Synthesis and Reactivity of Bicyclomycin C(3′) Amines

Hyeung-geun Park, Marco A. Vela, and Harold Kohn*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77204-5641

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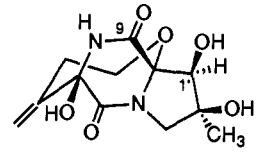
Abstract: The novel bicyclomycin C(3′) tertiary amines **6–8** were prepared in which morpholine, *N*-acetylpiperazine, and *N*-carboethoxypiperazine were installed at the C(3′) site in bicyclomycin (**1**), respectively. Previous attempts to synthesize bicyclomycin C(3′) amines were unsuccessful. Compounds **6–8** were found to be more reactive than **1** in neutral and basic solutions. Under these conditions, a novel ring fragmentation process occurred to give a monosubstituted hydantoin (i.e., **15**, **18**, **19**) and α-methylene-γ-butyrolactone (**17**). Pathways for the formation of hydantoins **15**, **18**, and **19** and butyrolactone **17** are proposed, and evidence is presented in support of these hypotheses. The potential significance of this ring fragmentation process in future drug design is discussed.

Bicyclomycin (**1**) is a commercial antibiotic possessing a broad spectrum of activity against Gram-negative bacteria.[1-4] Drug function is believed to occur by intercepting rho, an enzyme necessary for the synthesis of numerous RNA transcripts.[5] Chemical studies have provided support that drug–protein bonding proceeds by C(6) hemiaminal ring opening in **1** to furnish enone **2**,[6,7] followed by the Michael addition of a protein nucleophile to give initially **3** (Scheme 1).[8] Cysteine-202 in rho has been proposed as the likely bicyclomycin bonding site on the basis of biochemical and chemical studies and the proximity of this residue to point mutations in altered rhos that display bicyclomycin resistance.[5]

Mechanistic investigations have suggested that the distal C(1) triol moiety plays an integral role in both the activation process and the product-determining steps.[7d,9,10] Previous studies have highlighted the importance of the C(1′) hydroxyl group. Kinetic studies comparing the reactivities of the constrained [N(8)-C(3′)]-cyclized bicyclomycin derivative **4** versus **1** with sodium ethanethiolate demonstrated that thiolate addition proceeded more rapidly with **4** than with **1** and were consistent with the notion that intramolecular transfer of a proton from the C(1′) hydroxyl group to the C(9) amide system facilitated the initial hemiaminal bond cleavage step.[9] A comparable effect was cited for the addition of sodium methanethiolate to bicyclomycin mimic **5** at "pH" 12.5 in tetrahydrofuran–water (3:1) mixtures.[10]



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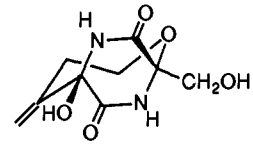

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In this paper attention is focused on the C(3′) substituent in bicyclomycin derivatives. Compounds **6–8** were prepared in which morpholine, *N*-acetylpiperazine, and *N*-carboethoxypiperazine have been installed at the C(3′) site. The reactivities of the bicyclomycin C(3′) amine derivatives **6–8** in water and in tetrahydrofuran–water mixtures were determined both in the absence and presence of the C(5)–C(5a) exomethylene trapping agent,[9] thiophenol. Compounds **6–8** have been shown to be more reactive than **1** at near neutral and basic pH values. Activation of these bicyclomycin C(3′) derivatives is believed to proceed by a novel pathway promoted by the C(3′) amine group. Our cumulative findings suggested new avenues for future drug design.

Results

Synthesis of Bicyclomycin C(3′) Amines 6–8. Preparation of **6–8** was accomplished by treatment of a methanolic solution of bicyclomycin 3′-*O*-methanesulfonate[11] (**9**) with excess morpholine, *N*-acetylpiperazine, and *N*-carboethoxypiperazine, respectively. Use of anhydrous tetrahydrofuran in place of methanol for the preparation of **6** led to lower yields of the desired product, while tetrahydrofuran–water (3:1) mixtures gave principally **10**.[9] Previous attempts to prepare bicyclomycin C(3′) amines (i.e., **11**, R = NHiPr; **12**, R = NH_2) led to the [N(8)-C(3)]-cyclized adduct **4** and undetermined products.[11] The spectral properties obtained for **6–8** were in accord with their proposed structures (Table 1).[12] A distinguishing feature in the ^1H NMR spectra for bicyclomycin C(3′) amines was the upfield shifts of the C(3′) methylene proton resonances versus **1**.

Chemical Properties of Bicyclomycin C(3′) Amines 6–8. (a) Stability of Compounds 6–8 versus Bicyclomycin. Our investigations on the reactivity of **6–8** began by monitoring the stability of each bicyclomycin C(3′) amine in buffered aqueous solutions

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(2) (a) Miyoshi, T.; Miyari, N.; Aoki, H.; Kohsaka, M.; Sakai, H.; Imanaka, H. *J. Antibiot.* **1972**, *25*, 569–575. (b) Kamiya, T.; Maeno, S.; Hashimoto, M.; Mine, Y. *Ibid.* **1972**, *25*, 576–581. (c) Nishida, M.; Mine, Y.; Matsubara, T. *Ibid.* **1972**, *25*, 582–593. (d) Nishida, M.; Mine, Y.; Matsubara, T.; Goto, S.; Kuwahara, S. *Ibid.* **1972**, *25*, 594–601.

(3) (a) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Takana, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K.; Matsuki, K.; Hoshino, T. *J. Antibiot.* **1972**, *25*, 610–612. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H. *Ibid.* **1973**, *26*, 479–484.

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(8) For an expanded mechanism, see: ref 7b,d.

(9) Vela, M. A.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 5223–5231.

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(12) For previous compilations of NMR spectral data for bicyclomycin derivatives, see: ref 7c,d and Kohn, H.; Abuzar, S.; Korp, J. D.; Zektzer, A. S.; Martin, G. E. *J. Heterocycl. Chem.* **1988**, *25*, 1511–1517.

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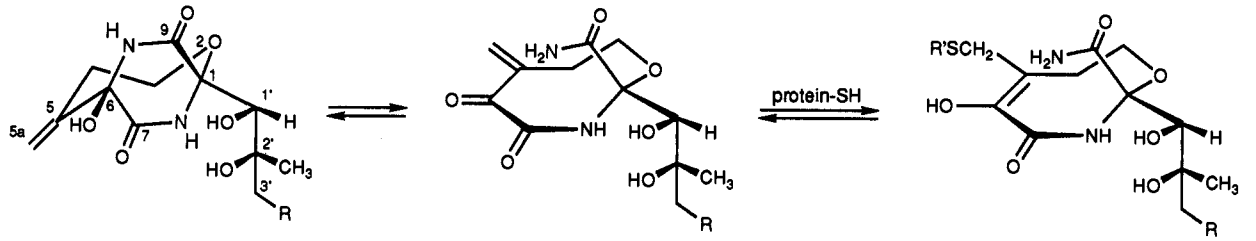
Scheme 1. Proposed Pathway for the Initial Formation of Bicyclomycin–Protein Adducts

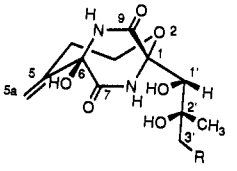


1 R = OH
6 R = N(CH2CH2)2O
7 R = N(CH2CH2)2NC(O)CH3
8 R = N(CH2CH2)2NC(O)OCH2CH3
9 R = OS(O2)CH3
11 R = NHiPr
12 R = NH2
21 R = SCH2CH3

2

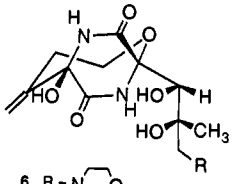
3 R' = protein

Table 1. Key ^1H and ^{13}C NMR Spectral Properties for Bicyclomycin and Select Bicyclomycin Derivatives[a]

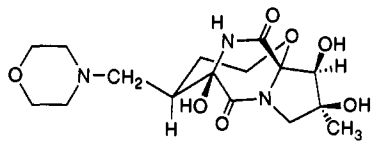


	^1H NMR[b]					^{13}C NMR[c]						
cmpd	C(5a)*H*H	C(5a)H*H'*	C(1')H	C(3')*H*H'	C(3')H*H'*	C(1)	C(5)	C(5a)	C(6)	C(1')	C(2')	C(3')
1	5.14 (s)	5.55 (s)	4.08 (s)	3.52 (d, J = 11.4 Hz)	3.65 (d, J = 11.4 Hz)	89.30	149.38	117.03	82.92	72.04	78.12	68.35
6	5.12 (s)	5.56 (s)	4.21 (s)	2.47 (d, J = 13.8 Hz)	2.55–2.75 (m)	88.85	149.54	116.65	83.05	75.47	77.79	66.40
7	5.12 (s)	5.55 (s)	4.21 (s)	2.50 (d, J = 13.8 Hz)	2.55–2.80 (m)	88.98	149.58	116.57	83.06	75.32	77.97	65.70
8	5.12 (s)	5.55 (s)	4.21 (s)	2.49 (d, J = 13.8 Hz)	2.55–2.75 (m)	88.88	149.53	116.66	83.03	75.30	77.92	65.83
20	5.15 (s)	5.58 (s)	5.42 (s)	2.50–2.70 (m)	2.50–2.70 (m)	87.48	148.98	117.01	83.14	85.74	76.43	66.39
21	5.11 (s)	5.55 (s)	4.22 (s)	2.75 (d, J = 13.4 Hz)	3.00 (d, J = 13.4 Hz)	90.95[d]	149.62	116.75	82.92	72.29	79.00	43.07
22	*e*	*e*	4.14 (s)	2.51 (d, J = 13.8 Hz)	2.55–2.75 (m)	88.95	46.80	15.87	84.54	75.35	77.85	66.44
23	5.17 (s)	5.59 (s)	5.24 (s)	4.23 (d, J = 9.8 Hz)	4.44 (d, J = 9.8 Hz)	88.67	148.92	117.43	82.94	81.34	76.52	74.91

[a] All spectra were recorded in CD_3OD. [b] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant in hertz. ^1H NMR spectra were recorded at 300 MHz. [c] ^{13}C NMR spectra were obtained at 75 MHz. [d] Data taken from ref 7d. The C(5a)CH_3 signal appeared at δ 1.06 (d, J = 6.9 Hz).



6 R = N(CH2CH2)2O
7 R = N(CH2CH2)2NC(O)CH3
8 R = N(CH2CH2)2NC(O)OCH2CH3



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as a function of pH (TLC analysis). In dilute acid (pH 5.0) all three compounds were stable, while at neutral pH each bicyclomycin C(3') amine was slowly consumed. Elevation of the pH from 7.0 to 9.0 led to increased rates of consumption for **6–8**. Of the three bicyclomycin C(3') amines, **6** was the most reactive. TLC analysis of the pH 9.0 reaction with **6** indicated that approximately 50% of the starting bicyclomycin derivative had been consumed within 12 h. A comparable result was obtained by monitoring the reactivity of **6–8** by ^1H NMR spectroscopy in buffered deuterium oxide solutions (pD 9.7). Integration of the downfield shift of the vinylic protons as a function of time permitted the determination of the $t_{1/2}$ values for the apparent first-order decay for these bicyclomycin C(3') amines at 22 °C (Figure 1, Table 2). The $t_{1/2}$ values for **6**, **7**, and **8** were 23.9, 57.8, and 31.5 h, respectively. Similar findings were observed upon dissolution of **6–8** in buffered tetrahydrofuran–water (3:1) mixtures ("pH" 5.0, 7.0, 9.0) at room temperature (5–7 days) (TLC analysis). All three compounds displayed increased

Table 2. Rates of Ring Fragmentation of Bicyclomycin C(3') Amines

cmpd	solvent	"pD"	k_1 (h^{-1})	$t_{1/2}$ (h)
6	D_2O	9.7	2.9×10^{-2}	23.9
6	THF-d_8–D_2O (3:1)	9.8	3.6×10^{-3}	192.5
7	D_2O	9.7	1.2×10^{-2}	57.8
8	D_2O	9.7	2.2×10^{-2}	31.5

reactivity in base than in acid, with **6** again being the most reactive. The rate of conversion of **6–8** to products in tetrahydrofuran–water was slower than in water alone. The $t_{1/2}$ value for **6** in tetrahydrofuran-d_8–deuterium oxide (3:1) at "pD" 9.8 was 192.5 h at 22 °C (^1H NMR, Figure 1, Table 2). The decreased reactivity of **6** in tetrahydrofuran-d_8–deuterium oxide mixtures versus deuterium oxide was opposite to the pattern previously observed for thiol-mediated bicyclomycin transformations.[7] In comparison to **6–8**, aqueous and tetrahydrofuran–water (3:1) mixtures containing bicyclomycin (**1**) were virtually unchanged (<10%) after 7 days at "pH" 5.0 and 7.0 (TLC analysis), while in basic deuterium oxide solutions (pD 9.7) **1** was slowly converted (7 days) to a series of yet unidentified products (NMR, TLC analyses).

Introduction of thiophenol (5 equiv) into tetrahydrofuran–water (3:1) solutions containing **6** led to a more complicated TLC profile but did not appreciably alter the apparent rate of consumption of this bicyclomycin C(3') amine. Only trace amounts of products were observed at "pH" 5.0 (7 days). Elevation of the "pH" of the solution led to increase utilization of **6**. At "pH" 9.0, approximately half of **6** was converted to

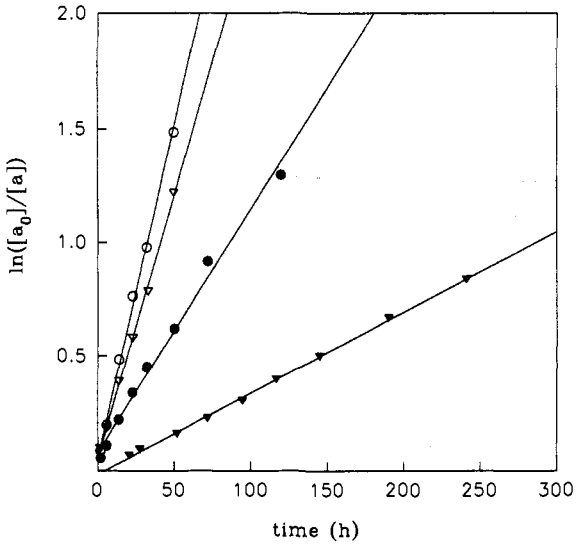
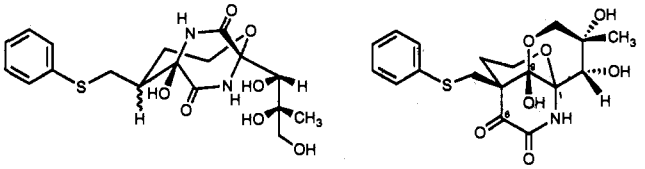


Figure 1. Kinetic plots for fragmentation of bicyclomycin C(3′) amines **6–8** in deuterium oxide and tetrahydrofuran-d_8–deuterium oxide (3:1) mixtures (deuterium oxide, **6** (○), **7** (●), **8** (▽); tetrahydrofuran-d_8–deuterium oxide, **6** (▲)).

products within 7 days (room temperature). Use of water (pH 9.0) in place of tetrahydrofuran–water (3:1) mixtures led to comparable results except that the reaction was complete within 2 days. Similar experiments were also conducted using bicyclomycin (**1**). At "pH" 5.0 and 7.0 in tetrahydrofuran–water (3:1) mixtures, no significant reaction was noted (7 days), while at "pH" 9.0 two new adducts were observed (1 day). Repetition of this experiment under semipreparative conditions permitted the identification of these compounds as **13** and **14**.[13]



13 **14**

(b) Elucidation of Bicyclomycin C(3′) Amines 6–8 Fragmentation Products. Structural determination of the individual products furnished in the bicyclomycin C(3′) amines **6–8** reactions both in the absence and presence of thiophenol was accomplished by performing select experiments on a larger scale. Dissolution of **6** in a tetrahydrofuran–water (3:1) mixture at "pH" 7.5 (5 days) led to the isolation of a major compound whose TLC R_f values in two different solvent systems matched those observed in the previous water and tetrahydrofuran–water experiments conducted at "pH" 7.0 and 9.0. Spectroscopic analyses (^{1}H and ^{13}C NMR, MS, X-ray) identified this compound as the monosubstituted hydantoin **15** (Figure 2). Formation of **15** resulted from cleavage of **6**. Identification of the other ring fragment in this transformation was aided by the introduction of thiophenol into the reaction *after* 5 days. This protocol led to the isolation of **16**[14] along with **15**, suggesting that **6** underwent ring scission to give originally **17** and **15**. Evidence that fragmentation of **6** led to the production of both **15** and **17** was secured from the NMR studies (Figure 1, Table 2). Addition of authentic samples of **15** and **17** to the NMR sample at the conclusion of the reaction led to an increase in the signals attributed to these compounds

(13) Comparable products have been previously isolated with other thiols; see: ref 7.

(14) Grieco, P. A.; Miyashita, M. *J. Org. Chem.* **1975**, *40*, 1181–1183.

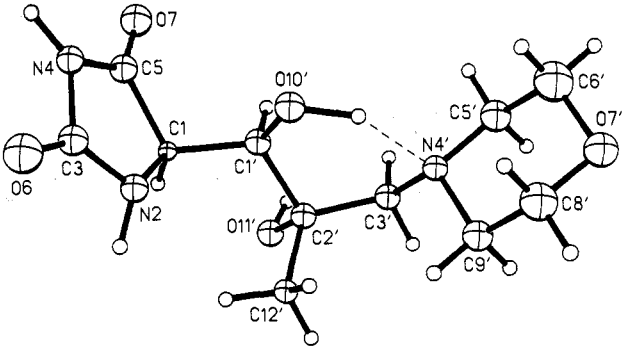
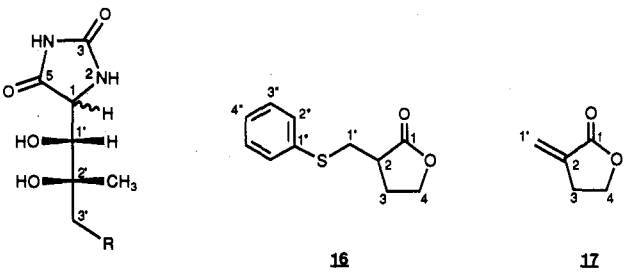


Figure 2. ORTEP drawing of **15** showing the atom-numbering scheme. The thermal ellipsoids are 40% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. The intramolecular hydrogen bond is indicated by a dashed line. Selected bond distances (Å): C(1)–N(2), 1.454 (15); C(1)–C(1′), 1.521 (14); N(2)–C(3), 1.334 (17); C(3)–O(6), 1.237 (16); N(4)–C(5), 1.376 (16); C(1)–C(5), 1.535 (16); C(5)–O(7), 1.241 (15); C(1′)–C(2′), 1.544 (16); C(1′)–O(10′), 1.435 (14). Selected angles (deg): N(2)–C(1)–C(5), 99.5(8); C(5)–C(1)–C(1′), 112.2(9); C(1)–N(2)–C(3), 113.3(10); N(2)–C(3)–N(4), 109.4(11); N(4)–C(3)–O(6), 119.8(12); C(3)–N(4)–C(5), 107.2(10); C(1)–C(5)–N(4), 110.2-(10); N(4)–C(5)–O(7), 127.9(11); C(1)–C(1′)–O(10′), 107.7(9); N(2)–C(1)–C(1′), 115.7(9); N(2)–C(3)–O(6), 130.6(12); C(1)–C(5)–O(7), 121.9(10).

and no new peaks. Confirmation that **17** could furnish **16** under the reaction conditions was attained by treatment of **17** with thiophenol at "pH" 7.0 in a tetrahydrofuran–water (3:1) mixture (2 h) to give **16** in 79% yield. The efficiency of this transformation was further gauged by treatment of a 1:1 binary mixture of **1** and **17** in a tetrahydrofuran–water (3:1) mixture with 1 equiv of thiophenol ("pH" 7.0, room temperature, 24 h). Under these conditions, only **17** was consumed, yielding the thiophenoxy adduct **16**. Similarly, bicyclomycin C(3′) amines **7** and **8** provided hydantoins **18** and **19**, respectively, along with **16** upon dissolution in tetrahydrofuran–water and then addition of thiophenol.



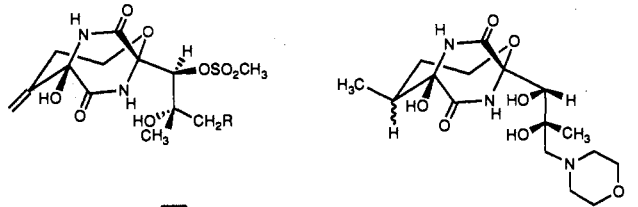
15 R = N⟨O

16 **17**

18 R = N⟨NC(O)CH$_3$

19 R = N⟨NC(O)OCH$_2$CH$_3$

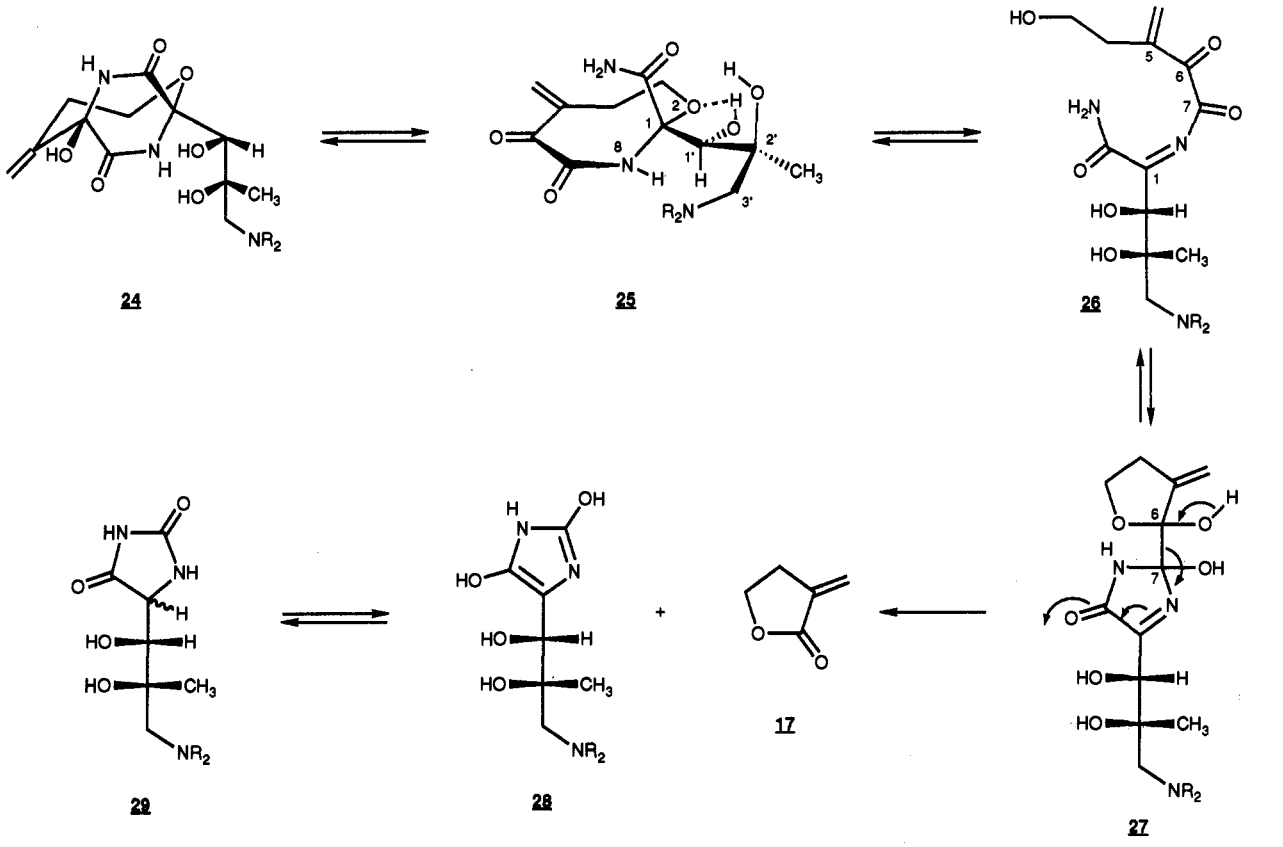
The novelty of this bicyclomycin fragmentation process led us to probe the structural features necessary for this transformation. Select derivatives were prepared in which the C(1′) hydroxyl (i.e., **20**), the C(3′) substituent (i.e., **21**[7d]), or the C(5)–C(5a)



20 R = N⟨O **22**

23 R = OS(O$_2$)CH$_3$

Scheme 2. Proposed Pathway for the Fragmentation of Bicyclomycin C(3′) Amines **6–8**



exomethylene unit (i.e., **22**) in **6** were modified. The C(1′) hydroxyl modified bicyclomycin C(3′) amine **20** was synthesized by treating bicyclomycin 1′-*O*,3′-*O*-dimethanesulfonate (**23**) with morpholine, while catalytic reduction (PtO_2, H_2) of **6** gave **22** in near quantitative yield. Dissolution of **20–22** in tetrahydrofuran–water (3:1) mixtures (room temperature (5 days); 32 °C (2 days); "pH" 7.2–7.5) led to no reaction in each case (TLC analysis) and the subsequent reisolation of starting material. These results implied that the C(1′) hydroxyl group, the C(3′) substituent, and the C(5)–C(5a) exomethylene unit all played important roles in the cleavage of the bicyclomycin ring system in **6–8**.

Discussion

Introduction of an amine moiety at the C(3′) position in bicyclomycin (**1**) led to a novel ring fragmentation process. Our results supported the pathway depicted in Scheme 2 in which hemiaminal ring opening of **24** to give enone **25** is followed by scission of the C(1)–O(2) bond to furnish **26**. Cleavage of the latter bond is believed to be assisted by the C(1′) hydroxyl unit, the prior generation of a conjugated enone system, and either abstraction of the N(8) proton by the C(3′) amine group or removal of the C(2′) hydroxyl proton by the C(3′) amine group, permitting the abstraction of the N(8) proton by the (incipient) C(2′) alkoxide species. Molecular model building and energy minimization[15] studies of ring-opened intermediate **25** (R_2N = morpholino) were in agreement with the proposed proton assistance by the C(1′) hydroxyl group. Also consistent with this pathway was the lack of reactivity of the C(3′) thioethyl derivative **21**, the C(1′) hydroxyl protected bicyclomycin C(3′) amine **20**, and the C(5)–C(5a) dihydrobicyclomycin adduct **22** under conditions that led to complete fragmentation of **6–8** and the neutral to basic requirements needed for ring cleavage. Generation of **26** permits intramolecular cyclization to take place both at the C(6) and C(7) carbonyl sites to give **27**.[16] Retroaldol fragmentation of **27** furnishes imidazole **28** and α-methylene-γ-butyrolactone (**17**). Tautomerization of **28** in the final step gives hydantoin **29**.

Results in accord with this scenario were obtained by inclusion of thiophenol in the original tetrahydrofuran–water (3:1) or water solutions containing **6**. Under these conditions, trace amounts of **15** and **16** were obtained along with two new products. Spectral analysis of one of these compounds was consistent with the dithiophenoxy adduct **30**. The remaining compound has been tentatively identified as **34**. We suspect that under the reaction conditions thiophenol addition to both the α,β-unsaturated carbonyl and imine systems in **31** (**26**) took place to give **32**, followed by retroaldol bond cleavage to eventually furnish **30** and **34** (Scheme 3).

The efficiency of the thiophenol addition to α-methylene-γ-butyrolactone (**17**) to afford **16** prompted us to determine the biochemical and biological properties of bicyclomycin C(3′) amines **6–8**. All three compounds did not noticeably inhibit rho-dependent hydrolysis of ATP[19] at concentration levels (400 μM) sufficient to block ATPase activity by bicyclomycin (% inhibition of ATPase activity: **1** (95%), **6** (20%), **7** (10%), **8** (10%)). Consistent with these findings, **6–8** exhibited no antibiotic activity against *Escherichia coli* W3350 cells at 1000 μg/mL concentration levels using a filter disc microbiological assay.[20] The minimal inhibitory concentration for **1** in this test was 250 μg/mL. No antimicrobial activity (>1000 μg/mL) for **1** and **6–8** was also detected against *Serratia marcescens* SM6 (G(−) rods), *Bacillus megaterium* ATCC 11478 (G(+) rods), and *Saccharomyces cerevisiae* MG 159B (yeast). Previous studies have shown that the antibiotic activities of bicyclomycin derivatives are sensitive to structural changes.[4,11,21] These results suggest

(15) The calculations were done using the program PCMODEL V(88.0) from Serena Software, Bloomington, IN.

(16) Similar hemiketal formation reactions have been previously observed in bicyclomycin transformations proceeding in aqueous acid[17] and base.[6c,d,18]

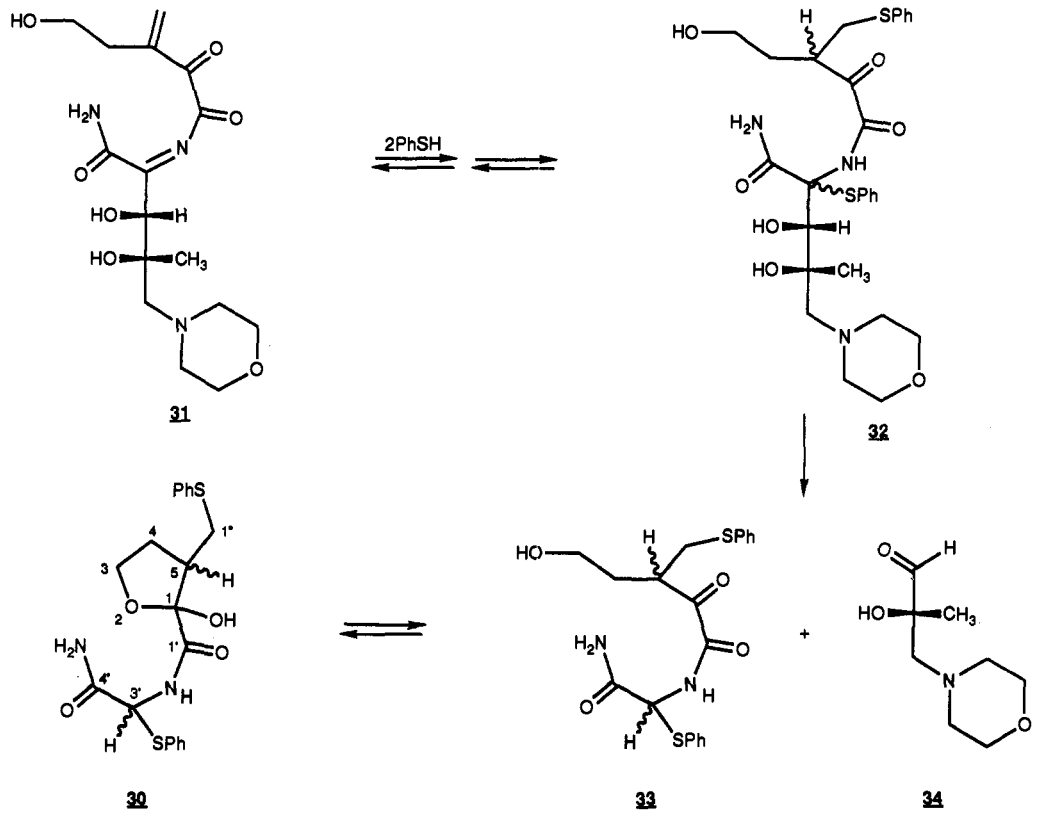
(17) Kohn, H.; Abuzar, S. *J. Org. Chem.* **1988**, *53*, 2769–2773.

(18) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1989**, *111*, 4895–4903.

(19) Sharp, J. A.; Galloway, J. L.; Platt, T. *J. Biol. Chem.* **1983**, *258*, 3482–3486.

(20) Ericsson, H. M.; Sherris, J. C. *Acta Pathol. Microbiol. Scand.* **1971**, Suppl. 217, 1–90.

Scheme 3. Proposed Pathway for the Generation of Compounds **30** and **34**



that the placement of a bulky residue at the C(3') site may have inhibited binding to rho.

Conclusions

The unique reactivity of compounds **6–8** documented the role of the appended C(1) unit in initiating the fragmentation of the [4.2.2] bicyclic ring system. This transformation has not been previously detected for bicyclomycin. The observed ring cleavage reaction for **6–8** may provide opportunities for future drug design. Unlike **1**, bicyclomycin C(3') amines undergo release of α-methylene-γ-butyrolactone (**17**). This compound is a more potent thiolate trapping agent than **1** at near neutral pH values, thereby possibly allowing protein inactivation to proceed more efficiently.[5] These cumulative findings suggest that bicyclomycin C(3') amines that bind to rho and undergo subsequent expulsion of **17** may display antibiotic activity.

Experimental Section

General Methods. FT-IR spectra were run on a Mattson Galaxy Series FT-IR 5000 infrared spectrophotometer. Absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on Nicolet NT-300 and General Electric QE-300 NMR instruments. Chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si, and coupling constants (J values) are in hertz. Low-resolution and high-resolution mass spectral investigations were conducted at the Baylor College of Medicine on VG ZAB-SEQ and VG JS250 instruments by Dr. Simon Gaskell and Mr. Ralph Orkiszewski. pH measurements were determined on a Radiometer pHM26 meter using a Radiometer G202 glass electrode.

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. Tetrahydrofuran was distilled from Na^0 and benzophenone. Thin-layer chromatography was run on precoated silica gel GHLF slides (20 × 20 cm; Analtech No. 21521).

Reaction of Bicyclomycin 3'-*O*-Methanesulfonate (9) with Morpholine in Tetrahydrofuran–Water (3:1). To a solution of **9** (50 mg, 0.13 mmol) in a tetrahydrofuran–water (3:1) mixture (1 mL) was added morpholine (23 mg, 0.26 mmol). The solution was stirred at room temperature (24 h), during which time two liquid phases formed. TLC analysis indicated the complete consumption of the starting material and the formation of **10** as the major product along with other unidentified, more polar adducts. The solvents were removed *in vacuo*, and the residue was dissolved in the minimum amount of methanol. Preparative TLC (10% methanol–chloroform, three developments) afforded **10**[9]: yield 12 mg (25%); R_f 0.60 (20% methanol–chloroform); ^1H NMR (CD_3OD) δ 1.45 (s, 3 H, C(2')CH_3), 1.47–1.54 (m, 1 H, C(4)HH'), 1.70–1.82 (m, 1 H, C(4)-HH'), 2.27 (dd, 1 H, C(5a) HH', J = 4.0, 13.0 Hz), 2.40–2.69 (m, 5 H, N(CH_2CH_2)$_2$O, C(5)H), 2.87 (dd, 1 H, C(5a)HH', J = 11.6, 13.0 Hz), 3.44–3.54 (m, 2 H, C(3')HH', C(3)HH'), 3.62–3.78 (m, 5 H, N(CH_2CH_2)$_2$O, C(3')HH'), 3.85 (s, 1 H, C(1')H), 3.85–3.95 (m, 1 H, C(3)HH'); ^{13}C NMR (CD_3OD) 27.03 (C(2')CH_3), 32.04 (C(4)), 44.07 (C(5)), 54.23 (N(CH_2CH_2)$_2$O), 58.44 (C(3')), 59.90 (C(5a)), 63.67 (C(3)), 67.79 (N(CH_2CH_2)$_2$O), 75.20 (C(2')), 81.77 (C(1')), 86.75 (C(6)), 93.72 (C(1)), 167.85 (C(7) or C(9)), 171.52 (C(9) or C(7)) ppm. The identity of **10** was verified by cospotting the reaction product with an authentic sample on a TLC plate.

Reaction of Bicyclomycin 3'-*O*-Methanesulfonate (9) with Morpholine in Dry Tetrahydrofuran. Preparation of 6. To a suspension of **9** (50 mg, 0.13 mmol) in dry tetrahydrofuran (5 mL) was added morpholine (23 mg, 0.26 mmol). The suspension was stirred at room temperature (48 h), then the solvents were removed *in vacuo*, and the residue was purified by preparative TLC (20% methanol–chloroform, two developments) to give compound **6** as a colorless oil: yield 15 mg (31%); R_f 0.50 (20% methanol–chloroform); ^1H NMR (CD_3OD) δ 1.32 (s, 3 H, C(2')CH_3), 2.46 (d, 1 H, C(3')HH', J = 13.8 Hz), 2.55–2.75 (m, 7 H, N(CH_2-CH_2)$_2$O, C(4)H_2, C(3')HH'), 3.65–3.95 (m, 6 H, N(CH_2CH_2)$_2$O, C(3)-H_2), 4.21 (s, 1 H, C(1')H), 5.12 (s, 1 H, C(5a)HH'), 5.55 (s, 1H, C(5a)HH'); the ^1H NMR assignments were confirmed by the COSY experiment; ^{13}C NMR (CD_3OD) 26.84 (C(2')CH_3), 36.89 (C(4)), 56.46 (N(CH_2CH_2)$_2$O), 65.42 (C(3) or C(3')), 66.46 (C(3') or C(3)), 67.82 (N(CH_2CH_2)$_2$O), 75.58 (C(1')), 77.78 (C(2')), 83.09 (C(6)), 88.95 (C(1)), 116.59 (C(5a)), 149.65 (C(5)), 168.90 (C(7) or C(9)), 172.88 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed by the APT experiment.

Reaction of Bicyclomycin 3'-*O*-Methanesulfonate (9) with Morpholine in Methanol. Preparation of 6. To a methanolic solution (2 mL) of **9** (48 mg, 0.13 mmol) was added morpholine (55 mg, 0.63 mmol). The reaction mixture was stirred at 45 °C (2 h), and then the solvent was removed *in vacuo*. The residue was dissolved in a minimum amount of

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methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **6**: yield 24 mg (51%); R_f 0.25 (10% methanol–chloroform); FT-IR (KBr) 3439, 3283, 1697, 1408, 1115, 1071 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.47 (d, 1 H, C(3′)HH′, J = 13.8 Hz), 2.55–2.75 (m, 7 H, N(CH_2CH_2)$_2$O, C(4)H_2, C(3′)HH′), 3.60–3.95 (m, 6 H, N(CH_2CH_2)$_2$O, C(3)H_2), 4.21 (s, 1 H, C(1′)H), 5.12 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 26.87 (C(2′)CH_3), 36.85 (C(4)), 56.40 (N(CH_2CH_2)$_2$O), 65.34 (C(3) or C(3′)), 66.40 (C(3′) or C(3)), 67.78 (N(CH_2CH_2)$_2$O), 75.47 (C(1′)), 77.79 (C(2′)), 83.05 (C(6)), 88.85 (C(1)), 116.65 (C(5a)), 149.54 (C(5)), 168.94 (C(7) or C(9)), 173.01 (C(9) or C(7)) ppm; MS (+FAB) 372 [M + 1]$^+$; M_r (+FAB) 372.176 99 [M + 1]$^+$ (calcd for $C_{16}H_{26}N_3O_7$ 372.177 07).

Reaction of Bicyclomycin 3′-*O*-Methanesulfonate (9) with *N*-Acetylpiperazine in Methanol. Preparation of 7. To a methanolic solution (2 mL) of **9** (20 mg, 0.053 mmol) was added *N*-acetylpiperazine (34 mg, 0.265 mmol). The solution was stirred at 45 °C (2.5 h), and then the solvent was removed *in vacuo*. The residue was dissolved in a minimum amount of methanol, and the excess *N*-acetylpiperazine was removed by preparative TLC (20% methanol–chloroform) and then the residue purified by preparative TLC (10% methanol–chloroform) to afford **7**: yield 6.3 mg (29%); R_f 0.22 (10% methanol–chloroform); FT-IR (KBr) 3414 (br), 1688, 1618, 1427 (br), 1125 (br), 1072 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.07 (s, 3 H, CH_3C(O)), 2.50 (d, 1 H, C(3′)HH′, J = 13.8 Hz), 2.55–2.80 (m, 7 H, N(CH_2CH_2)$_2$NC(O)CH_3, C(4)H_2, C(3′)HH′), 3.50–3.70 (m, 4 H, N(CH_2CH_2)$_2$NC(O)CH_3), 3.75–3.95 (m, 2 H, C(3)H_2), 4.21 (s, 1 H, C(1′)H), 5.12 (s, 1 H, C(5a)HH′), 5.55 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 21.04 (CH_3C(O)), 26.65 (C(2′)CH_3), 36.87 (C(4)), 42.59 and 47.32 (N(CH_2CH_2)$_2$NC(O)CH_3), 55.52 and 55.91 (N(CH_2CH_2)$_2$NC(O)CH_3), 65.41 (C(3)), 65.70 (C(3′)), 75.32 (C(1′)), 77.97 (C(2′)), 83.06 (C(6)), 88.98 (C(1)), 116.57 (C(5a)), 149.58 (C(5)), 168.90 (C(7) or C(9)), 171.59 (CH_3C(O)), 173.01 (C(9) or C(7)) ppm; MS (+FAB) 413 [M + 1]$^+$; M_r (+FAB) 413.202 92 [M + 1]$^+$ (calcd for $C_{18}H_{29}N_4O_7$ 413.203 63).

Reaction of Bicyclomycin 3′-*O*-Methanesulfonate (9) with *N*-Carboethoxypiperazine in Methanol. Preparation of 8. To a methanolic solution (3 mL) of **9** (50 mg, 0.13 mmol) was added *N*-carboethoxypiperazine (62 mg, 0.39 mmol). The reaction mixture was stirred at 55 °C (3 h), and then the solvent was removed *in vacuo*. The residue was dissolved in a minimum amount of methanol, and the excess *N*-carboethoxypiperazine was removed by preparative TLC (20% methanol–chloroform) and the residue purified by preparative TLC (10% methanol–chloroform) to afford **8**: yield 9 mg (16%); R_f 0.60 (20% methanol–chloroform); FT-IR (KBr) 3453 (br), 1690, 1437 (br), 1252, 1128 cm^{-1}; ^1H NMR (CD_3OD) δ 1.24 (t, 3 H, $NCO_2CH_2CH_3$, J = 7.0 Hz), 1.33 (s, 3 H, C(2′)C H_3), 2.49 (d, 1 H, C(3′)HH′, J = 13.8 Hz), 2.55–2.75 (m, 7 H, N(CH_2CH_2)$_2$$NCO_2$-$CH_2CH_3$, C(4)$H_2$, C(3′)H$H$′), 3.45–3.60 (m, 4 H, N(CH_2CH_2)$_2$$NCO_2$-$CH_2CH_3$), 3.75–3.95 (m, 2 H, C(3)H_2), 4.10 (q, 2 H, $NCO_2CH_2CH_3$, J = 7.0 Hz), 4.21 (s, 1 H, C(1′)H), 5.12 (s, 1 H, C(5a)HH′), 5.55 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 14.92 ($NCO_2CH_2CH_3$), 26.70 (C(2′)CH_3), 36.84 (C(4)), 44.61 (N(CH_2CH_2)$_2$$NCO_2CH_2CH_3$), 55.58 (N($CH_2CH_2$)$_2$$NCO_2CH_2CH_3$), 62.68 (N($CH_2CH_2$)$_2$$NCO_2CH_2CH_3$), 65.34 (C(3)), 65.83 (C(3′)), 75.30 (C(1′)), 77.92 (C2′)), 83.03 (C(6)), 88.88 (C(1)), 116.66 (C(5a)), 149.53 (C(5)), 157.22 ($NCO_2CH_2CH_3$), 168.97 (C(7) or C(9)), 173.07 (C(9) or C(7)) ppm; MS (+FAB) 443 [M + 1]$^+$; M_r (+FAB) 443.214 15 [M + 1]$^+$ (calcd for $C_{19}H_{31}N_4O_8$ 443.214 19).

Fragmentation of Compound 6. A solution of **6** (3.5 mg, 0.01 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was stirred at room temperature under Ar (5 days), during which time the "pH" of the solution remained at 7.5. Thiophenol (5.5 mg, 0.05 mmol) was then added and the reaction stirred for an additional 2 h. TLC analysis indicated the presence of **15** and **16** along with starting material **6**. The solvent was removed *in vacuo*, and the residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **15** (0.95 mg, 37%), **16** (0.42 mg, 21%), and **6** (0.5 mg, 14%).

Compound 15: R_f 0.25 (10% methanol–chloroform); ^1H NMR (CD_3-OD) δ 1.28 (s, 3 H, C(2′)CH_3), 2.42 (d, 1 H, C(3′)HH′, J = 14.1 Hz), 2.55–2.75 (m, 5 H, N(CH_2CH_2)$_2$O, C(3′)HH′), 3.65–3.75 (m, 4 H, N(CH_2CH_2)$_2$O), 3.96 (d, 1 H, C(1′)H, J = 1.1 Hz), 4.48 (d, 1 H, C(1)H, J = 1.1 Hz); ^{13}C NMR (DMSO-d_6) 22.27 (C(2′)CH_3), 55.17 (N(CH_2-CH_2)$_2$O), 59.42 (C(1)), 65.81 (C(3′)), 66.36 (N(CH_2CH_2)$_2$O), 73.92 (C(1′)), 74.50 (C(2′)), 158.65 (C(3)), 176.37 (C(5)) ppm; MS (+FAB) 274 [M + 1]$^+$; M_r (+FAB) 274.140 35 [M + 1]$^+$ (calcd for $C_{11}H_{20}N_3O_5$ 274.140 30).

Compound 16: R_f 0.60 (10% methanol–chloroform); ^1H NMR ($CDCl_3$) δ 2.10–2.20 (m, 1 H, C(3)HH′), 2.45–2.60 (m, 1 H, C(3)HH′), 2.75–3.00 (m, 2 H, C(2)H, C(1′)HH′), 3.55–3.65 (m, 1 H, C(1′)HH′), 4.15–4.30 (m, 1 H, C(4)HH′), 4.35–4.50 (m, 1 H, C(4)HH′), 7.20–7.50 (m, 5 H, Ar H); MS (+FAB) 209 [M + 1]$^+$; M_r (+FAB) 209.063 66 [M + 1]$^+$ (calcd for $C_{11}H_{13}O_2S$ 209.063 63).

Fragmentation of Compound 7. The previously described procedure was employed using **7** (3 mg, 0.007 mmol) and thiophenol (4 mg, 0.035 mmol). Preparative TLC (20% methanol–chloroform) afforded **18** (0.4 mg, 17%), **16** (0.3 mg, 20%), and starting material **7** (1.5 mg, 50%).

Compound 18: R_f 0.30 (20% methanol–chloroform); ^1H NMR (CD_3-OD) δ 1.18 (s, 3 H, C(2′)CH_3), 2.07 (s, 3 H, N(CH_2CH_2)$_2$NC(O)CH_3), 2.42 (d, 1 H, C(3′)HH′, J = 14.1 Hz), 2.50–2.75 (m, 5 H, N(CH_2-CH_2)$_2$NC(O)CH_3, C(3′)HH′), 3.50–3.65 (m, 4 H, N(CH_2CH_2)$_2$NC-(O)CH_3), 3.93 (d, 1 H, C(1′)H, J = 2.3 Hz), 4.32 (d, 1 H, C(1)H, J = 2.3 Hz); ^{13}C NMR (DMSO-d_6) 21.13 (NC(O)CH_3), 21.98 (C(2′)CH_3), 41.04 and 45.85 (N(CH_2CH_2)$_2$NC(O)CH_3), 54.43 and 54.84 (N(CH_2-CH_2)$_2$NC(O)CH_3), 60.09 (C(1)), 65.23 (C(3′)), 73.63 (C(1′)), 74.12 (C(2′)), 168.08 (NC(O)CH_3) ppm; the C(3) and C(5) carbonyl carbon signals were not observed; MS (+FAB) 315 [M + 1]$^+$; M_r (+FAB) 315.166 78 [M + 1]$^+$ (calcd for $C_{13}H_{22}N_4O_5$ 315.166 85).

Compound 16: R_f 0.60 (10% methanol–chloroform); ^1H NMR ($CDCl_3$) δ 2.05–2.10 (m, 1 H, C(3)HH′), 2.40–2.60 (m, 1 H, C(3)HH′), 2.75–3.00 (m, 2 H, C(2)H, C(1′)HH′), 3.55–3.65 (m, 1 H, C(1′)HH′), 4.15–4.25 (m, 1 H, C(4)HH′), 4.35–4.45 (m, 1 H, C(4)HH′), 7.20–7.50 (m, 5 H, Ar H).

Fragmentation of Compound 8. Using the protocol previously described for **6**, compound **8** (3 mg, 0.007 mmol) and thiophenol (3.8 mg, 0.034 mmol) provided **19** (0.7 mg, 30%), **16** (0.4 mg, 25%), and **8** (1.1 mg, 37%) after preparative TLC (10% methanol–chloroform).

Compound 19: R_f 0.35 (10% methanol–chloroform); ^1H NMR (CD_3-OD) δ 1.18 (s, 3 H, C(2′)CH_3), 1.24 (t, 3 H, $NCO_2CH_2CH_3$, J = 7.0 Hz), 2.40 (d, 1 H, C(3′)HH′, J = 14.1 Hz), 2.50–2.70 (m, 5 H, N(CH_2-CH_2)$_2$$NCO_2CH_2CH_3$, C(3′)H$H$′), 3.40–3.50 (m, 4 H, N(CH_2CH_2)$_2$-$NCO_2CH_2CH_3$), 3.97 (d, 1 H, C(1′)H, J = 0.8 Hz), 4.10 (q, 2 H, $NCO_2CH_2CH_3$, J = 7.0 Hz), 4.39 (d, 1 H, C(1)H, J = 0.8 Hz); ^{13}C NMR (DMSO-d_6) 14.55 ($NCO_2CH_2CH_3$), 21.86 (C(2′)CH_3), 43.51 (N(CH_2CH_2)$_2$$NCO_2CH_2CH_3$), 54.33 (N($CH_2CH_2$)$_2$$NCO_2CH_2CH_3$), 60.12 ($NCO_2CH_2CH_3$), 60.56 (C(1)), 65.35 (C(3′)), 73.58 (C(1′)), 74.12 (C(2′)), 154.52 ($NCO_2CH_2CH_3$) ppm; the C(3) and C(5) carbonyl carbon signals were not observed; MS (+FAB) 345 [M + 1]$^+$; M_r (+FAB) 345.177 35 [M + 1]$^+$ (calcd for $C_{14}H_{25}N_4O_6$ 345.177 41).

Compound 16: R_f 0.60 (10% methanol–chloroform); ^1H NMR ($CDCl_3$) δ 2.05–2.10 (m, 1 H, C(3)HH′), 2.40–2.60 (m, 1 H, C(3)HH′), 2.75–3.00 (m, 2 H, C(2)H, C(1′)HH′), 3.55–3.65 (m, 1 H, C(1′)HH′), 4.15–4.25 (m, 1 H, C(4)HH′), 4.35–4.45 (m, 1 H, C(4)HH′), 7.20–7.50 (m, 5 H, Ar H).

Fragmentation Studies of Compounds 6–8 in Deuterium Oxide. NMR kinetic experiments were performed in 0.1 M K_2DPO_4–deuterium oxide solutions (0.5 mL, "pD" 9.7) containing **6–8** (2 mg). The reaction temperature was maintained at 22 ± 1 °C. The pD of the solution was determined from the observed pH meter reading by using the relationship pD = pH meter reading + 0.4.[22] The reactions were monitored by ^1H NMR spectroscopy using a General Electric QE-300 NMR instrument. Each transformation was monitored for at least two half-lives, during which time at least six measurements were made. The relative amounts of **6–8** and product at each time point were calculated from the ^1H NMR spectra by comparing the integrated area for the vinylic hydrogens in **6–8** versus the exomethylene signals in **17**. Standard data plots of $\ln(a_0/a)$ versus time, where a_0 is the initial amount of the bicyclomycin derivative (i.e., **6–8**) and a is the remaining amount of starting material, yielded linear slopes (Figure 1) from which the first-order rate constants (k_1) were calculated. At the conclusion of the experiment, verification of the reaction products was accomplished by the addition of authentic samples (i.e., **15**, **17**, **18**, **19**) to the NMR sample and observing the selective increase of the signals corresponding to each of these compounds. Duplicate kinetic runs were performed and the results averaged (Table 2). A similar protocol (single kinetic experiment) was employed for **6** using a buffered (K_2DPO_4) tetrahydrofuran-d_8–deuterium oxide (3:1, "pD" 9.8) solution (Table 2).

Reaction of α-Methylene-γ-butyrolactone (17) with Thiophenol. To a solution of **17** (15 mg, 0.15 mmol) in a tetrahydrofuran–water (3:1) mixture (3 mL) was added thiophenol (84 mg, 0.76 mmol). The reaction was stirred at room temperature (2 h), during which time the "pH" of the solution remained at 7.0. The solvent was removed *in vacuo*, and the residue was dissolved in a minimum amount of chloroform and then purified by preparative TLC (methylene chloride) to afford **16**: yield 25 mg (79%); R_f 0.50 (methylene chloride); ^1H NMR ($CDCl_3$) δ 2.00–2.20

(22) Bates, R. G. *Determination of pH: Theory and Practice*, 2nd ed.; Wiley: New York, 1973; pp 375–376.

(m, 1 H, C(3)*H*H'), 2.40–2.57 (m, 1 H, C(3)H*H'*), 2.65–2.90 (m, 2 H, C(2)H, C(1')*H*H'), 3.50–3.60 (m, 1 H, C(1')H*H'*), 4.05–4.30 (m, 1 H, C(4)*H*H'), 4.30–4.40 (m, 1 H, C(4)H*H'*), 7.20–7.45 (m, 5 H, Ar H); the ^{1}H NMR assignments were confirmed by the COSY spectrum; ^{13}C NMR ($CDCl_3$) 28.27 (C(3)), 34.54 (C(2)), 39.48 (C(1')), 66.56 (C(4)), 126.82 (C(2'')Ar), 129.14 (C(4'')Ar), 129.99 (C(3'')Ar), 134.58 (C(1'')-Ar), 177.51 (C(1)) ppm.

Competition Study of Bicyclomycin (1) and α-Methylene-γ-butyrolactone (17) with Thiophenol. Equimolar amounts of **1** (21.6 mg, 0.07 mmol) and **17** (7.0 mg, 0.07 mmol) in a tetrahydrofuran–water (3:1) mixture were treated with thiophenol (7.8 mg, 0.07 mmol). The solution ("pH" 7.0) was stirred at room temperature (24 h). The solvents were removed *in vacuo*. The residue was dissolved in a minimum amount of methanol and then purified by preparative TLC to afford **1** (16 mg, 74%) and **16** (11 mg, 74%).

Compound 1: R_f 0.20 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2')CH_3), 2.58–2.65 (m, 2 H, C(4)H_2), 3.50 (d, 1 H, C(3')*H*H', J = 11.4 Hz), 3.67 (d, 1 H, C(3')H*H'*, J = 11.4 Hz), 3.75–3.96 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1')H), 5.13 (s, 1 H, C(5a)-*H*H'), 5.55 (s, 1 H, C(5a)H*H'*).

Compound 16: R_f 0.60 (10% methanol–chloroform); ^{1}H NMR ($CDCl_3$) δ 2.05–2.20 (m, 1 H, C(3)*H*H'), 2.40–2.55 (m, 1 H, C(3)H*H'*), 2.75–2.95 (m, 2 H, C(2)H, C(1')*H*H'), 3.55–3.65 (m, 1 H, C(1')H*H'*), 4.15–4.25 (m, 1 H, C(4)*H*H'), 4.35–4.45 (m, 1 H, C(4)H*H'*), 7.20–7.50 (m, 5 H, Ar H).

Preparation of Bicyclomycin 1'-*O*,3'-*O*-Dimethanesulfonate (23). Bicyclomycin (100 mg, 0.33 mmol) was dissolved in anhydrous pyridine (3 mL), and CH_3SO_2Cl (182 mg, 1.65 mmol) was added, after which time the temperature was raised to 45 °C (5 h). The solvent was removed *in vacuo*. The residue was subjected to flash chromatography on SiO_2 (5% methanol–chloroform) to provide a pale yellow solid: yield 57 mg (38%); mp 148–150 °C; R_f 0.40 (10% methanol–chloroform); FT-IR (KBr) 3470, 3252, 1705, 1397, 1352, 1177 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.52 (s, 3 H, C(2')CH_3), 2.58–2.68 (m, 2 H, C(4)H_2), 3.08 (s, 3 H, C(3')OSO_2CH_3 or C(1')OSO_2CH_3), 3.11 (s, 3 H, C(1')OSO_2CH_3 or C(3')OSO_2CH_3), 3.85–4.05 (m, 2 H, C(3)H_2), 4.23 (d, 1 H, C(3')*H*H', J = 9.8 Hz), 4.44 (d, 1 H, C(3')H*H'*, J = 9.8 Hz), 5.17 (s, 1 H, C(5a)-*H*H'), 5.24 (s, 1 H, C(1')H), 5.59 (s, 1 H, C(5a)H*H'*); ^{13}C NMR (CD_3OD) 25.04 (C(2')CH_3), 36.40 (C(4)), 37.47 (C(3')OSO_2CH_3 or C(1')OSO_2CH_3), 39.46 (C(1')OSO_2CH_3 or C(3')OSO_2CH_3), 66.08 (C(3)), 74.91 (C(3')), 76.52 (C(2')), 81.34 (C(1')), 82.94 (C(6)), 88.67 (C(1)), 117.43 (C(5a)), 148.92 (C(5)), 167.06 (C(7) or C(9)), 171.82 (C(9) or C(7)) ppm; MS (+FAB) 459 [M + 1]$^+$; M_r (+FAB) 459.074 50 [M + 1]$^+$ (calcd for $C_{14}H_{23}N_2O_{11}S_2$ 459.074 33).

Reaction of Bicyclomycin 1'-*O*,3'-*O*-Dimethanesulfonate (23) with Morpholine. Preparation of 20. To a methanolic solution (2 mL) of **23** (15 mg, 0.033 mmol) was added morpholine (8.6 mg, 0.099 mmol). The reaction solution was stirred at 45 °C (2 h), and then the solvent was removed *in vacuo*. The residue was dissolved in a minimum amount of methanol and purified by preparative TLC (10% methanol–chloroform) to afford **20**: yield 7 mg (48%); R_f 0.50 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.41 (s, 3 H, C(2')CH_3), 2.50–2.70 (m, 8 H, N(CH_2-CH_2)$_2$O, C(4)H_2, C(3')H_2), 3.04 (s, 3 H, C(1')OSO_2CH_3), 3.60–4.00 (m, 6 H, N(CH_2CH_2)$_2$O, C(3)H_2), 5.15 (s, 1 H, C(5a)*H*H'), 5.42 (s, 1 H, C(1')H), 5.58 (s, 1 H, C(5a)H*H'*); ^{13}C NMR (CD_3OD) 28.53 (C(2')-CH_3), 36.68 (C(4)), 39.40 (C(1')OSO_2CH_3), 55.84 (N(CH_2CH_2)$_2$O), 65.78 (C(3)), 66.39 (C(3')), 67.61 (N(CH_2CH_2)$_2$O), 76.43 (C(2')), 83.14 (C(6)), 85.74 (C(1')), 87.48 (C(1)), 117.01 (C(5a)), 148.98 (C(5)), 167.46 (C(7) or C(9)), 172.74 (C(9) or C(7)); MS (+FAB) 450 [M + 1]$^+$; M_r (+FAB) 450.154 54 [M + 1]$^+$ (calcd for $C_{17}H_{28}N_3O_9S$ 450.154 63).

Preparation of Dihydrobicyclomycin C(3') Morpholine (22). To a methanolic solution (3 mL) of **6** (7 mg, 0.019 mmol) was added PtO_2 (1 mg, 0.004 mmol). The mixture was stirred at room temperature under an atmosphere of H_2 (1 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of one major product: yield 7.2 mg (~100%); R_f 0.40 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.06 (d, 3 H, C(5a)H_3, J = 6.9 Hz), 1.32 (s, 3 H, C(2')CH_3), 1.60–1.75 (m, 1 H, C(4)*H*H'), 1.95–2.10 (m, 1 H, C(4)H*H'*), 2.10–2.25 (m, 1 H, C(5)H), 2.51 (d, 1 H, C(3')*H*H', J = 13.8 Hz), 2.55–2.75 (m, 7 H, N(CH_2CH_2)$_2$O, C(4)H_2, C(3')H*H'*), 3.65–3.80 (m, 5 H, C(3)*H*H', N(CH_2CH_2)$_2$O), 4.00 (dd, 1 H, C(3)H*H'*, J = 8.7, 13.8 Hz), 4.14 (s, 1 H, C(1')H); the ^{1}H NMR assignments were confirmed by the COSY experiment; ^{13}C NMR (CD_3OD) 15.87 (C(5a)), 26.80 (C(2')CH_3), 34.94 (C(4)), 46.80 (C(5)), 56.44 (N(CH_2CH_2)$_2$O), 62.87 (C(3)), 66.44 (C(3')), 67.88 (N(CH_2CH_2)$_2$O), 75.35 (C(1')), 77.85 (C(2')), 84.54 (C[illegible] 88.95 (C[illegible]), 168.58 (C[illegible]) or C(9)), 172.36 (C(9) or C(7)) ppm; additional low-intensity signals were observed and may be attributed to a diastereomer of **22**; MS (+FAB) 374 [M + 1]$^+$; M_r (+FAB) 374.193 18 [M + 1]$^+$ (calcd for $C_{16}H_{28}N_3O_7$ 374.192 73).

Stability Study of Compound 20. A solution of **20** (3 mg, 0.007 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was stirred first at room temperature (5 days) and then at 32 °C (2 days) under Ar. The "pH" of the solution (7.5) remained unchanged during this interval. TLC analysis indicated no significant consumption of **20**. The solvents were removed *in vacuo*, and the residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **20** (1.3 mg, 43%): R_f 0.50 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.41 (s, 3 H, C(2')CH_3), 2.50–2.70 (m, 8 H, N(CH_2CH_2)$_2$O, C(4)H_2, C(3')H_2), 3.03 (s, 3 H, C(1')OSO_2CH_3), 3.65–4.00 (m, 6 H, N(CH_2CH_2)$_2$O, C(3)H_2), 5.15 (s, 1 H, C(5a)*H*H'), 5.42 (s, 1 H, C(1')H), 5.58 (s, 1 H, C(5a)H*H'*).

Stability Study of Compound 21. A solution of **21**[7d] (3 mg, 0.009 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was stirred first at room temperature (5 days) and then at 32 °C (2 days) under Ar. The "pH" of the solution (7.5) remained unchanged during this interval. TLC analysis indicated no significant consumption of **21**. The solvents were removed *in vacuo*, and the residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **21** (1.4 mg, 47%): R_f 0.40 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.21 (t, 3 H, SCH_2CH_3, J = 7.2 Hz), 1.37 (s, 3 H, C(2')CH_3), 2.55–2.65 (m, 4 H, C(4)H_2, SCH_2CH_3), 2.75 (d, 1 H, C(3')*H*H', J = 13.4 Hz), 3.00 (d, 1 H, C(3')H*H'*, J = 13.4 Hz), 3.75–3.95 (m, 2 H, C(3)H_2), 4.22 (s, 1 H, C(1')H), 5.11 (s, 1 H, C(5a)*H*H'), 5.55 (s, 1 H, C(5a)H*H'*).

Stability Study of Compound 22. A solution of **22** (4 mg, 0.011 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was stirred first at room temperature (5 days) and then at 32 °C (2 days) under Ar. The "pH" of the solution (7.2) remained unchanged during this interval. TLC analysis indicated no significant consumption of **22**. The solvents were removed *in vacuo*, and the residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **22** (3.0 mg, 75%): R_f 0.40 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.06 (d, 3 H, C(5a)H_3, J = 6.9 Hz), 1.31 (s, 3 H, C(2')CH_3), 1.60–1.75 (m, 1 H, C(4)*H*H'), 1.95–2.07 (m, 1 H, C(4)H*H'*), 2.10–2.25 (m, 1 H, C(5)H), 2.51 (d, 1 H, C(3')*H*H', J = 14.1 Hz), 2.55–2.75 (m, 7 H, N(CH_2CH_2)$_2$O, C(4)H_2, C(3')H*H'*), 3.65–3.80 (m, 5 H, C(3)*H*H', N(CH_2CH_2)$_2$O), 3.99 (dd, 1 H, C(3)H*H'*, J = 8.7, 13.8 Hz), 4.14 (s, 1 H, C(1')H).

Reaction of Bicyclomycin C(3') Morpholine (6) with Thiophenol in Tetrahydrofuran–Water (3:1). To a solution of **6** (4 mg, 0.011 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was added thiophenol (6 mg, 0.054 mmol). The "pH" of the reaction was initially adjusted to 9.0 with a dilute aqueous NaOH solution. The reaction was stirred at room temperature (7 days), during which time the "pH" of the solution dropped to 7.8. TLC analysis (10% methanol–chloroform) indicated the presence of one major spot along with trace amounts of **6**, **15**, **16**, and several unidentified compounds. The solvents were removed *in vacuo*, and the residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **30** (1.1 mg, 24%): R_f 0.60 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.80–1.95 (m, 1 H, C(4)*H*H'), 2.15–2.35 (m, 2 H, C(4)H*H'*, C(5)H), 2.80–2.90 (m, 1 H, C(1'')*H*H'), 3.05–3.35 (m, 1 H, C(1'')H*H'*), 3.70–3.95 (m, 1 H, C(3)-*H*H'), 4.00–4.15 (m, 1 H, C(3)H*H'*), 5.67 (m, 1 H, C(3')H), 7.10–7.65 (m, 10 H, Ar H); the ^{1}H NMR assignments were confirmed by the COSY experiment; MS (+FAB) 419 [M + 1]$^+$; M_r 419.109 71 (calcd for $C_{20}H_{23}N_2O_4S_2$ 419.109 93).

Reaction of Bicyclomycin C(3') Morpholine (6) with Thiophenol in Water. To an aqueous solution (2 mL) of **6** (7.5 mg, 0.02 mmol) was added thiophenol (12 mg, 0.109 mmol). The "pH" was initially adjusted with a dilute aqueous NaOH solution to 9.0. The reaction was stirred at room temperature (2 days), during which time the "pH" of the solution dropped to 8.4. TLC analysis (10% methanol–chloroform) indicated the presence of two major spots along with trace amounts of **15** and **16**, and several unidentified compounds. The reaction mixture was extracted with methylene chloride (3 × 2 mL), the methylene chloride layers were combined, and the solvent was removed *in vacuo*. The residue was dissolved in methanol and then purified by preparative TLC (10% methanol–chloroform) to afford **30** (1.9 mg, 23%) and **34** (0.5 mg, 14%).

Compound 30: R_f 0.60 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.80–1.95 (m, 1 H, C(4)*H*H'), 2.15–2.35 (m, 2 H, C(4)H*H'*, C(5)H), 2.80–2.90 (m, 1 H, C(1'')*H*H'), 3.05–3.35 (m, 1 H, C(1'')-H*H'*), 3.70–3.95 (m, 1 H, C(3)*H*H'), 4.00–4.15 (m, 1 H, C(3)H*H'*), 5.67 (m, 1 H, C(3')H), 7.10–7.65 (m, 10 H, Ar H); ^{13}C NMR ($CDCl_3$) 30.01,

30.07 (C(4)), 32.41, 32.54 (C(1″)), 47.38, 47.70 (C(5)), 57.21, 57.45 (C(3′)), 68.18, 68.36 (C(3)), 101.64, 101.77 (C(1)), 126.05 (C(4′)Ar), 128.77, 128.92 (C(2′)Ar), 129.04, 129.34 (C(3′)Ar), 134.78, 134.89 (C(1′)Ar), 168.73, 168.84 (C(1′) or C(4′)), 170.69 (C(1′) or C(4′)); MS (+FAB) 419 $[M + 1]^+$.

Compound 34: R_f 0.80 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.39 (s, 3 H, C(2)CH_3), 2.35–2.65 (m, 6 H, C(3)H_2, N(CH_2CH_2)$_2$O), 3.59–3.70 (m, 4 H, N(CH_2CH_2)$_2$O); the aldehyde proton was not detected; MS (+FAB) 174 $[M + 1]^+$; M_r 174.113 19 (calcd for $C_8H_{16}NO_3$ 174.113 02).

Reaction of Bicyclomycin (1) with Thiophenol. A solution of **1** (10 mg, 0.033 mmol) and thiophenol (18 mg, 0.16 mmol) in a tetrahydrofuran–water (3:1) mixture (2 mL) was stirred at room temperature (24 h) under Ar. The "pH" was initially adjusted with a dilute aqueous NaOH solution to 9.3. During the course of the reaction the "pH" value dropped to 9.0. TLC analysis (10% methanol–chloroform) indicated one major and one minor compound and unreacted **1**. The solvents were removed *in vacuo*, and the residue was purified by preparative TLC (10% methanol–chloroform) to give **13** (6.2 mg, 46%) and **14** (1.4 mg, 13%).

Compound 13: R_f 0.26 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.32 (s, 3 H, C(2′)CH_3), 2.05–2.35 (m, 3 H, C(4)H_2, C(5)H), 2.56 (dd, 1 H, C(5a)*H*H′, J = 12.0, 13.8 Hz), 3.45–3.60 (m, 1 H, C(3′)-*H*H′), 3.62–3.80 (m, 3 H, C(3)*H*H′, C(5a)H*H*′, C(3′)H*H*′), 3.95–4.05 (m, 2 H, C(3)H*H*′, C(1′)H), 7.10–7.40 (m, 5 H, Ar H); the ^{1}H NMR assignments were confirmed by the COSY experiment; ^{13}C NMR (CD_3OD) 24.19 (C(2′)*C*H$_3$), 29.86 (C(5a)), 33.06 (C(4)), 51.77 (C(5)), 62.10 (C(3)), 68.50 (C(3′)), 72.13 (C(1′)), 78.12 (C(2′)), 83.58 (C(6)), 89.37 (C(1)), 126.94 (C(4′)Ar), 129.86 (C(2′)Ar), 130.03 (C(3′)Ar), 137.31 (C(1′)Ar), 168.65 (C(7) or C(9)), 172.10 (C(9) or C(7)) ppm; MS (+FAB) 413 $[M + 1]^+$; M_r (+FAB) 413.137 95 (calcd for $C_{18}H_{25}N_2O_7S$ 413.138 25).

Compound 14: R_f 0.58 (10% methanol–chloroform); ^{1}H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2′)CH_3), 2.05 (dd, 1 H, C(4)*H*H′, J = 2.7, 14.0 Hz), 2.34 (dt, 1 H, C(4)H*H*′, J = 6.6, 14.0 Hz), 3.25 (d, 1 H, C(5a)*H*H′, J = 13.7 Hz), 3.50 (d, 1 H, C(5a)H*H*′, J = 13.7 Hz), 3.60 (d, 1 H, C(3′)*H*H′, J = 12.3 Hz), 3.73 (dt, 1 H, C(3)*H*H′, J = 2.7, 14.0 Hz), 3.92–4.25 (m, 3 H, C(1′)H, C(3)H*H*′, C(3′)H*H*′), 7.07–7.22 (m, 5 H, Ar H); the ^{1}H NMR assignments were confirmed by the COSY experiment; MS (+FAB) 396 $[M + 1]^+$; M_r (+FAB) 396.111 31 (calcd for $C_{18}H_{22}NO_7S$ 396.111 70).

Crystallographic Procedure for Compound 15. A small colorless square column obtained after recrystallization from methanol having approximate dimensions 0.06 × 0.08 × 0.45 mm was mounted in a random orientation on a Nicolet R3m/V automatic diffractometer. 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, are listed in Table 3. The Laue symmetry 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 change. During data reduction Lorentz and polarization corrections were applied; however, no correction for absorption was made due to the small absorption coefficient.

The structure was solved by the SHELXTL direct methods program, which revealed the positions of most of the atoms in the molecule. Remaining nonhydrogen atoms were located in subsequent difference Fourier synthesis, after which all hydrogens were entered in ideal calculated positions and constrained to riding motion, with a single variable isotropic temperature factor for all of them. Since the sample crystal was extremely thin, there was relatively little observed data, and therefore only isotropic temperature factors were used for all atoms. In the final cycles of refinement, the four hydrogens not attached to carbon were allowed to move independently. H11′ did not stay in a reasonable position, however, and so finally it was fixed in a location deemed appropriate for hydrogen bonding to a neighboring O10′. Although the molecule is chiral, it contains no significant anomalous scatterer, and thus the absolute configuration could not be determined experimentally. Therefore, the configuration was arbitrarily fixed so as to match that of the known starting material, which is *S* at C(2′). After all shift/esd ratios were less than 0.1, convergence was reached at the agreement factors listed in Table 3. 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 0.3 e/Å^3. All calculations were made using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs.

Table 3. Data Collection and Processing Parameters for Compound **15**

space group	$P2_12_12_1$ (orthorhombic)
cell constants	a = 6.237(4) Å
	b = 10.052(5) Å
	c = 20.099(12) Å
	V = 1260 Å^3
molecular formula	$C_{11}H_{19}N_3O_5$
formula weight	273.33
formula units per cell	Z = 4
density	ρ = 1.44 g cm^{-3}
absorption coefficient	μ = 1.07 cm^{-1}
radiation (Mo Kα)	λ = 0.71073 Å
collection range	$4° \leq 2\theta \leq 40°$
scan width	$\Delta\theta = 1.20 + (K\alpha_2 - K\alpha_1)°$
scan speed range	1.5–15.0° min^{-1}
total data collected	718
independent data, $I > 3\sigma(I)$	454
total variables	87
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.057
$R_w = [\Sigma w(\|F_o\| - \|F_c\|)^2/\Sigma w\|F_o\|^2]^{1/2}$	0.047
weights	$w = \sigma(F)^{-2}$

Inhibitory Properties of Bicyclomycin (1) and Bicyclomycin Derivatives 6–8 in the Poly(C)-Dependent ATPase Rho Assay.[19] The poly(C)-dependent ATPase activity of rho[19,23] at 32 °C was assayed measuring the amount of [^{32}P] inorganic phosphate hydrolyzed from ATP after separation on Baker-Flex cellulose PEI TLC plates (J. T. Baker, Inc., Phillipsburg, NJ) using 0.75 M potassium phosphate, pH 3.5, as the mobile phase. Reactions were initiated by addition of ATP (250 μM) and 1-μCi [γ-^{32}P]ATP to a 0.2-mL solution containing buffer (50 mM Tris·HCl, pH 7.9, 50 mM KCl, 12 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 0.14 mg of bovine serum albumin), poly(C) (24 μM), rho (80 nM), and various concentrations (10–400 μM) of the test compound. The TLC plates were exposed to XAR5 X-ray film and visualized. TLC bands were cut and counted by liquid scintillation. Relative percent activities of rho were calculated from the initial velocities.

Antimicrobial Assay of Bicyclomycin (1) and Bicyclomycin Derivatives 6–8. Centrifuged cells (*E. coli* W3350, *S. marcescens* SM6, *B. megaterium* ATCC 11478, *S. cerevisiae* MG 159B) from overnight LB broth cultures (50 mL) were suspended in LB broth (4 mL), and then 100 μL of cells was diluted into 2 mL of broth and mixed. The solution was poured onto 15-mL-volume LB agar plates. The LB agar plate was gently rocked to distribute the cells evenly over the plate surface, and any excess cell solution was removed with a pipet. The plate was dried (15 min) in the incubator at 37 °C. An antibiotic-assay disk (Aldrich, Z13409-0, 1/4 in.), containing 20 μL of the test compound (1000, 2000, 4000, 8000, 16 000 μg/mL), was placed on the agar surface. The plates were incubated at 37 °C (20 h). Data plots of the zone of inhibited bacterial growth (cm^3) versus log C, where C is the concentration of test compound (μg/mL), yielded linear slopes to provide the minimal inhibitory concentrations (MIC) for **1**, **6**, **7**, and **8**.

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Supplementary Material Available: Tables giving a complete listing of atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, and hydrogen-bonding parameters for compound **15** (2 pages); table of observed and calculated structure factors for compound **15** (3 pages). This material is contained in many 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.

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