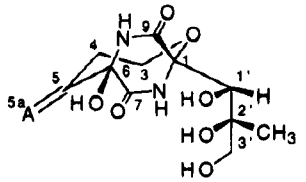
Bicyclomycin Oxidative Transformations. Synthesis and Chemical Properties of Bicyclomycin-5-norketone†

Zhuming Zhang, Hyeung-guen Park, and Harold Kohn*

Department of Chemistry, University of Houston, Houston, Texas 77204-5641

Received January 31, 1995 (Revised Manuscript Received May 30, 1995)

Bicyclomycin (**1**) is a structurally unique antibiotic[1-4] with a novel chemical mechanism of activation.[5] We and others have theorized that bicyclomycin function is associated with the covalent attachment of a nucleophilic protein residue (e.g., cysteine, histidine, lysine) to the C(5)–C(5a) exomethylene group in **1**.[6-9] In 1993, we

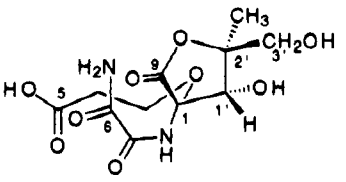


1 A = CH_2
2 A = O

demonstrated that the principal bicyclomycin target in *Escherichia coli* is the rho transcription termination factor.[10] The bicyclomycin binding site in rho has not been identified. Structure–activity studies show that alterations of most functional groups within **1** lead to near total loss of biological activity.[11,12] Only select C(5)-modified bicyclomycins retain notable antimicrobial activities.[11] These compounds were synthesized using bicyclomycin-5-norketone (**2**).[11] We synthesized **2** in order to prepare C(5)-substituted bicyclomycin photoaffinity reagents and enzyme inactivators designed to identify the bicyclomycin binding domain. We report, herein, the oxidation of **1** to **2** and related products and the oxidative and reductive chemistry of bicyclomycin-5-norketone (**2**).

Results and Discussion

A. Oxidative Transformations of Bicyclomycin (1) and Bicyclomycin-5-norketone (2). Müller and co-workers reported the large-scale synthesis (48 g) of bicyclomycin-5-norketone (**2**) in an 81% yield by ozonolysis of a methanolic solution of **1**, followed by the addition of dimethyl sulfide.[11] Attempts to repeat this transformation on a small scale (0.1 g) produced a complex mixture. NMR analysis of the products in CD_3OD indicated the presence of **2** along with other adducts. Maintenance of the CD_3OD solution at room temperature (2 d) resulted in **3** as the sole detectable product. In the



3

^1H NMR spectrum for **3** the C(1′) methine proton (δ 4.66) appeared downfield from the corresponding proton in **1** (δ 4.08), and in the ^{13}C NMR spectrum the two carboxamide carbonyl signals were observed at 162.00 and 162.66 ppm. The structural identity of **3** was determined from the ^1H, ^{13}C, COSY, HMQC, and HMBC NMR experiments and X-ray crystallographic analysis (Figure 1).

Substitution of EtOH in place of MeOH in the ozonolysis reaction of **1** led to partial precipitation of **2**. This modification provided both improved yields of bicyclomycin-5-norketone (**2**) (91%) and enriched samples (>87%, ^1H NMR analysis). Attempts to purify **2** by recrystallization (methanol–ethyl acetate[11]) were unsuccessful.[13] Structural support for **2** was provided by the NMR spectra taken in DMF-d_7. In the ^1H NMR spectrum of **2** the C(4) methylene protons (δ 2.72–2.80, 3.05–3.13) appeared downfield compared with those in **1** (DMF-d_7, δ 2.55–2.58), while in the corresponding ^{13}C NMR spectrum the C(5) carbonyl resonance was located at 203.88 ppm. A similar ^1H NMR spectrum for **2** was observed in THF-d_8. However, within 1 day **2** was converted to **4**, a major new product, along with a small amount of **3**. The ^{13}C NMR spectrum for **4** contained signals at 155.23, 157.14, and 169.35 ppm for the piperazinetrione ring system,[14] and the isolated mixture exhibited a $[M + 1]^+$ signal in the high resolution +CI mass spectrum consistent with **4**. Addition of triphenylphosphine to a freshly prepared THF-d_8 sample of **6** diminished the extent of conversion of **2** to **4** and led to the production of triphenylphosphine oxide.[15]

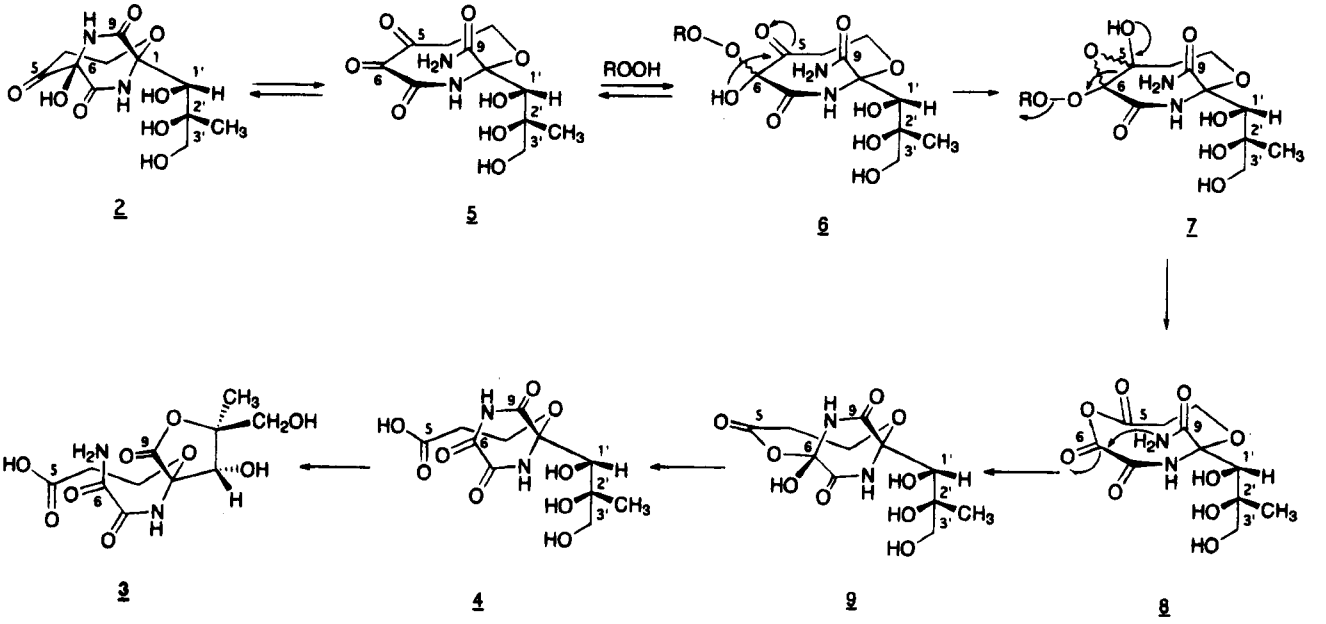
The conversion of **2** to **3** and **4** in the CD_3OD and THF-d_8 solutions, respectively, and the detection of triphenylphosphine oxide when triphenylphosphine was added to THF-d_8 solutions of **2** indicated that either oxidants entrained in the ozonolysis product mixture or present in the reaction solvents were responsible for these

† To aid the discussion of the observed bicyclomycin transformations we have retained the numbering system employed for bicyclomycin in all the chemical drawings and the NMR assignments.

(1) Tanaka, N. *Antibiotics* (NY) **1979** *5*, 18.
(2) (a) Miyoshi, T.; Miyari, N.; Aoki, H.; Kohsaka, M.; Sakai, H.; Imanaka, H. *J. Antibiot.* **1972**, *25*, 569. (b) Kamiya, T.; Maeno, S.; Hashimoto, M.; Mine, Y. *J. Antibiot.* **1972**, *25*, 576. (c) Nishida, M.; Mine, Y.; Matsubara, T. *J. Antibiot.* **1972**, *25*, 582. (d) Nishida, M.; Mine, Y.; Matsubara, T.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1972**, *25*, 594.
(3) (a) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Takana, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K.; Matuski, K.; Hoshino, T. *J. Antibiot.* **1972**, *25*, 610. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H. *J. Antibiot.* **1973**, *26*, 479.
(4) Williams, R. M.; Durham, C. A. *Chem. Rev.* **1988**, *88*, 511 and references therein.
(5) Abuzar, S.; Kohn, H. *J. Org. Chem.* **1989**, *54*, 4000.
(6) Someya, A.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402.
(7) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733.
(8) Pisabarro, A. G.; Canada, F. J.; Vasquez, D.; Arriaga, P.; Rodriguez-Tebar, A. *J. Antibiot.* **1986**, *34*, 914.
(9) (a) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089. (b) Kohn, H.; Abuzar, S. *J. Am. Chem. Soc.* **1988**, *110*, 3661. (c) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1990**, *112*, 3114.
(10) Zweifka, A.; Kohn, H.; Widger, W. R. *Biochemistry* **1993**, *32*, 3564.
(11) Müller, B. W.; Zak, O.; Kump, W.; Tosch, W.; Wacker, O. *J. Antibiot.* **1979**, *32*, 689.
(12) (a) Zhang, Z.; Kohn, H. J. *Chem. Soc., Chem. Commun.* **1994**, 1343. (b) Zhang, Z.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 9815.
(13) Addition of thiourea after the introduction of dimethyl sulfide during the workup of **2** did not prevent the conversion of **2** to **3**.
(14) Aoyama, H.; Ohnota, M.; Sakamoto, M.; Omote, Y. *J. Org. Chem.* **1986**, *51*, 247.
(15) Zhang, Z. Ph.D. Thesis, University of Houston, 1994.

0022-3263/95/1960-5346$09.00/0

Scheme 1. Proposed Pathway for Formation of Compound 3



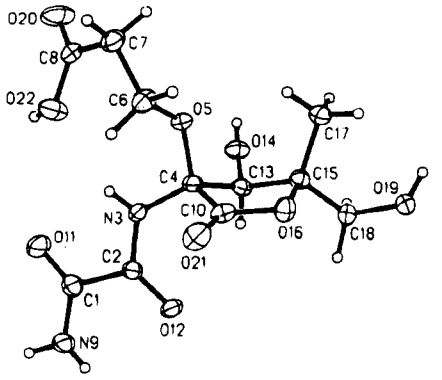
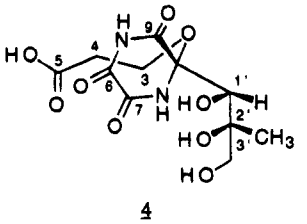


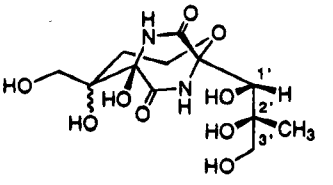
Figure 1. ORTEP drawing for **3** showing the atom numbering scheme. The thermal ellipsoids are 50% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. Selected bond distances (Å) are as follows: C(1)–O(11), 1.221(4); C(1)–C(2), 1.546(4); C(2)–O(12), 1.228(4); C(2)–N(3), 1.336(4); N(3)–C(4), 1.447(4); C(4)–C(10), 1.552(4); C(10)–O(16), 1.337(4); O(16)–C(15), 1.477(4); C(15)–C(13), 1.546(4); C(13)–C(14), 1.520(4). Selected angles (deg) are as follows: C(2)–C(1)–O(11), 120.6(3); C(2)–C(1)–N(9), 113.6(3); C(1)–C(2)–O(12), 123.7(3); C(2)–N(3)–C(4), 124.3(3); C(4)–C(10)–O(16), 109.2(3); C(10)–O(16)–C(15), 112.0(2); O(16)–C(15)–C(13), 102.4(2); C(15)–C(13)–C(4), 103.2(2); C(13)–C(4)–C(10), 101.3(2).



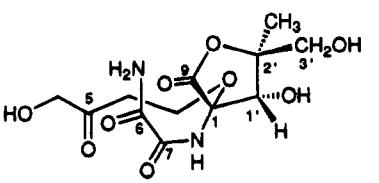
4

transformations. Several likely candidates exist and include the hydroperoxides (i.e., $H_2C(OOH)OCH_3$) formed when MeOH is added to carbonyl oxides[16] generated during the Criegee cleavage of the initial ozonide.[17] In agreement with this notion, addition of H_2O_2 (2 equiv) to a freshly prepared CD_3OD solution of **2** gave **3** as the predominant product within 1 day.[15] An oxidative pathway for the conversion of **2** to **3** and **4** can be proposed based, in part, on a study of the peracid oxidation of α-diketones to acid anhydrides[18] (Scheme 1). In this mechanism, the hemiketal opening of **2** gives **5**. Peroxide addition at either of the two ketone carbonyl sites yields initially an alcohol (**6**) and then an epoxide (**7**) intermediate. Subsequent carbon–carbon fragmentation produces anhydride **8**, which permits intramolecular cyclization by the C(9) amide group to give piperazinedione **9**. Ring fragmentation of **9** yields **4** and then **3** by an intramolecular lactonization process. Other mechanisms for the oxidation of **2** to **3** and **4** are conceivable.[18,19]

Further evidence that the bicyclomycin ring framework can undergo oxidative fragmentation was obtained from the reaction of bicyclomycin with H_2O_2 and catalytic amounts of OsO_4. In addition to isolating the known hexol **10**,[11] we obtained lactone **11**. Key spectral data for



10



11

11 included the four resonances in the ^{13}C NMR spectrum

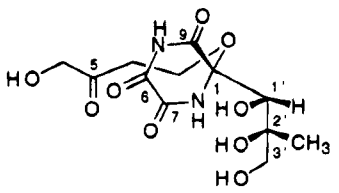
(16) (a) Sander, W. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 344. (b) Brunelle, W. *Chem. Rev.* **1991**, *91*, 335.

(17) (a) Criegee, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 745. (b) Kuczkowski, R. L. *Acc. Chem. Res.* **1983**, *16*, 42. (c) Murray, R. W. *Acc. Chem. Res.* **1968**, *1*, 313.

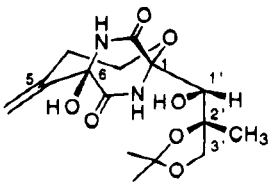
(18) Cullis, P. M.; Arnold, J. R. P.; Clarke, M.; Howell, R.; DeMira, M.; Naylor, M.; Nicholls, D. *J. Chem. Soc., Chem. Commun.* **1987**, 1088.

(19) (a) Suenram, R. D.; Lovas, F. J. *J. Am. Chem. Soc.* **1978**, *100*, 5117. (b) Ogata, Y.; Sawaki, Y.; Shiroyama, M. *J. Org. Chem.* **1977**, *42*, 4061.

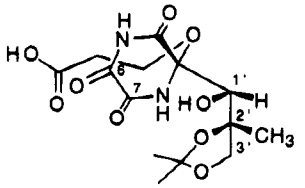
at 162.18, 162.74, 169.18, and 210.53 ppm for the two oxamide, lactone, and ketone carbonyl carbons and the observation of the parent ion ([M + 1]$^+$) in the high resolution +CI mass spectrum. We suspect that under the reaction conditions, **1** is converted to piperazinetrione **12**. Generation of **12** facilitates intramolecular lactonization and bond cleavage to give **11**. Similarly, treatment of the C(2′),C(3′) diol-protected bicyclomycin acetonide[20] (**13**) with OsO_4 and $NaIO_4$ gave piperazinetrione **14**.



12

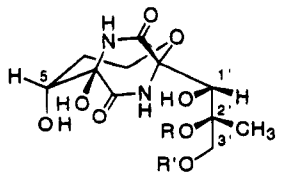


13



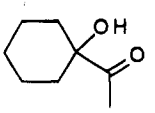
14

B. Reductive Transformations of Bicyclomycin-5-norketone (2). $NaCNBH_3$ reduction of **2** led to the stereospecific production of alcohol **15** (^{13}C NMR analysis).[21] Compound **15** was also generated upon catalytic reduction (Pd/C, H_2, 1 atm). The facility of this process mirrored the catalytic reduction of 1-acetylcyclohexanol[22] (**16**). Correspondingly, pentol **15** could be prepared in near quantitative yield by ozonolysis of **1** at −78 °C in MeOH, followed by direct catalytic hydrogenation (10% Pd/C, 30 psi, 3 h).[23] Attempts to determine the orientation of the C(5) hydroxyl group by selective, one-dimensional nOe experiments in DMF-d_7 were unsuccessful. This problem was resolved by sequential ozonolysis and catalytic reduction of bicyclomycin acetonide (**13**) to give alcohol **17**. X-ray crystallographic analysis of **17** (supplementary materials, Figure 2) revealed the stereochemical orientation at C(5) as (*R*). When the acetonide group in **17** was removed, **15** resulted, which was identical to the compound obtained by catalytic or chemical reduction of **2** (TLC and NMR analyses).



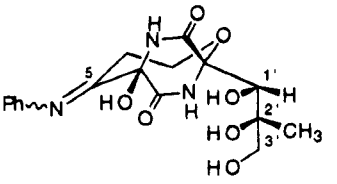
15 R, R′ = H

17 R, R′ = $C(CH_3)_2$

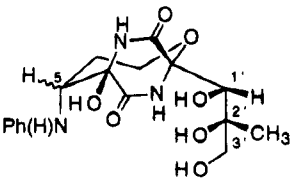


16

Addition of aniline to an ethanolic solution of **2** generated the Schiff base **18** *in situ*. Both catalytic (Pd/C, H_2) and chemical ($NaCNBH_3$) reduction of **18** provided amine **19**, as a single isomer (^{13}C NMR analysis), along with **15**. Attempts to determine the stereochemical orientation of the C(5) anilino group in **19** by selective, one-dimensional NOE experiments in DMF-d_7 were also unsuccessful.



18



19

Conclusions

A small-scale synthesis of bicyclomycin-5-norketone (**2**) was developed based on the ozonolysis procedure of Müller and co-workers.[11] Compound **2** is easily oxidized and reduced. The previously determined structure–activity relationship for C(5)-modified bicyclomycins and the facility with which bicyclomycin-5-norketone undergoes chemical functionalization indicated that **2** could be useful in the design of bicyclomycin photoaffinity reagents and enzyme inactivators.

Experimental Section

General Methods. The mass spectral studies were conducted at the University of Texas at Austin by Dr. M. Moini on a Finnegan MAT TSQ-70 instrument. The ozonolysis experiments were conducted using a Welsbach Model T-23 ozonator. The solvents and reactants were of the best commercial grade available and were used without further purification unless noted.

Preparation of (3S,4S,5S)-3-(2-Carboxyethoxy)-4-hydroxy-5-(hydroxymethyl)-5-methyl-3-oxamido-γ-lactone[24] (3). Ozonolysis of Bicyclomycin (1) in MeOH. A methanolic solution (35 mL) of **1** (100 mg, 0.33 mmol) was treated with O_3 at −78 °C. After approximately 2 min the solution became blue, and O_3 was introduced into the reaction for an additional 5 min. Dimethyl sulfide (27 μL, 0.36 mmol) was added, and the solution was allowed to slowly warm to room temperature. The solvent was evaporated *in vacuo*, and the oily residue was dissolved in MeOH (5 mL) and allowed to stand at room temperature (2 d). The solvent was removed *in vacuo*, dried, and then recrystallized from a MeOH–THF (1:1) binary mixture to give **3** as a white solid: yield 93 mg (88%); mp 179–181 °C; FT-IR (KBr) 3468, 3306, 2984, 2943, 1784, 1717, 1669 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.37 (s, 3 H), 2.55–2.75 (m, 2 H), 3.55 (d, J = 12.6 Hz, 1 H), 3.64 (d, J = 12.6 Hz, 1 H), 3.72–3.85 (m, 1 H), 4.02–4.15 (m, 1 H), 4.66 (s, 1 H); ^{13}C NMR (CD_3-

(20) Kamiyama, T.; Maeno, S.; Kitaura, Y. *Belg. Pat.* 847, 475.
(21) Use of $NaBH_4$ in MeOH led to decomposition of **2**.
(22) Stacy, G. W.; Mikulec, R. A. *J. Am. Chem. Soc.* **1954**, *76*, 524.
(23) (a) Diaper, D. G. M.; Mitchell, D. L. *Can. J. Chem.* **1960**, *38*, 1976. (b) Sousa, J. A.; Bluhm, A. L. *J. Org. Chem.* **1960**, *25*, 108.

(24) The IUPAC nomenclature system has been used for the names of each compound. The numbering system for the structural depictions for compounds **3**, **4**, **11**, and **14** corresponds to that employed for bicyclomycin.

OD) 17.42, 34.74, 61.79, 66.31, 72.44, 86.08, 88.47, 162.00, 162.66, 169.14, 175.11 ppm; the proposed structure was consistent with the COSY, HMQC, HMBC NMR data; MS (+CI) 321 $[M + 1]^+$; M_r (+CI) 321.092 59 $[M + 1]^+$ (calcd for $C_{11}H_{17}N_2O_9$ 321.093 41). The proposed structure was verified by X-ray crystallography (Figure 1).

Preparation of 8,10-Diaza-6-hydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-5-oxo-1-oxabicyclo[4.2.2]-decane-7,9-dione. Generation of Bicyclomycin-5-norketone (2). An anhydrous ethanolic solution (35 mL) of **1** (150 mg, 0.50 mmol) was treated with O_3 at −78 °C until a blue color appeared (approximately 2 min). The solution was degassed with Ar (10 min), and then dimethyl sulfide (300 μL, 4.1 mmol) was added at −78 °C. The solution was allowed to slowly warm to 0 °C during which time the solution became cloudy. The solvent was removed *in vacuo*, and the residue was triturated with ethyl acetate and dried under vacuum to give **2** as a white solid: yield 137 mg (91%, 87% purity (^{1}H NMR analysis)); mp 161−164 °C (lit.[11] mp 171−175 °C); FT-IR (KBr) 3393, 3113, 2984, 2934, 1728, 1690 cm^{-1} (lit.[11] IR (KBr) 3425, 3330, 3270, 1705, 1670 cm^{-1}); ^{1}H NMR (DMF-d_7) δ 1.35 (s, 3 H), 2.72−2.80 (m, 1 H), 3.05−3.13 (m, 1 H), 3.49 (dd, J = 5.9, 10.9 Hz, 1 H), 3.71 (dd, J = 5.9, 10.9 Hz, 1 H), 3.86−4.05 (m, 2 H), 4.21 (d, J = 7.8 Hz, 1 H), 4.65 (t, J = 5.9 Hz, 1 H), 5.40 (d, J = 7.8 Hz, 1 H), 5.45 (s, 1 H), 7.09 (s, 1 H), 8.98 (s, 1 H), 9.35 (s, 1 H); ^{13}C NMR (DMF-d_7) 24.30, 42.54, 59.54, 67.87, 71.75, 76.53, 85.57, 89.67, 165.88, 169.77, 203.88 ppm; the proposed structure was in agreement with the COSY, HMQC, and HMBC NMR data; MS (+CI) 305 $[M + 1]^+$; M_r (+CI) 304.090 58 $[M]^+$ (calcd for $C_{11}H_{16}N_2O_8$ 304.090 67).

Preparation of (1′*S*,2′*S*,6*S*)-6-(Carboxyethoxy)-6-(2-methyl-1,2,3-trihydroxypropyl)piperazine-2,3,5-trione[24] (4). Compound **2** (5 mg, 0.016 mmol) was dissolved in THF-d_8 (0.5 mL) and then monitored by NMR spectroscopy.

Compound **2**: ^{1}H NMR (THF-d_8) after 10 min: δ 1.31 (s, 3 H), 2.60−2.80 (m, 1 H), 2.98−3.15 (m, 1 H), 3.45−3.62 (m, 2 H), 3.88-3.94 (m, 2 H), 4.07 (d, J = 7.2 Hz, 1 H), 4.80 (d, J = 7.2 Hz, 1 H), 4.84 (s, 1 H), 6.55 (s, 1 H), 8.31 (s, 1 H), 9.10 (s, 1 H); the C(3′)OH was not observed and is believed to overlap with the solvent peak. A small amount of **4** (~10%) was also detected. Compound **4**: ^{1}H NMR δ 1.23 (s, 3 H); the other signals overlapped with nearby peaks.

Compound **4**: ^{1}H NMR (THF-d_8) after 30 h: δ 1.23 (s, 3 H), 2.48−2.61 (m, 2 H), 3.42 (d, J = 11.1 Hz, 1 H), 3.45−3.60 (m, 1 H), 3.67 (d, J = 11.1 Hz, 1 H), 3.81 (d, J = 7.4 Hz, 1 H), 3.84−4.00 (m, 1 H), 4.31 (br s, 1 H), 4.80 (br s, 1 H), 5.11 (d, J = 7.4 Hz, 1 H), 8.54 (s, 2 H), 11.10 (s, 1 H); ^{13}C NMR 24.14, 34.95, 61.32, 67.40, 76.29, 76.67, 92.58, 155.23, 157.14, 169.35, 172.59 ppm. Noticeable amounts of **2** (~9%) and **3** (~21%) were also present in the NMR sample. Compound **2**: ^{1}H NMR δ 1.31 (s, 3 H), 2.60−2.80 (m, 1 H), 2.98−3.15 (m, 1 H), 4.08 (d, J = 7.2 Hz, 1 H); the remaining peaks were not clearly discerned and overlapped with other signals. Compound **3**: ^{1}H NMR δ 1.34 (s, 3 H); the remaining peaks were not clearly discerned and overlapped with other signals; ^{13}C NMR 17.32, 34.86, 61.22, 66.31, 72.83, 85.05, 88.96, 161.57, 162.45, 168.70, 172.50 ppm. The identity of **3** was verified by the selective increase of the signals attributed to **3** after the addition of an authentic sample of **3** to the NMR solution.

The THF-d_8 solution was stirred for a total of 48 h under Ar, and then the solvent was removed *in vacuo* to give **4**: yield 5 mg (~70% purity, ^{1}H NMR analysis); MS (+CI) 321 $[M + 1]^+$; M_r (+CI) 321.092 21 $[M + 1]^+$ (calcd for $C_{11}H_{17}N_2O_9$ 321.093 41). Attempted purification of the reaction mixture by preparative TLC led to the production of **3**.

Oxidation of Bicyclomycin (1) with OsO_4−H_2O_2. To an aqueous solution (1 mL) of **1** (50 mg, 0.165 mmol) and OsO_4 (1 mg, 0.004 mmol) was added H_2O_2 (~30%, 0.5 mL). The solution was stirred at 0 °C (1 h) and then concentrated *in vacuo*. The residue was purified by preparative TLC (30% MeOH−$CHCl_3$) to give **10** (11 mg, 20%) and **11** (22 mg, 44%).

8,10-Diaza-5,6-dihydroxy-5-(hydroxymethyl)-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]-decane-7,9-dione (10): mp 180 °C dec (lit.[11] mp 180−185 °C dec); R_f 0.10 (30% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.32 (s, 3 H), 1.85 (dd, J = 8.1, 16.4 Hz, 1 H), 2.09 (dd, J = 8.1, 16.4 Hz, 1 H), 3.53 (d, J = 12.0 Hz, 1 H), 3.54 (d, J = 11.4 Hz, 1 H), 3.70 (d, J = 11.4 Hz, 1 H), 3.77 (d, J = 12.0 Hz, 1 H), 3.80−4.01 (m, 2 H), 4.04 (s, 1 H); ^{13}C NMR (CD_3OD) 24.19, 36.26, 61.74, 66.02, 68.47, 72.28, 78.10, 80.60, 85.19, 89.78, 162.63, 172.29 ppm; MS (+CI) 337 $[M + 1]^+$; M_r (+CI) 337.124 63 $[M + 1]^+$ (calcd for $C_{12}H_{21}N_2O_9$ 337.124 71).

(3*S*,4*S*,5*S*)-3-(4-Hydroxy-3-oxobutoxy)-4-(hydroxymethyl)-5-methyl-3-oxamido-γ-lactone[24] (11): mp 175 °C dec; R_f 0.30 (30% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.65−2.90 (m, 2 H), 3.53 (d, J = 12.6 Hz, 1 H), 3.63 (d, J = 12.6 Hz, 1 H), 3.73−3.83 (m, 1 H), 4.05−4.15 (m, 1 H), 4.23 (s, 2 H), 4.64 (s, 1 H); ^{13}C NMR (CD_3OD) 17.43, 38.48, 60.50, 66.34, 69.17, 72.59, 86.06, 88.58, 162.18, 162.74, 169.18, 210.53 ppm; MS (+CI) 335 $[M + 1]^+$; M_r (+CI) 335.108 61 $[M + 1]^+$ (calcd for $C_{12}H_{19}N_2O_9$ 335.109 06).

Preparation of (1′*S*,2′*S*,6*S*)-6-(Carboxyethoxy)-6-(1-hydroxy-2,3-*O*,*O*-isopropylidene-2-methyl-2,3-dioxapropyl)piperazine-2,3,5-trione[24] (14). Oxidation of Bicyclomycin C(2′),C(3′)-Acetonide (13) with OsO_4−$NaIO_4$. To a dioxane solution (1 mL) containing **13** (10 mg, 0.029 mmol) were successively added a 2-methyl-2-propanol solution of OsO_4 (2.5%, 5 μL) and an aqueous solution (1 mL) of $NaIO_4$ (19 mg, 0.088 mmol), and then the mixture was stirred at room temperature (2 h). The reaction mixture was filtered, and the residue was concentrated *in vacuo* and purified by preparative TLC (20% MeOH−$CHCl_3$) to give **14** (7 mg, 67%): mp 168 °C; R_f 0.10 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3450, 3297 (br), 1711, 1588, 1427, 1384, 1257, 1093 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.32 (s, 3 H), 1.33 (s, 3 H), 1.36 (s, 3 H), 2.38−2.60 (m, 2 H), 3.50−3.60 (m, 1 H), 3.74−3.80 (m, 3 H), 4.08 (d, J = 8.4 Hz, 1 H); ^{13}C NMR (CD_3OD) 23.69, 26.57, 28.08, 37.77, 63.08, 72.61, 78.04, 84.61, 91.56, 110.67, 157.67, 160.30, 171.51 ppm; the remaining signal was not detected; MS (+CI) 361 $[M + 1]^+$; M_r (+CI) 361.125 78 $[M + 1]^+$ (calcd for $C_{14}H_{21}N_2O_9$ 361.124 71).

Preparation of 8,10-Diaza-5,6-dihydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]decane-7,9-dione (15). $NaCNBH_3$ Reduction of Bicyclomycin-5-norketone (2). To an ethanolic solution (10 mL) of **2** (25 mg, 0.08 mmol) was added $NaCNBH_3$ (6 mg, 0.10 mmol) and HOAc (6 μL, 0.10 mmol). The solution was stirred at room temperature (4 h) during which time the solution became cloudy. TLC analysis indicated the presence of one major product. The solvent was removed

in vacuo, and the residue was dissolved in a minimum amount of MeOH and purified by preparative TLC (40% MeOH-$CHCl_3$). Compound **15** was obtained as a solid: yield 14 mg (57%); mp 157–159 °C; R_f 0.10 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.82–2.02 (m, 1 H), 2.06–2.25 (m, 1 H), 3.54 (d, J = 11.4 Hz, 1 H), 3.67 (d, J = 11.4 Hz, 1 H), 3.68–3.82 (m, 1 H), 3.91–3.98 (m, 1 H), 4.03 (s, 1 H), 4.04–4.16 (m, 1 H); ^{13}C NMR (CD_3OD) 24.17, 34.77, 60.39, 68.36, 72.28, 78.08, 79.05, 83.69, 89.50, 168.98, 171.44 ppm.

Preparation of 8,10-Diaza-5,6-dihydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]decane-7,9-dione (15). Catalytic Reduction of Bicyclomycin-5-norketone (2). To an ethanolic solution (10 mL) of **2** (50 mg, 0.16 mmol) was added Pd/C (10%, 5 mg). The mixture was stirred at room temperature under an atmosphere of H_2 (24 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of only **15**. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC (20% MeOH–$CHCl_3$) to give **15** as a solid: yield 36 mg (72%); mp 159–160 °C; R_f 0.10 (20%MeOH–$CHCl_3$); FT-IR (KBr) 3399, 3273, 2947, 1690 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.86–2.00 (m, 1 H), 2.05–2.21 (m, 1 H), 3.54 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.67–3.79 (m, 1 H), 3.90–3.99 (m, 1 H), 4.02 (s, 1 H), 4.08–4.20 (m, 1 H); ^{13}C NMR (CD_3OD) 24.14, 34.74, 60.39, 68.35, 72.25, 78.10, 79.01, 83.69, 89.50, 168.98, 171.44 ppm; MS (+CI) 307 $[M + 1]^+$; M_r (+CI) 307.113 03 $[M + 1]^+$ (calcd for $C_{11}H_{19}N_2O_8$ 307.114 14).

Preparation of 8,10-Diaza-5,6-dihydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]decane-7,9-dione (15). To an anhydrous methanolic solution (35 mL) of **1** (70 mg, 0.23 mmol) maintained at −78 °C was passed O_3 until a blue color appeared (~2 min). The solution was transferred to a hydrogenation vessel, and then a catalytic amount of 10% Pd/C (10 mg) was added to the solution at −78 °C. The mixture was evacuated and then H_2 (30 psi) introduced into the reaction chamber. This procedure was repeated three times. The reaction was then stirred under H_2 (30 psi) for 3 h during which time the solution was slowly warmed to room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of one major product: yield 72 mg (~100%); mp 159–161 °C; R_f 0.10 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3399, 3273, 2947, 1690 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.85–2.00 (m, 1 H), 2.05–2.20 (m, 1 H), 3.54 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.67–3.79 (m, 1 H), 3.90–3.99 (m, 1 H), 4.02 (s, 1 H), 4.08–4.20 (m, 1 H); ^{13}C NMR (CD_3OD) 24.14, 34.73, 60.39, 68.35, 72.25, 78.10, 79.01, 83.69, 89.50, 168.98, 171.44 ppm.

Preparation of 8,10-Diaza-5,6-dihydroxy-1-(1-hydroxy-2,3-*O,O*-isopropylidene-2-methyl-2,3-dioxapropyl)-2-oxabicyclo[4.2.2]decane-7,9-dione (17). To an anhydrous methanolic solution (30 mL) of **13** (60 mg, 0.175 mmol) maintained at −78 °C was passed O_3 until a blue color appeared. The solution was transferred to a hydrogenation vessel, and then a catalytic amount of 10% Pd/C (10 mg) was added to the solution at −78 °C. The mixture was evacuated and then H_2 (30 psi) introduced into the reaction chamber. This procedure was repeated three times. The reaction was then stirred under H_2 (30 psi) for 3 h during which time the solution was slowly warmed to room temperature. The reaction mixture was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of one major product: yield 58 mg (96%); mp 194–197 °C; R_f 0.36 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3512, 3431, 3306, 2988, 1688 cm^{-1}; ^1H NMR (CD_3OD) δ 1.36 (s, 3 H), 1.45 (s, 6 H), 1.75–2.00 (m, 1 H), 2.07–2.20 (m, 1 H), 3.71 (d, J = 8.4 Hz, 1 H), 3.73–3.82 (m, 1 H), 3.85–3.90 (m, 1 H), 4.11 (s, 1 H), 4.10–4.20 (m, 1 H), 4.47 (d, J = 8.4 Hz, 1 H); ^{13}C NMR (CD_3OD) 25.05, 26.79, 28.23, 34.74, 61.52, 73.11, 79.04, 83.69, 86.43, 89.22, 111.62, 168.54, 170.99 ppm; MS (+CI) 347 $[M + 1]^+$; M_r (+CI) 347.145 89 $[M + 1]^+$ (calcd for $C_{14}H_{23}N_2O_8$ 347.145 44). The proposed structure was verified by X-ray crystallography (supporting information, Figure 2).

Preparation of 8,10-Diaza-5,6-dihydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]decane-7,9-dione (15). Deprotection of 17. A MeOH–H_2O solution (1:1, 5 mL) of **17** (36 mg, 0.10 mmol) was acidified ("pH" 1.8) with dilute aqueous H_2SO_4 (0.2 N) and then heated at 60 °C (1 h). The solution was neutralized with a saturated $NaHCO_3$ aqueous solution. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC (20% MeOH–$CHCl_3$) to give **15** as a white solid: yield 15 mg (47%); R_f 0.10 (cospotted with an authentic sample, 20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.87–2.00 (m, 1 H), 2.05–2.22 (m, 1 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.68 (d, J = 11.4 Hz, 1 H), 3.69–3.80 (m, 1 H), 3.87–3.95 (m, 1 H), 4.02 (s, 1 H), 4.10–4.22 (m, 1 H); ^{13}C NMR (CD_3OD) 24.20, 34.88, 60.42, 68.50, 72.37, 78.12, 79.15, 83.71, 89.62, 169.03, 171.53 ppm.

Preparation of 5-Anilino-8,10-diaza-6-hydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]-decane-7,9-dione (19). Catalytic Reductive Amination of Bicyclomycin-5-norketone (2)–Aniline Mixtures. An anhydrous ethanolic solution (20 mL) of **2** (26 mg, 0.086 mmol) and aniline (16 μL, 0.18 mmol) was stirred at room temperature (1 h) followed by the addition of Pd/C catalyst (10%, 5 mg). The mixture was stirred at room temperature under an atmosphere of H_2 (24 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of two major products. The residue was purified by preparative TLC (20% MeOH–$CHCl_3$) to give **15** (11 mg, 42%) and **19** (14 mg, 43%).

Compound 15: mp 157–159 °C; R_f 0.10 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.86–2.00 (m, 1 H), 2.05–2.20 (m, 1 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.68–3.80 (m, 1 H), 3.90–3.99 (m, 1 H), 4.02 (s, 1 H), 4.08–4.20 (m, 1 H); the identity of **15** was verified by cospotting on TLC with an authentic sample.

Compound 19: mp 144–146 °C; R_f 0.45 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3401, 3287, 2940, 1688 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.92–2.20 (m, 2 H), 3.54 (d, J = 11.1 Hz, 1 H), 3.70 (d, J = 11.1 Hz, 1 H), 3.72–3.82 (m, 2 H), 4.06 (s, 1 H), 4.05–4.18 (m, 1 H), 6.62–6.70 (m, 3 H), 7.10 (t, J = 7.8 Hz, 2 H); ^{13}C NMR (CD_3OD) 24.19, 32.73, 61.75, 64.71, 68.49, 72.19, 78.17, 83.20, 89.64, 114.83, 118.93, 130.08, 148.71, 168.20, 171.88 ppm; MS (+CI) 382 $[M + 1]^+$; M_r (+CI) 382.160 99 $[M + 1]^+$ (calcd for $C_{17}H_{24}N_3O_7$ 382.161 43).

Preparation of 5-Anilino-8,10-diaza-6-hydroxy-1-(2-methyl-1,2,3-trihydroxypropyl)-2-oxabicyclo[4.2.2]-decane-7,9-dione (19). $NaCNBH_3$ Reductive Amination of C(5) Bicyclomycin-5-norketone (2)–Aniline Mixtures. To an anhydrous ethanolic solution (10 mL)

Table 1. Data Collection and Processing Parameters for Compound 3

	compd **3**
space gr	$P2_12_12_1$ (orthorhombic)
cell constants	
a (Å)	7.530(1)
b (Å)	11.333(2)
c (Å)	16.722(3)
v (Å^3)	1427
molecular formula	$C_{11}H_{16}N_2O_9$
formula wt	320.29
formula units per cell Z	4
density ϱ (g-cm^{-3})	1.49
absorp coeff μ (cm^{-1})	1.23
T (°C)	−50
radiatn (Mo Kα) λ (Å)	0.710 73
collection range (deg)	$4 \le 2\Theta \le 55$
scan width $\Delta\Theta$ (deg)	$1.25 + (K\alpha_2 - K\alpha_1)$
scan speed range (deg-min^{-1})	1.5−15.0
total data collected	1899
independent data, $I > 3\sigma(I)$	1563
total variables	218
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.035
$R_w = [\Sigma w(\|F_o\| - \|F_c\|)^2/\Sigma w\|F_o\|^2]^{1/2}$	0.031
weights w	σ (F)$^{-2}$

of **2** (50 mg, 0.16 mmol) was added aniline (26 μL, 0.29 mmol). The solution was stirred at room temperature (3 h) followed by the addition of $NaCNBH_3$ (19 mg, 0.30 mmol). The solution became cloudy after 10 min and was stirred at room temperature for an additional 20 h. The solvent was removed, and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to give **15** (25 mg, 51%) and **19** (13 mg, 21%).

Compound 15: mp 157−159 °C; R_f 0.10 (20% MeOH−$CHCl_3$, cospot with authentic sample); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 1.86−2.01 (m, 1 H), 2.05−2.22 (m, 1 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.68 (d, J = 11.4 Hz, 1 H), 3.67−3.81 (m, 1 H), 3.90−4.00 (m, 1 H), 4.03 (s, 1 H), 4.04−4.20 (m, 1 H).

Compound 19: mp 144−146 °C; R_f 0.45 (20% MeOH−$CHCl_3$, cospot with an authentic sample); ^{1}H NMR δ 1.33 (s, 3 H), 1.90−2.20 (m, 2 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.68 (d, J = 11.4 Hz, 1 H), 3.71−3.82 (m, 2 H), 4.06 (s, 1 H), 4.05−4.20 (m, 1 H), 6.61−6.70 (m, 3 H), 7.10 (t, J = 7.8 Hz, 2 H); ^{13}C NMR (CD_3OD) 24.20, 32.79, 61.84, 64.76, 68.51, 72.32, 78.15, 83.25, 89.65, 114.90, 118.99, 130.08, 148.71, 168.14, 171.87 ppm.

Crystallographic Procedure for (3*S*,4*S*,5*S*)-3-(2-(Carboxyethoxy)-4-hydroxy-5-(hydroxymethyl)-5-methyl-3-oxamido-γ-lactone [24] (3). A colorless square block having approximate dimensions 0.25 × 0.25 × 0.50 mm was mounted in a random orientation on a Nicolet R3m/V automatic diffractometer. The sample was placed in a stream of dry N_2 gas at −50 °C, and the radiation used was Mo Kα monochromatized by a highly ordered graphite crystal. Final cell constants, as well as other information pertinent to data collection and refinement for **3**, are listed in Table 1. The Laue symmetry for **3** was determined to be *mmm*, and from the systematic absences noted the space group was shown unambiguously to be $P2_12_12_1$. Intensities were measured using the ω scan technique, with the scan rate depending on the count obtained in rapid prescans of each reflection. Two standard reflections were monitored after every 2 h or every 100 data collected, and these showed no significant variation. During data reduction Lorentz and polarization corrections were applied; however, no correction for absorption was made due to the very small absorption coefficient.

The structure was solved by the SHELXTL direct methods program, which revealed the positions of all of the non-hydrogen atoms in the molecule. The usual sequence of isotropic and anisotropic refinement was followed, after which all hydrogens were located in difference maps. Those hydrogens attached to carbon were moved to ideal calculated positions and constrained to riding motion. The hydrogens attached to nitrogen or oxygen in **3** were allowed to refine independently. A single variable isotropic temperature factor was used for all hydrogens.

The absolute configuration of **3** was arbitrarily set so as to match that of the known starting material, which is *S* at both C(13) and C(15). After all shift/esd ratios were less than 0.1 convergence was reached at the agreement factors listed in Table 1. No unusually high correlations were noted between any of the variables in the last cycle of full-matrix least-squares refinement, and the final difference density map showed a maximum peak of about 0.2 e/Å^3 for **3**. All calculations were made using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs. The author has deposited atomic coordinates for **3** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Acknowledgment. We thank the National Institutes of Health (Grant No. GM37934) and the Robert A. Welch Foundation (Grant No. E-607) for their support of this research. Special thanks is given to Dr. James D. Korp for carrying out the X-ray crystallographic studies of **3** and **17**. We express our appreciation to Dr. M. Kawamura and the Fujisawa Pharmaceutical Co., Ltd., Japan, for providing us with a gift of bicyclomycin.

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of compounds **2**−**4**, **10**, **11**, **14**, **15**, **17**, **19**, an ORTEP drawing (Figure 2) for **17** showing the atom numbering scheme, and Tables 2−5 and 7−10 for compounds **3** and **17**, respectively, providing a complete listing of atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, and hydrogen-bonding parameters (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO950193M