Solvolysis Study of Cycliciminomitomycins

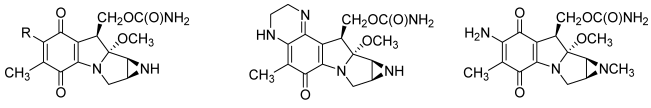
Younghwa NA

College of Pharmacy, Catholic University of Daegu; Gyeongsan, Gyeongbuk, 712–702, Korea.
Received November 10, 2006; accepted December 8, 2006

The solvolysis rates for the substituted C(7)-cyclohexylamino- or C(8)-cyclohexyliminomitomycins 8—19 were determined in buffered methanolic solutions (0.06 M bis-Tris·HCl, pH: 5.5) at 25 °C and then compared with mitomycin C (1) and porfiromycin. Kinetic studies showed that C(8)-cyclohexyliminomitomycins 8—13 underwent solvolysis 150—230 times faster than mitomycin C (1) to give C(1)-methoxymitosene products. The solvolysis rates were slightly faster than that reported for 6. The C(7)-(2′-hydroxy)cyclohexylaminomitomycins 16—19 exhibited comparable solvolysis rates with 1 and porfiromycin.

Key words cycliciminomitomycin; solvolysis; mitomycin

The clinical agent, mitomycin C (**1**), isolated from *Streptomyces caespitosus* in 1956, is the most effective anticancer antibiotic within a series of pharmacologically active compounds known as mitomycins.[1—4] Mitomycin C (**1**) is the prototype of a major class of bio-reductive alkylating agents and can be activated by reductive conditions[5,6] and mild acidic conditions.[7—9] The toxicity of **1** and the resistance of tumor cells to **1** have compromised the clinical utility of mitomycin C. An intensive effort to identify more effective mitomycin derivatives has led to the discovery of KW-2149 (**3**) and BMS-181174 (**4**). Both compounds exhibited improved *in vivo* and *in vitro* pharmacological profiles compared to **1** and had been advanced to clinical trial. But the exact activation pathway for **3** and **4** has not been clearly disclosed yet. Mechanistic proposals have suggested that both agents share a common intermediate, thiol **5**, a species that can be generated by an external thiol-mediated disulfide cleavage pathway.



1 R = NH_2
2 R = OCH_3
3 R = $N(H)(CH_2)_2SS(CH_2)_2N(H)C(O)(CH_2)_2CH(NH_2)CO_2H$
4 R = $N(H)(CH_2)_2SS$-(*p*-nitrophenyl)
5 R = $N(H)(CH_2)_2SH$
7 R = $N(H)(CH_2)_2N(CH_3)_2$

6 **Porfiromycin**

In a previous study, Wang and Kohn prepared a series of C(7) substituted diaminomitomycins and examined their chemical reactivities.[10] One of these was the C(8) cyclized iminomitomycin **6** which was obtained by reacting mitomycin A (**2**) with ethylene diamine.[11] These researchers found that **6** ($t_{1/2}$: 0.1 d) underwent aziridine ring cleavage to give C(1) methoxy substituted mitosene products more than *ca.* 100 times faster (MeOH, pH: 5.5) than **1** ($t_{1/2}$: 13.7 d). In contrast, mitomycin **7**, generated from **2** and *N*,*N*-dimethylethylenediamine, underwent solvolysis ($t_{1/2}$: 11.3 d) at a rate comparable to **1**. The C(8)-iminomitomycin **6** showed significant anticancer activity in animal tests.[12—14] This finding provided preliminary support for the proposed activation pathway for KW-2149 and BMS-181174, which proceeded through a C(8) cyclized adduct (Chart 1). According to the literature,[9] acidic conditions can activate mitomycins to DNA binding and cross-linking process. DNA mono-alkylation and cross-linking pathways are the molecular basis of the cytotoxicty of mitomycin C (**1**). The report of Wang and Kohn prompted us to investigate if other C(8)-iminomitomycins also show increased solvolysis rates compared to **1** and if structural effects within the imino unit affected solvolysis. These structural considerations guided our choice to select cyclohexyl ring fused C(8)-cyclohexyliminomitomycins (Group 1; Chart 2). We subjected that fusion of strained cyclohexyl ring in the C(8)-iminomitomycin could disturb the delocalized conjugated electron system in the mitomycin and thus increase aziridine ring opened mitosene formation, which might implicate enhancing biological activity. We, previously, reported the preparation of C(8)-cyclohexyliminomitomycins and related mitomycins.[15] In this report we describe the chemical reactivity of these compounds and related compounds under mild acidic conditions.

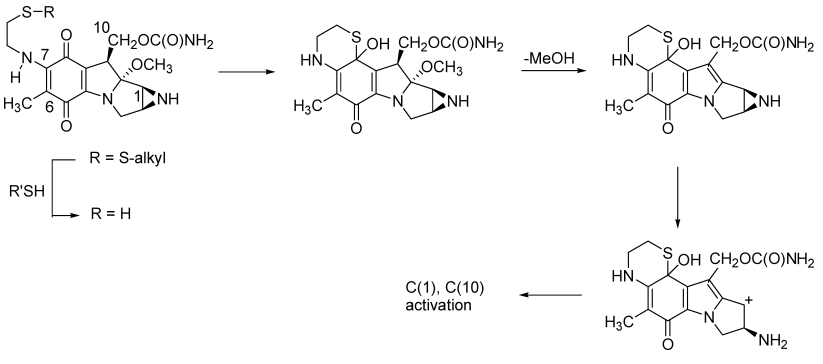


Chart 1. The Kohn and Wang Proposal for the Mode of Action of Mitomycin KW-2149 and BMS-181174[11]

e-mail: yna7315@cu.ac.kr
© 2007 Pharmaceutical Society of Japan

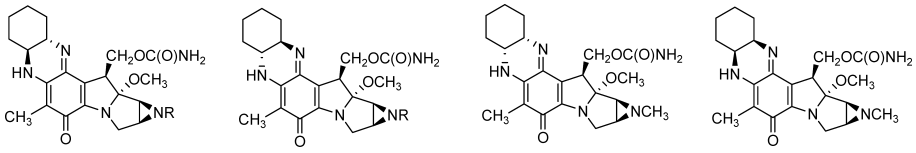


Chart 2. Group 1

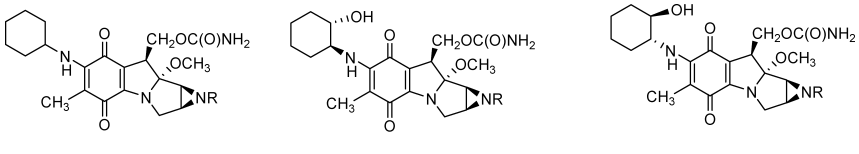


Chart 3. Group 2

Results and Discussion

We knew that the reduction potential required for the conversion of the quinone ring to the hydroquinone (leucomitomycin) system was modulated by the C(7) substituent.[3] We attempted to extend the scope of this study to mitomycins possessing different functional groups, like OH, in cyclohexyl ring other than an amine which led to the C(8)-cyclohexyliminomitomycins to determine if these moieties can alter the mitomycin activation process (Group 2; Chart 3). The parent compounds, 7-*N*-cyclohexylmitomycin C (**14**) and 7-*N*-cyclohexylporfiromycin (**15**),[15] served as our controls.

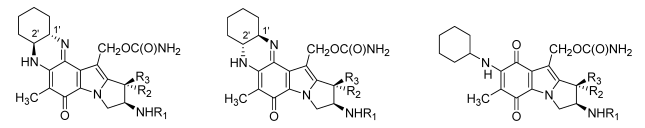
The solvolysis rates for the substituted C(7)-cyclohexylamino- or C(8)-cyclohexyliminomitomycins **8**—**19**[15] were determined in buffered methanolic solutions (0.06 M bis-Tris·HCl, pH: 5.5) at 25 °C and then compared with mitomycin C (**1**) and porfiromycin. All reactions were monitored by UV–visible spectroscopy (200—800 nm). The pseudo first-order kinetic data were calculated using Eq. 1:

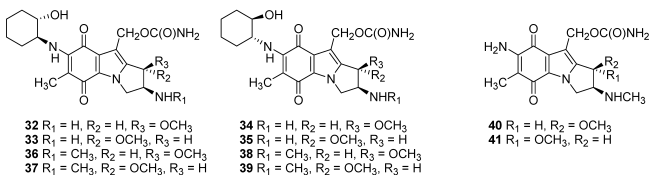
$$\ln(A_i - A_f/A - A_f) = kt \quad (1)$$

(A_i: initial concentration; A_f: final concentration; A: concentration at a given time).

The reactions were done in duplicate and the pseudo first-order kinetic plots gave linear responses ($R^2 > 0.99$) giving the corresponding k_{obs} and $t_{1/2}$ values listed in Table 1.

All of the solvolysis reactions generated the corresponding *cis*- and *trans*-1-methoxymitosenes (HPLC and TLC analyses). The solvolysis reaction products were identified by co-injection of authentic samples in the HPLC and co-spot of authentic samples in the TLC. Authentic samples of the reaction products were prepared by dissolving the mitomycin test substrates in "pH" 3—5.5 buffered MeOH or methanolic HCl solutions. The solvolytic products were identified by their distinctive NMR, UV–visible and mass spectroscopic properties. In the reaction, *cis*- and *trans*-mitosenes were formed with almost same ratio (*cis*- : *trans*- = 1 : 1). For the C(8)-iminomitosenes **20**—**27**, the ^{13}C-NMR spectra exhibited a resonance at *ca.* 153 ppm for the C(8) carbon. Further support for the imino structure was obtained from the mass spectra that showed a molecular ion peak 18 mass units ($-H_2O$) less than the expected signal for the corresponding non-cyclized mitosene. The ^{1}H-NMR spectra permitted us to distinguish the *cis*- and *trans*-1-methoxymitosenes. We found that for the *cis*-isomers the C(1)H–C(2)H and the C(2)H–C(3)H$_\beta$ vicinal coupling constants were moderate (*ca.* 4—7 Hz) and the C(2)H–C(3)H$_\alpha$ coupling interaction was large (*ca.* 9 Hz), whereas in the *trans*-isomers the C(1)H–C(2)H vicinal coupling was nearly zero, the C(2)H–C(3)H$_\beta$ vicinal coupling was small (*ca.* 0—1 Hz) and the C(2)H–C(3)H$_\alpha$ coupling interaction was moderate (*ca.* 4—5 Hz).[16] In the ^{13}C-NMR spectra, the C(1) carbon signal for the *trans*-isomers was shifted downfield (*ca.* 6 ppm) from the corresponding value for the *cis*-isomers. These observations were consistent with previous findings.[17]





The solvolysis studies (Table 1) showed that C(8)-cyclohexyliminomitomycins **8**—**13** underwent C(1) solvolysis 150—230 times faster than mitomycin C (**1**) at "pH" 5.5 (25 °C). The rates for **8**—**13** were nearly two times faster than that for **6**. We observed that the C(1′)-(*S*), C(2′)-(*S*) isomers **8** and **10** solvolyzed slightly faster (*ca.* 1.25—1.5-fold) than the corresponding C(1′)-(*R*), C(2′)-(*R*) isomers **9** and **11**. Correspondingly, the parent cyclohexylaminomitomycins **14** and **15** and the 2-hydroxycyclohexylamine substituted mitomycins **16**—**19** underwent solvolysis at rates comparable to **1** and porfiromycin. We once again observed a small rate enhancement (*ca.* 1.4 fold) for the C(1′)-(*S*), C(2′)-(*S*)-*trans*-isomer **16** compared to C(1′)-(*R*), C(2′)-(*R*)-*trans*-isomer **18**. Finally, we found that the C(8)-iminomitomycins and C(7)

Table 1. Methanolysis Results of C(7)-Cyclohexylaminomitomycins and C(8)-Cyclohexyliminomitomycins[a)]

Compounds	k_{obs} (d^{-1})	$t_{1/2}$ (d)
8	13.9	5.0×10^{-2}
9	9.2	7.5×10^{-2}
10	15.1	4.6×10^{-2}
11	11.9	5.8×10^{-2}
12 and **13**	9.2	7.5×10^{-2}
14	5.0×10^{-2}	13.3
15	3.4×10^{-2}	20.0
16	8.0×10^{-2}	8.6
17	6.0×10^{-2}	12.2
18	3.5×10^{-2}	19.8
19	3.8×10^{-2}	18.5
6[b)]	7.2	0.1
1[b)]	5.0×10^{-2}	13.7
Porfiromycin[c)]	4.0×10^{-2}	17.0

a) Reactions were run in buffered methanol (0.06 M-bis-Tris·HCl) solution (pH: 5.5) at 25 °C. All reactions were run in duplicate and the values averaged. The data were obtained using Cary 3Bio Varian UV–visible spectrophotometer and the reactions monitored at 370±3 nm. *b*) Ref. 9. *c*) The reaction was monitored at 359 nm.

substituted mitomycins all underwent solvolysis at slightly faster (*ca.* 1.25—1.5 fold) rates than the corresponding porfiromycins (*i.e.*, **1** *vs.* porfiromycin, **10** *vs.* **8**, **11** *vs.* **9**, **14** *vs.* **15**, **16** *vs.* **18**, and **17** *vs.* **19**).

Our studies provided two important findings. First, the solvolysis rates recorded for **8**—**13** confirmed the initial report of Kohn and Wang.[11)] These workers showed that the C(8)-iminomitomycin **6** underwent aziridine ring opening and solvolysis more rapidly than the corresponding reaction with mitomycin C (**1**). We suspect that the diminished delocalization of the N(4a) electrons with the C(5a)–C(8a)–C(8)–O(8) α,β-unsaturated carbonyl systems in **8**—**13** contributes to this rate increase. The net effect of this diminished resonance interaction is to facilitate the N(4a) electron expulsion of the C(9a) methoxy group (Chart 4). This is an important first step in mitosene production and aziridine ring cleavage. Second, we observed that the rates of solvolysis for **16**—**19** either were the same or slightly less than the rates for **14**, **15** and **1**. This finding indicated that the C(2′) hydroxyl moiety in cyclohexyl ring did not appreciably interact with the C(8) carbonyl system and facilitate mitomycin solvolysis. Finally, we observed a consistent, small increase in the rates for solvolysis of the mitomycin derivatives compared to their porfiromycin analogues (**10** *vs.* **8**, **11** *vs.* **9**, **14** *vs.* **15**, **16** *vs.* **18**, and **17** *vs.* **19**). This finding suggested that the rate differences stemmed from basicity difference of the N(1a) nitrogen in mitomycin C (**1**) and porfiromycin.

Kinetic studies showed that C(8)-cyclohexyliminomitomycins **8**—**13** underwent solvolysis 150—230 times faster than mitomycin C (**1**) to give C(1)-methoxymitosene products. The solvolysis rates were slightly faster than that reported for **6**. The C(7)-(2′-hydroxy)cyclohexylaminomitomycins **16**—**19** exhibited comparable solvolysis rates with **1** and porfiromycin. These findings suggested that strained ring fused C(8) cyclohexyliminomitomycins solvolyzed more rapidly than C(8)-iminomitomycins in acid due to the more efficient perturbation of resonance interactions of the N(4a) lone pair electrons with the C(5a)–C(8a)–C(8)–O(8) α,β-unsaturated carbonyl system.

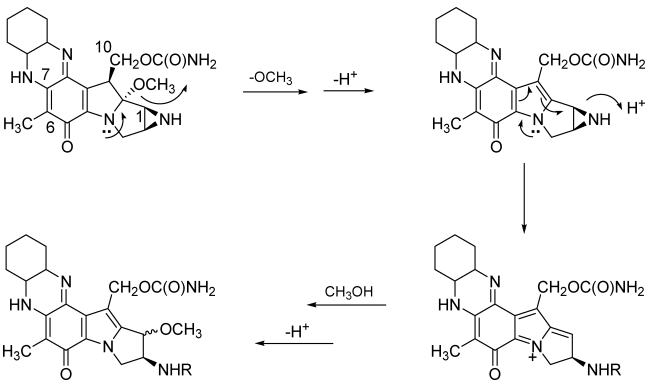


Chart 4. Proposed Pathway for Mitosene Production for C(8)-Cyclohexyliminomitomycins

Experimental

General Methods FT-IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. Absorption values are expressed in wavenumbers (cm^{-1}). ^{1}H- 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 10000 (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: instrument A: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector; instrument B: 515 A pump, 515 B pump, Millennium chromatography manager, Waters 996 photodiode array detector, Rheodyne 7725i manual injector. In both cases the column was fitted with a mbondapak guardpak pre-column. The product analyses were conducted with a C_{18} μBondapak (stainless steel) column (3.9×300 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, 10% B to 45% A, 55% B in 30 min. The flow rate was 1 ml/min, and the eluent was monitored at 313 and 365 nm on instrument A, and from 200 to 400 nm on instrument B. 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 (20×20 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.

General Method for the Synthesis of *cis*- and *trans*-C(1)-Methoxymitosenes 20—27 The mitomycin was dissolved in a buffered methanolic solution (0.06 M bis-Tris·HCl, "pH" 5.5, 2 ml) and then stirred at room temperature (1 d). The solvent was removed under reduced pressure and the residue was purified using PTLC (10% MeOH : $CHCl_3$) to provide the desired compounds.

By using this method, the following compounds were prepared.

Synthesis of Compounds 20 and 21 Using **8** (2 mg, 4.6 μmol) provided **20** and **21** (1 d). The red products were purified by PTLC (3×10% MeOH : $CHCl_3$).

Compound **20** (0.5 mg, 27%): HPLC t_R: 25.8 min (instrument B); *Rf* 0.36 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 210, 256, 316, 491 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.19—1.32 (m, C(4′)HH′, C(5′)HH′), 1.40—1.48 (m, C(3′)HH′, C(6′)HH′), 1.54—1.58 (m, C(4′)HH′), 1.64—168 (m, C(5′)HH′), 1.96—2.02 (m, C(3′)HH′), 2.12 (s, C(6)CH_3), 2.38 (s, N(1a)CH_3), 2.45—2.50 (m, C(6′)HH′), 2.93 (ddd, J=2.7, 11.7, 11.7 Hz, C(2′)H), 3.28 (ddd, J=2.7, 11.7, 11.7 Hz, C(1′)H), 3.51 (s, C(1)OCH_3), 3.58—3.62 (m, C(2)H), 4.01 (dd, J=9.0, 11.5 Hz, C(3)HH′), 4.88 (dd, J=7.5, 11.5 Hz, C(3)HH′), 5.84 (1/2ABq, J=13.2 Hz, C(10)HH′), 6.11 (1/2ABq, J=13.2 Hz, C(10)HH′), 6.57 (s, C(7)NH), the signal for C(1)H is

believed to be beneath solvent peak; MS (+CI, methane) *m/e* 428 $[M+1]^+$; M_r (+CI, methane) 428.229 68 $(M)^+$ (Calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Compound **21** (0.6 mg, 30%): HPLC t_R: 24.5 min (instrument B); *Rf* 0.36 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 210, 255, 316, 493 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.17—1.48 (m, C(4′)HH′, C(5′)HH′, C(3′)HH′, C(6′)HH′), 1.52—1.56 (m, C(4′)HH′), 1.62—1.66 (m, C(5′)HH′), 1.95—1.99 (m, C(3′)HH′), 2.10 (s, C(6)CH_3), 2.42 (s, N(1a)CH_3), 2.42—2.45 (m, C(6′)HH′), 2.93 (ddd, *J*=3.0, 12.3, 12.3 Hz, C(2′)H), 3.23—3.30 (m, C(1′)H), 3.55 (s, C(1)OCH_3), 3.86 (d, *J*=5.1 Hz, C(2)H), 4.51 (d, *J*=12.9 Hz, C(3)HH′), 4.63 (dd, *J*=5.1, 12.9 Hz, C(3)HH′), 5.05 (s, C(1)H), 5.90 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 5.98 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 6.45 (s, C(7)NH); MS (+CI, methane) *m*/e 428 $[M+1]^+$; M_r (+CI, methane) 428.229 08 $(M)^+$ (Calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Synthesis of Compounds 22 and 23 Using **9** (7 mg, 16 μmol) provided enriched mixtures of **22** and **23** (1 d). The red products were purified by PTLC (3×10% MeOH : $CHCl_3$) to provide pure **22** and a sample that was enriched in **23**.

Compound **22** (2 mg, Pure, 29%): HPLC t_R: 28.2 min (instrument B); *Rf* 0.29 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 256, 316, 491 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.19—1.38 (m, C(4′)HH′, C(5′)HH′, C(3′)HH′, C(6′)HH′), 1.54—1.59 (m, C(4′)HH′), 1.67—1.74 (m, C(5′)HH′), 1.97—2.01 (m, C(3′)HH′), 2.10 (s, C(6)CH_3), 2.44—2.50 (m, C(6′)HH′), 2.65 (s, N(1a)CH_3), 2.98 (ddd, *J*=3.9, 11.4, 11.4 Hz, C(2′)H), 3.27 (ddd, *J*=3.9, 11.4, 11.4 Hz, C(1′)H), 3.62 (s, C(1)OCH_3), 4.01—4.08 (m, C(2)H), 4.30 (dd, *J*=9.3, 12.0 Hz, C(3)HH′), 5.16 (dd, *J*=7.8, 12.0 Hz, C(3)HH′), 5.20 (d, *J*=4.8 Hz, C(1)H), 5.92 (1/2ABq, *J*=13.5 Hz, C(10)HH′), 5.94 (1/2ABq, *J*=13.5 Hz, C(10)HH′), 6.53 (s, C(7)NH); MS (+CI, methane) *m/e* 428 $[M+1]^+$; M_r (+CI, methane) 428.228 42 $(M+1)^+$ (Calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Compound **23** (2.5 mg, 2 : 1 Mixture of **23** and **22**, Respectively, 36%): HPLC t_R: 27.5 min (instrument B); *Rf* 0.32 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 211, 256, 316, 492 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.19—1.43 (m, C(4′)HH′, C(5′)HH′, C(3′)HH′, C(6′)HH′), 1.57—1.62 (m, C(4′)HH′), 1.66—1.70 (m, C(5′)HH′), 1.98—2.01 (m, C(3′)HH′), 2.09 (s, C(6)CH_3), 2.37—2.42 (m, C(6′)HH′), 2.59 (s, N(1a)CH_3), 2.94 (ddd, *J*=3.3, 12.3, 12.3 Hz, C(2′)H), 3.24 (ddd, *J*=3.3, 12.3, 12.3 Hz, C(1′)H), 3.55 (s, C(1)OCH_3), 4.10 (br s, C(2)H), 4.67—4.72 (m, C(3)H_2), 5.45 (s, C(1)H), 5.80 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 5.98 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 6.49 (s, C(7)NH); MS (+CI, methane) *m/e* 428 $[M+1]^+$; M_r (+CI, methane) 428.229 41 $(M+1)^+$ (Calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Synthesis of Compounds 24 and 25 Using **10** (10 mg, 24 μmol) provided enriched mixtures of **24** and **25** (1 d). The red products were purified by PTLC (5×10% MeOH : $CHCl_3$) to provide samples that were enriched in **24** and **25**.

Compound **24** (4.3 mg, 4 : 1 Mixture of **24** and **25**, Respectively, 43%): HPLC t_R: 26.8 min (instrument B); *Rf* 0.24 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 210, 256, 314, 490 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.14—1.30 (m, C(4′)HH′, C(5′)HH′), 1.34—1.45 (m, C(3′)HH′, C(6′)HH′), 1.52—1.58 (m, C(4′)HH′), 1.67 (br d, *J*=11.4 Hz, C(5′)HH′), 1.98 (br d, *J*=11.7 Hz, C(3′)HH′), 2.10 (s, C(6)CH_3), 2.44 (br d, *J*=13.2 Hz, C(6′)HH′), 2.93 (ddd, *J*=2.4, 11.7, 12.0 Hz, C(2′)H), 3.28 (ddd, *J*=2.4, 11.7, 11.7 Hz, C(1′)H), 3.58 (s, C(1)OCH_3), 4.35—4.40 (m, C(2)H, C(3)HH′), 5.05 (d, *J*=4.2 Hz, C(1)H), 5.69 (d, *J*=14.7 Hz, C(3)HH′), 5.82 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.07 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.63 (s, C(7)NH); ^{13}C-NMR (pyridine-d_5, 75 MHz) 8.8 (C(6)$\underline{C}H_3$), 25.0 (C(4′)), 26.2 (C(5′)), 31.8 (C(3′)), 33.4 (C(6′)), 51.7 (C(3)), 55.0 (C(2′)), 57.3 (C(1)O$\underline{C}H_3$), 58.4 (C(10)), 60.4 (C(2)), 63.5 (C(1′)), 75.7 (C(1)), 107.1 (C(6)), 114.7 (C(8a)), 127.0 (C(9)), 138.7 (C(9a)), 142.6 (C(7)), 153.3 (C(8)), 158.8 (C(10a)), 177.3 (C(5)) ppm, the C(5a) signal is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 413 $[M]^+$; M_r (+CI, methane) 413.206 10 $(M)^+$ (Calcd for $C_{21}H_{27}N_5O_4$, 413.206 31).

Compound **25** (3.5 mg, 3 : 1 Mixture of **25** and **24**, Respectively, 35%): HPLC t_R: 26.2 min (instrument B); *Rf* 0.21 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 210, 256, 314, 488 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.16—1.30 (m, C(4′)HH′, C(5′)HH′), 1.35—1.43 (m, C(3′)HH′, C(6′)HH′), 1.55 (br d, *J*=12.9 Hz, C(4′)HH′), 1.70 (br d, *J*=13.5 Hz, C(5′)HH′), 2.00 (br d, *J*=11.7 Hz, C(3′)HH′), 2.09 (s, C(6)CH_3), 2.41 (br d, *J*=11.7 Hz, C(6′)HH′), 2.94 (ddd, *J*=3.0, 10.8, 11.4 Hz, C(2′)H), 3.25 (ddd, *J*=3.0, 11.4, 11.7 Hz, C(1′)H), 3.59 (s, C(1)OCH_3), 4.63 (d, *J*=4.2 Hz, C(2)H), 4.80 (d, *J*=4.2, 13.2 Hz, C(3)HH′), 4.90 (d, *J*=13.2 Hz, C(3)HH′), 5.62 (s, C(1)H), 5.87 (s, C(10)H_2), 6.48 (s, C(7)NH); ^{13}C-NMR (pyridine-d_5, 75 MHz) 8.8 (C(6)$\underline{C}H_3$), 24.4 (C(4′)), 26.2 (C(5′)), 31.9 (C(3′)), 33.4 (C(6′)), 53.0 (C(3)), 55.0 (C(2′)), 57.1 (C(1)O$\underline{C}H_3$), 60.4 (C(10)), 61.7 (C(2)), 63.3 (C(1′)), 82.0 (C(1)), 107.3 (C(6)), 113.2 (C(8a)), 126.6 (C(9)), 139.3 (C(9a)), 142.7 (C(7)), 158.9 (C(10a)) ppm, the C(8), C(5a) and C(5) signals were not observed; MS (+CI, methane) *m/e* 413 $[M]^+$; M_r (+CI, methane) 413.205 67 $(M)^+$ (Calcd for $C_{21}H_{27}N_5O_4$, 413.206 31).

Synthesis of Compounds 26 and 27 Using **11** (9.5 mg, 23 μmol) provided **26** and **27** (1 d). The red products were purified by PTLC (3×10% MeOH : $CHCl_3$).

Compound **26** (3.4 mg, 36%): HPLC t_R: 26.9 min (instrument B); *Rf* 0.24 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 256, 316, 485 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.14—1.30 (m, C(4′)HH′, C(5′)HH′), 1.34—1.40 (m, C(3′)HH′, C(6′)HH′), 1.51—1.58 (m, C(4′)HH′), 1.67 (br d, *J*=14.4 Hz, C(5′)HH′), 1.98 (br d, *J*=11.7 Hz, C(3′)HH′), 2.11 (s, C(6)CH_3), 2.45 (br d, *J*=11.7 Hz, C(6′)HH′), 2.96 (ddd, *J*=3.9, 11.7, 11.7 Hz, C(2′)H), 3.27 (ddd, *J*=3.9, 11.7, 11.7 Hz, C(1′)H), 3.57 (s, C(1)OCH_3), 4.094.19 (m, C(2)H, C(3)HH′), 4.83 (d, *J*=4.2 Hz, C(1)H), 4.95 (dd, *J*=5.4, 9.6 Hz, C(3)HH′), 5.95 (s, C(10)H_2), 6.56 (s, C(7)NH); ^{13}C-NMR (pyridine-d_5, 75 MHz) 8.8 (C(6)$\underline{C}H_3$), 25.0 (C(4′)), 26.3 (C(5′)), 31.9 (C(3′)), 33.4 (C(6′)), 52.4 (C(3)), 55.1 (C(2′)), 57.1 (C(1)O$\underline{C}H_3$), 59.4 (C(10)), 60.3 (C(2)), 63.4 (C(1′)), 76.0 (C(1)), 107.4 (C(6)), 114.5 (C(8a)), 127.0 (C(9)), 139.4 (C(9a)), 142.4 (C(7)), 153.4 (C(8)), 158.9 (C(10a)), 177.4 (C(5)) ppm, the C(5a) signal is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 413 $[M]^+$; M_r (+CI, methane) 413.205 18 $(M)^+$ (Calcd for $C_{21}H_{27}N_5O_4$, 413.206 31).

Compound **27** (3.5 mg, 37%): HPLC t_R: 26.2 min (instrument B); *Rf* 0.21 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 214, 256, 316, 490 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.18—1.30 (m, C(4′)HH′, C(5′)HH′), 1.36—1.48 (m, C(3′)HH′, C(6′)HH′), 1.58 (br d, *J*=12.9 Hz, C(4′)HH′), 1.79 (br d, *J*=11.4 Hz, C(5′)HH′), 2.00 (br d, *J*=11.4 Hz, C(3′)HH′), 2.09 (s, C(6)CH_3), 2.43 (br d, *J*=12.0 Hz, C(6′)HH′), 2.97 (ddd, *J*=3.6, 11.1, 11.4 Hz, C(2′)H), 3.25 (ddd, *J*=3.6, 11.4, 11.7 Hz, C(1′)H), 3.55 (s, C(1)OCH_3), 4.49 (d, *J*=4.8 Hz, C(2)H), 4.69 (d, *J*=12.9 Hz, C(3)HH′), 4.79 (dd, *J*=4.8, 12.9 Hz, C(3)HH′), 5.44 (s, C(1)H), 5.81 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.96 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.50 (s, C(7)NH), the ^{1}H-NMR assignments were consistent with the COSY spectrum; ^{13}C-NMR (pyridine-d_5, 75 MHz) 8.8 (C(6)$\underline{C}H_3$), 25.0 (C(4′)), 26.2 (C(5′)), 31.8 (C(3′)), 33.4 (C(6′)), 53.8 (C(3)), 55.1 (C(2′)), 57.0 (C(1)O$\underline{C}H_3$), 60.4 (C(10)), 61.7 (C(2)), 63.4 (C(1′)), 82.1 (C(1)), 107.0 (C(6)), 115.0 (C(8a)), 126.6 (C(9)), 138.6 (C(9a)), 142.6 (C(7)), 153.2 (C(8)), 158.9 (C(10a)), 177.3 (C(5)) ppm, the C(5a) signal is believed to be beneath the solvent peaks; MS (+CI, methane) *m/e* 413 $[M]^+$; M_r (+CI, methane) 413.206 28 $(M)^+$ (Calcd for $C_{21}H_{27}N_5O_4$, 413.206 31).

General Method for the Synthesis of *cis*- and *trans*-C(1) Methoxymitosenes 28—41 The mitomycin was dissolved in a methanolic solution (5 ml) and then the "pH" was adjusted to *ca.* 3.0 with a methanolic 2 M HCl solution. The reaction solution was stirred at room temperature (2—5 d) and then the solvent was removed under reduced pressure and the residue purified using PTLC (10% MeOH : $CHCl_3$) to provide the desired compounds.

By using this method, the following compounds were prepared.

Synthesis of Compounds 28 and 29 Using **14** (8 mg, 18 μmol) provided enriched mixtures of **28** and **29** (5 d). The red products were purified by PTLC (3×10% MeOH : $CHCl_3$) to provide samples that were enriched in **28** and **29**.

Compound **28** (2.5 mg, 1.5 : 1 Mixture of **28** and **29**, Respectively, 33%): HPLC t_R: 33.4 min (instrument B); *Rf* 0.35 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 254, 316, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.06—1.27 (m, C(2′)HH′, C(3′)HH′, C(4′)HH′), 1.42—1.47 (m, C(4′)HH′), 1.58—1.63 (m, C(3′)HH′), 1.86—1.90 (m, C(2′)HH′), 2.19 (s, C(6)CH_3), 3.53 (s, C(1)OCH_3), 3.70—3.77 (m, C(1′)H), 3.83—4.03 (m, C(2)H, C(3)HH′), 4.70 (d, *J*=4.8 Hz, C(1)H), 4.74 (dd, *J*=4.8, 10.8 Hz, C(3)HH′), 5.76 (s, C(10)H_2), 6.72 (d, *J*=9.0 Hz, C(7)NH); MS (+CI, methane) *m/e* 417 $[M+1]^+$; M_r (+CI, methane) 417.213 80 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_5$, 417.213 80).

Compound **29** (2.5 mg, 1.5 : 1 Mixture of **29** and **28**, Respectively, 33%): HPLC t_R: 32.4 min (instrument B); *Rf* 0.32 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 254, 316, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.12—1.29 (m, C(2′)HH′, C(3′)HH′, C(4′)HH′), 1.42—1.47 (m, C(4′)HH′), 1.59—1.64 (m, C(3′)HH′), 1.86—1.90 (m, C(2′)HH′), 2.17 (s, C(6)CH_3), 3.52 (s, C(1)OCH_3), 3.69—3.77 (m, C(1′)H), 4.26 (d, *J*=5.4 Hz, C(2)H), 4.37 (d, *J*=13.2 Hz, C(3)HH′), 4.60 (dd, *J*=5.4, 13.2 Hz, C(3)HH′), 4.91 (s, C(1)H), 5.72 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.78 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.08 (d, *J*=9.9 Hz, C(7)NH); MS (+CI, methane) *m/e* 417 $[M+1]^+$; M_r (+CI, methane) 417.213 29 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_5$, 417.213 80).

Synthesis of Compounds 30 and 31 Using **15** (6 mg, 184 μmol) com-

pounds **30** and **31** (2 d) were obtained as red products after purification by PTLC (3×10% MeOH : $CHCl_3$).

Compound **30** (2 mg, 38%): HPLC t_R: 37.9 min (instrument B); *Rf* 0.44 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 214, 255, 314, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.13—1.26 (m, C(2′)HH′, C(3′)HH′, C(4′)HH′), 1.43—1.46 (m, C(4′)HH′), 1.59—1.62 (m, C(3′)HH′), 1.87—1.90 (m, C(2′)HH′), 2.20 (s, C(6)CH_3), 2.39 (s, NCH_3), 3.50 (s, C(1)OCH_3), 3.59—3.61 (m, C(2)H), 3.73—3.77 (m, C(1′)H), 3.90 (dd, *J*=9.3, 12.0 Hz, C(3)HH′), 4.74 (dd, *J*=7.2, 12.0 Hz, C(3)HH′), 5.00 (d, *J*=4.5 Hz, C(1)H), 5.78 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.82 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.13 (d, *J*=9.3 Hz, C(7)NH); MS (+CI, methane) *m/e* 431 $[M+1]^+$; M_r (+CI, methane) 431.229 95 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_5$, 431.229 45).

Compound **31** (2.5 mg, 38%): HPLC t_R: 34.9 min (instrument B); *Rf* 0.44 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 213, 256, 313, 530 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.11—1.29 (m, C(2′)HH′, C(3′)HH′, C(4′)HH′), 1.43—1.47 (m, C(4′)HH′), 1.58—1.62 (m, C(3′)HH′), 1.84—1.89 (m, C(2′)HH′), 2.17 (s, C(6)CH_3), 2.43 (s, NCH_3), 3.53 (s, C(1)OCH_3), 3.69—3.77 (m, C(1′)H), 3.85 (d, *J*=5.4 Hz, C(2)H), 4.40 (d, *J*=13.2 Hz, C(3)HH′), 4.55 (dd, *J*=5.4, 13.2 Hz, C(3)HH′), 4.99 (s, C(1)H), 5.73 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 5.79 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 6.08 (d, *J*=9.9 Hz, C(7)NH); MS (+CI, methane) *m/e* 431 $[M+1]^+$; M_r (+CI, methane) 431.229 60 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_5$, 431.229 45).

Synthesis of Compounds 32 and 33 Using **16** (8 mg, 19 μmol) compounds **32** and **33** (5 d) were obtained as red products after purification by PTLC (2×10% MeOH : $CHCl_3$).

Compound **32** (1.5 mg, 19%): HPLC t_R: 25.6 min (instrument B); *Rf* 0.15 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 211, 254, 314, 535 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.25—1.36 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.57—1.69 (m, C(4′)HH′, C(5′)HH′), 2.09—2.14 (m, C(6′)HH′), 2.15—2.22 (m, C(3′)HH′), 2.32 (s, C(6)CH_3), 3.56 (s, C(1)OCH_3), 3.66—3.69 (m, C(2′)H), 3.94—3.98 (m, C(1′)H), 4.16 (dd, *J*=8.4, 12.3 Hz, C(3)HH′), 4.25—4.29 (m, C(2)H), 4.82 (dd, *J*=7.2, 12.3 Hz, C(3)HH′), 4.88 (d, *J*=4.8 Hz, C(1)H), 5.72 (s, C(10)H_2), 6.42 (d, *J*=10.2 Hz, C(7)NH); MS (+CI, methane) *m/e* 433 $[M+1]^+$; M_r (+CI, methane) 433.208 31 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

Compound **33** (2.6 mg, 33%): HPLC t_R: 23.7 min (instrument B); *Rf* 0.18 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 210, 254, 314, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.24—1.27 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.54—1.58 (m, C(4′)HH′), 1.60—1.64 (m, C(5′)HH′), 2.03—2.05 (m, C(6′)HH′), 2.15 (br d, *J*=12.5 Hz, C(3′)HH′), 2.27 (s, C(6)CH_3), 3.48 (s, C(1)OCH_3), 3.64—3.67 (m, C(2′)H), 3.90—3.93 (m, C(1′)H), 4.36 (d, *J*=4.5 Hz, C(2)H), 4.51 (d, *J*=13.1 Hz, C(3)HH′), 4.58 (dd, *J*=4.5, 13.1 Hz, C(3)HH′), 5.12 (s, C(1)H), 5.64 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.68 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.34 (d, *J*=9.6 Hz, C(7)NH); ^{13}C-NMR (pyridine-d_5, 150 MHz) 10.5 (C(6)CH_3), 24.5 (C(5′) or C(4′)), 24.6 (C(4′) or C(5′)), 33.4 (C(6′)), 35.0 (C(3′)), 53.9 (C(3)), 56.6 (C(1′)), 58.4 (C(1)OCH_3), 60.1 (C(10)), 61.3 (C(2)), 74.1 (C(2′)), 81.7 (C(1)), 107.5 (C(6)), 116.1 (C(8a)), 121.8 (C(9)), 129.8 (C(9a)), 139.1 (C(7)), 147.1 (C(5a)), 158.1 (C(10a)), 178.1 (C(8)), 179.7 (C(5)) ppm; MS (+CI, methane) *m/e* 433 $[M+1]^+$; M_r (+CI, methane) 433.207 36 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

Synthesis of Compounds 34 and 35 Using **17** (4.5 mg, 10 μmol) compounds **34** and **35** (5 d) were obtained as red products after purification by PTLC (2×10% MeOH : $CHCl_3$).

Compound **34** (1 mg, 22%): HPLC t_R: 25.7 min (instrument B); *Rf* 0.08 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 211, 254, 315, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.22—1.26 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.54—1.58 (m, C(4′)HH′), 1.62—1.65 (m, C(5′)HH′), 2.05—2.07 (m, C(6′)HH′), 2.15 (br d, *J*=12.7 Hz, C(3′)HH′), 2.29 (s, C(6)CH_3), 3.51 (s, C(1)OCH_3), 3.66—3.68 (m, C(2′)H), 3.93—3.95 (m, C(1′)H), 4.07 (dd, *J*=8.9, 12.0 Hz, C(3)HH′), 4.17—4.19 (m, C(2)H), 4.79 (dd, *J*=7.4, 12.0 Hz, C(3)HH′), 4.81 (d, *J*=5.3 Hz, C(1)H), 5.67 (1/2ABq, *J*=13.0 Hz, C(10)HH′), 5.70 (1/2ABq, *J*=13.0 Hz, C(10)HH′), 6.40 (d, *J*=9.6 Hz, C(7)NH); MS (+CI, methane) *m/e* 433 $[M+1]^+$; M_r (+CI, methane) 433.207 75 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

Compound **35** (1 mg, 22%): HPLC t_R: 24.7 min (instrument B); *Rf* 0.15 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 254, 314, 535 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.21—1.26 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.51—1.58 (m, C(4′)HH′), 1.61—1.64 (m, C(5′)HH′), 2.03—2.05 (m, C(6′)HH′), 2.22 (br d, *J*=13.1 Hz, C(3′)HH′), 2.26 (s, C(6)CH_3), 3.50 (s, C(1)OCH_3), 3.63—3.67 (m, C(2′)H), 3.88—3.91 (m, C(1′)H), 4.52 (d, *J*=4.3 Hz, C(2)H), 4.66 (dd, *J*=4.3, 13.2 Hz, C(3)HH′), 4.69 (d, *J*=13.2 Hz, C(3)HH′), 5.41 (s, C(1)H), 5.64 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.66 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.35 (d, *J*=9.6 Hz, C(7)NH); MS (+CI, methane) *m/e* 433 $[M+1]^+$; M_r (+CI, methane) 433.208 04 $(M+1)^+$ (Calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

Synthesis of Compounds 36 and 37 Using **18** (5 mg, 11 μmol) compounds **36** and **37** (2 d) were obtained as red products after purification by PTLC (2×10% MeOH : $CHCl_3$).

Compound **36** (2 mg, 46%): HPLC t_R: 26.4 min (instrument B); *Rf* 0.21 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 215, 255, 315, 533 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.26—1.32 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.62—1.67 (m, C(4′)HH′, C(5′)HH′), 2.09—2.15 (m, C(6′)HH′), 2.17—2.24 (m, C(3′)HH′), 2.34 (s, C(6)CH_3), 2.42 (s, NCH_3), 3.50 (s, C(1)OCH_3), 3.66—3.74 (m, C(2′)H, C(2)H), 3.92 (dd, *J*=9.3, 12.0 Hz, C(3)HH′), 3.97—4.00 (m, C(1′)H), 4.76 (dd, *J*=7.2, 12.0 Hz, C(3)HH′), 4.90 (d, *J*=4.8 Hz, C(1)H), 5.73 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 5.79 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 6.44 (d, *J*=9.9 Hz, C(7)NH); ^{13}C-NMR (pyridine-d_5, 150 MHz) 11.0 (C(6)CH_3), 25.0 (C(5′) or C(4′)), 25.1 (C(4′) or C(5′)), 33.9 (C(6′)), 34.7 (NCH_3), 35.5 (C(3′)), 50.8 (C(3)), 57.0 (C(1)OCH_3), 59.1 (C(10)), 60.6 (C(2)), 67.2 (C(1′)), 71.3 (C(2′)), 72.6 (C(1)), 106.9 (C(6)), 116.5 (C(8a)), 121.4 (C(9)), 130.7 (C(9a)), 139.6 (C(7)), 147.7 (C(5a)), 158.6 (C(10a)), 178.5 (C(8)), 180.4 (C(5)) ppm; MS (+CI, methane) *m/e* 447 $[M+1]^+$; M_r (+CI, methane) 447.224 99 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Compound **37** (2 mg, 40%): HPLC t_R: 24.8 min (instrument B); *Rf* 0.30 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 213, 254, 314, 533 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.23—1.31 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.62—1.70 (m, C(4′)HH′, C(5′)HH′), 2.08—2.10 (m, C(6′)HH′), 2.16—2.21 (m, C(3′)HH′), 2.31 (s, C(6)CH_3), 2.42 (s, NCH_3), 3.51 (s, C(1)OCH_3), 3.69—3.74 (m, C(2′)H), 3.83 (d, *J*=5.4 Hz, C(2)H), 3.94—3.98 (m, C(1′)H), 4.39 (d, *J*=13.2 Hz, C(3)HH′), 4.50 (dd, *J*=5.4, 13.2 Hz, C(3)HH′), 4.98 (s, C(1)H), 5.69 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 5.74 (1/2ABq, *J*=13.2 Hz, C(10)HH′), 6.38 (d, *J*=9.9 Hz, C(7)NH); ^{13}C-NMR (pyridine-d_5, 75.0 MHz) 10.1 (C(6)CH_3), 24.1 (C(5′) or C(4′)), 24.3 (C(4′) or C(5′)), 32.9 (C(6′)), 33.7 (NCH_3), 34.4 (C(3′)), 52.0 (C(3)), 56.1 (C(1)OCH_3), 58.1 (C(10)), 59.9 (C(2)), 69.6 (C(1′)), 74.0 (C(2′)), 80.2 (C(1)) ppm, the signals in the downfield area were not observed; MS (+CI, methane) *m/e* 447 $[M+1]^+$; M_r (+CI, methane) 447.224 45 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Synthesis of Compounds 38 and 39 Using **19** (3 mg, 7 μmol) compounds **38** and **390** (1 d) were obtained as red products after purification by PTLC (2×10% MeOH : $CHCl_3$).

Compound **38** (1 mg, 28%): HPLC t_R: 26.5 min (instrument B); *Rf* 0.27 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 212, 255, 314, 533 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 1.24—1.37 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.62—1.70 (m, C(4′)HH′, C(5′)HH′), 2.09—2.14 (m, C(6′)HH′), 2.15—2.20 (m, C(3′)HH′), 2.34 (s, C(6)CH_3), 2.37 (s, NCH_3), 3.48 (s, C(1)OCH_3), 3.55—3.59 (m, C(2)H), 3.67—3.73 (m, C(2′)H), 3.88 (dd, *J*=9.3, 12.3 Hz, C(3)HH′), 3.95—4.01 (m, C(1′)H), 4.72 (dd, *J*=7.5, 12.3 Hz, C(3)HH′), 4.86 (d, *J*=4.5 Hz, C(1)H), 5.73 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 5.78 (1/2ABq, *J*=12.6 Hz, C(10)HH′), 6.43 (d, *J*=9.9 Hz, C(7)NH); MS (+CI, methane) *m/e* 447 $[M+1]^+$; M_r (+CI, methane) 447.223 17 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Compound **39** (1 mg, 48%): HPLC t_R: 27.3 min (instrument B); *Rf* 0.20 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 214, 255, 314, 531 nm; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ 1.23—1.32 (m, C(3′)HH′, C(4′)HH′, C(5′)HH′, C(6′)HH′), 1.59—1.68 (m, C(4′)HH′, C(5′)HH′), 2.07—2.14 (m, C(6′)HH′), 2.17—2.21 (m, C(3′)HH′), 2.32 (s, C(6)CH_3), 2.43 (s, NCH_3), 3.52 (s, C(1)OCH_3), 3.65—3.71 (m, C(2′)H), 3.93—3.98 (m, C(1′)H), 3.84 (d, *J*=4.8 Hz, C(2)H), 4.38 (d, *J*=13.5 Hz, C(3)HH′), 4.52 (dd, *J*=4.8, 13.5 Hz, C(3)HH′), 4.98 (s, C(1)H), 5.70 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 5.76 (1/2ABq, *J*=12.9 Hz, C(10)HH′), 6.39 (d, *J*=9.6 Hz, C(7)NH); MS (+CI, methane) *m/e* 447 $[M+1]^+$; M_r (+CI, methane) 447.224 39 $(M+1)^+$ (Calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Synthesis of Compounds 40 and 41 Using porfiromycin (8 mg, 24 μmol) compounds **40** and **41** (3 d) were obtained as red products after purification by PTLC (3×10% MeOH : $CHCl_3$).

Compound **40** (3 mg, 38%): HPLC t_R: 22.1 min (instrument B); *Rf* 0.28 (10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 250, 304, 517 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 2.16 (s, C(6)CH_3), 2.63 (s, NCH_3), 3.56 (s, C(1)OCH_3), 4.06—4.10 (m, C(2)H), 4.21 (dd, *J*=9.0, 12.0 Hz, C(3)HH′), 5.06 (dd, *J*=7.5, 12.0 Hz, C(3)HH′), 5.18 (d, *J*=4.8 Hz, C(1)H), 5.78 (1/2ABq, *J*=12.3 Hz, C(10)HH′), 5.86 (1/2ABq, *J*=12.3 Hz, C(10)HH′); MS (+CI, methane) *m/e* 349 $[M+1]^+$; M_r (+CI, methane) 349.151 81 $(M+1)^+$ (Calcd for $C_{16}H_{21}N_4O_5$, 349.151 20).

Compound **41** (3 mg, 38%): HPLC t_R: 21.1 min (instrument B); *Rf* 0.28

(10% MeOH–$CHCl_3$); UV–vis (MeOH) λ_{max} 250, 307, 518 nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 2.14 (s, C(6)CH_3), 2.67 (s, $\underline{N}CH_3$), 3.55 (s, C(1)OCH_3), 4.20 (d, J=5.1 Hz, C(2)H), 4.65 (d, J=13.8 Hz, C(3)$\underline{H}H'$), 4.76 (dd, J=5.1, 13.8 Hz, C(3)$H\underline{H}'$), 5.52 (s, C(1)H), 5.72 (1/2ABq, J=12.3 Hz, C(10)$\underline{H}H'$), 5.77 (1/2ABq, J=12.3 Hz, C(10)$H\underline{H}'$); MS (+CI, methane) m/e 349 $[M+1]^+$; M_r (+CI, methane) 349.151 17 $(M+1)^+$ (Calcd for $C_{16}H_{21}N_4O_5$, 349.151 20).

Acknowledgements Author expresses sincere gratitude to Professor Harold Kohn at University of North Carolina, Chapel Hill, U.S.A., for his generous help for this research.

References

1) Hata T., Sano Y., Sugawara R., Matsumae A., Kanamori K., Shima T., Hoshi T., *J. Antibiot. Ser. A*, **9**, 141—146 (1956).
2) Carter S. C., Crooke S. T., "Mitomycin C. Current Status and New Developments," Academic Press, New York, 1979.
3) Remers W. A., Dorr R. T., "Alkaloids, Chemical and Biological Perspective," Vol. 6, ed. by Pelletter S. W., Wiley and Sons, New York, 1988, pp. 1—74.
4) Tomasz M., "Topics in Molecular and Structural Biology: Molecular Aspects of Anticancer Drug-DNA Interactions," Vol. 2, ed. by Neidle S., Waring M., Macmillan, New York, 1994, pp. 312—347.
5) Iyer V. N., Szybalski W., *Proc. Natl. Acad. Sci. U.S.A.*, **50**, 355—362 (1963).
6) Szybalski W., Iyer V. N., "Antibiotics, I. Mechanism of Action," ed. by Gottlieband D., Shaw P. D., Springer-Verlag, New York, 1967, pp. 211—245.
7) Tomasz M., Lipman R., *J. Am. Chem. Soc.*, **101**, 6063—6067 (1979).
8) McClelland R. A., Lam K., *J. Am. Chem. Soc.*, **107**, 5182—5186 (1985).
9) Lown J. W., Begleiter A., Johnson D., Morgan A. R., *Can. J. Biochem.*, **54**, 110—119 (1976).
10) Wang S., Kohn S., *J. Org. Chem.*, **62**, 5404—5412 (1997).
11) Kohn H., Wang S., *Tetrahedron Lett.*, **37**, 2337—2340 (1996).
12) Saito Y., Kasai M., Shirahata K., Kono M., Morimoto M., Asizawa T., U.S. Patent 4853385 (1989).
13) Saito Y., Kasai M., Shirahata K., Kono M., Morimoto M., Asizawa T., Eur. Patent EP 287855 (1988).
14) Saito Y., Kasai M., Shirahata K., Kono M., Morimoto M., Asizawa T., Jpn. Patent 63246379 (1987) [*Chem. Abstr.*, **111**, 7145*v* (1989)].
15) Na Y., Kohn H., *Heterocycles*, **55**, 1347—1363 (2001).
16) Hornemann U., Iguchi K., Keller P. J., Vu H. M., Kozlowski J. F., Kohn H., *J. Org. Chem.*, **48**, 5026—5033 (1983).
17) Wang S., "Studies on the Mode of Action of the Antitumor Antibiotic Mitomycin KW-2149 and BMS-181174," Ph. D. Dissertation, University of Houston, August, 1998.