Synthesis, DNA Cross-Linking Activity, and Cytotoxicity of Dimeric Mitomycins

Younghwa Na,[†] Ven-Shun Li,[†] Yuka Nakanishi,[‡] Kenneth F. Bastow,[‡] and Harold Kohn*[,†,‡]

Department of Chemistry, University of Houston, Houston, Texas 77204-5641, and Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360

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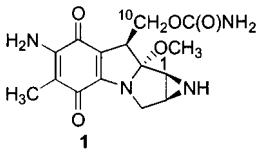
Dimeric DNA cross-linking compounds have emerged as important new antitumor agents. We report the synthesis and biochemical evaluation of a select set of dimeric mitomycins in which the two mitomycin units are tethered at either the mitomycin C(7) amino or the aziridine N(1a) positions. Significantly, mitomycin C (**1**) itself is the prototypical bioreductive DNA cross-linking agent. DNA cross-linking experiments using a denaturing-gel-electrophoresis-based assay showed that the extent of DNA cross-linking for select dimeric mitomycins can exceed that of the parent compound, mitomycin C, and that the reaction proceeds, in part, at the two distal C(1) sites in the mitomycins. The efficiency of DNA cross-linking depended on the nature of the linker and the position of linker unit's attachment. When we compared the efficiency of DNA cross-linking for the dimeric mitomycins with their in vitro cytotoxicities in cultured human tumor cells, we observed a poor correlation. The mitomycins that gave the highest levels of DNA cross-linked adducts displayed the weakest cytotoxicities. These findings determined that the denaturing-gel-electrophoresis-based assay was a poor predictor of cytotoxic activity.

In recent years there has been increasing interest in the design of novel dimeric agents that target DNA.[1,2] Both intercalating and irreversible alkylating agents have been prepared to identify compounds that modify DNA with enhanced sequence selectivity and efficiency. Special attention has been given to dimeric alkylating agents that can induce DNA interstrand cross-links (ISC) and disrupt cellular processes necessary for cell maintenance and replication.

In this study we asked if tethering two mitomycin C[3] (**1**) units could lead to efficient interstrand DNA cross-linking transformations. At first glance, mitomycin C may seem to be an unlikely candidate because it is an archetypical DNA cross-linking agent.[4] Mitomycin C function is initiated by enzymatic reduction that permits DNA adduction at the C(1) and the C(10) sites. DNA modification proceeds selectively at the N(2) aminoguanine (G*) site[5,6] and occurs preferentially at 5′CG* sequences.[7−10] Mitomycin C C(1) and C(10) modification within 5′CG*•5′CG* sequences produces DNA ISC.[6] The mitomycin C ISC adduct is considered to be the key biological event leading to cell cytotoxicity.[4] Significantly, the efficiency of this DNA cross-linking transformation is low because of competing reactions at C(1) and C(10)[11−13] and the diminished reactivity of the C(10) site.[14] Surprisingly, while over 600 mitomycins have been prepared in an effort to generate more efficient agents, few dimeric mitomycins have been synthesized[16−19] and evaluated in either cell-[18] or DNA[19]-based assays.



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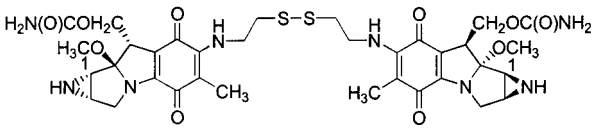
In this paper, we report the synthesis of two classes of dimeric mitomycins. Both series were evaluated in a DNA cross-linking assay,[20] and the extent of DNA cross-linking for individual compounds was compared with their in vitro activities in human tumor cell lines. We document that select dimeric mitomycins can efficiently cross-link DNA and that the DNA cross-linking efficiency depends on the structural scaffold that connects the two mitomycin units. However, DNA ISC in vitro by dimeric mitomycins is not a predictor of cytotoxic activity against human tumor cell lines. We observed that only those compounds that contained an aryl unit tethered directly to the mitomycin C(7) amino substituent displayed equivalent or improved cytotoxic activity compared to **1**, yet these compounds provided only trace levels of DNA ISC under the test conditions.

Results

1. Experimental Plan. 1.1. Selection of Compounds. Our choice of compounds was based on a recent report by Hartley and co-workers showing that 7-*N*,7′-*N*′-dithiobis(2,1-ethanediyl)bismitomycin (**2**),[19] a metabolite of KW-2149 (**3**),[21] generated higher levels of drug−DNA cross-links upon treatment with glutathione than **3**.[18] No information was provided on the site of



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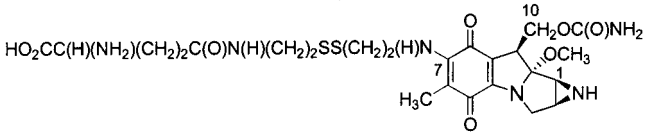

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* To whom correspondence should be addressed. Phone: (919) 966-2680. Fax: (919) 843-7835. E-mail:harold_kohn@unc.edu.
[†] University of Houston.
[‡] University of North Carolina.

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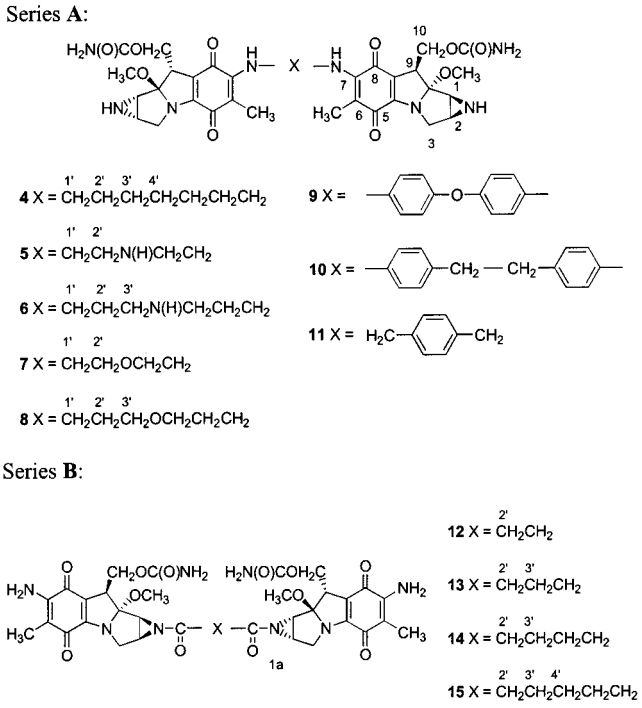


Figure 1. Dimeric mitomycins.

drug modification. We have modeled the putative ISC adduct from **2** and DNA after reductive activation where the DNA cross-linking process is envisaged to proceed at the two distal mitomycin C(1) sites with DNA modification occurring at guanine N(2).[22] The DNA ISC was separated by five base pairs, and it induced little DNA helical distortion. Significantly, the linker that bridges the two mitomycin units in **2** contains a chemically labile disulfide unit.

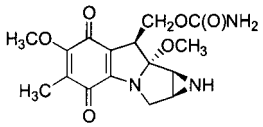
We selected two series (A, B) of dimeric mitomycins for synthesis and evaluation (Figure 1). Series A and B differed in their point of attachment of the linker with the mitomycin. In series A, the mitomycins were attached at the mitomycin C(7) nitrogen residue, while in series B we tethered the mitomycins at the aziridine N(1a) position. Both sets of linkers were designed to be chemically inert under the conditions used to reductively activate the dimeric mitomycins. Within each series we chose to vary the length of the linker to determine whether a preferred length existed for DNA ISC. Linkers in series A contained a hydrocarbon backbone (**4**) or incorporated either a heteroatom (i.e., N(H), O) (**5**−**8**) or an aromatic unit (**9**−**11**) within the backbone to learn if these additional structures aided drug−DNA modification and cytotoxic activity. The inclusion of aromatic units within the **9** and **10** linkers introduced an important structural change. We expected that both of these dimeric mitomycins would undergo reductive activation more readily than mitomycin C (**1**), C(7) alkylamine dimeric mitomycins **4**-**8**, and **11**. Studies by Remers and co-workers have shown that the reduction potential for C(7)-*anilino*mitomycins ($E_{1/2}$ = −0.28 − −0.37 V) were significantly lower than that of C(7)-*alkyl*aminomitomycins ($E_{1/2}$ = −0.41 − −0.45 V).[23] In series B (**12**−**15**), the linker consisted of a diacyl polymethylene unit, where the length of the alkyl chain was systematically changed from ethylene to pentylene.

1.2. Biochemical and Biological Evaluation. 1.2.1. DNA Cross-Linking Experiments. To test whether the dimeric mitomycins generated DNA ISC upon reductive activation, we used *Eco*RI linearized pBR322 plasmid DNA and 1.2% alkaline agarose gel electrophoresis.[20] The DNA cross-linking experiments were predicated on earlier experimental findings. First, we chose to activate the dimeric mitomycins chemically with sodium dithionite[24,25] rather than enzymatically. We have previously shown that not all mitomycin derivatives are efficiently activated by the enzymatic reductive system xanthine oxidase/NADH[26] and that both sodium dithionite and xanthine oxidase/NADH provided the same DNA bonding profiles for mitomycin C (**1**) itself. Accordingly, we have assumed that the extent of DNA ISC formation observed for similar dimeric mitomycins reflected the ability of the test substrate to modify complementary DNA strands rather than a reflection of the extent of mitomycin reductive activation. Second, mitomycin C−DNA modification studies[9,10,26,27] showed that **1**−DNA cross-linking processes did not proceed at appreciable levels when moderately low levels of **1** (≤0.2 mM) were used with ≤1 equiv of $Na_2S_2O_4$ and the reaction temperature was maintained at 0 °C. Under these conditions, DNA modification gave only mitomycin C−DNA monoadducts. Correspondingly, when either higher amounts of drug or higher amounts of reductant were employed, DNA ISC were observed. These results predict that dimeric mitomycins treated with 1 equiv of $Na_2S_2O_4$ *per* mitomycin unit at 0 °C would undergo preferential C(1) activation. If correct, then detection of DNA ISC under these conditions suggests that DNA modification preferentially occurred at the two *distal* mitomycin C(1) sites.

1.2.2. In Vitro Cytotoxic Activity. To test whether dimeric mitomycins were inhibitors of human tumor cell line replication and to compare their activities to monomeric mitomycins, target compounds were evaluated side-by-side with control compounds using a 96-well plate tissue culture assay.[28] Final numbers of attached cells after treatment and fixation were measured spectrophotometrically by staining with sulforhodamine B, an anionic protein stain.

2. Synthesis. 2.1. Series A Dimeric Mitomycins. Dimeric mitomycins **4**−**11** were prepared by treating concentrated mitomycin A (**16**) (28−40 mM) MeOH or CH_2Cl_2 solutions with the corresponding diamine (0.5−1.0 equiv). HPLC (TLC) analysis of the reaction indicated the initial production of the monomeric mitomycins followed by formation of the desired dimeric adducts. In several cases, we isolated both the monoadducts (**17**−**19**) and the dimeric products. Our spectral data for the mono- and di-adducts were consistent with the proposed structures and included the detection of molecular ion peaks in the low- and high-resolution mass spectra (CI). For the N(7) phenyl dimeric mitomycins **9** and **10**, we observed a downfield shift (∼2.5 ppm) in the ^{13}C NMR for the mitomycin C(6) methyl carbon compared with the other dimeric mitomycins and **1**. This chemical shift value is comparable to that reported for **20**.[29] We suspect that this shift reflects the diminished delocalization of the C(7) nitrogen electrons with the quinone ring, a

factor that likely contributes to the lower reduction potential observed for **20**, compared with **1**.[23a]



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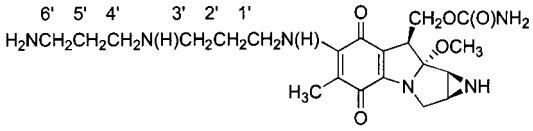

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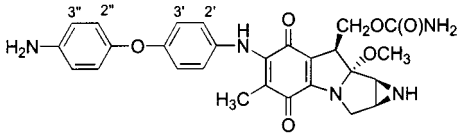

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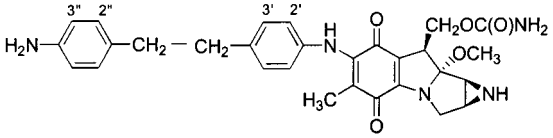

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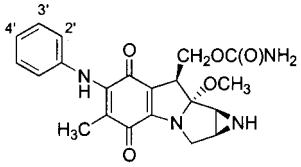

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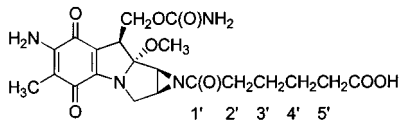
2.2. Series B Dimeric Mitomycins. Series B dimeric mitomycins **12**-**15** were synthesized from **1** (14−18 mM) and the corresponding alkyl diacyl dichlorides (1 equiv) in basic (TEA) THF solutions. The reactions were rapid (<30 min), and the products were purified using TLC. Using adipoyl chloride with **1**, we isolated **21** along



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with dimeric mitomycin **14**. The spectroscopic data (MS, NMR, UV) for **12**−**15** were in agreement with their proposed dimeric mitomycin structures. In the ^{1}H NMR we observed that the mitomycin C(1) and C(2) methine protons appeared as doublets at ∼δ 3.8 and ∼δ 3.6, respectively. These chemical shift values are downfield (Δ(ppm): ∼0.9) from those observed for **1** and are consistent with the introduction of an electron-withdrawing acyl group at the N(1a) position. Mitomycins

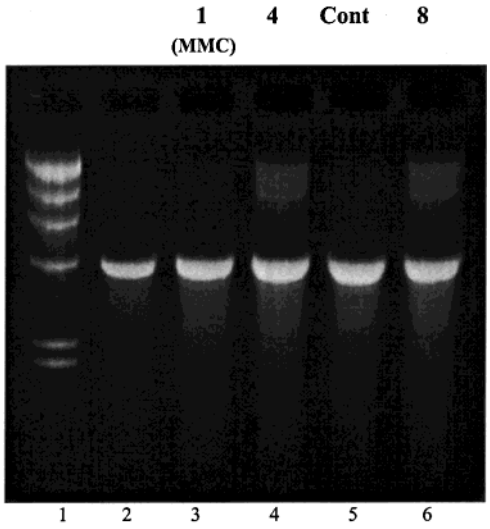


Figure 2. Denaturing 1.2% alkaline agarose gel for **1**, **4**, and **8** in DNA cross-linking experiments for mitomycin C (**1**), dimeric mitomycins **4** and **8** using *Eco*RI-linearized pBR322 plasmid DNA and $Na_2S_2O_4$. A total of 1 equiv of $Na_2S_2O_4$ was used for MMC (**1**) (0.2 mM) or dimeric mitomycins (0.2 mM). All reactions were incubated (1 h) at 0 °C. Lane 1: λ Hind III DNA molecular weight marker. Lane 2: control (only linearized pBR322). Lane 3: MMC (**1**) + $Na_2S_2O_4$. Lane 4: **4** + $Na_2S_2O_4$. Lane 5: only $Na_2S_2O_4$. Lane 6: **8** + $Na_2S_2O_4$.

12−**15** exhibited a peak at ∼184.0 ppm in the ^{13}C NMR spectra, which has been assigned to the N(1a)-carbonyl carbon.

3. Biochemical and Biological Evaluation of Dimeric Mitomycins. 3.1. Introduction. Two different assays were used to determine the effectiveness of the dimeric mitomycins. The first measured the ability of the dimeric mitomycins to efficiently cross-link complementary DNA strands using linearized pBR322 DNA.[20] The second determined the anticancer activities of the dimeric mitomycins in a limited series of human tumor cell lines.[28] We recognized that the measurements were independent of each other and that high efficiency (activity) in one assay did not ensure high efficiency (activity) in the second. We were anxious to identify compounds that proved effective in both assays.

3.2. DNA Cross-Linking Experiments. The ability of dimeric mitomycins to cross-link complementary *Eco*RI-linearized pBR322 DNA was determined using denaturing 1.2% alkaline gel electrophoresis according to a previously reported method.[20] The size of the DNA product(s) was estimated using λ DNA digested with Hind III as a molecular weight marker. We first examined the reactivity of series A dimeric mitomycins **4**−**11** with DNA under reductive conditions. We divided this set into compounds containing a saturated alkyl linker without (**4**) or with an embedded heteroatom (**5**−**8**) (Figures 2 and 3) and those with an aromatic unit (or units) within the linker (**9**−**11**) (Figure 4). In these experiments, we employed 0.2 mM of the test compound and 1 equiv of $Na_2S_2O_4$ *per* dimeric mitomycin as the reductant. Mitomycin C (0.2 mM) served as the standard, and 1 equiv of $Na_2S_2O_4$ was employed for drug activation.

Dimeric mitomycins **4**−**8** generated appreciable amounts of DNA ISC (Figures 2 and 3). Significantly, we observed only trace levels of DNA ISC with **1** at this

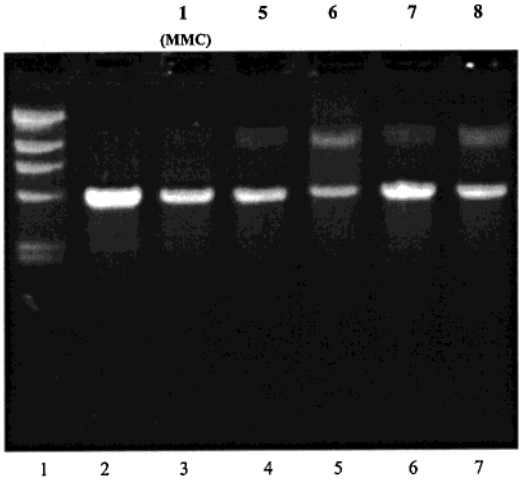


Figure 3. Denaturing 1.2% alkaline agarose gel for **5**−**8** in DNA cross-linking experiments for dimeric mitomycins **5**−**8** using *Eco*RI-linearized pBR322 plasmid DNA and $Na_2S_2O_4$. A total of 1 equiv of $Na_2S_2O_4$ was used for dimeric mitomycin (0.2 mM) or MMC (**1**) (0.2 mM). All reactions were incubated (1 h) at 0 °C. Lane 1: λ Hind III DNA molecular weight marker. Lane 2: control (only linearized pBR322). Lane 3: MMC (**1**) + $Na_2S_2O_4$. Lane 4: **5** + $Na_2S_2O_4$. Lane 5: **6** + $Na_2S_2O_4$. Lane 6: **7** + $Na_2S_2O_4$. Lane 7: **8** + $Na_2S_2O_4$.

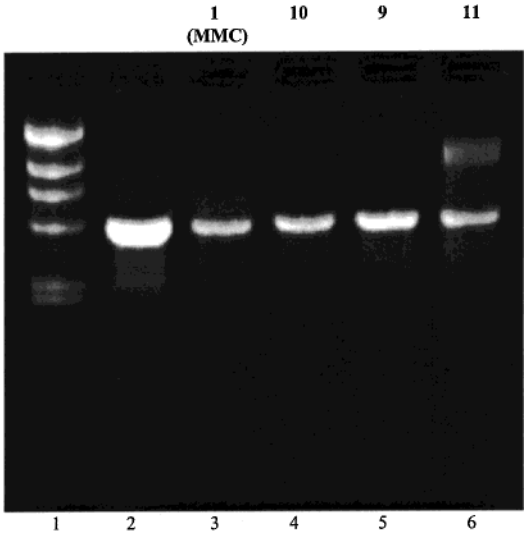


Figure 4. Denaturing 1.2% alkaline agarose gel for **9**−**11** in DNA cross-linking experiments for dimeric mitomycins **9**−**11** using *Eco*RI-linearized pBR322 plasmid DNA and $Na_2S_2O_4$. A total of 1 equiv of $Na_2S_2O_4$ was used for dimeric mitomycin (0.2 mM) or MMC (**1**) (0.2 mM). All reactions were incubated (1 h) at 0 °C. Lane 1: λ Hind III DNA molecular weight marker. Lane 2: control (only linearized pBR322). Lane 3: MMC (**1**) + $Na_2S_2O_4$. Lane 4: **10** + $Na_2S_2O_4$. Lane 5: **9** + $Na_2S_2O_4$. Lane 6: **11** + $Na_2S_2O_4$.

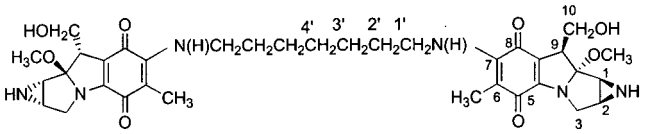
concentration (0.2 mM) (Figures 2 and 3, lane 3). Of the four dimeric mitomycins with central hereroatoms, the two dimeric mitomycins with slightly larger linkers (**6**, **8**) were more efficient in cross-linking DNA than those with shorter linkers (**5**, **7**) (Figure 3, lanes 5 and 7 vs lanes 4 and 6). Side-by-side comparison of the comparable-size dimeric mitomycins **4**, **6**, and **8** indicated that including a nitrogen atom within the linker (**6**) increased the relative percentage of cross-linked products, compared with hydrocarbon **4**, while including an oxygen atom (**8**) did not appreciably alter the levels of DNA cross-linked products (Figures 2 and 3).

We compared the ISC efficiencies of **4** and **1** (data not shown; Figures 5 and 6 of Supporting Information). For **1** we observed no band(s) corresponding to interstrand cross-links when 1 equiv of $Na_2S_2O_4$ was used and the concentration of **1** was below 0.2 mM (Figure 6, lane 3). Correspondingly, when we used concentrations of **4** as low as 0.05 mM and only 1 equiv of $Na_2S_2O_4$ *per* dimeric mitomycin, we detected DNA adducts whose approximate molecular weight matched that expected for the cross-linked DNA adducts (Figure 5, lane 3). Increasing the amount of $Na_2S_2O_4$ by 2 so that the number of equivalents of $Na_2S_2O_4$ were the same as the mitomycin subunits led to increased levels of DNA interstrand cross-linked products at lower **4** concentrations (i.e., 0.025 mM) (Figure 5, lane 6).

Figure 4 shows the effect of aryl substituents within the linkers on DNA cross-linking efficiency. Only **11** exhibited appreciable levels of DNA products that corresponded to ISC (Figure 4, lane 6). Dimeric mitomycin **11**, like **4**−**8**, contained an *alkyl* substituent attached to the mitomycin C(7) amino moiety.

The second dimeric series included mitomycins tethered at the N(1a) site (**12**−**15**). We observed no DNA cross-linked adducts after $Na_2S_2O_4$ treatment (data not shown; Figure 7 of Supporting Information).

The detection of mitomycin DNA ISC with **4**−**8** and **11** does not, by itself, signify that DNA modification proceeded at the two distal C(1) sites in the dimers. ISC formation may have proceeded at the mitomycin C(1) and C(10) sites. To learn if cross-linking reactions can proceed at only the C(1) sites, we prepared 7-*N*,7′-*N*′-(1,7-heptanediyl)di-*C*(10)-decarbamoylmitomycin (**22**).



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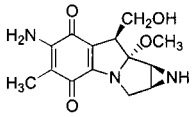

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Compound **22**, unlike **4**, can only cross-link DNA at the two C(1) sites. Dimeric mitomycin **22** provides a stringent test because $Na_2S_2O_4$-mediated C(10)-decarbamoylmitomycin C[30] (**23**)−DNA C(1) monoadduction processes occur with lower efficiency than the corresponding reaction with mitomycin C.[9,10] Accordingly, we varied the concentrations of **22** (0.1−0.45 mM), increased the number of equivalents of $Na_2S_2O_4$ to 2, and elevated the reaction temperature (22 °C) in an effort to promote ISC. We found that **22** generated product(s) that corresponded to DNA ISC but that the efficiency of these reactions was considerably less than that observed with **4** (data not shown; Figure 8 of Supporting Information). These findings indicated that the two distal mitomycin C(1) sites were sufficient for the generation of DNA ISC.

3.3. In Vitro Cytotoxic Activities. The anticancer activities of dimeric mitomycins **5**−**15** in select human tumor cell lines were determined and are summarized in Table 1. Results for mitomycin C (**1**) and mitomycin

Table 1. In Vitro Anticancer Activity Test Against Cultured Human Tumor Cell Lines for Compounds **5**−**15**

compd	tumor cell growth IC_{50} [a] (μg/mL)		
	A549[b]	KB	U-87MG
5	>1 (48)[c]	0.98	>1 (46)
6	>1 (31)	>1 (32)	>1 (12)
7	>10 (43)	*d*	>10 (22)
8	>10 (40)	>1 (8)	>1 (8)
9	0.062	0.064	0.32
10	0.095	0.215	0.51
11	>1 (24)	>1 (21)	>1 (15)
12	0.67	>1 (46)	>1 (35)
13	0.67	>1 (44)	>1 (38)
14	0.76	>1 (39)	>1 (31)
15	0.68	>1 (41)	>1 (30)
mitomycin A (**16**)	0.006	0.008	0.009
mitomycin C (**1**)	0.08	0.10	0.97

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%. [b] Tumor origin and description of cell lines are in the Experimental Section. [c] If the test compound did not inhibit less than or equal to 50% of the tumor cell replication at the highest test concentration used, then the percent inhibition observed is given in the parenthesis. [d] Not determined.

A (**16**) were compatible with previous reports using continuous exposures of 1−3 days.[31,32] We found that dimers **5**−**8** tethered at the mitomycin C(7) nitrogen site and containing alkyl linkers with a central heteroatom (NH, O) displayed lower activities against A549 (lung), KB (epidermoid), and U-87MG (glioblastoma) tumor cell lines than did **1**; the biggest differences were seen in the A549 and KB tumor cell lines. Within these dimeric mitomycins, the two compounds that contained an amino moiety in the linker (**5**, **6**) displayed enhanced activity compared with the dimers with an internal ether unit (**7**, **8**). Correspondingly, considerable cytotoxicity was observed for two of the three dimers with an aromatic unit (or units) within the linker. Compounds **9** and **10** exhibited anticancer activity comparable to **1** in the A549 and KB cell lines and enhanced activity, compared with **1**, in the U87-MG cell line. Mitomycin **11**, however, displayed decreased activity, compared with **1**, across all three cell lines. Finally, we observed that the N(1a) acyl dimeric mitomycins **12**−**15** exhibited minimal anticancer activity when compared with mitomycin C (**1**). These results showed that dimeric mitomycins tethered at the mitomycin C(7) sites that contained an C(7) *aryl*amino unit were more cytotoxic in in vitro tests than their C(7) *alkyl*amino counterparts.

The excellent activities observed for **9** and **10** led us to retest these two compounds and to expand the human tumor cell line panel to include MCF-7 (breast), HCT-8 (ileocecal), PC-3 (prostate), and HepG2 (hepatocellular). In this battery of tests we included 7-*N*-phenylmitomycin C[29] (**20**) as a reference compound because both **9** and **10** contained mitomycin C(7) *aryl*amino units. The anticancer test data are listed in Table 2. We found that dimeric mitomycins **9** and **10** and monomeric mitomycin **20** all displayed similar activities (mean IC_{50}'s of 0.11, 0.20, and 0.09 μg/mL, respectively). The activities of **9**, **10**, and **20** were typically found to be between the activities of **1** and **16** (mean IC_{50}'s of 0.63 and 0.01 μg/mL, respectively). The similar profiles for these compounds suggested that they functioned by similar pathways.

Discussion

In this study, we asked whether dimeric mitomycins could efficiently cross-link DNA and whether these adducts exhibit improved anticancer activities compared with mitomycin C. We examined dimeric mitomycins containing alkyl, alkylamino, alkyl ether, alkylaryl, arylalkyl, and acylalkyl linkers. Together, these dimeric mitomycins permitted us to vary the distance, lipophilicity, and basicity of the linker and to determine the potential role of this unit in biochemical processes.

Two classes of compounds (series A and B) were prepared. In series A, we attached the two mitomycins at the mitomycin C(7) amino substituent. In B, the two mitomycin units were connected by tethering at the mitomycin aziridine N(1a) position.

We learned that 7-*N*,7′-*N*′-(1,7-heptanediyl)dimitomycin (**4**) produced DNA ISC at concentrations that were 3-fold to 10-fold lower than mitomycin C (Figures 5 and 6) and that the efficiency of this process was modulated by the introduction of a substituent within the linker (Figures 2-4). We showed that strategic placement of nitrogen, oxygen, and aromatic units within the C(7) amino linker led to increased levels of DNA ISC compared with **1** and that the nitrogen substituent was the most effective in promoting DNA ISC. Inspection of the data set provided additional information concerning the effect of structure on DNA adduction. Enhanced levels of DNA interstrand modification occurred when the linker spanned seven (**6**, **8**) rather than five atoms (**5**, **7**) (Figure 3). Attachment of an aromatic unit to the mitomycin C(7) amino substituent led to dimeric mitomycins (**9**, **10**) that gave trace levels of DNA ISC under the test conditions (Figure 4). Finally, we saw that linking the two mitomycins through the aziridine N(1a) position led to dimeric mitomycins (**12**−**15**) that were ineffective in generating DNA ISC upon $Na_2S_2O_4$ treatment.

Together, our findings provide preliminary support for the concept that dimeric mitomycins can serve as DNA interstrand cross-linking agents. We have demonstrated that select dimeric mitomycins (**4**−**8**, **11**) are

Table 2. In Vitro Anticancer Activity Test Against Cultured Human Tumor Cell Lines for Compounds **9**, **10**, and **20**

compd	tumor cell growth IC_{50} [a] (μg/mL)							
	U-87MG[b]	KB	A549	MCF-7	HCT-8	PC-3	hepG2	mean IC_{50}[c]
9	0.22 ± 0.09	0.13 ± 0.06	0.06 ± 0.01	0.14	0.06	0.08	0.05	0.11 ± 0.06
10	0.42 ± 0.13	0.20 ± 0.01	0.08 ± 0.01	0.21	0.11	0.20	0.16	0.20 ± 0.10
20	0.26 ± 0.04	0.13	0.02 ± 0.01	0.06	0.004	0.11	0.05	0.09 ± 0.09
1	1.57 ± 0.59	0.17 ± 0.09	0.14 ± 0.05	0.45	0.15	1.55	0.36	0.63 ± 0.59
16	0.008 ± 0.001	0.008	0.006 ± 0.003	0.045	0.006	0.020	0.008	0.014 ± 0.013

[a] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [b] Tumor origin and description of cell lines are in the Experimental Section. [c] Mean IC_{50} ± standard deviation (n = 12) for all seven human tumor cell lines.

more efficient than mitomycin C in cross-linking complementary DNA strands but that the DNA cross-linking efficiency depends on the nature (length, substitution, basicity, lipophilicity) of the linker and the manner in which the mitomycins are tethered (C(7) vs N(1a)). Our finding that $Na_2S_2O_4$ activation of 7-*N*,7-*N*′-(1,7-heptanediyl)-*C*(10)-decarbamoyldimitomycin (**22**) led to DNA ISC provided support for the notion that DNA modification proceeded, in part, at the two distal C(1) sites.

The cytotoxic activities of the dimeric mitomycins were determined using human cultured tumor cell lines. The results for **5**−**15** provided clear trends. We found that only those dimeric mitomycins (**9**, **10**) that contained an aryl unit appended directly to the mitomycin C(7) amino substituent displayed activity equal to or greater than **1**. Dimeric mitomycins (**5**−**8**, **11**) appended at the C(7) amino site by an alkyl, alkyl ether, alkylamino, and alkylaryl linker and dimeric mitomycins joined at the N(1a) sites (**12**−**15**) were less active than **1**. Several factors likely account for the observed anticancer activities (Tables 1 and 2). First, only dimeric mitomycins **9** and **10** displayed potent cytotoxicities. The cytotoxic activities of these compounds mirrored that of **20** and were between the cytotoxic activities of **1** and **16**. Because monomeric mitomycins **1**, **16**, and **20** are all likely to express activity, in part, by cross-linking DNA through their C(1) and C(10) sites, we assume that comparable processes were occurring with **9** and **10**. Second, improved activities in the in vitro test were observed for mitomycins (**9**, **10**, **16**, **20**) that displayed or were likely to display lower quinone reduction potentials than **1** (for **1**, $E_{1/2} = -0.45$ V; for **16**, $E_{1/2} = -0.21$ V; for **20**, $E_{1/2} = -0.31$ V).[23] We suspect that this is the dominant reason for their excellent cytotoxicities. Third, introduction of an acyl unit at the aziridine N(1a) site (**12**−**15**) led to loss of drug activity compared with **1**. We hypothesize that this is due to the decreased basicity of the aziridine nitrogen in these compounds. Protonation of this site is considered to be an important prerequisite for C(1) site activation and DNA adduction.[4] Most surprising to us was the poor cytotoxicities of dimeric mitomycins (**5**−**8**, **11**) containing C(7) amino alkyl units. These compounds were expected to undergo quinone reduction and mitomycin activation as efficiently as **1**. We are uncertain whether to attribute this loss of cytotoxic activity to the linkers because the lipophilicity (hydrophilicity) of the bridging units varied widely within this series. Previous studies have shown that mitomycin C(7) amino substitution can lead to both improved and reduced IC_{50} values in in vitro assays.[33] We observed that the in vitro cytotoxicities for **5**−**8** differed considerably from the cytotoxicities reported by Kono and co-workers[18] for dimeric mitomycins containing a labile disulfide linker. These dimeric mitomycin disulfides showed in vitro cytotoxic activity against HeLa-S_3 cells equal to or better than **1**. The linker size in the dimeric mitomycins with disulfide units were comparable to **5**−**8**, suggesting that alternative pathways for mitomycin function exist for the dimeric mitomycin disulfides compared to **5**−**8**.

An important finding of this study was the poor correlation between the two bioactivity assays. We found that the compounds that gave the highest levels of DNA cross-linked adducts (**5**−**8**, **11**) were among the weakest cytotoxic agents, while compounds that displayed potent cytotoxicities in in vitro tests (**9**, **10**) were unable to cross-link complementary DNA strands under the test conditions. We are left to conclude that reductive activation coupled with the denaturing-gel-electrophoresis-based assay is not a predictor of cytotoxic activity. It is certainly possible that the ease of enzymatic reductive activation correlates with the cytotoxic profile or that DNA adduction is just one of many parameters that govern mitomycin efficacy. Evaluation of these possibilities awaits in-depth mechanistic studies with selected compounds.

Conclusions

We have shown that dimeric mitomycins can cross-link DNA. Although the structure of the DNA ISC has yet to be determined in terms of the site of mitomycin covalent attachment and DNA sequence selectivity, our results have shown that the extent of DNA cross-link formation can exceed that of **1**, that the reactions proceed, in part, at the distal C(1) positions, and that the efficiency of this process depends on the nature of the linker and the position of attachment of the linker unit. Although most of the compounds studied are less cytotoxic than **1**, we expect that additional structural elaboration of the linker unit may provide dimeric mitomycins with improved DNA cross-linking efficiency and/or improved antitumor potential.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were taken on General Electric QE 300 MHz and Bruker AMX 600 MHz NMR instruments. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane, and coupling constants (J values) are in hertz. Low-resolution and high-resolution (CI) mass spectral investigations were conducted at the University of Texas at Austin by Dr. M. Moini. The low-resolution mass studies were run on a Finnegan MAT-TSQ-70 instrument, and the high-resolution mass studies were conducted on a Micromass ZAB-E spectrometer tuned to a resolution of 10 000 (10% valley definition). pH measurements were determined on a Radiometer pHM26 meter using a Radiometer G202 glass electrode.

HPLC analyses were conducted with the following Waters Associate Units: 515 A pump, 515 B pump, Millennium chromatography manager, Waters 996 photodiode array detector, Rheodyne 7725i manual injector. The column was fitted with a μBondapak Guardpak precolumn. The product analyses were conducted with a C_{18} μBondapak (stainless steel) column (3.9 mm × 300 mm) using the following linear gradient condition: 90% A (aqueous 0.025 M triethylammonium acetate, pH 6.5), 10% B (acetonitrile) isocratic for 5 min, then from 90% A and 10% B to 45% A and 55% B in 30 min. The flow rate was 1 mL/min, and the eluent was monitored from 200 to 400 nm. The HPLC solvents were filtered (aqueous solution with Millipore HVLP, 0.45 mm; acetonitrile with Millipore HV, 0.45 mm) and degassed before utilization. Thin-layer chromatography was run on general purpose silica gel plates (20 cm × 20 cm; Aldrich no. Z12272-6). Deionized water was obtained with a Milli-Q (18 MΩs) water system (Millipore). The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. Tetrahydrofuran was distilled from Na metal and benzophenone. The Ar used was O_2-free (O_2 < 5 ppm). UV−visible spectra were obtained using a Cary-3Bio UV−visible spectrophotometer. *Eco*RI enzyme and pBR322 plasmid DNA were purchased from New England Biolab, Inc.

General Method for the Synthesis of Dimitomycins 4 and 22. To a methanolic solution of **16** was added the requisite diamine. The reaction solution was stirred at room tempera-

ture (6−12 h), the solvent was removed under reduced pressure, and the residue was purified by PTLC (10% MeOH−$CHCl_3$).

The following compounds were prepared by using this procedure.

7-*N*,7′-*N*′-(1,7-Heptanediyl)dimitomycin C (4).[16b] Using **16** (6 mg, 17 μmol) and 1,7-diaminoheptane (1.1 mg, 8.5 μmol) in MeOH (0.3 mL) gave **4** as a gray solid (5 mg, 75%): HPLC t_R, 30.3 min; R_f 0.23 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 220, 368 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.35−1.39 (m, C(3′)H_2, C(4′)H), 1.61 (quint, J = 6.3 Hz, C(2′)H_2), 2.02 (s, C(6)-CH_3), 2.82 (d, J = 3.9 Hz, C(2)H), 2.90 (d, J = 3.9 Hz, C(1)H), 3.21 (s, C(9a)OCH_3), 3.52 (d, J = 12.9 Hz, C(3)HH′), 3.53 (merged with C(3)HH′, C(1′)H_2), 3.62 (dd, J = 4.5, 10.5 Hz, C(9)H), 4.30 (d, J = 12.9 Hz, C(3)HH′), 4.48 (dd, J = 10.5, 10.5 Hz, C(10)HH′), 4.70 (dd, J = 4.5, 10.5 Hz, C(10)HH′), 6.35 (t, J = 6.0 Hz, C(7)NH), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.0 (C(6)CH$_3$), 26.7 (C(3′)), 29.1 (C(4′)), 30.9 (C(2′)), 32.8 (C(2)), 36.7 (C(1)), 42.9 (C(9)), 45.2 (C(1′)), 50.0 (C(9a)OCH$_3$), 50.1 (C(3)), 62.7 (C(10)), 103.6 (C(6)), 106.3 (C(9a)), 109.7 (C(8a)), 147.4 (C(7)), 156.1 (C(5a)), 156.7 (C(10a)), 176.2 (C(8)), 178.9 (C(5)); MS (+CI, methane) m/e 763 [M − 1]$^+$; M_r (+CI, methane) 763.340 65 (M − 1)$^+$ (calcd for $C_{37}H_{47}N_8O_{10}$ 763.341 52).

7-*N*,7′-*N*′-(1,7-Heptanediyl)di-*C*(10)-decarbamoylmitomycin C (22). Using *C*(10)-decarbamoylmitomycin A[34] (1.8 mg, 5.9 μmol) and 1,7-diaminoheptane (0.4 mg, 3.0 μmol) in MeOH (0.15 mL) gave **22** as a gray solid (5 mg, 75%): HPLC t_R, 30.8 min; R_f 0.36 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 221, 369 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32−1.40 (br s, C(3′)H_2, C(4′)H), 1.62 (quint, J = 6.3 Hz, C(2′)H_2), 2.04 (s, C(6)CH_3), 2.87 (s, C(2)H, C(1)H), 3.21 (s, C(9a)OCH_3), 3.39 (t, J = 5.4 Hz, C(9)H), 3.50−3.59 (m, C(3)HH′, C(1′)H_2), 4.01 (d, J = 5.4 Hz, C(10)H_2), 4.30 (d, J = 12.9 Hz, C(3)HH′), 6.38 (t, J = 6.0 Hz, C(7)NH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 10.0 (C(6)-CH$_3$), 26.8 (C(3′)), 29.1 (C(4′)), 30.9 (C(2′)), 33.8 (C(2)), 36.1 (C(1)), 45.2 (C(1′)), 46.4 (C(9)), 50.0 (C(9a)OCH$_3$), 50.1 (C(3)), 62.5 (C(10)), 103.6 (C(6)), 107.2 (C(9a)), 113.3 (C(8a)), 147.5 (C(7)), 156.3 (C(5a)), 177.3 (C(8)), 178.9 (C(5)); MS (+CI, methane) m/e 677 [M − 1]$^+$; M_r (+CI, methane) 677.328 54 (M − 1)$^+$ (calcd for $C_{35}H_{45}N_6O_8$ 677.329 89).

7-*N*,7′-*N*′-(Iminobis(2,1-ethanediyl))bismitomycin C (5). To an anhydrous pyridine solution (0.4 mL) of **16** (6.9 mg, 20 μmol) was added diethylenetriamine (1.1 μL, 10 μmol, 0.5 equiv) in pyridine (0.1 mL) under Ar, and the reaction solution was stirred overnight at room temperature. The reaction solution was applied to a neutral alumina pipet column (14.5 cm) and then eluted first with pyridine and then with 1% MeOH−pyridine. The initial eluted product was collected, and the solvent was removed in vacuo to give **5** as a blue-gray solid (2.9 mg, 40%): HPLC t_R, 23.7 min; UV−vis (MeOH) λ_{max} 220, 369 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.17 (s, C(6)CH_3), 2.74 (br d, J = 4.2 Hz, C(2)H), 3.13 (d, J = 4.2 Hz, C(1)H), 3.22 (s, C(9a)OCH_3), 3.30 (t, J = 5.7 Hz, C(2′)H_2), 3.59 (dd, J = 1.5, 12.9 Hz, C(3)HH′), 4.05 (dt, J = 5.7, 6.3 Hz, C(1′)H_2), 3.96 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.50 (d, J = 12.9 Hz, C(3)-HH′), 5.02 (dd, J = 10.5, 11.1 Hz, C(10)HH′), 5.36 (dd, J = 4.5, 10.5 Hz, C(10)HH′), 7.42 (t, J = 6.3 Hz, C(7)NH), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) δ 10.1 (C(6)CH$_3$), 32.5 (C(2)), 36.7 (C(1)), 44.1 (C(9)), 44.5 (C(1′)), 49.3 (C(2′)), 49.5 (C(9a)-OCH$_3$), 50.6 (C(3)), 62.4 (C(10)), 103.4 (C(6)), 106.8 (C(9a)), 110.5 (C(8a)), 147.6 (C(7)), 156.2 (C(5a)), 158.3 (C(10a)), 176.7 (C(8)), 178.9 (C(5)); MS (+ES) m/e 720.7 [M − 17]$^+$ (100%), 738.4 [M + 1]$^+$ (80%).

Synthesis of 7-*N*,7′-*N*′-(Iminobis(3,1-propanediyl))bismitomycin C (6) and 7-*N*-(3′-(3-Aminopropyl)aminopropyl)mitomycin C (17). To a CH_2Cl_2 solution (1.5 mL) of **16** (6.3 mg, 18 μmol) was added a CH_2Cl_2 solution of *N*-(3-aminopropyl)-1,3-propanediamine (1.1 μL, 9 μmol, 0.5 equiv) in CH_2Cl_2 (1 mL). The reaction solution was stirred at room temperature (20 h), and then the solvent was reduced under low pressure. The residue was purified using PTLC (20% MeOH−$CHCl_3$) to give **6** and **17** as blue-gray solids.

Compound 6:[16b] yield, 3.6 mg, 52%; HPLC t_R, 23.9 min; R_f 0.15 (50% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 220, 368 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.75 (quint, J = 6.3 Hz, C(2′)H_2), 2.16 (s, C(6)CH_3), 2.65 (t, J = 6.3 Hz, C(3′)H_2), 2.74 (br s, C(2)H), 3.14 (br s, C(1)H), 3.23 (s, C(9a)OCH_3), 3.61 (d, J = 12.6 Hz, C(3)HH′), 3.68 (dt, J = 6.0, 6.3 Hz, C(1′)H_2), 4.02 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.58 (d, J = 12.6 Hz, C(3)HH′), 5.10 (dd, J = 10.8, 11.1 Hz, C(10)HH′), 5.40 (dd, J = 4.5, 10.8 Hz, C(10)HH′), 7.47 (t, J = 6.0 Hz, C(7)NH), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) δ 10.5 (C(6)CH$_3$), 31.4 (C(2′)), 33.1 (C(2)), 37.2 (C(1)), 44.5 (C(9)), 44.7 (C(1′)), 48.3 (C(3′)), 50.1 (C(9a)OCH$_3$), 51.1 (C(3)), 63.0 (C(10)), 103.8 (C(6)), 107.3 (C(9a)), 111.0 (C(8a)), 148.3 (C(7)), 156.7 (C(5a)), 158.6 (C(10a)), 177.3 (C(8)), 179.3 (C(5)); MS (+CI, methane) m/e 766 [M + 1]$^+$; M_r (+CI, methane) 766.350 63 (M + 1)$^+$ (calcd for $C_{36}H_{48}N_9O_{10}$ 766.352 41).

Compound 17: yield, 3.9 mg, 47%; HPLC t_R, 21.3 min; R_f 0.05 (50% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 220, 368 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.64−1.67 (m, C(5′)H_2), 1.73−1.76 (m, C(2′)H_2), 2.18 (s, C(6)CH_3), 2.57−2.66 (m, C(4′)-H_2), 2.67−2.71 (m, C(3′)H_2), 2.75 (br s, C(2)H), 2.95−3.03 (m, C(6′)H_2), 3.16 (br s, C(1)H), 3.22 (s, C(9a)OCH_3), 3.60−3.64 (m, C(3)HH′, C(1′)H_2), 4.01 (dd, J = 4.2, 10.5 Hz, C(9)H), 4.60 (d, J = 12.6 Hz, C(3)HH′), 5.14 (dd, J = 10.5, 10.5 Hz, C(10)HH′), 5.37 (dd, J = 4.2, 10.5 Hz, C(10)HH′), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 150 MHz) δ 10.5 (C(6)CH$_3$), 27.3 (C(5′) or C(2′)), 27.7 (C(2′) or C(5′)), 32.8 (C(2)), 36.9 (C(1)), 44.5 (C(6′)), 45.3 (C(1′), C(9)), 49.8 (C(9a)OCH$_3$), 50.8 (C(4′) or C(3′)), 50.9 (C(3′) or C(4′)), 51.0 (C(3)), 62.7 (C(10)), 107.0 (C(6)), 107.9 (C(9a)), 110.7 (C(8a)), 148.3 (C(7)), 156.6 (C(5a)), 158.3 (C(10a)), 177.2 (C(8)), 179.0 (C(5)); MS (+CI, methane) m/e 448 [M]$^+$; M_r (+CI, methane) 448.243 65 (M)$^+$ (calcd for $C_{21}H_{32}N_6O_5$ 448.243 42).

General Method for the Synthesis of Dimitomycins 7 and 8. To a methanolic solution of **16** (1 equiv, 28−32 mM) was added a methanolic solution of the requisite diamine (0.5 equiv). The reaction solution was stirred at room temperature (4−20 h). The solvent was concentrated under reduced pressure, and then the residue was purified using PTLC (10% MeOH−$CHCl_3$). In the case of compound **7**, 7.5 equiv of TEA was used.

The following compounds were prepared by using this procedure.

7-*N*,7′-*N*′-(Oxybis(2,1-ethanediyl))bismitomycin C (7). Using **16** (5 mg, 14 μmol), oxybis(ethylamine)·2HCl (1.3 mg, 7 μmol, 0.5 equiv), and TEA (15 μL, 105 μmol) in MeOH (0.5 mL) gave **7** (20 h) as a blue-gray solid (4.7 mg, 88%): HPLC t_R, 25.5 min; R_f 0.47 (30% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 220, 368 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.00 (s, C(6)-CH_3), 2.81 (d, J = 4.2 Hz, C(2)H), 2.89 (d, J = 4.2 Hz, C(1)H), 3.20 (s, C(9a)OCH_3), 3.52 (d, J = 12.9 Hz, C(3)HH′), 3.60 (dd, J = 4.5, 10.2 Hz, C(9)H), 3.64 (t, J = 6.0 Hz, C(2′)H_2), 3.78 (dt, J = 6.0, 6.0 Hz, C(1′)H_2), 4.27 (d, J = 12.9 Hz, C(3)HH′), 4.49 (dd, J = 10.2, 10.5 Hz, C(10)HH′), 4.68 (dd, J = 4.5, 10.5 Hz, C(10)HH′), 6.52 (t, J = 6.0 Hz, C(7)NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.1 (C(6)CH$_3$), 32.9 (C(2)), 36.7 (C(1)), 43.0 (C(9)), 44.9 (C(1′)), 50.0 (C(9a)OCH$_3$), 50.1 (C(3)), 62.8 (C(10)), 70.3 (C(2′)), 104.5 (C(6)), 106.4 (C(9a)), 110.3 (C(8a)), 147.4 (C(7)), 155.7 (C(5a)), 156.8 (C(10a)), 176.3 (C(8)), 179.2 (C(5)); MS (+ES) m/e 739.1 [M + 1]$^+$ (100%).

7-*N*,7′-*N*′-(Oxybis(3,1-propanediyl))bismitomycin C (8). Using **16** (5.5 mg, 16 μmol) and bisaminopropyl ether (1.1 μL, 8 μmol, 0.5 equiv) in MeOH (0.5 mL) gave **8** (4 h) as a blue-gray solid (4.8 mg, 80%): HPLC t_R, 26.3 min; R_f 0.23 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 221, 368 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.92 (quint, J = 6.0 Hz, C(2′)H_2), 2.03 (s, C(6)CH_3), 2.80 (d, J = 4.2 Hz, C(2)H), 2.90 (d, J = 4.2 Hz, C(1)H), 3.20 (s, C(9a)OCH_3), 3.51 (d, J = 12.9 Hz, C(3)HH′), 3.61 (dd, J = 4.2, 10.5 Hz, C(9)H), 3.54 (t, J = 6.0 Hz, C(3′)-H_2), 3.75 (dt, J = 6.0, 6.0 Hz, C(1′)H_2), 4.30 (d, J = 12.9 Hz, C(3)HH′), 4.46 (dd, J = 10.5, 10.5 Hz, C(10)HH′), 4.69 (dd, J = 4.2, 10.5 Hz, C(10)HH′), 6.87 (t, J = 6.0 Hz, C(7)NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.0 (C(6)CH$_3$), 30.7 (C(2′)), 32.8

(C(2)), 36.8 (C(1)), 43.0 (C(9)), 43.6 (C(1′)), 50.0 (C(9a)O*C*H$_3$), 50.1 (C(3)), 62.8 (C(10)), 69.5 (C(3′)), 103.4 (C(6)), 106.5 (C(9a)), 111.0 (C(8a)), 147.7 (C(7)), 156.0 (C(5a)), 156.8 (C(10a)), 176.4 (C(8)), 178.8 (C(5)); MS (+CI, methane) *m/e* 767 [M + 1]$^+$; M_r (+CI, methane) 767.335 94 (M + 1)$^+$ (calcd for $C_{36}H_{47}N_8O_{11}$ 767.336 43).

General Method for the Synthesis of Dimitomycins 9−11. To a methanolic solution of **16** (1 equiv, 28−40 mM) was added a methanolic solution of the requisite diamine (0.5−1.0 equiv), and the reaction solution was stirred at room temperature (1−2 days). The solvent was reduced under low pressure, and then the residue was purified using PTLC (10% MeOH−CHCl$_3$).

The following compounds were prepared by using this procedure.

7-*N*,7′-*N*′-(Oxybis(1,4-phenylenyl))bismitomycin C (9) and 7-*N*-[*p*-(4-Aminophenoxy)phenyl]mitomycin C (18). Using **16** (6 mg, 17 μmol) and 4,4′-oxydianiline (2.8 mg, 14 μmol, 0.8 equiv) in MeOH (0. 5 mL) gave **9** and **18** (2 days) as solids.

Compound 9: yield, 1.5 mg, 21%; HPLC t_R, 33.1 min; R_f 0.18 (10% MeOH−CHCl$_3$); UV−vis (MeOH) λ_{max} 213, 264, 380 nm; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.49 (s, C(6)CH$_3$), 2.86 (dd, J = 1.5, 4.5 Hz, C(2)H), 2.94 (d, J = 4.5 Hz, C(1)H), 3.25 (s, C(9a)OCH$_3$), 3.54 (dd, J = 1.5, 12.9 Hz, C(3)*H*H′), 3.70 (dd, J = 4.5, 10.5 Hz, C(9)H), 4.26 (d, J = 12.9 Hz, C(3)H*H*′), 4.59 (dd, J = 10.5, 10.8 Hz, C(10)*H*H′), 4.76 (dd, J = 4.5, 10.8 Hz, C(10)H*H*′), 6.96 (d, J = 9.0 Hz, 2 ArC(3′)H), 7.00 (d, J = 9.0 Hz, 2 ArC(2′)H), 7.71 (s, C(7)NH); ^{13}C NMR (CDCl$_3$, 150 MHz) δ 12.1 (C(6)*C*H$_3$), 32.6 (C(2)), 36.5 (C(1)), 43.0 (C(9)), 49.8 (C(3), C(9a)O*C*H$_3$), 62.6 (C(10)), 106.2 (C(6)), 108.5 (C(9a)), 110.7 (C(8a)), 118.9 (2 Ar(C(3′)), 125.3 (2 Ar(C(2′)), 134.8 (ArC(1′)), 143.6 (C(7)), 154.3 (C(5a)), 155.0 (ArC(4′)), 156.4 (C(10a)), 176.8 (C(8)), 180.2 (C(5)); MS (+CI, methane) *m/e* 834 [M]$^+$; M_r (+CI, methane) 834.298 89 (M)$^+$ (calcd for $C_{42}H_{42}N_8O_{11}$ 834.297 31).

Compound 18: yield, 5.5 mg, 62%; HPLC t_R, 30.6 min; R_f 0.31 (10% MeOH−CHCl$_3$); UV−vis (MeOH) λ_{max} 258, 378 nm; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.46 (s, C(6)CH$_3$), 2.85 (dd, J = 1.5, 4.5 Hz, C(2)H), 2.93 (d, J = 4.5 Hz, C(1)H), 3.25 (s, C(9a)-OCH$_3$), 3.51 (dd, J = 1.5, 12.9 Hz, C(3)*H*H′), 3.67 (dd, J = 4.5, 10.8 Hz, C(9)H), 4.26 (d, J = 12.9 Hz, C(3)H*H*′), 4.57 (dd, J = 10.8, 10.8 Hz, C(10)*H*H′), 4.75 (dd, J = 4.5, 10.8 Hz, C(10)-H*H*′), 6.69 (d, J = 8.7 Hz, 2 ArC(2″)H), 6.87 (d, J = 7.2 Hz, 2 ArC(2′)H), 6.88 (d, J = 7.2 Hz, 2 ArC(3′)H), 6.94 (d, J = 8.7 Hz, 2 ArC(3″)H), 7.72 (s, C(7)NH); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 12.6 (C(6)*C*H$_3$), 32.9 (C(2)), 36.8 (C(1)), 43.2 (C(9)), 50.0 (C(9a)O*C*H$_3$), 50.1 (C(3)), 62.8 (C(10)), 106.5 (C(6)), 108.1 (C(9a)), 110.7 (C(8a)), 116.5 (2 ArC(3′)), 117.5 (2 ArC(2′)), 121.3 (2 ArC(2″)), 125.6 (ArC(3″)), 133.7 (ArC(1′)), 143.1 (ArC(1″)), 144.0 (C(7)), 148.7 (ArC(4″)), 155.3 (C(5a)), 156.6 (ArC(4′)), 156.7 (C(10a)), 177.1 (C(8)), 180.5 (C(5)); MS (+CI, methane) *m/e* 518 [M + 1]$^+$; M_r (+CI, methane) 518.202 94 (M + 1)$^+$ (calcd for $C_{27}H_{28}N_5O_6$ 518.203 96).

7-*N*,7′-*N*′-(Ethylenebis(1,4-phenylenyl))bismitomycin C (10) and 7-*N*-[*p*-(2-(4-Aminophenyl)ethyl)phenyl]mitomycin C (19). Using **16** (8.3 mg, 24 μmol) and 4,4′-ethylenedianiline (5 mg, 24 μmol, 1 equiv) in MeOH (0.6 mL) gave **10** and **19** (16 h) as green solids.

Compound 10: yield, 4 mg, 40%; HPLC t_R, 35.0 min; R_f 0.27 (10% MeOH−CHCl$_3$); UV−vis (MeOH) λ_{max} 215, 262, 369 nm; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.44 (s, C(6)CH$_3$), 2.86 (dd, J = 1.5, 4.5 Hz, C(2)H), 2.89 (s, 2 ArC(4′)CH$_2$), 2.93 (d, J = 4.5 Hz, C(1)H), 3.25 (s, C(9a)OCH$_3$), 3.54 (dd, J = 1.5, 12.9 Hz, C(3)*H*H′), 3.68 (dd, J = 4.5, 10.5 Hz, C(9)H), 4.26 (d, J = 12.9 Hz, C(3)H*H*′), 4.56 (dd, J = 10.5, 10.5 Hz, C(10)*H*H′), 4.74 (dd, J = 4.5, 10.5 Hz, C(10)H*H*′), 6.89 (d, J = 8.1 Hz, 2 ArC-(2′)H), 7.04 (d, J = 8.1 Hz, 2 ArC(3′)H), 7.72 (s, C(7)NH), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (CDCl$_3$, 75 MHz) δ 12.4 (C(6)*C*H$_3$), 32.9 (C(2)), 36.8 (C(1)), 37.5 (ArC(4′)*C*H$_2$), 43.2 (C(9)), 50.0 (C(9a)O*C*H$_3$), 50.1 (C(3)), 62.8 (C(10)), 106.5 (C(6)), 108.7 (C(9a)), 110.9 (C(8a)), 124.0 (2 ArC(2′)), 129.1 (2 ArC(3′)), 137.5 (ArC(4′)), 138.5 (ArC(1′)), 143.8 (C(7)), 155.3 (C(5a)), 156.7 (C(10a)), 177.2 (C(8)), 180.6 (C(5)); MS (+CI, methane) *m/e* 863 [M + 17]$^+$; M_r (+CI, methane) 863.334 16 (M + 1)$^+$ (calcd for $C_{44}H_{47}N_8O_{11}$ 863.336 43); MS (+ES) *m/e* 869 [M + Na]$^+$ (100%).

Compound 19: yield, 6.8 mg, 54%; HPLC t_R, 33.3 min; R_f 0.41 (10% MeOH−CHCl$_3$); UV−vis (MeOH) λ_{max} 237, 257, 380 nm; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 1.39 (s, C(6)CH$_3$), 2.78 (t, J = 6.3 Hz, C(4″)CH$_2$ or C(4′)CH$_2$), 2.80 (t, J = 6.3 Hz, C(4′)-CH$_2$ or C(4″)CH$_2$), 2.82 (br d, J = 4.5 Hz, C(2)H), 2.97 (br s, C(1)H), 3.25 (s, C(9a)OCH$_3$), 3.50 (dd, J = 1.5, 12.9 Hz, C(3)-*H*H′), 3.65 (dd, J = 4.5, 10.5 Hz, C(9)H), 4.16 (d, J = 12.9 Hz, C(3)H*H*′), 4.34 (dd, J = 10.5, 10.5 Hz, C(10)*H*H′), 4.75 (dd, J = 4.5, 10.5 Hz, C(10)H*H*′), 6.63 (d, J = 8.4 Hz, 2 ArC(2″)H), 6.86 (d, J = 8.4 Hz, 2 ArC(3″)H), 6.92 (d, J = 8.4 Hz, 2 ArC-(3′)H), 7.09 (d, J = 8.4 Hz, 2 ArC(2′)H); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 12.6 (C(6)*C*H$_3$), 33.7 (C(2)), 37.8 (C(1)), 38.4 (C(4″)-*C*H$_2$), 39.1 (C(4′)*C*H$_2$), 44.6 (C(9)), 50.3 (C(9a)O*C*H$_3$), 51.0 (C(3)), 63.3 (C(10)), 106.0 (C(6)), 109.3 (C(9a)), 111.6 (C(8a)), 117.0 (2 ArC(2″)), 125.0 (2 ArC(2′)), 130.1 (2 ArC(3′)), 130.3 (2 ArC(3″)), 132.9 (ArC(4′)), 138.9 (ArC(4″)), 140.4 (ArC(1′)), 146.0 (C(7)), 146.3 (ArC(1″)), 156.6 (C(5a)), 159.7 (C(10a)), 178.4 (C(8)), 181.1 (C(5)); MS (+CI, methane) *m/e* 530 [M + 1]$^+$; M_r (+CI, methane) 530.238 00 (M + 1)$^+$ (calcd for $C_{29}H_{32}N_5O_5$ 530.240 35).

7-*N*,7′-*N*′-(*p*, *p*′-Xylylenyl)bismitomycin C (11). Using **16** (5 mg, 14 μmol) and *p*-xylylenediamine (0.95 mg, 7 μmol, 0.5 equiv) in MeOH (0.5 mL) gave **11** (16 h) as a dark-gray solid (4.5 mg, 80%): HPLC t_R, 28.6 min; UV−vis (MeOH) λ_{max} 219, 368 nm; R_f 0.34 (10% MeOH−CHCl$_3$); ^{1}H NMR (CD$_3$OD, 300 MHz) δ 1.90 (s, C(6)CH$_3$), 2.84 (d, J = 3.6 Hz, C(2)H), 2.97 (d, J = 3.6 Hz, C(1)H), 3.21 (s, C(9a)OCH$_3$), 3.46 (d, J = 12.9 Hz, C(3)*H*H′), 3.57 (dd, J = 4.2, 11.0 Hz, C(9)H), 4.14 (d, J = 12.9 Hz, C(3)H*H*′), 4.28 (dd, J = 10.5, 11.0 Hz, C(10)*H*H′), 4.66 (dd, J = 4.2, 10.5 Hz, C(10)H*H*′), 4.78 (s, C(7)N(H)CH$_2$), 7.25 (s, 2 ArH); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 9.9 (C(6)*C*H$_3$), 32.7 (C(2)), 36.7 (C(1)), 43.1 (C(9)), 48.7 (C(7)N(H)*C*H$_2$), 50.0 (C(9a)O*C*H$_3$), 50.1 (C(3)), 62.8 (C(10)), 104.6 (C(6)), 106.4 (C(9a)), 110.2 (C(8a)), 127.9 (2 ArC(2′)), 138.2 (ArC(1′)), 147.1 (C(7)), 155.7 (C(5a)), 156.6 (C(10a)), 176.3 (C(8)), 179.4 (C(5)); MS (+CI, methane) *m/e* 771 [M + 1]$^+$; M_r (+CI, methane) 771.309 19 (M + 1)$^+$ (calcd for $C_{38}H_{43}N_8O_{10}$ 771.310 22).

General Method for the Synthesis of *N*(1a),*N*′(1a)-Diacyldimitomycin C Compounds 12−15. To a suspension of **1** (14−17 mM) in dry THF were added TEA (8 equiv) and the appropriate diacyl chloride (1 equiv). The reaction mixture was stirred at room temperature (30 min), the precipitated solid was filtered, and the solvent was removed under low pressure. The residue was purified using PTLC (10% MeOH−CHCl$_3$).

The following compounds were prepared by using this procedure.

***N*(1a),*N*′(1a)-(1,4-Butanedionyl)dimitomycin C (12).** Using **1** (5.6 mg, 17 μmol), TEA (19 μL, 134 μmol, 8 equiv), and succinyl chloride (1.8 μL, 17 μmol) in dry THF (1 mL) provided **12** as a gray solid (2.2 mg, 35%): HPLC t_R, 24.5 min; R_f 0.19 (10% MeOH−CHCl$_3$); UV−vis (MeOH) λ_{max} 214, 356 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.05 (s, C(6)CH$_3$), 2.71 (d, J = 12.6 Hz, C(2′)*H*H′), 2.90 (d, J = 12.6 Hz, C(2′)H*H*′), 3.17 (s, C(9a)OCH$_3$), 3.59 (dd, J = 1.5, 12.9 Hz, C(3)*H*H′), 3.62 (dd, J = 1.5, 4.2 Hz, C(2)H), 3.83 (d, J = 4.2 Hz, C(1)H), 4.10 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.62 (dd, J = 10.8, 11.1 Hz, C(10)-*H*H′), 4.78 (d, J = 12.9 Hz, C(3)H*H*′), 5.67 (dd, J = 4.5, 10.8 Hz, C(10)H*H*′), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 150 MHz) δ 9.0 (C(6)*C*H$_3$), 31.6 (C(2′)), 40.8 (C(2)), 43.6 (C(9)), 43.9 (C(1)), 50.0 (C(9a)O*C*H$_3$), 50.1 (C(3)), 62.6 (C(10)), 105.3 (C(6)), 107.2 (C(9a)), 111.8 (C(8a)), 156.2 (C(5a)), 159.1 (C(10a)), 178.3 (C(8)), 179.4 (C(5)), 184.7 (N(1a)*C*(O)), the signal for C(7) was not detected and is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 751 [M + 1]$^+$; M_r (+CI, methane) 751.267 95 (M + 1)$^+$ (calcd for $C_{34}H_{39}N_8O_{12}$ 751.268 74).

***N*(1a),*N*′(1a)-(1,5-Pentanedionyl)dimitomycin C (13).** Using **1** (9 mg, 27 μmol), TEA (30 μL, 216 μmol, 8 equiv), and glutaryl chloride (3.4 μL, 27 μmol) in dry THF (2 mL) provided **13** as a gray solid (5 mg, 49%): HPLC t_R, 25.3 min; R_f 0.29

(10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 214, 356 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.98 (quint, J = 6.9 Hz, C(3′)H), 2.05 (s, C(6)CH_3), 2.59 (t, J = 6.9 Hz, C(2′)H_2), 3.19 (s, C(9a)-OCH_3), 3.53 (d, J = 4.8 Hz, C(2)H), 3.60 (d, J = 13.2 Hz, C(3)-*H*H′), 3.85 (d, J = 4.8 Hz, C(1)H), 4.10 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.68 (dd, J = 11.1, 11.1 Hz, C(10)*H*H′), 4.78 (d, J = 13.2 Hz, C(3)H*H*′), 5.64 (dd, J = 4.5, 11.1 Hz, C(10)H*H*′), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) δ 9.3 (C(6)*C*H_3), 20.4 (C(3′)), 35.6 (C(2′)), 40.4 (C(2)), 43.6 (C(9)), 43.7 (C(1)), 50.0 (C(9a)O*C*H_3), 50.1 (C(3)), 62.6 (C(10)), 105.0 (C(6)), 106.9 (C(9a)), 111.4 (C(8a)), 155.6 (C(5a)), 158.5 (C(10a)), 177.5 (C(8)), 178.6 (C(5)), 183.9 (N(1a)*C*(O)), the signal for C(7) was not detected and is believed to be beneath the solvent peaks; MS (+ES) *m/e* 787 $[M + Na]^+$ (100%).

***N*(1a),*N*′(1a)-(1,6-Hexanedionyl)dimitomycin C (14) and 21.** Using **1** (11 mg, 33 μmol), TEA (37 μL, 263 μmol, 8 equiv), and adipoyl chloride (4.8 μL, 33 μmol) in dry THF (2 mL) provided compounds **14** and **21** as gray solids.

Compound 14: yield, 4.5 mg, 35%; HPLC t_R, 25.7 min; R_f 0.46 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 214, 357 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.65 (t, J = 6.6 Hz, C(3′)-H_2), 2.06 (s, C(6)CH_3), 2.50 (t, J = 6.6 Hz, C(2′)H_2), 3.21 (s, C(9a)OCH_3), 3.51 (dd, J = 1.5, 4.5 Hz, C(2)H), 3.63 (dd, J = 1.5, 13.2 Hz, C(3)*H*H′), 3.83 (d, J = 4.5 Hz, C(1)H), 4.11 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.67 (dd, J = 10.8, 11.1 Hz, C(10)-*H*H′), 4.82 (d, J = 13.2 Hz, C(3)H*H*′), 5.67 (dd, J = 4.5, 10.8 Hz, C(10)H*H*′), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) δ 9.3 (C(6)*C*H_3), 24.6 (C(3′)), 36.3 (C(2′)), 40.2 (C(2)), 43.5 (C(9)), 43.6 (C(1)), 50.0 (C(9a)O*C*H_3), 50.1 (C(3)), 62.6 (C(10)), 104.9 (C(6)), 106.9 (C(9a)), 111.3 (C(8a)), 155.5 (C(5a)), 158.5 (C(10a)), 177.5 (C(8)), 178.5 (C(5)), 184.0 (N(1a)*C*(O)), the signal for C(7) was not detected and is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 779 $[M + 1]^+$; M_r (+CI, methane) 779.301 22 $(M + 1)^+$ (calcd for $C_{36}H_{43}N_8O_{12}$ 779.300 04).

Compound 21: yield, 4.2 mg, 28%; HPLC t_R, 18.5 min; R_f 0.10 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 214, 356 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.78−1.82 (m, C(3′)H_2, C(4′)-H_2), 2.07 (s, C(6)CH_3), 2.47 (t, J = 6.6 Hz, C(5′)H_2), 2.59 (t, J = 6.9 Hz, C(2′)H_2), 3.22 (s, C(9a)OCH_3), 3.52 (br d, J = 4.5 Hz, C(2)H), 3.64 (dd, J = 1.2, 13.2 Hz, C(3)*H*H′), 3.87 (d, J = 4.5 Hz, C(1)H), 4.14 (dd, J = 4.5, 11.1 Hz, C(9)H), 4.71 (dd, J = 10.8, 11.1 Hz, C(10)*H*H′), 4.84 (d, J = 13.2 Hz, C(3)H*H*′), 5.73 (dd, J = 4.5, 10.8 Hz, C(10)H*H*′); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 9.3 (C(6)*C*H_3), 25.0 (C(4′) or C(3′)), 25.5 (C(3′) or C(4′)), 35.0 (C(2′)), 36.5 (C(5′)), 40.2 (C(2)), 43.6 (C(9)), 43.7 (C(1)), 49.9 (C(9a)O*C*H_3), 50.0 (C(3)), 62.6 (C(10)), 105.0 (C(6)), 107.0 (C(9a)), 111.4 (C(8a)), 155.6 (C(5a)), 158.4 (C(10a)), 176.1 (C(O)OH), 177.5 (C(8)), 178.6 (C(5)), 184.0 (N(1a)*C*(O)), the signal for C(7) was not detected and is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 463 $[M + 1]^+$; M_r (+CI, methane) 463.182 52 $(M + 1)^+$ (calcd for $C_{21}H_{27}N_4O_8$ 463.182 89).

***N*(1a),*N*′(1a)-(1,7-Heptanedionyl)dimitomycin C (15).** Using **1** (11 mg, 33 μmol), TEA (36.7 μL, 263 μmol, 8 equiv), and pimeloyl chloride (5.3 μL, 33 μmol) in dry THF (2 mL) provided **15** as a gray solid: yield, 9.1 mg, 70%; HPLC t_R, 26.5 min; R_f 0.29 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 214, 357 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.36 (quint, J = 7.5 Hz, C(4′)H), 1.50−1.65 (m, C(3′)H_2), 2.07 (s, C(6)CH_3), 2.55 (t, J = 7.5 Hz, C(2′)H_2), 3.23 (s, C(9a)OCH_3), 3.51 (dd, J = 1.5, 4.5 Hz, C(2)H), 3.65 (dd, J = 1.5, 13.2 Hz, C(3)*H*H′), 3.83 (d, J = 4.5 Hz, C(1)H), 4.08 (dd, J = 4.2, 11.1 Hz, C(9)H), 4.62 (dd, J = 10.8, 11.1 Hz, C(10)*H*H′), 4.77 (d, J = 13.2 Hz, C(3)-H*H*′), 5.59 (dd, J = 4.2, 10.8 Hz, C(10)H*H*′); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 9.4 (C(6)*C*H_3), 25.2 (C(3′)), 29.3 (C(4′)), 36.5 (C(2′)), 40.3 (C(2)), 43.8 (C(9), (C(1)), 49.9 (C(9a)O*C*H_3), 50.1 (C(3)), 62.7 (C(10)), 105.0 (C(6)), 106.7 (C(9a)), 111.0 (C(8a)), 155.6 (C(5a)), 159.0 (C(10a)), 177.4 (C(8)), 178.5 (C(5)), 184.6 (N(1a)*C*(O)), the signal for C(7) was not detected and is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 792 $[M]^+$; M_r (+CI, methane) 792.310 89 $(M)^+$ (calcd for $C_{37}H_{44}N_8O_{12}$ 792.307 87).

Method for the Anticancer Activity Test. The human tumor cell line panel included A549 (lung carcinoma, CCL 185), KB (epidermoid carcinoma, CL 17), U-87MG (glioblastoma, HTB14), MCF-7 (breast adenocarcinoma, HTB 22), HCT-8 (ileocecal adenocarcinoma, CCL 244), PC-3 (prostate adenocarcinoma, CRL 1435), and HepG2 (hepatocellular carcinoma, HB 8065). Cell lines were adapted for growth in RPMI-1640 medium supplemented with 25 mM HEPES, 2% (w/v) $NaHCO_3$, 10% (v/v) fetal bovine serum, and 100 μg/mL Kanamycin. Cultures were maintained in a 5% CO_2, humidified atmosphere at 37 °C. Compounds were tested as inhibitors of human tumor cell line replication using the sulforhodamine B microtiter plate assay with only minor modification.[28] After 3 days of continuous exposure, cultures were fixed with 10% (w/v) trichloroacetic acid and stained with 0.4% (w/v) sulforhodamine B in 1% acetic acid. Acid-fast dye was dissolved in 20 mM Tris pH 8.0 buffer, and optical density was recorded at 562 nm using a UV max spectrophotometer (Molecular Devices, Menlo Park, CA). Each determination was for duplicate treatments, and variation between replicates was less than 5%. Experimental variation between several independent determinations is given as mean ± SD. Only experiments where A_{562} of untreated cultures was less than two units was used for subsequent analysis. The IC_{50} value, the concentration of compound that inhibited cell line replication by 50% relative to control, was interpolated from dose−response graphs fitted to data using Prizm (GraphPad software, San Diego, CA).

General Protocol for Alkaline Agarose Gel Electrophoresis.[20] The agarose gels were prepared by adding 1.2 g of agarose to an aqueous 0.1 M NaCl solution (100 mL) containing EDTA (2 mM) ("pH" 8.0). The suspension was heated in a microwave oven until all of the agarose was dissolved (1 min). The gel was poured and was allowed to cool and solidify (1 h) at room temperature. The gel was soaked (1 h) in an alkaline running buffer (40 mM NaOH aqueous solution (50 mL) containing EDTA (1 mM)). The comb was removed, and the buffer was refreshed prior to electrophoresis. Agarose loading dye was added to the sample (5 μL), and the samples were loaded into the wells. The gel was run for 2−4 h. The gel was then neutralized in an aqueous 100 mM aqueous Tris pH 7.0 buffer solution containing NaCl (150 mM) (45 s), which was refreshed every 15 s. The gel was visualized by one of two methods. (1) The gel was stained in a 5.0×10^{-3} mM ethidium bromide and 0.1 M aqueous Tris pH 7.5 buffer solution (100 mL) containing 150 mM NaCl (20 min). The background staining was then removed by soaking the gel in an aqueous 50 mM NH_4OAc solution containing 10 mM β-mercaptoethanol. Gels were visualized by UV and photographed using Polaroid film 667 (Figures 5, 6, and 8 of Supporting Information). (2) The gel was stained with a 5.0×10^{-3} mM ethidium bromide and 0.1 M aqueous Tris pH 7.5 buffer solution (100 mL) containing 150 mM NaCl (100 mL) (20 min). Gels were visualized by UV and photographed using Eagle Eye II Still video system (Stratagene) (Figures 2−4 and Figure 7 of Supporting Information).

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Supporting Information Available: Figures 5−8 of denaturing 1.2% alkaline agarose gels for **1**, **4**, **12**−**15**, and **22** and a table listing mass spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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