5), 5.91 (br s, 2), 5.41 (br s, 2), 4.89 (d of d, J = 10.7, J' = 4.2, 1), 4.22 (d of d, J = 10.7, J' = 10.7, 1), 3.90 (d, J = 4.2, 1), 3.65–3.62 (m, 1), 3.64 (d of d, J = 11.0, J' = 4.2, 1), 3.20 (s overlapping with a br s, 5), 1.75 (s, 3); ^{13}C NMR (CD_3CN) 193.0, 178.88, 177.0, 157.87, 155.50, 149.0, 129.78, 129.58, 126.93, 124.60, 111.50, 107.65, 105.16, 62.37, 50.34 (2 C), 47.52, 47.35, 42.50, 8.47 ppm; MS, m/e (relative intensity) (EI) 135 (76), 93 (100), 77 (52); MS, m/e (relative intensity) (CI) 136 (100), 94 (96).

7-Amino-9a-methoxy-1a-(methylthiocarbamoyl)mitosane (39). To a solution of 100 mg (299 μmol) of mitomycin C in 1,4-dioxane was added 22.5 μL (24.1 mg, 329 μmol) of methyl isothiocyanate. The solution was then heated to 95 °C for 5 h and then concentrated to give 180 mg of a purple solid. Flash column chromatography (92.5/7.5 $CHCl_3$/MeOH) gave 67 mg (55%) of **39** as a purple amorphous solid: mp 170–172 °C dec; R_f 0.38 (9/1 $CHCl_3$/MeOH); IR ($CHCl_3$) 3500, 3420, 3365, 3000, 1730, 1650, 1605, 1565, 1065 cm^{-1}; ^1H NMR (CD_3CN) δ 8.11 (br q, J = 4.6, 1), 5.98 (br s, 2), 5.55 (br s, 2), 4.85 (d of d, J = 10.5, J' = 4.5, 1), 4.44 (d, J = 13.4, 1), 4.16 (d of d, J = 10.5, J' = 10.5, 1), 3.71 (d, J = 4.7, 1), 3.60 (d of d, J = 10.5, J' = 4.5, 1), 3.48 (d of d, J = 13.4, J' = 1.6, 1), 3.38 (d of d, J = 4.7, J' = 1.6, 1), 3.20 (s, 3), 2.94 (d, J = 4.6, 3), 1.70 (s, 3); ^{13}C NMR (CD_3CN) 195.05, 178.73, 176.84, 157.72, 155.47, 149.14, 111.03, 107.32, 105.00, 62.15, 50.23, 50.07, 46.17, 43.33, 42.27, 32.86, 8.34 ppm.

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Registry No. 1, 50-07-7; 14, 75-55-8; 15, 109334-11-4; 16, 109334-12-5; 17, 21384-48-5; 18, 73680-89-4; 19, 22039-86-7; 20, 109334-13-6; 21, 109334-14-7; 23, 21384-49-6; 24, 151-56-4; 27, 109334-15-8; 28, 109334-16-9; 29, 56177-17-4; 30, 109334-17-0; 31, 109334-18-1; 32, 109334-19-2; 33, 109334-20-5; 34, 109334-21-6; 35, 109334-22-7; 36, 109334-23-8; 37, 109334-24-9; 38, 18887-04-2; 39, 18886-99-2; 40, 109334-25-0; 41, 109334-26-1; ClC(S)OPh, 1005-56-7; $ClCO_2Ph$, 1885-14-9; PhNCO, 103-71-9; $ClCO_2Me$, 79-22-1; ClC(O)SPh, 13464-19-2; MeSc(O)Cl, 18369-83-0; MeNCO, 624-83-9; PhNCS, 103-72-0; $ClCS_2Ph$, 16911-89-0; $ClCS_2Me$, 16696-91-6; MeNCS, 556-61-6.

Supplementary Material Available: ^1H (Tables IV and V) and ^{13}C (Tables VI and VII) NMR spectral data for mitomycin derivatives **30–36** and **38–41**; select in vitro (Table VIII) and in vivo (Table IX) test results for 1-methylaziridine analogues, and in vitro test results for mitomycin C derivatives **30–36** and **38–40** (Table X) (11 pages). Ordering information is given on any current masthead page.