rotations of each intermediate were recorded: **9a**, $[\alpha]_D$ 31.4° (*c* 0.873, 95% ethanol); **10a**, $[\alpha]_D$ 29.5° (*c* 0.818, 95% ethanol). The HPLC analysis of the final product **2a** showed an identical retention time as that of **2a** obtained from the resolution of intermediate **7a,b** as shown on Scheme I. The spectral properties were also identical: $[\alpha]_D$ 31.3° (*c* 0.713, 95% ethanol); high-resolution MS (as TMS derivative), *m/z* calcd for $C_{30}H_{50}O_4Si_2$ 530.3247, found 530.3248.

[[1(*S*)-(3-Cyclohexylpropyl)-2,3,3a,4,9,9a-hexahydro-2-(*S*)-hydroxy-(3a*R*,9a*R*)-1*H*-benz[*f*]inden-5-yl]oxy]acetic Acid (2b). Compound **7b** (obtained from **16b**) was converted to **2b** in an identical manner as previously described for the conversion of **7b** (obtained via resolution of **7a,b**) to **2b**. The specific rotations of each intermediate were recorded: **9b**, $[\alpha]_D$ −31.8° (*c* 0.916, 95% ethanol); **10b**, $[\alpha]_D$ −30.0° (*c* 0.83, 95% ethanol). The HPLC analysis of the final product **2b** showed an identical retention time as that of **2b** obtained from the resolution of intermediate **7a,b** as shown in Scheme I. The spectral properties were also identical: $[\alpha]_D$ −31.4° (*c* 0.72, 95% ethanol); high-resolution MS (as TMS derivative), *m/z* calcd for $C_{30}H_{50}O_4Si_2$ 530.3247, found 530.3237.

Acknowledgment. Helpful discussions with Dr. D. R. Morton are acknowledged. The supply of the intermediates as well as the technical assistance of D. L. Alexander, A. W. Harrison, P. G. Brewer, and D. P. Carvell are gratefully acknowledged. We also thank C. Lancaster, A. S. Olafsson, and K. P. Kolbasa for their assistance in providing the biological data.

Novel Mitomycin C Amidines:[1] Synthesis and Their Reactions with Amines

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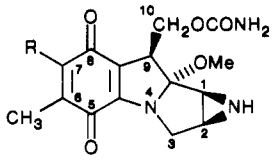
Reactions of formamide acetals (e.g., DMFDMA, **10**, **12**) with mitomycin C (**1**) has afforded novel amidine derivatives (e.g., **7–9**, **11**, **13**). Investigation of reactions of amines with bisamidine **8** in both polar and nonpolar solvents (e.g., MeOH vs $CHCl_3$) has led to the discovery that **8**, in its reactions with primary amines in methanol, behaves as a mitomycin A (**2**) equivalent to afford 7-N-substituted mitosanes (e.g., **16–19**). In contrast, bisamidine **8** undergoes a selective deamidination reaction with primary amines in chloroform to afford monoamidine **14**.

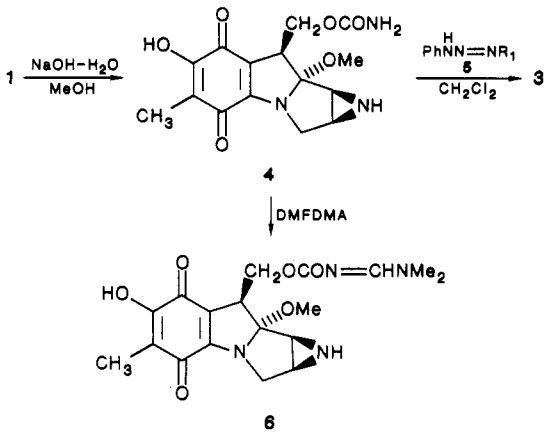
Fermentation-derived[3] antineoplastic antibiotics, mitomycin C (**1**) and mitomycin A (**2**),[4] are of great significance in cancer chemotherapy. While **1** is currently[5] in clincal use for the management of a variety of neoplasms, mitomycin A (**2**) is continuing to play a pivotal role in analogue research[6] which is directed toward discovery of new clinical agents endowed with less myelosuppressive properties and a broader spectrum of antitumor activity.



1: R = NH_2
2: R = OCH_3
3: R = OR_1

Recently,[7] we reported a practical approach to the synthesis of **2** and its analogues, namely 7-alkoxymitosanes **3**[8] from mitomycin C. The key reaction of this process

Scheme I



(Scheme I) involves O-alkylation of 7-hydroxymitosane (**4**) with an appropriate triazene (**5**) in a nonpolar solvent. During a similar attempt to methylate **4** with another well-established methylating agent, namely *N,N*-dimethylformamide dimethyl acetal (DMFDMA),[9] an amidine derivative, **6**, was obtained as the sole product in place of the desired product **2**. This finding was not surprising in light of the fact that DMFDMA is known to react with amines, amides, and urethanes to yield corresponding amidines. However, the observed functionalization[10] of the carbamoyl moiety of **4** is unprecedented. This encouraged us to investigate the reactions of formamide acetals with mitomycin C, which bears potentially three reactive amino

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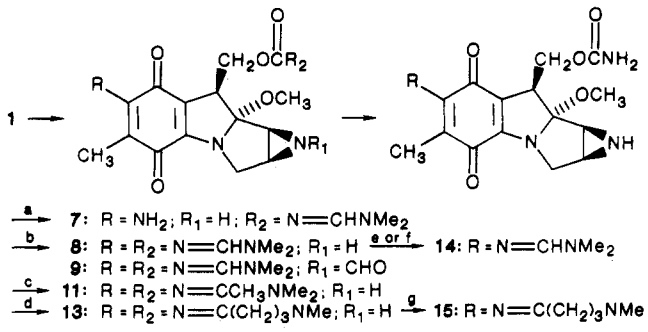
(2) Present address: Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876.
(3) Remers, W. A. In *Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol 1, pp 221–276 and references therein.
(4) According to the trivial system of nomenclature, which has found wide use in mitomycin literature, mitomycin C (**1**) is named as 7-amino-9a-methoxymitosane and mitomycin A (**2**) as 7,9a-dimethoxymitosane.
(5) Carter, S. K.; Crooke, S. T. *Mitomycin C, Current Status and New Developments*; Academic: New York, 1979; Chapter 15.
(6) Sami, S. M.; Iyengar, S. E.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701 and references cited therein.
(7) Vyas, D. M.; Benigni, D.; Partyka, R. A.; Doyle, T. W. *J. Org. Chem.* **1986**, *51*, 4307.
(8) Sami, S. M.; Iyengar, B. S.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1987**, *30*, 168.
(9) For a review on the chemistry of formamide acetals, see: Abdulla, R. F.; Brinkmeyer, R. S. *Tetrahedron* **1979**, *35*, 1675.
(10) Under reductive conditions, thionucleophiles are known to displace the carbamoyl moiety. See: Bean, M.; Kohn, H. *J. Org. Chem.* **1985**, *50*, 293.

0022-3263/87/1952-5601$01.50/0

Scheme II.[a] Mitomycin C Amidines



a (a) DMFDMA (8 equiv), $CHCl_3$, CH_3OH, 60 °C, 1 h; (b) DMFDMA (50 equiv), $CHCl_3$, CH_3OH, 60 °C, 18 h; (c) *N,N*-dimethylacetamide dimethyl acetal (**10**), MeOH, 75–80 °C, 2 h; (d) 2,2-dimethoxy-1-methylpyrrolidine (**12**), MeOH, 55 °C, 5 h; (e) $PhCHNH_2$, $CHCl_3$, 65 °C, 8 h; (f) *n*-$BuNH_2$, $CHCl_3$, 56 °C, 4 h; (g) *n*-$BuNH_2$, $CHCl_3$, reflux, 28 h.

functionalities. In this paper we report the results of our investigations, which have led to the syntheses of hitherto unknown novel amidine derivatives of mitomycin C and the discovery of their new reactions with amines, which may prove to be of practical utility in analogue research and in other areas of general organic chemistry.

Results and Discussions

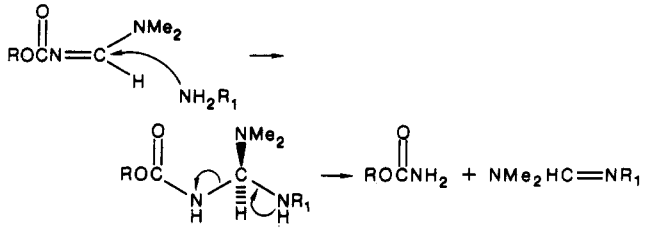
Synthesis of Mitomycin C Amidines. In light of the literature precedent[9] that formamide acetals react with amines, amides, and urethanes, our investigation began with studying the reaction of DMFDMA and mitomycin C (**1**) under mild and controlled conditions so as to ascertain if any chemoselective amidination of **1** took place. Thus, when **1** was treated with an 8 molar excess of DMFDMA in chloroform at 50 °C for ~1 h, one major compound was isolated in 65% yield. The ^1H NMR spectrum of this compound was indicative of the fact that no N-formylation of the aziridine nitrogen had taken place. Moreover, in the UV spectrum, the λ_{max} at 365 precluded the possibility that any reaction at the C-7 amino functionality had occurred. Thus, on the basis of this evidence, this purple compound was characterized as the carbamoylamidine derivative **7**. Only one of the two possible geometrical isomers was formed, on the basis of the ^1H NMR spectral data.

Interestingly, when **1** was treated with a 50-fold excess of DMFDMA in chloroform under slightly forcing conditions (60 °C, 24 h), two green compounds were isolated in an overall yield of 78% and in the ratio of 2:1 (Scheme II). The major product, on the basis of its ^1H NMR and UV spectra, was assigned the bisamidine structure **8**, whereas the minor product was designated as the *N*-formyl bisamidine derivative **9**. The N-formylation in **9** was clearly reflected in its ^1H NMR spectrum, where the diagnostic downfield shift of ~0.5–1.0 ppm associated with the aziridine nitrogen acylation was evident in the chemical shifts of methine protons H-1 and H-2 with respect to the shifts of corresponding protons in **8**.

It is noteworthy in the above reactions that there is a definitive preferential reactivity demonstrated by the carbamate functionality toward DMFDMA to that of the C-7 amino functionality (a vinylogous amide). This behavior contrasts with the reported[11] preferential reactivity by the C-7 amino moiety, when mitomycin C anion was treated with a formimidium chloride.[12] The observed

(11) Kaneko, T.; Wong, H.; Doyle, T. W. *Tetrahedron Lett.* **1985**, *26*, 3923.

Scheme III



difference in reactivity pattern can be rationalized on the basis of hard and soft acid–base theory.[13]

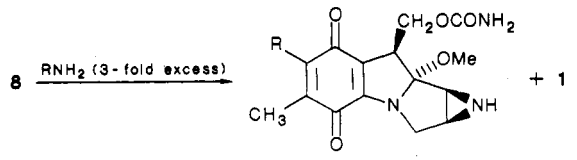
The scope and generality of the formamide acetal reaction with mitomycin C (**1**) was further demonstrated by the reactivity of *N,N*-dimethylacetamide dimethyl acetal (**10**)[14] and 2,2-dimethoxy-1-methylpyrrolidine (**12**)[15] with **1** to yield bisamidines **11** and **13**, respectively (Scheme II). In all of these amidine formation reactions of **1**, only one of the two possible geometrical isomers was formed.

Reactions of Amidines with Amines. Amidine functionality in organic chemistry has provided unique synthetic opportunities[9] in the areas of heterocyclic synthesis,[16] nucleoside chemistry[17] asymmetric synthesis,[18] diacylamine synthesis,[19] and prodrug synthesis.[20] Our focus was on employing some of the known amidine transformations, especially their reactions with nucleophiles, to gain entry into novel mitomycin derivatives for their potential application as chemotherapeutic agents. At the outset we decided to investigate the reactions of amines on bisamidine **8**. Reactions of primary amines and amino-containing nucleophiles (NH_2OH, H_2NNH_2) on amidines and acylamidines respectively have been well documented.[22,16] Thus, when **8** was treated with an excess (3-fold) of a primary amine, namely benzhydrylamine in a nonpolar solvent, chloroform at 65 °C for ~8 h, a major green product was isolated in 72% yield. To this was assigned the monoamidine structure **14**, on the basis of its ^1H NMR and UV spectral data (Scheme II). The same product was isolated as the major product when **8** was treated with a less hindered amine like *n*-butylamine in chloroform. The observed reactions can be regarded as an amidine exchange reaction (Scheme III), whereby the carbamoylamidine moiety of **8** is converted to the carbamoyl functionality by the reacting amine (RNH_2) to afford monoamidine **14**. It is noteworthy that this exchange reaction takes place selectively at the C-10 amidine moiety of **8**. Hindered carbamoylamidines on the other hand, as in the case of compound **13**, required forcing conditions (reflux, 28 h) with *n*-butylamine to yield the selective deamidination product **15** (scheme II).

The most extensively studied reactions of amidines are its hydrolysis reactions,[21] both in acidic and basic media. In the present study, our attempts to achieve selective

(12) Iminium salts have been employed for syntheses of amidines of Rifamycin. See: Marsili, L.; Franceschi, G.; Ballabio, M.; Oronzo, A.; Vigevani, A. *J. Antibiot.* **1983**, *36*, 1495.
(13) Ho, T.-L. *Hard and Soft Acids and Basic Principles in Organic Chemistry*; Academic: New York, 1977.
(14) Purchased from Fluka Chemical Corp.
(15) Meerwein, H.; Florian, W.; Schon, N.; Stopp. G. *Justus Liebigs Ann. Chem.* **1961**, *641*, 1.
(16) Lin, Y.; Lang, S. A.; Lovell, M. F.; Perkinson, N. A. *J. Org. Chem.* **1979**, *44*, 4160.
(17) Zemlicka, J.; Holy, J. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159.
(18) Meyers, A. I. *Aldrichimica Acta* **1985**, *18*, 59.
(19) Lin, Y.; Lang, S. A. *Synthesis* **1980**, 119.
(20) Lund, F.; Tybring, L. *Nature (London) New Biol.* **1972**, *236*, 135.
(21) DeWolfe, R. H. *Chemistry of Amidines and Imidates*; Wiley: New York, 1975; pp 349–384.
(22) Beck, J. R.; Gajewski, R. P. *J. Heterocycl. Chem.* **1976**, *13*, 605.

Table I



entry	RNH_2	7-aminomitosane[a] (% yield)
1	$CH_3CH_2CH_2NH_2$	16, R = $NHCH_2CH_2CH_3$ (45)
2	$HOCH_2CH_2NH_2$	17, R = $NHCH_2CH_2OH$ (37)
3	$PhCH_2SCH_2CH_2NH_2$	18, R = $NHCH_2CH_2SCH_2Ph$ (30)
4	$(Ph)_2CHNH_2$	14, R = $N{=}CHNMe_2$ (41)
		19, R = $NHCH(Ph)_2$ (8)
5	t-$BuNH_2$	no reaction

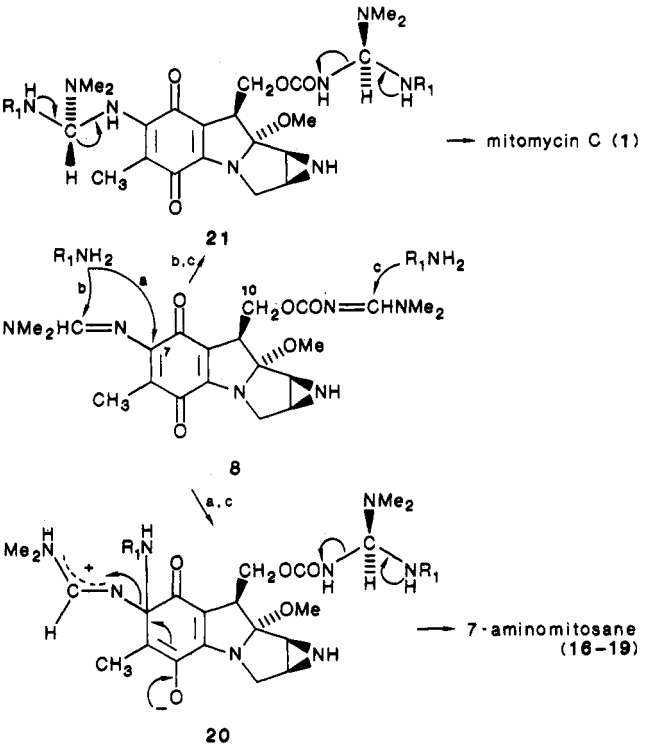
[a] Mitomycin C production was observed (TLC) in all cases but was isolated only in one case (entry 4) in 20% yield.

hydrolysis of 8 to 14 in aqueous methanol proved unsuccessful.[23,24] However, the above-mentioned deamidination reactions on bisamidines 8 and 13 with primary amines provided a successful methodology that can be used as an alternative to the conventional hydrolytic procedures.

Much to our surprise, reactions of bisamidine 8 took a different course when treated with a primary amine in a more polar solvent such as methanol. For example, when 8 was treated with a ~3 molar excess of *n*-propylamine in methanol for 24 h at room temperature, two products in the ratio ~1:1 were isolated. The less polar compound, on the basis of its ^1H NMR, UV, and elemental analysis data was identified as the 7-(propylamino)mitosane **16**. The more polar compound was identified as mitomycin C (1) on the basis of comparison of its spectral data with that of the authentic sample. This reaction may prove to be of significant utility in the field of mitomycin chemistry, since bisamidine 8 behaves as a mitomycin A equivalent in its reactions with amines. Thus, for example, reaction of simple amines with 8 yielded 7-N-substituted mitosanes (e.g. **17–19**, Table I). This new methodology circumvents the use of mitomycin A, which is difficult to prepare in large quantities. In analogy with the mitomycin A reaction, this amidine–amine reaction can be regarded as a nucleophilic displacement reaction governed by the usual steric requirements. For example, a bulky amine like benzhydrylamine (entry 4) yielded mainly the deamidination product 14 and a small amount of the displacement product **19**. This aspect is further substantiated by the fact that *tert*-butylamine (entry 5) has no effect on 8.

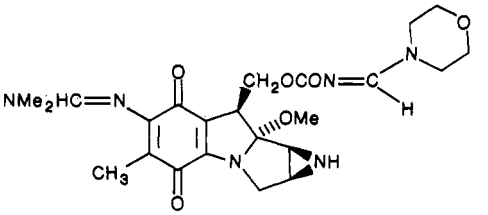
The observation that a change of solvent from chloroform to methanol in the above described reactions of 8 with primary amines affects the course of the reaction is not surprising, since it is well documented[7] that nucleophilic displacement reactions at C-7 of 2 proceed only in polar solvents like methanol. For example, mitomycin A (2) undergoes displacement with various anilines in methanol,[6] whereas it is inert to aniline in methylene chloride. Mechanistically (Scheme IV), the driving force for the displacement reaction of the bisamidine 8 by primary amines (RNH_2) is the basicity of the amidine moiety at C-7 to yield an intermediate **20**, which subsequently leads to formation of 7-substituted mitosane. This reaction is further driven to completion by the law of mass action in that an excess of primary amine (at least 3 molar excess) is required to drive the reaction to products at a significant rate. Formation of mitomycin C in these reactions can be

Scheme IV

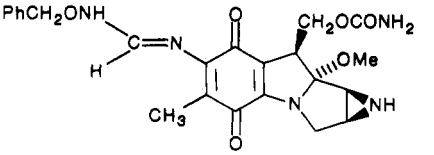


envisioned through the intermediate **21**. In both intermediates, **20** and **21**, the fate of the C-10 carbamoylamidine moiety in the presence of the reacting primary amine is similar to that depicted in Scheme III.

Amidines are known to undergo an amine exchange reaction when treated with secondary amines.[18] Accordingly, when bisamidine 8 was treated with an excess of secondary amine morpholine in methanol, an unsymmetrical bisamidine **22** was obtained as the sole reaction product. The site of amine exchange reaction on 8 was unequivocally established to be at the C-10 position because treatment of monoamidined 14 with morpholine formamide dimethyl acetal afforded **22** as the sole product. The above selective amine exchange reaction at C-10 is surprising and can be explained by assuming either that the C-7 amidine moiety in 8 is involved in conjugation with the quinone moiety or that the secondary amine is sterically restricted to react at the C-7 amidine carbon.



22



23

So far, in the above described amidine exchange and/or amine exchange reactions of 8, the C-7 amidine moiety has remained unaffected. However, when the monoamidine 14 was treated with *O*-benzylhydroxylamine in methanol at room temperature, a major orange-brown product was

(23) UV follow up of the hydrolysis reaction (MeOH–H_2O) of 8 indicated formation of mitomycin C.

(24) Chen, C.; Coppola, W. A.; Johns, W. H.; Bogardus, J. B.; Lipper, R. A. *J. Pharm. Sci.* **1986**, *75*, 208.

isolated, and to this was assigned structure **23** on the basis of its UV, ^{1}H NMR, and elemental analysis data. With hydroxylamine (NH_2OH), a reaction with **14** was clearly observed by TLC and a color change (green to orange); however, the instability of the products precluded isolation. Reactions of hydroxylamines with amidines have been very well documented[25] and widely employed in heterocyclic synthesis.[26]

In conclusion, the present investigation has clearly defined the reactivity of mitomycin C toward formamide acetals and has unravelled some novel transformations, probably unique to mitomycin amidines, e.g., displacement of the 7-amidino functionality by amines. However, these reaction types may find some utility in organic chemistry. The biological activity of the analogues described herein will be described at length separately.

Experimental Section

Melting points (degrees centigrade) were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded either on a Varian XL 100 or a Bruker WM 360 spectrometer in pyridine-d_5 unless otherwise stated. IR spectra were obtained on a Beckman 4240 spectrophotometer. UV spectra were recorded on a Varian-Cary 219 spectrophotometer. Thin-layer chromatography (TLC) was carried out on 0.25-mm E. Merck precoated silica gel plates (60F-254) with UV light as visualizing agent. Flash chromatography was performed with Woelm silica gel (32–63 μm). Solvents were evaporated under reduced pressure and at temperatures below 40 °C.

7-Amino-N^{10}-[(dimethylamino)methylene]-9a-methoxymitosane (7). To a solution of mitomycin C (1; 200 mg, 0.6 mmol) in chloroform (10 mL) and methanol (2 mL) was added *N,N*-dimethylformamide dimethyl acetal (DMFDMA, 0.64 mL, 4.8 mmol), and the solution was stirred at ~50 °C for 50 min. TLC (CH_2Cl_2/MeOH, 9:1) revealed a trace amount of unreacted mitomycin C (R_f 0.22) and a major new blue zone at R_f 0.33 with a trace amount of green component at R_f 0.42. The solution was concentrated to a syrup, which was flash chromatographed over silica gel (25 g) with 20:1 CH_2Cl_2/MeOH as the eluting solvent. The major component (R_f 0.33) was isolated and precipitated from methylene chloride and *n*-pentane as an amorphous solid (**7**; 148 mg, 64%): ^{1}H NMR (pyridine-d_5) δ 2.02 (s, 3 H), 2.76 (br m, 4 H), 2.86 (s, 3 H), 3.21 (s, 3 H), 3.28 (d, 1 H, J = 4 Hz), 3.62 (dd, 1 H, J = 2, 13 Hz), 3.94 (br s), 4.14 (dd, 1 H, J = 4, 10 Hz), 4.56 (d, 1 H, J = 13 Hz), 5.12 (t, 1 H, J = 10 Hz), 5.52 (dd, 1 H, J = 4, 10 Hz); IR (KBr) 3430, 3320, 3280, 2930, 1675, 1615, 1650, 1230, 1115 cm^{-1}; UV (λ_{max}, H_2O) 364, 244, 219 nm. Anal. Calcd for $C_{18}N_{23}N_5O_5$: C, 55.48; H, 5.91; N, 17.98; Found: C, 54.70; H, 6.41; N, 17.95.

7-[[(Dimethylamino)methylene]amino]-N^{10}-[(dimethylamino)methylene]-9a-methoxymitosane (8) and 7-[[(Dimethylamino)methylene]amino]-N^{10}-[(dimethylamino)methylene]-N^{1a}-formyl-9a-methoxymitosane (9). To a suspension of mitomycin C (1; 500 mg, 1.50 mmol) in chloroform (25 mL) was added DMFDMA (9.6 mL, 72 mmol), and the suspension was stirred at 50 °C for 41 h. TLC (CH_2Cl_2/MeOH, 20:1) revealed a complete conversion of mitomycin C to two new green components (R_f 0.16 and 0.22). The dark green solution was concentrated to a dark syrup, which was then subjected to flash chromatography on silica gel with CH_2Cl_2/MeOH (20:1) as the eluting solvent.

The major component (R_f 0.16) was isolated as an amorphous green solid, which after precipitation from diethyl ether and hexane was identified as **8** (340 mg, 52%): mp 155–157 °C; ^{1}H NMR (pyridine-d_5) δ 2.18 (s, 3 H), 2.70 (br, 1 H), 2.76 (s, 3 H), 2.82 (s, 3 H), 2.86 (s, 6 H), 3.22 (s, 3 H), 3.30 (br, 1 H), 3.60 (d, 1 H, J = 12 Hz), 4.12 (dd, 1 *H*, J = 4, 10 Hz), 4.43 (d, 1 H, J = 12 Hz), 4.90 (br, 1 H), 5.10 (t, 1 H, J = 10 Hz), 5.52 (dd, 1 H, J = 4, 10 Hz), 7.85 (s, 1 H), 8.64 (s, 1 H); IR (KBr) 3300, 2930, 1675, 1620, 1545, 1230, 1060 cm^{-1}; UV (H_2O, λ_{max}) 244 and 390 nm. Anal. Calcd for $C_{21}H_{28}N_6O_5$: C, 56.71; H, 6.08; N, 18.90. Found: C, 56.20; H, 6.28; N, 17.88.

The minor component (R_f 0.22) was also isolated as an amorphous green solid upon precipitation from diethyl ether and hexane and identified as compound **9** (180 mg, 25%): ^{1}H NMR (pyridine-d_5) δ 2.20 (s, 3 H), 2.60–3.00 (3 s, 9 H), 3.20 (s, 3 H), 3.65 (m, 2 H), 4.04 (d, 1 H, J = 4 Hz), 4.16 (dd, 1 H, J = 4, 12 Hz), 4.60 (d, 1 H, J = 13 Hz), 4.86 (t, 1 H, J = 12 Hz), 4.90 (br, 1 H), 5.48 (dd, 1 H, J = 12 Hz), 7.90 (s, 1 H), 8.64 (s, 1 H), 9.06 (s, 1 H); IR (KBr) 2490, 2860, 1698, 1630, 1600, 1540, 1250, 1060 cm^{-1}; UV (H_2O, λ_{max}) 244 and 390 nm. Anal. Calcd for $C_{22}H_{28}N_6O_6$: C, 55.89; H, 5.93; N, 17.78. Found: C, 55.41; H, 5.96; N, 16.99.

7-[[1-(Dimethylamino)ethylidene]amino]-N^{10}-[1-(dimethylamino)ethylidene]-9a-methoxymitosane (11). To a suspension of mitomycin C (600 mg, 1.79 mmol) in methanol (2 mL) was added *N,N*-dimethylacetamide dimethyl acetal (3 mL). The suspension was heated at 75–80 °C with stirring for 2 h. TLC (CH_2Cl_2/MeOH, 10:1) revealed the consumption of mitomycin C and appearance of a major green component. The reaction mixture was concentrated to a syrup under reduced pressure. This syrup was flash chromatographed on silica gel (40 g) with the following sequence gradient elution: 1% MeOH in CH_2Cl_2 (200 mL) and 2% MeOH in CH_2Cl_2 (400 mL) to afford the title compound **11** (110 mg, 13%) as an amorphous solid: mp 124–125 °C (precipitation from acetone (2 mL) and excess hexane); IR (KBr) 3440, 3295, 2925, 1770, 1660, 1620, 1580, 1550, 1300, 1055 cm^{-1}; UV (H_2O, λ_{max}) 235 and 364 nm. Anal. Calcd for $C_{23}H_{32}N_6O_5$: C, 58.46; H, 6.83; N, 17.79. Found: C, 58.89; H, 6.89; N, 17.64.

7-[(1-Methyl-2-pyrrolidinylidene)amino]-N^{10}-(1-methyl-2-pyrrolidinylidene)-9a-methoxymitosane (13). To a suspension of mitomycin C (1; 280 mg, 0.34 mmol) in methanol (20 mL) was added 2,2-dimethoxy-1-methylpyrrolidine (1.5 g, 10.3 mmol), and the suspension was heated with stirring at 55 °C for 5 h. TLC (alumina plates, CH_2Cl_2/MeOH, 97:3) revealed the formation of a major green component with traces of mitomycin C remaining. Excess of reagent and solvent were removed by distillation in vacuo at 40 °C. The residue was chromatographed over Woelm alumina (GIII, 25 g) with gradient elution with CH_2Cl_2 (200 mL) and 1% MeOH in CH_2Cl_2 (100 mL). Fractions containing pure green component were pooled and concentrated to yield the title compound **13** (53 mg 32%) as an amorphous solid: IR (KBr) 3300, 3220, 2940, 1660, 1620, 1550, 1290, 1055 cm^{-1}; UV (MeOH, λ_{max}) 239 and 354 nm. Anal. Calcd for $C_{25}H_{32}H_6O_5 \cdot 0.85H_2O$: C, 58.60; H, 6.64; N, 16.42. Found: C, 58.63; H, 6.46; N, 16.50.

Reaction of Benzhydrylamine with Bisamidine 8 in Chloroform. 7-[[(Dimethylamino)methylene]amino]-9a-methoxymitosane (14). To a solution of bisamidine **8** (34.07 g, 76.6 mmol) in chloroform (133 mL) was added benzhydrylamine (62 mL, 360 mmol), and the solution was stirred at 55 °C for 6 h. TLC (10% MeOH in CH_2Cl_2) revealed that no detectable starting material (R_f 0.25) was left, and a major new zone at R_f 0.20 had developed. The reaction mixture was concentrated, and the resulting residue was chromatographed over Wolem neutral alumina (900 g) with the following sequence of gradient elution: methylene chloride (4 L), 1% MeOH in CH_2Cl_2 (2 L), and 2% MeOH in CH_2Cl_2 (4 L). The title product (R_f 0.20) was isolated as an amorphous solid (21.5 g, 72%): mp 170–171 °C; ^{1}H NMR (pyridine-d_5) δ 2.18 (s, 3 H), 2.70 (br, 1 H), 2.80 (s, 3 H), 2.88 (s, 3 H), 3.08 (br, 1 H), 3.24 (s, 3 H), 3.56 (br d, 1 H, J = 12 Hz), 4.00 (dd, 1 H), 4.44 (d, 1 H, J = 12 Hz), 5.06 (t, 1 H, J = 10 Hz), 5.56 (dd, 1 H, J = 4, 10 Hz), 7.58 (br, 2 H), 7.88 (s, 1 H); IR (KBr) 3300–3450, 2960–2910, 1715, 1620, 1535, 1050 cm^{-1}; UV (H_2O, λ_{max}) 226 and 390 nm. Anal. Calcd for $C_{18}H_{23}N_5O_5$: C, 55.48; H, 5.91; N, 17.98. Found: C, 55.11; H, 5.88; N, 17.70.

Reactions of Benzhydrylamine with Bisamidine 8 in Methanol. To a solution of bisamidine **8** (600 mg, 1.35 mmol) in anhydrous methanol (10 mL) was added benzhydrylamine (2.2 mL, 10.8 mmol), and the solution was stirred at 54 °C for 4 h. The progress of the reaction was monitored by TLC (CH_2Cl_2/MeOH, 9:1), and after 4 h it revealed that all of the starting material (R_f 0.32) was consumed and three new products at R_f 0.37 (blue), 0.29 (green), and 0.16 (purple) had appeared. The reaction mixture was concentrated under reduced pressure, and

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(26) Tishler, M. *Heterocycles* **1983**, *20*, 1591.

the resulting residue was subjected to flash chromatography over silica gel (25 g) with CH_2Cl_2MeOH (20:1, v:v) as eluting solvent.

The product at R_f 0.37 was identified as 7-[(diphenylmethyl)amino]-9a-methoxymitosane (**19**; 51 mg, 8%): ^{1}H NMR (pyridine-d_5) δ 2.07 (s, 3 H), 2.13 (br, 1 H), 2.73 (m, 1 H), 3.12 (m, 1 H), 3.22 (s, 3 H), 3.60 (dd, 1 H, J = 2, 12 Hz), 4.00 (dd, 1 H, J = 4, 10 Hz), 4.48 (d, 1 H, J = 12 Hz), 5.05 (t, 1 H), 5.40 (dd, 1 H, J = 4, 10 Hz), 6.44 (d, 1 H, J = 7 Hz); UV (MeOH, λ_{max}) 368, 244, and 210 (sh) nm. Anal. Calcd for $C_{28}H_{28}N_4O_5 \cdot 0.8H_2O$: C, 65.27; H, 5.76; N, 10.88. Found: C, 65.05; H, 5.94; N, 11.25.

The major green component at R_f 0.29 was characterized as 7-[[(dimethylamino)methylene]amino]-9a-methoxymitosane (**14**; 215 mg, 41%).

The most polar component at R_f 0.16 was identified as mitomycin C (**1**; 90 mg, 20%), which was identical in all respects (IR, UV, ^{1}H NMR) with an authentic sample of **1**.

Reaction of *n*-Butylamine with Bisamidine 8 in Chloroform. To a solution of **8** (264 mg, 0.6 mmol) in anhydrous chloroform (1 mL) was added *n*-butylamine (0.23 mL, 2.4 mmol). The reaction mixture was heated at 55 °C for 4 h. TLC (CH_2Cl_2/MeOH, 10:1) indicated disappearance of starting material and the formation of a polar green component as a major zone and minor traces of mitomycin C. The major green component after isolation was identified as the monoamidine **14**.

7-[(1-Methyl-2-pyrrolidinylidene)amino]-9a-methoxymitosane (15). To a solution of **13** (80 mg, 0.16 mmol) in chloroform (15 mL) was added *n*-butylamine (0.48 mL), and the solution was refluxed for 28 h. TLC (alumina, 2% MeOH in CH_2Cl_2) revealed a major new green zone (R_f 0.60) and a few impurities. The solution was concentrated, and the residual syrup was chromatographed on alumina (50 g) with the following sequence of gradient elution: 1% MeOH in CH_2Cl_2 (200 mL) and 2% MeOH in CH_2Cl_2 (400 mL). The title compound was isolated as an amorphous solid (24 mg 39%): ^{1}H NMR (pyridine-d_5) δ 1.72 (q, 2 H), 2.04 (s, 3 H), 2.16 (q, 2 H), 2.72 (br, 1 H), 2.84 (s, 3 H), 3.12 (m, 3 H), 3.24 (s, 3 H), 3.60 (dd, 1 H, J = 2, 14 Hz), 4.00 (dd, 1 H, J = 6, 12 Hz), 4.40 (d, 1 H, J = 14 Hz), 5.04 (t, 1 H, J = 14 Hz), 5.38 (dd, 1 H, J = 6, 12 Hz), 7.48 (br, 2 H). Anal. Calcd for $C_{20}H_{25}N_5O_5 \cdot 0.6H_2O$: C, 56.35; H, 6.20; N, 16.43. Found: C, 56.19; H, 6.29; N, 15.69.

7-(*n*-Propylamino)-9a-methoxymitosane (16). To a solution of bisamidine **8** (330 mg, 0.74 mm) in anhydrous methanol (10 ml) was added *n*-propylamine (1.0 mL). The reaction mixture was stirred at room temperature for 6 h and then at 0–4 °C for 16 h. TLC (silica gel, CH_2Cl_2/MeOH, 10:1) indicated that the green starting material was converted to two new compounds (R_f 0.40 and 0.30). The lower R_f component (R_f 0.30) corresponded to that of mitomycin C. Evaporation of solvent and flash chromatography of the residue over silica gel with CH_2Cl_2/MeOH (30:1) as eluting solvent afforded the title compound **16** (125 mg, 44.5%) as an amorphous solid (precipitated from CH_2Cl_2 and hexane): mp 69–71 °C; ^{1}H NMR (pyridine-d_5) δ 0.80 (t, 3 H), 1.42 (m, 2 H), 2.11 (s, 3 H), 2.74 (br, 1 H), 3.12 (br, 1 H), 3.22 (s, 3 H), 3.36 (q, 2 H), 3.60 (d, 1 H, J = 12 Hz), 3.96 (dd, 1 H, J = 4, 11 Hz), 4.54 (d, 1 H, J = 12 Hz), 5.00 (m, 3 H), 5.36 (dd, 1 H, J = 4, 11 Hz), 6.90 (t, 1 H); IR (KBr) 3440, 3300, 2960, 2940, 1715, 1630, 1600, 1550, 1510, 1220, 1060 cm^{-1}; UV (H_2O, λ_{max}) 222 and 372 nm. Anal. Calcd for $C_{18}H_{24}N_4O_5$: C, 57.40; H, 6.38; N, 14.88. Found: C, 57.28; H, 6.41; N, 14.80.

7-[(2-Hydroxyethyl)amino]-9a-methoxymitosane (17). To a solution of bisamidine (**8**; 330 mg, 0.74 mmol) in anhydrous methanol (5 mL) was added ethanolamine (2 mL), and the resulting solution was stirred at room temperature for 2 h. TLC (silica gel; CH_2Cl_2/MeOH, 10:1) indicated complete conversion of starting green component to two bluish components, as observed in the case of compound **16**. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (5 × 60 mL). The combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated to a bluish-purple residue, which upon flash chromatography with CH_2Cl_2/MeOH (10:1) as the eluting solvent afforded the newly formed blue compound **17** (besides mitomycin C) as an amorphous solid (105 mg, 37%): ^{1}H NMR (pyridine-d_5) δ 2.14 (s, 3 H), 2.81 (br, 1 H), 3.18 (d, 1 H, J = 4 Hz), 3.24 (s, 3 H), 3.65 (dd, 1 H, J = 4, 12 Hz), 3.70–4.20 (m, 5 H), 4.52 (d, 1 H, J = 13 Hz), 4.96 (t, 1 H), 7.38 (t, 1 H), 7.58 (br, 1 H); IR (KBr) 3300–3500, 2930, 1710, 1630, 1600, 1540, 1510, 1210, 1055 cm^{-1}; UV (H_2O λ_{max}) 221 and 371 nm. Anal. Calcd for $C_{17}H_{22}N_4O_6 \cdot H_2O$: C, 51.50; H, 6.00; N, 14.10. Found: C, 51.30; H, 5.88; N, 14.80.

7-[[2-(Benzylthio)ethyl]amino]-9a-methoxymitosane (18). To a solution of bisamidine **8** (200 mg, 0.45 mmol) in anhydrous methanol (2 mL) was added *S*-benzyl-2-aminoethanethiol (0.5 mL), and the resulting solution was stirred at room temperature for 16 h. The residue obtained upon evaporation of the solvent at reduced pressure was flashed chromatographed (silica gel, 40 g) with 6% MeOH in CH_2Cl_2 as the eluting solvent. The title compound was isolated as an amorphous solid (65 mg, 30%): ^{1}H NMR (pyridine-d_5) δ 2.04 (s, 3 H), 2.04 (br, 1 H), 2.73 (t, 3 H), 2.74 (br, 1 H), 3.12 (br, 1 H), 3.22 (s, 3 H), 3.60 (dd, 1 H, J = 2, 12 Hz), 3.60–3.86 (4 H), 4.00 (dd, 1 H, J = 4, 10 Hz), 4.53 (d, 1 H, J = 12 Hz), 5.04 (t, 1 H), 5.38 (dd, 1 H, J = 4, 10 Hz), 7.20–7.60 (5 H); IR (KBr) 3460, 3300, 2940, 1725, 1640, 1565, 1515, 1460, 1335, 1230, 1070 cm^{-1}; UV (MeOH, λ_{max}) 368 nm. Anal. Calcd for $C_{24}H_{28}N_9O_5S$: C, 59.49; H, 5.82; N, 11.56. Found: C, 59.72; H, 5.94; N, 11.08.

Reaction of Bisamidine 8 with Morpholine in Methanol. 7-[[(Dimethylamino)methylene]amino]-*N*-[(4-morpholinyl)methylene]-9a-methoxymitosane (22). To a solution of bisamidine **8** (485 mg, 1.09 mmol) in anhydrous methanol (8 mL) was added morpholine (1 mL), and the reaction mixture was stirred at room temperature for 3 h. The solution was concentrated to a syrup under reduced pressure. Flash chromatography of this syrup over silica gel with 5% MeOH in CH_2Cl_2 afforded the major green component as an amorphous solid (322 mg, 61%): ^{1}H NMR (pyridine-d_5) δ 1.80 (br, 1 H), 2.12 (s, 3 H), 2.74 (m, 1 H), 2.84 (s, 3 H), 2.88 (s, 3 H), 3.20 (s, 3 H), 3.20–3.84 (m, 12 H), 4.04 (dd, 1 H, J = 4, 11 Hz), 4.39 (d, 1 H, J = 14 Hz), 5.04 (t, 1 H), 5.43 (dd, 1 H, J = 4, 11 Hz), 7.83 (s, 1 H), 8.38 (s, 1 H); IR (KBr) 3440, 3305, 2920, 1675, 1620, 1540, 1445, 1375, 1310, 1275, 1060 cm^{-1}; UV (MeOH, λ_{max}) 248 and 386 nm. Anal. Calcd for $C_{23}H_{30}N_6O_6$: C, 56.78; H, 6.22; N, 17.27. Found: C, 54.86; H, 6.38; N, 16.18.

Reaction of Monoamidine 14 with Morpholine Formamide Dimethyl Acetal. To a solution of **14** (116 mg, 0.3 mmol) in chloroform (5 mL) and anhydrous methanol (5 mL) was added morpholine formamide dimethyl acetal (0.6 mL). The reaction mixture was stirred at ~55 °C for 48 h. The progress of the reaction was monitored by TLC (10% MeOH in CH_2Cl_2). At the completion of the reaction, almost all of the starting material (R_f 0.43) was converted to a major green material (R_f 0.57). The reaction mixture was concentrated to an oil, which was chromatographed on alumina (Woelm, GIII) with CH_2Cl_2 (50 mL), 1% MeOH in CH_2Cl_2 (50 mL), and 2% MeOH in CH_2Cl_2 as the eluting solvents. The compound (R_f 0.57) was obtained as an amorphous solid (96 mg, 67%) whose ^{1}H NMR spectrum (pyridine-d_5) was identical with that of compound **22**.

7-[[[(Benzyloxy)amino]methylene]amino]-9a-methoxymitosane (23). To a solution of monoamidine **14** (100 mg, 0.26 mmol) in methanol (2 mL) containing triethylamine (0.5 mL) was added *O*-benzylhydroxylamine hydrochloride (400 mg, 2.5 mmol). The reaction mixture was stirred at room temperature for 2.5 h. TLC (MeOH/CH_2Cl_2, 1:10) revealed formation of a major orange-brown zone in advance of the green zone of the starting material. The reaction mixture was concentrated to a syrupy residue, which was flash chromatographed (20 g, silica gel) with CH_2Cl_2/MeOH (20:1) as an eluting solvent. The major orange-brown zone constituting the title compound was collected as an amorphous solid (80 mg, 66%): m.p. 96–97 °C; ^{1}H NMR (pyridine-d_5) δ 1.92 (s, 3 H), 2.12 (m, 1 H), 2.72 (m, 1 H), 3.12 (m, 1 H), 3.20 (s, 3 H), 3.52 (br d, 1 H, J = 12 Hz), 3.96 (dd, 1 H, J = 4, 10 Hz), 4.12 (d, 1 H, J = 12 Hz), 5.00 (t, 1 H), 5.28 (2 H), 5.36 (dd, 1 H, J = 4, 10 Hz), 7.80 (d, 1 H); IR (KBr) 3460, 3300, 2945, 2920, 1745, 1720, 1570, 1275, 1220, 1060 cm^{-1}; UV (MeOH, λ_{max}) 209, 245, and 376 nm. Anal. Calcd for $C_{23}H_{25}N_5O_6$: C, 59.10; H, 5.35; N, 14.97. Found: C, 58.43; H, 5.48; N, 14.62.

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