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Chemical, Biochemical, and Biological Studies on Select C(1) Triol Modified Bicyclomycins

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Abstract: To determine the importance of the C(1) triol group to bicyclomycin (**1**)-mediated transformations we prepared the bicyclomycin diastereomers **6** (C(1′)-*R*, C(2′)-*S*) and **7** (C(1′)-*S*, C(2′)-*R*), in which the stereochemical configuration at C(1′) and C(2′) in the triol group in **1** (C(1′)-*S*, C(2′)-*S*) was reversed, and the C(1′) ketone analogue **8** (C(2′)-*S*), in which the stereogenic center at C(1′) in **1** was removed. Synthesis of **6** and **8** proceeded from C(1′) ketobicyclomycin C(2′), C(3′) acetonide (**10**). Reduction ($NaBH_4$, $CeCl_3$) of **10** produced a diastereomeric mixture, that, after separation and removal of the acetonide protecting group, gave **6**. Correspondingly, deprotection of **10** gave **8**. Bicyclomycin analogue **7** was prepared by dissolving the known bicyclomycin C(2′), C(3′) epoxide (**13**) in dilute methanolic sulfuric acid; this process produced the novel [O(9)–C(2′)]cyclized bicyclomycin (**14**). Compound **14** formed with inversion of the C(2′) center. Subsequent aqueous acid hydrolysis yielded **7**. Data documenting the proposed reaction pathways and structures for compounds **6–8** are presented. The stability of bicyclomycin analogues **6–8** and **1** in deuterium oxide (pD 5.6–5.8, 7.4, 9.2–9.4) and in DMF-d_7 solutions were examined. Compounds **7** and **8** were stable under these conditions (room temperature, 14 days), whereas bicyclomycin underwent noticeable change only in basic deuterium oxide. Correspondingly, **6** was rapidly converted ($t_{1/2} < 30$ h) to a new set of products in both acidic and basic deuterium oxide as well as in DMF-d_7. The facility of these conversions have been attributed in part to the role of the C(1) triol substituent in the ring opening of the C(6) hemiketal group in **6**. All three bicyclomycin analogues reacted with ethanethiol at the C(5)–C(5a) exomethylene unit at rates comparable to **1** in buffered ("pH" 8.0–8.5) THF–H_2O (3:1) mixtures. The products generated from **6** and **7** were similar to those previously determined for **1**, except for the configuration of the C(1′) and C(2′) substituents, whereas **8** yielded the novel piperidine adduct **33**. The ethanethiol–**8** reaction proceeded easily in spite of earlier projections that the C(1′) hydroxyl group in bicyclomycin was required for exomethylene modification. Similarly the corresponding C(2′), C(3′) acetonide of **8**, **10**, readily underwent reaction with ethanethiol. Significantly, compounds **6** and **7** only partially (25–35%) inhibited rho-dependent hydrolysis of ATP at the concentration levels observed to block ATPase activity by **1**, and no inhibition of ATP hydrolysis was detected for **8**. Our previous studies established that the primary site of bicyclomycin action in *Escherichia coli* is the cellular protein transcription termination factor rho. Similarly, none of the three compounds exhibited antibiotic activity at a concentration of 1200 μg/mL, using a filter disc assay. These cumulative results suggested that key interactions existed between the C(1) triol group in bicyclomycin and the antibiotic binding site in rho, which are necessary for drug utilization and function.

Bicyclomycin (**1**) is a structurally distinctive, commercial antibiotic[1–4] whose primary site of action in *Escherichia coli* is the essential cellular protein transcription termination factor rho.[5] Studies in 1988 showed that modification of bicyclomycin by nucleophiles proceeded in mixed aqueous–tetrahydrofuran solutions under mild conditions to give piperidinediones **5**.[6] A novel

* Abstract published in *Advance ACS Abstracts*, October 1, 1994.
(1) Tanaka, N. *Antibiotics (N.Y.)* **1979**, *5*, 18–25.
(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. *J. Antibiot.* **1972**, *25*, 576–581. (c) Nishida, M.; Mine, Y.; Matsubara, T. *J. Antibiot.* **1972**, *25*, 582–593. (d) Nishida, M.; Mine, Y.; Matsubara, T.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1972**, *25*, 594–601.

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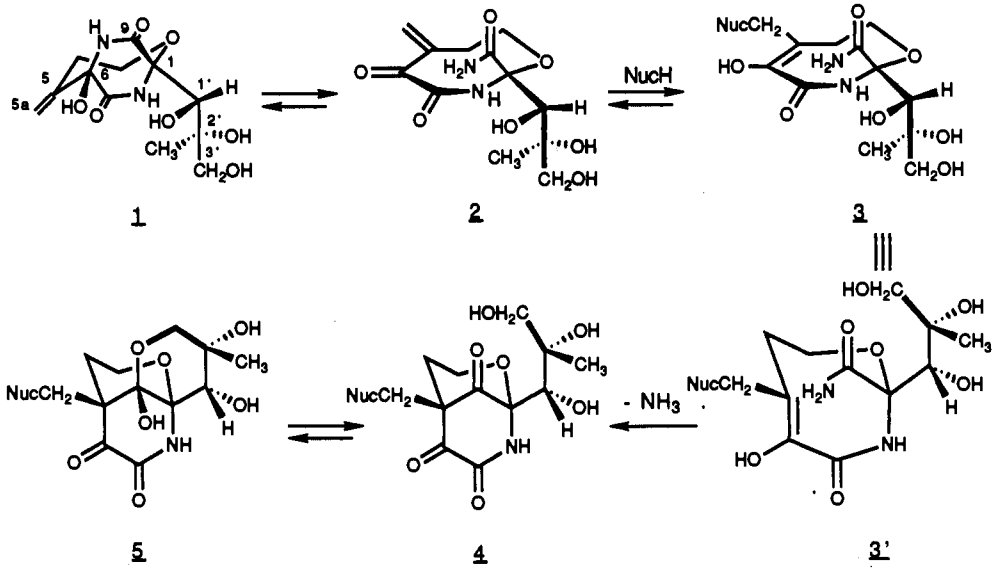
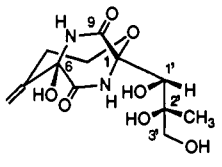
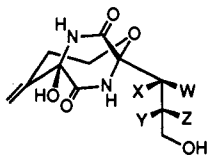
Scheme 1. Proposed Mechanism for the Mode of Action of Bicyclomycin (**1**)



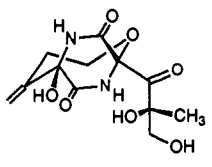
pathway (Scheme 1) for this reaction was advanced that emphasized the importance of the C(6) hemiketal, C(9) amide, and C(5)–C(5a) exomethylene functional groups and the constrained [4.2.2] bicyclic ring system in the conversion of **1** to **5**.[6] Complementary studies have documented the role of these structural units in bicyclomycin activation and bonding processes.[7-10] By comparison, less is known about the role of the appended C(1) triol group in bicyclomycin transformations. Our primary source of information of these issues comes from our studies[9,12,13] and those of Williams[11] that demonstrated that opening of the C(6) hemiketal ring in bicyclomycin derivatives and model compounds can be assisted by intramolecular hydrogen bond interactions between the C(1′) hydroxyl group and the C(9) amide unit. Here we report that the C(1) triol group in bicyclomycin plays an essential role in the drug-rho recognition process.[14] Comparison of the chemical, biochemical, and biological activities of bicyclomycin (**1**) with the two bicyclomycin isomers **6** and **7** and the C(1) ketone analogue **8** demonstrated that the chemical nature and stereochemical configuration of the appended C(1) unit was critical for efficient inactivation of rho.



1



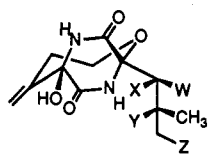
6 W = OH, X = H, Y = OH, Z = CH_3
7 W = H, X = OH, Y = CH_3, Z = OH

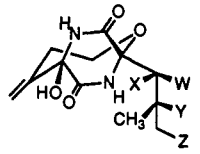


8

Results

a. Choice of Substrates and Synthesis. Our study comprised the bicyclomycin derivatives **6** (C(1′)-*R*, C(2′)-*S*) and **7** (C(1′)-*S*, C(2′)-*R*), in which the stereochemical configuration at C(1′) and C(2′) in **1** (C(1′)-*S*, C(2′)-*S*) were reversed, and the C(1′) ketone analogue **8** (C(2′)-*S*), in which the stereogenic center at C(1′) in **1** had been removed. Synthesis of the C(1′) epimer **6** and the C(1′) ketone **8** analogues required the initial ketalization of bicyclomycin to give acetonide **9**.[15] Attempted oxidation of **9** using either pyridinium chlorochromate or pyridinium dichromate in dichloromethane (room temperature, 24 h) led to the recovery of **9** (^{1}H NMR, TLC analyses). Swern oxidation,[16] however, produced the desired compound **10** along with two unidentified adducts. A 60% yield of **10** was obtained by adding trifluoroacetic anhydride to a dichloromethane solution of **9** and dimethyl sulfoxide at −78 °C and then adding triethylamine (1.2–1.5 equiv). The identity of **10** was confirmed by X-ray crystallographic analysis (supplementary material). Deprotection of **10** with trifluoroacetic acid provided the bicyclomycin C(1′) ketone analogue **8**.





1 W = H, X = OH, Y = OH, Z = OH
9 W = H, X = OH, Y,Z = $OC(CH_3)_2O$
* **6** W = OH, X = H, Y = OH, Z = OH
* **8** W,X = O, Y = OH, Z = OH
10 W,X = O, Y,Z = $OC(CH_3)_2O$
11 W = OH, X = H, Y,Z = $OC(CH_3)_2O$
12 W = H, X = OH, Y = OH, Z = OSO_2CH_3
13 W = H, X = OH, Y,Z = O

* **7** W = H, X = OH, Y = OH, Z = OH
17 W = H, X = OH, Y,Z = $OC(CH_3)_2O$
18 W = H, X, Z = $OC(CH_3)_2O$, Y = OH

Several reductive procedures were examined for the conversion of **10** to **11**. Use of $NaBH_4$ gave **9** as the major product along with a small amount of **11** (TLC and ^{1}H NMR analyses). Comparable results were obtained with $LiAlH_4$, $NaAlH_2(OCH_2CH_2OCH_3)_2$, lithium 9-BBN hydride, and $(i\text{-}Bu)_2AlH$. However, reduction of **10** with $NaBH_4$ and $CeCl_3$ under Luche conditions[17] rendered a 55:45 mixture of **11** and **9** that was separated by preparative TLC. X-ray crystallographic analysis of acetonide **11** (supplementary material) confirmed that reduction gave, in part, the C(1′)-*R* isomer **11**. Deprotection of the acetonide linkage

(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*, 610–612. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai H. *J. Antibiot.* **1973**, *26*, 479–484.

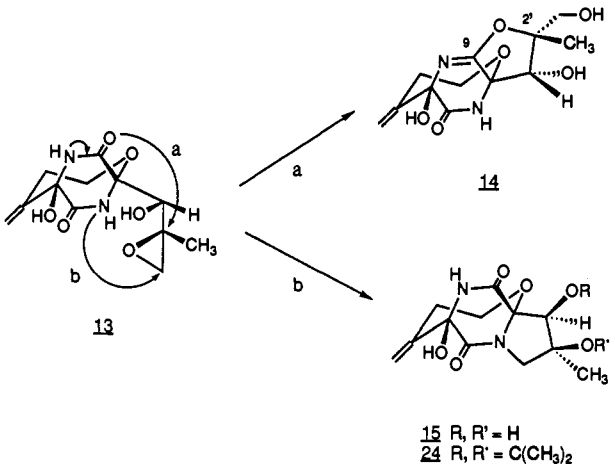
(4) Williams, R. M.; Durham, C. A. *Chem. Rev.* **1988**, *88*, 511–540, and references cited therein.

(5) Zweifka, A.; Kohn, H.; Widger, W. R. *Biochemistry* **1993**, *32*, 3564–3570.

(6) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089–4090.

in **11** with a dilute H_2SO_4 methanol–water solution furnished the C(1′) bicyclomycin stereoisomer **6**.

The remaining bicyclomycin analogue (**7**) chosen for study was prepared by converting **1** to the known C(3′) mesylate **12**[18] and then to the epoxide **13**.[18] Treatment of **13** with dilute H_2SO_4 in tetrahydrofuran–water (3:1) gave the novel [C(9)O–C(2′)] cyclized adduct **14** along with the known [N(8)–C(3′)]cyclized bicyclomycin (**15**).[12,18]



Using dilute methanolic sulfuric acid solutions in place of tetrahydrofuran–water (3:1) gave improved yields of **14**. X-ray crystallographic analysis of **14** (Figure 1) provided the evidence that cyclization proceeded with stereochemical inversion at C(2′). Hydrolysis of **14** with a dilute H_2SO_4 tetrahydrofuran–water (3:1) solution produced **7** and carboxylic acid **16**. The C(2′) stereochemical configuration was confirmed by treating **7** with 2,2-dimethoxypropane and *p*-toluenesulfonic acid to give acetonides **17** and **18** and then determining the X-ray crystallographic structure of **18** (supplementary material). Evidence in favor of carboxylic acid **16** was the observation of a $[M-1]^-$ parent ion in the high resolution FAB mass spectrum and signal detection at 83.01 and 97.88 ppm in the ^{13}C NMR spectrum, consistent with the proposed C(1′) ether and C(6) hemiketal carbon atoms, respectively.[19] Further evidence was provided by the HMBC experiment, which showed a diagnostic three-bond connectivity between the C(1′) proton and the hemiketal C(6) carbon.

Our finding that both **7** and **16** existed in the product mixture from **14** suggested that hydrolysis of **14** proceeded through imidate **19** (Scheme 2). Subsequent hydration would give **20**, which can undergo ring opening to give **21** and then ring closure to yield **7**, or undergo loss of ammonia to provide lactone **22**. Hydrolysis of **22**, followed by hemiketal formation yielded **16**.

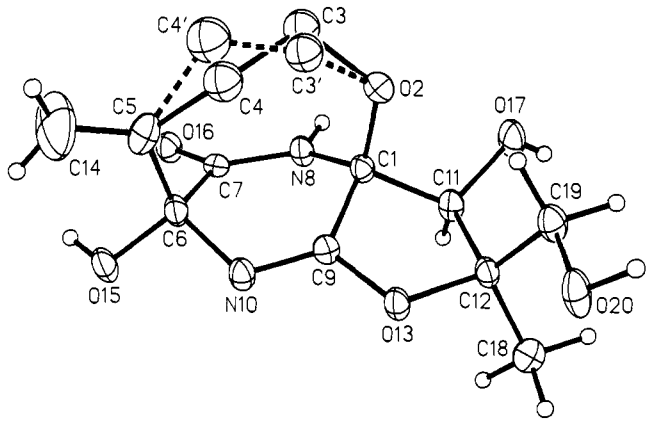
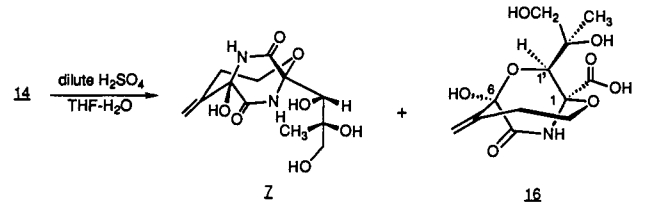
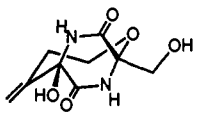


Figure 1. ORTEP drawing for **14** showing the atom numbering scheme. The thermal ellipsoids are 30% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. Both orientations of the disordered moiety are shown. To determine atom labels for molecule 2, add 20 to the numbers shown. Selected bond distances (Å) are as follows: C(1)–C(9), 1.516 (6); C(9)–O(13), 1.341 (4); C(12)–O(13), 1.482 (5); C(11)–C(12), 1.561 (5); C(1)–C(11), 1.530 (5). Selected angles (deg) are as follows: C(9)–O(13)–C(12), 111.8 (3); C(11)–C(12)–O(13), 102.2 (3); C(1)–C(11)–C(12), 103.9 (3); C(9)–C(1)–C(11), 100.5 (3); C(1)–C(9)–O(13), 110.5 (3).



b. Chemical Reactivity of Bicyclomycin Derivatives 6–8. Bicyclomycin function is believed to occur when a nucleophilic residue in rho is added to the exomethylene group in **1**.[20] In 1992, we demonstrated that thiolate addition occurred more rapidly with the constrained [N(8)–C(3′)]cyclized bicyclomycin derivative **15** than with the corresponding acetonide analogue **24**.[12] This finding was consistent with the notion that the C(1′) hydroxyl group facilitated C(6) hemiketal ring opening, permitting thiol addition to the α,β-unsaturated carbonyl system. Williams and co-workers reported a comparable finding for thiolate addition to bicyclomycin model compounds (i.e., **25**) at higher pH values.[11] This investigation led Williams to conclude that the C(1′) hydroxyl group was necessary for thiol C(5)–C(5a) exomethylene modification.[11] Accordingly, the chemical reactivities of **6–8** with ethanethiol were evaluated to determine the effect of C(1) triol modification on the functionalization of the C(5)–C(5a) exomethylene group.



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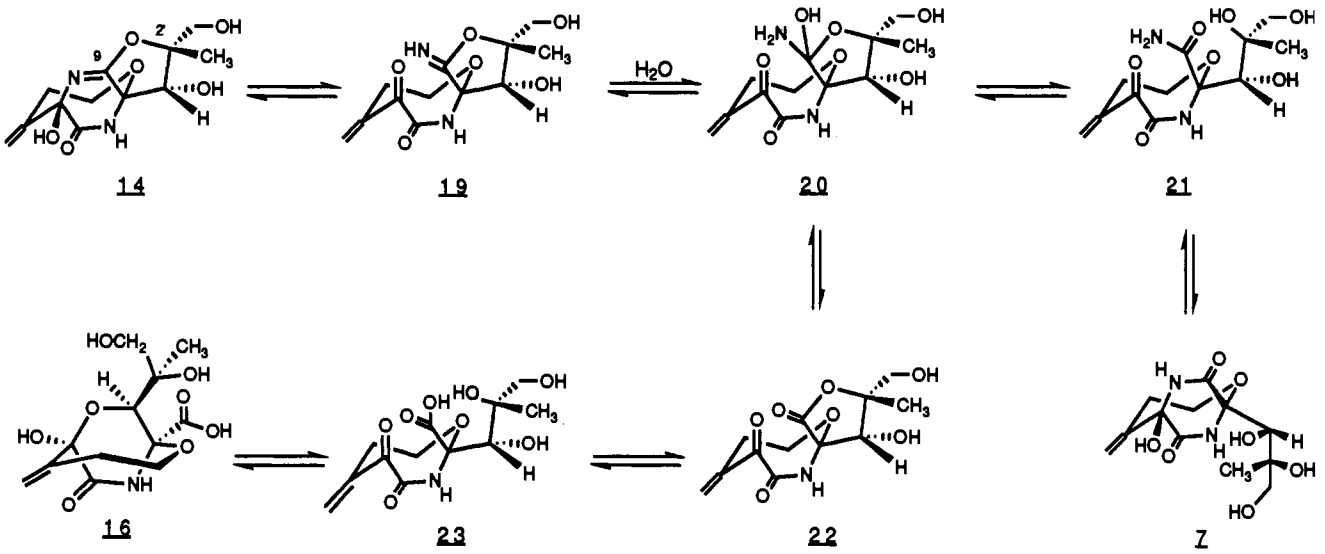
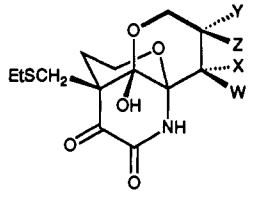
Our earlier studies on thiol-mediated bicyclomycin processes showed that the product type depended upon solution "pH".[6,7,9,21] In this study, we examined the reactivity of **1** and **6–8** with ethanethiol using the mildest conditions (tetrahydrofuran–water (3:1), "pH" 8.0–8.5, room temperature, 1–2 days) previously found to affect exomethylene functionalization.[6,9] These conditions produced a single product **26** (**5**) with **1**. Treatment of **6** with ethanethiol under comparable conditions gave piperidinedione

(7) Abuzar, S.; Kohn, H. *J. Org. Chem.* **1989**, *54*, 4000–4003.
(8) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1989**, *111*, 4895–4903.
(9) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1990**, *112*, 3114–3121.
(10) Oh, Y. S.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 3662–3672.
(11) (a) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1987**, *109*, 4028–4035. (b) Williams, R. M.; Tomizama, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 6419–6421.
(12) Vela, M. A.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 5223–5231.
(13) Park, H.; Vela, M. A.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 471–478.
(14) For a preliminary account of this study, see: Zhang, Z.; Kohn, H. *J. Chem. Soc., Chem. Commun.* **1994**, 1343–1344.
(15) Kamiyama, T.; Maeno, S.; Kitaura, Y. Belg. Pat. 847, 475.
(16) Omura, K.; Sharma, A. K.; Swern, D. *J. Org. Chem.* **1976**, *41*, 957–962.
(17) (a) Luche, J.-L.; Rodriguez-Hahn, L.; Crabbe', P. *J. Chem. Soc., Chem. Commun.* **1978**, 601–602. (b) Li, K.; Hamann, L. G.; Koreeda, M. *Tetrahedron Lett.* **1992**, *33*, 6569–6570.
(18) Müller, B. W.; Zak, O.; Kump, W.; Tosch, W.; Wacker, O. *J. Antibiot.* **1979**, *32*, 689–705.
(19) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972.
(20) Someya, S.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402–407.
(21) Kohn, H.; Abuzar, S. *J. Am. Chem. Soc.* **1988**, *110*, 3661–3663.

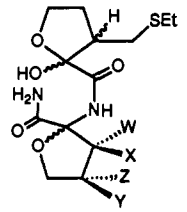
Scheme 2. Proposed Pathway for the Hydrolysis of Compound **14**



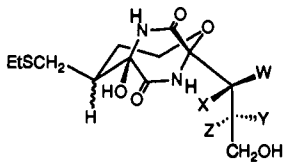
27 along with the ring opened adduct **28**. Correspondingly, **7** yielded **29** and **30**. The structures of **27** and **28** and of **29** and **30** differed only in the stereochemistry at the C(1′) or C(2′) sites, respectively, from the ethanethiol addition products **26** (**5**), **31**, and **32** previously obtained from **1**.[6,9,21] In agreement with these assignments, the observed ^1H and ^{13}C NMR chemical shift values corresponded closely within each structural class of compounds (i.e., **26** and **27** and **29**; **28** and **31**; and **30** and **32**).[6,9,21]



27 W = OH, X = H, Y = OH, Z = CH_3
29 W = H, X = OH, Y = CH_3, Z = OH
26 W = H, X = OH, Y = OH, Z = CH_3



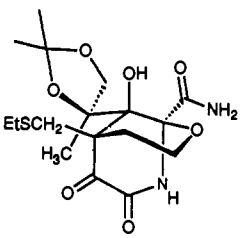
28 W = OH, X = H, Y = OH, Z = CH_3
31 W = H, X = OH, Y = OH, Z = CH_3



30 W = H, X = OH, Y = CH_3, Z = OH
32 W = H, X = OH, Y = OH, Z = CH_3

Treatment of the C(1′) ketone **8** with ethanethiol produced only **33**. The ^1H, ^{13}C, HMQC, and HMBC experiments and the X-ray crystallographic analysis of the tetra-*n*-butylammonium salt of **33** (Figure 2) confirmed the proposed structural assignment. We suspect that formation of **33** proceeded by a pathway similar to the one described in Scheme 1 for **5** (**26**), except that enol **35** underwent an intramolecular aldol condensation at the C(1′) carbonyl site instead of a Claisen condensation at the C(9) amide center (Scheme 3). Several likely pathways exist for the hydrolysis of the C(9) amide group, one of which proceeds through intermediate lactone formation with the appended C(1′) diol group.

Our finding that all three C(1) triol-modified bicyclomycin derivatives readily reacted with ethanethiol led us to the relative reactivities of **6**–**8** versus **1**. Pairing equimolar mixtures of bicyclomycin with the C(1) triol-modified derivatives **6**–**8** and treating the pairs with ethanethiol (4–6 equiv) (0.1 M Tris·HCl tetrahydrofuran–water (3:1), "pH" 8.1–8.6, room temperature) demonstrated that **6**–**8** consumption proceeded at rates comparable to bicyclomycin (i.e., **6** vs **1**, 2.5:1; **7** vs **1**, 0.7:1; **8** vs **1**, 5:1). The speed of ethanethiol-mediated consumption of **8**[22] was surprising in light of earlier findings that showed the C(1′) hydroxyl group can promote thiolate bonding to the exomethylene group in bicyclomycin derivatives.[11,12] Further, we determined that the presence of the C(1′) hydroxyl group was not mandatory for thiolate addition by treatment of the C(1′) ketone acetonide derivative **10** with ethanethiol (22 equiv) (tetrahydrofuran–water (3:1) "pH" 10.5–9.2, room temperature, 24 h). Under these conditions, we observed near complete conversion of **10** to **37**, despite the fact that no free hydroxyl groups exist on the appended C(1) substituent.[22] This finding prompted us to conduct three additional ethanethiol competition experiments to compare the reactivities of acetonides **9** and **10** with **1** and **6**–**8**. We observed that (1) bicyclomycin reacted with ethanethiol slightly faster than **9** (**9** vs **1**, 0.9:1); (2) **10** was consumed at a faster rate than **1** (**10** vs **1**, 1.8:1); and (3) **8** underwent C(5)–C(5a) exomethylene functionalization more rapidly than **10** (**8** vs **10**, 2.5:1). The major ethanethiol product generated from **9** was the known C(5a)-substituted adduct **38**.[12] The cumulative competition experiments gave the following order of reactivity toward ethanethiol ("pH" 8.1–8.6) for the bicyclomycin-derived substrates in this investigation: **8** > **10** ~ **6** > **1** ~ **9** > **7**.



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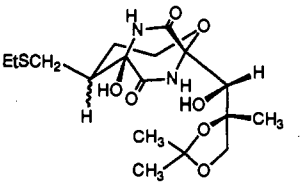


38

We conducted a parallel study of the C(1) triol modified compounds **6**–**8** reactivity (stability) in the *absence* of thiols in both aprotic and protic solvents (Table 1, Figure 3). Experimentally (^1H NMR analysis) we observed that **1**, **7**, and **8** were stable in DMF-d_7 solutions at room temperature for up to 14 days, whereas **6** reacted rapidly ($t_{1/2}$ = 5.2 h). The major product from **6** in DMF-d_7 has been tentatively identified as the diastereomeric bis-spiro adduct **39**. Key signals in the ^{13}C NMR

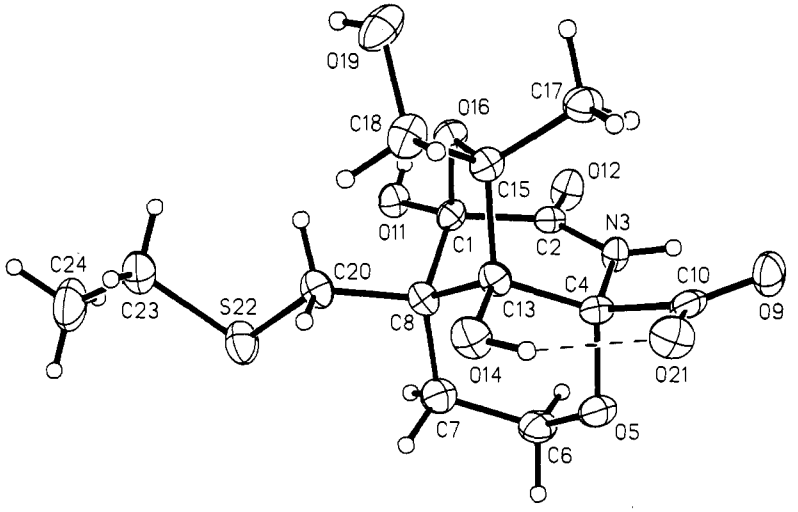
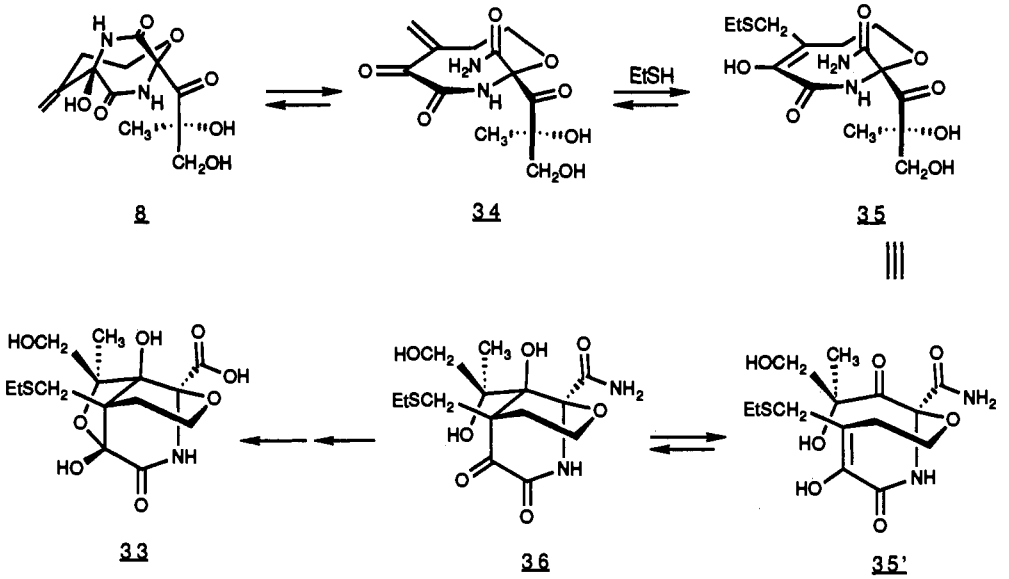


Figure 2. ORTEP drawing of the modified bicyclomycin carboxylate moiety of the tetra-*n*-butylammonium salt of **33** showing the atom numbering scheme. Thermal ellipsoids are 40% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. The intramolecular hydrogen bond is indicated by the thin dashed line. Selected bond distances (Å) are as follows: C(1)–C(2), 1.540 (5); C(2)–N(3), 1.348 (5); N(3)–C(4), 1.453 (4); C(4)–C(13), 1.554 (5); C(8)–C(13), 1.561 (5); C(1)–C(8), 1.541 (5). Selected angles (deg) are as follows: C(2)–C(1)–C(8), 113.6 (3); C(1)–C(2)–N(3), 115.9 (3); C(2)–N(3)–C(4), 124.3 (3); N(3)–C(4)–C(13), 109.5 (3); C(4)–C(13)–C(8), 106.8 (3); C(1)–C(8)–C(13), 96.6 (3).

Scheme 3. Proposed Mechanism for the Formation of Compound **33**



spectrum for **39** were the resonances located at 83.30–92.56 ppm for C(1) and C(6) and the signals at 113.98–114.47 ppm and 145.18 ppm for the exomethylene group. Consistent with the proposed structural assignment was the observation of the $[M + 1]^+$ peak in the high resolution +FAB mass spectrum. Dissolution of **1** and **6–8** in buffered deuterium oxide solutions (pD 5.6–5.8, 7.4, 9.2–9.4) led to a different reactivity pattern for each compound. Bicyclomycin did not undergo any appreciable change at pD 5.6 and 7.4 after 14 days but was converted to a series of new adducts at pD 9.3 ($t_{1/2}$ = 118 h). Compound **6** was moderately stable at pD 7.4 ($t_{1/2}$ = 97.2 h) but underwent change at pD 5.6 ($t_{1/2}$ = 29.8 h) and pD 9.2 ($t_{1/2}$ = 27.4 h). Finally, bicyclomycin derivatives **7** and **8** remained virtually unchanged at all three pD values (14 days).

The differential reactivity of bicyclomycin and its C(1′) isomer **6** in mildly acidic solution was further verified by treating an equimolar mixture of **1** and **6** with aniline (5 equiv) at pH 5.5. Under these conditions **6** was converted to **40**, but bicyclomycin did not react. An authentic sample of **40** was obtained by treating **6** with aniline at "pH" 10.7 in tetrahydrofuran water.

(c) Biochemical and Biological Activities of 6–8. The ease with which the C(5)–C(5a) exomethylene group in compounds **6–8** was modified by ethanethiol raised the possibility that these three bicyclomycin derivatives would inhibit rho-dependent termination events and display antibiotic activity. Accordingly, we first evaluated the ability of **6–8** to react with rho using the

Table 1. Stability ($t_{1/2}$) of Bicyclomycin (**1**) and Bicyclomycin Derivatives **6–8** in the Absence of Nucleophiles[a]

	medium			
compd	DMF-d_7 (h)	D_2O (pD 5.6–5.8) (h)	D_2O (pD 7.4) (h)	D_2O (pD 9.2–9.4) (h)
1	nc[b]	nc	nc	118
6	5.2	29.8	97.2	27.4
7	nc	nc	nc	nc[c]
8	nc	nc	nc	nc

[a] The stability of the substrates in the various solvents versus time were monitored by ^1H NMR spectroscopy by integrating the resonance of the C(2′)CH_3 signal in the starting material versus the corresponding peaks in the products. The $t_{1/2}$ values are in hours. [b] No change after 14 days = nc. [c] Less than 10% of the starting material was converted to products after 14 days.

(22) Within the limitations of detection of the NMR experiment no evidence was observed that the C(1′) carbonyl carbon in compounds **8** and **10** were hydrated in either D_2O or tetrahydrofuran-d_8-D_2O (3:1) (^{13}C NMR analysis).

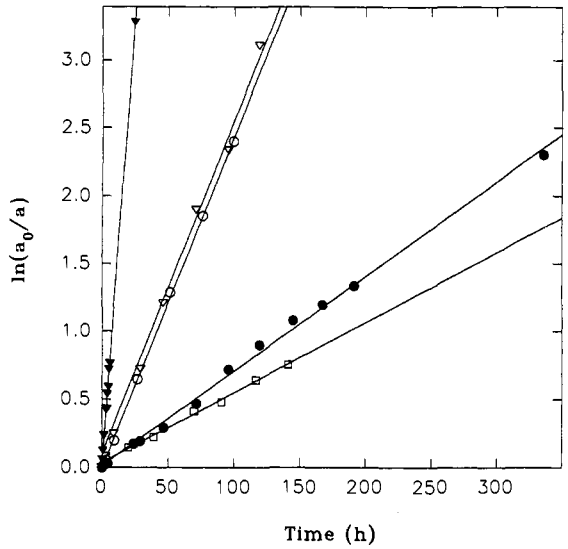
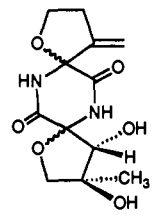


Figure 3. Stability of (1′*S*, 2′*S*)-bicyclomycin (**1**) and (1′*R*, 2′*S*)-bicyclomycin (**6**): (□) **1** (pD 9.3); (○) **6** (pD 5.6); (●) **6** (pD 7.4); (▽) **6** (pD 9.2); and (▼) **6** (DMF-d_7).



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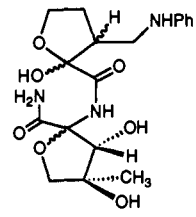

40

rho-dependent poly(C)-stimulated ATPase assay.[23] In Figure 4, we plotted the observed initial rates for ATP hydrolysis against concentration levels for **1**, **6**, **7**, and **8** and showed that none of the bicyclomycin C(1) triol modified derivatives **6**–**8** effectively inhibited rho, as compared with **1**. For example, the percentage of ATPase activity inhibition at 400 μmol concentrations were **1** (95%), **6** (35%), **7** (25%), and **8** (0%). Correspondingly, when we used a filter disc microbiological assay,[24] **6**–**8** did not exhibit noticeable antibiotic activity against *Escherichia coli* W3350 cells, whereas **1** produced a significant zone of inhibited bacterial cell growth (minimal inhibitory concentration (MIC): **1**, 250 μg/mL; **6**, >1200 μg/mL; **7**, >1200 μg/mL; **8**, >1200 μg/mL).

Discussion

Treatment of bicyclomycin (**1**) and the two isomers (**6** and **7**) with ethanethiol at near neutral "pH" values led to corresponding piperidinediones **26**, **27**, and **29**, respectively. We have proposed that **26** (**5**) formation proceeds by intramolecular Claisen condensation of the initially formed enol **3** (Scheme 1).[6,9] We expect the pathways for the production of **27** and **29** to be

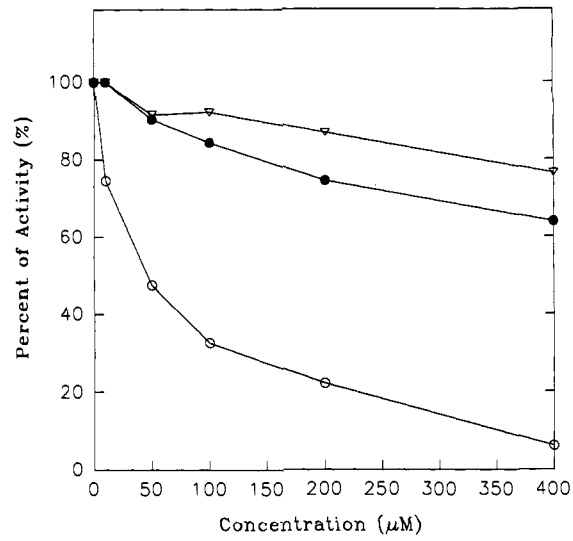

Figure 4. Percentage of ρ-dependent poly(C)-stimulated ATPase activity as a function of substrate concentration: (○) **1**; (●) **6**; and (▽) **7**.

comparable. Compound **33** was obtained as the sole product from C(1′) ketone **8** and ethanethiol, suggesting that an intramolecular aldol condensation (**35** → **36**) at the C(1′) ketone group occurred preferentially to Claisen condensation at the C(9) amide system (Scheme 3). Substantial amounts of other C(5a)-modified products were isolated in the **6**- and **7**-ethanethiol reactions. In the case of **6**, the ring opened adduct **28** was obtained, while **7** yielded the addition product **30**. Similar compounds have been obtained from **1** but only at higher "pH" values (i.e., ring opened adduct **31**: water, pH 12.5; C(5a) substituted adduct **32**: tetrahydrofuran–water (3:1), "pH" 10).[6,9,21] We have tentatively attributed the difference in the chemical reactivity of **6** and **7** versus **1** in part to the catalytic roles provided by the appended C(1) triol groups in these transformations. Hydrogen bond interactions of this group with the bicyclic piperazinedione ring system may facilitate the following: the C(6) hemiketal ring opening; the C(1)–O(2) bond cleavage; and the intramolecular Claisen condensation of the initially formed ethanethiol addition product. The associative nature of the C(1) triol group and the preferred conformational states of the bicyclomycin intermediates in the reaction pathway (or pathways) do not permit us to predict the relative importance of these interactions for compounds **1**, **6**, and **7**. It was interesting that bicyclomycin provided the cleanest reaction profiles of the three.

The reactivity of **8** toward ethanethiol was unexpected. This bicyclomycin derivative contained no C(1′) hydroxyl group.[22] Even more surprising was the finding that acetonide **10** was converted to **37**. The latter compound contained no free hydroxyl groups on the appended C(1) substituent.[22] We have attributed the ease of **8** and **10** functionalization to the multistep nature of the transformations. We suspect that the decrease in the equilibrium constant for the C(6) hemiketal ring opening of **8** to **34**, due to the absence of a hydrogen bond from the C(1′) hydroxyl group, is offset by the enhanced rate of the aldol (**35** → **36**), compared with the Claisen condensation reaction required for piperidine ring formation. The ease with which ethanethiol modified the C(5)–C(5a) exomethylene groups of **8** and **10** requires that the assumed *minimum* structural requirement for thiol addition to include a C(1′) hydroxyl group be reconsidered.[11]

Additional evidence that the structure of the C(1) triol group can influence the reactivity of the [4.2.2]piperazinedione ring system was derived from the relative reactivity (stability) of **1** and **6**–**8** in both aprotic and protic solvents in the absence of thiols. Only **6** was unstable in DMF-d_7, yielding **39**. In buffered deuterium oxide solutions, **1** was stable in moderately acidic and neutral pD solutions, but it underwent change in base. A different pattern was observed for **6**. This isomer was moderately stable

(23) Sharp, J. A.; Galloway, J. L.; Platt, T. *J. Biol. Chem.* **1983**, *258*, 3482–3486.
(24) Ericsson, H. M.; Sherris, J. C. *Acta Pathol. Microbiol. Scand.* **1971**, Suppl. 217, 1–90.
(25) Bates, R. G. *Determination of pH: Theory and Practice.* 2nd ed.; Wiley: New York, 1973, pp 375–376.

in neutral pD solutions but was rapidly consumed in acid and base. Finally, neither **7** nor **8** underwent significant change at pD 5–9. The observed differential reactivity patterns for compounds **1**, **6**, and **7** may be explained by the specific roles that the associated C(1) triol group can provide in the C(6) hemiketal ring opening and the C(1)–O(2) bond cleavage steps.

The demonstration that **6–8** along with **1** readily reacted with ethanethiol permitted us to test whether these new bicyclomycin derivatives could inhibit rho and display antimicrobial activity. We evaluated the ability of **6–8** to inhibit the bicyclomycin target, rho, using the rho dependent poly(C)-stimulated ATPase assay (Figure 4). None of the three newly synthesized compounds could block ATPase activity at the same concentrations as bicyclomycin. A parallel finding was obtained for the antibiotic activities of **1** and **6–8** using a filter disc microbiological assay. The MIC values for **6–8** against *Escherichia coli* W3350 cells exceeded 1200 μg/mL, whereas the value for **1** was 250 μg/mL. These findings permit us to suggest that the C(1) triol group in bicyclomycin plays a critical role in *in vivo* processes by permitting binding of **1** to rho. This interaction coupled with the proposed catalytic roles in the C(5)–C(5a) bonding step underscore the importance of this group in the antibiotic for inhibiting termination of vital transcripts in *Escherichia coli* organisms.

Conclusions

Our studies demonstrated that the structure of the C(1) triol substituent in the bicyclomycin adduct modulates the chemical, biochemical, and biological reactivities of the substrate. Our finding that **1** and **6–8** all underwent thiol addition but that **6–8** did not significantly inhibit rho-dependent ATPase activity or display antibiotic activity suggested that a strong molecular complementarity existed between bicyclomycin and a binding pocket in rho. These results document the importance of the C(1) triol group for future drug design. Studies are now underway to determine the site of bicyclomycin binding and bonding.

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, General Electric QE-300 NMR, and Bruker AMX 600 MHz instruments. Chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si, and coupling constants (J values) are in Hertz. The FAB mass spectral studies conducted at Baylor College of Medicine were performed by Dr. Simon Gaskell and Mr. Ralph Orkiszewski on a VG ZAB-SEQ instrument. All chemical ionization and FAB mass spectral investigations conducted at the University of Texas at Austin were performed by Dr. M. Moini on a Finnegan MAT TSQ-70 instrument. 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, and dichloromethane was distilled from phosphorus pentoxide. Thin-layer chromatography were run on precoated silica gel GHLF slides (20 × 20 cm; Analtech No. 21521).

Preparation of Bicyclomycin C(2′), C(3′) Acetonide (9). A dimethylformamide solution (1.5 mL) containing **1** (100 mg, 0.33 mmol), 2, 2–dimethoxypropane (1.00 mL, 8.13 mmol), and a few crystals of *p*-toluenesulfonic acid monohydrate was heated at 80 °C (2 h) under Ar. The solvent was removed *in vacuo*, and the residue was taken up in ethyl acetate (10 mL), then successively washed with an aqueous saturated $NaHCO_3$ solution (1 × 10 mL) and saturated brine (2 x 10 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure and dried overnight under vacuum. Compound **9** was obtained as a white solid: mp 188–194 °C (lit.[15] mp 201–204 °C); yield 72 mg (64%); R_f 0.49 (10% CH_3OH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.42 (s, 3 H, C(2′)-CH_3), 1.46 (s, 3 H, $C(CH_3)_2$), 1.48 (s, 3 H, $C(CH_3)_2$), 2.59–2.76 (m, 2 H, $C(4)H_2$), 3.76 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 3.82–4.03 (m, 2 H, $C(3)H_2$), 4.19 (s, 1 H, C(1′)H), 4.49 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 5.18 (s, 1 H, C(5a)HH′), 5.62 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 24.92 (C(2′)CH_3), 26.81 ($C(CH_3)_2$), 28.11 ($C(CH_3)_2$), 36.56 (C(4)), 66.51 (C(3)), 73.27 (C(3′)), 73.38 (C(1′)), 82.88 (C(6)), 86.38 (C(2′)), 88.94 (C(1)), 111.62 ($C(CH_3)_2$), 116.80 (C(5a)), 149.37 (C(5)), 168.52 (C(9)), 172.21 (C(7)) ppm; the assignment was consistent with the APT ^{13}C NMR spectrum.

Preparation of C(1′) Ketobicyclomycin C(2′), C(3′) Acetonide (10). A room temperature dichloromethane solution (35 mL) of **9**[15] (80 mg, 0.24 mmol) and dimethyl sulfoxide (150 μL, 2.1 mmol) was degassed with Ar, and then the temperature was lowered to −78 °C (15 min). Trifluoroacetic anhydride (220 μL, 1.6 mmol) was added, and the solution was stirred at −78 °C (1 h) before triethylamine (370 μL, 2.6 mmol) was added. The solution was allowed to warm to room temperature, and the solvent was evaporated under reduced pressure. The remaining oily residue was dissolved in dichloromethane (30 mL) and washed with H_2O (3 × 10 mL). The aqueous layer was extracted with dichloromethane (1 × 30 mL). The organic layers were combined, and the solvent was evaporated *in vacuo*. The residue was purified by preparative TLC using 10% methanol–chloroform to give **10** as a white solid: mp 154–158 °C; yield 48 mg (60%); R_f 0.52 (10% CH_3OH–$CHCl_3$); IR (Nujol) 1742, 1692 cm^{-1}; ^1H NMR (CD_3OD) δ 1.36 (s, 3 H, $C(CH_3)_2$), 1.40 (s, 3 H, $C(CH_3)_2$), 1.59 (s, 3 H, C(2′)CH_3), 2.56–2.70 (m, 2 H, $C(4)H_2$), 3.80–3.88 (m, 1 H, C(3)HH′), 3.92 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 3.98–4.06 (m, 1 H, C(3)HH′), 4.15 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 5.14 (s, 1 H, C(5a)-HH′), 5.57 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 25.96 (C(2′)CH_3), 26.22 ($C(CH_3)_2$), 27.63 ($C(CH_3)_2$), 36.74 (C(4)), 65.92 (C(3)), 73.63 (C(3′)), 83.14 (C(6)), 87.79, 87.95 (C(1), C(2′)), 112.42 ($C(CH_3)_2$), 116.76 (C(5a)), 149.82 (C(5)), 166.93 (C(9)), 172.18 (C(7)), 202.90 (C(1′)) ppm; the proposed structural assignment was in agreement with the HMQC and HMBC NMR spectral data; MS (+FAB) 341 [M + 1]$^+$; M_r (+CI) 341.134 14 [M + 1]$^+$ (calcd for $C_{15}H_{21}N_2O_7$ 341.134 88). The proposed structure was confirmed by X-ray crystallographic analysis.

Preparation of C(1′) Ketobicyclomycin (8). An aqueous methanolic solution (1:1, 2 mL) containing **10** (20 mg, 0.059 mmol) and a few drops of trifluoroacetic acid ("pH" 1.5) was heated at 65 °C (1 h) under Ar. The reaction mixture was neutralized with a saturated $NaHCO_3$ aqueous solution, and the solvent was removed *in vacuo*. The residue was dissolved in methanol and purified by preparative TLC using 20% methanol–chloroform as the eluent to provide **8** as a white solid: mp 184–187 °C; yield 10 mg (56%); R_f 0.39 (20% CH_3OH–$CHCl_3$); IR (KBr) 3422, 3270, 2940, 1688 cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2′)CH_3), 2.62–2.69 (m, 2 H, $C(4)H_2$), 3.45 (d, J = 10.5 Hz, 1 H, C(3′)HH′), 3.74 (d, J = 10.5 Hz, 1 H, C(3′)HH′), 3.80–3.90 (m, 1 H, C(3)HH′), 4.02–4.12 (m, 1 H, C(3)HH′), 5.16 (s, 1 H, C(5a)HH′), 5.58 (s, 1 H, C(5a)-HH′); ^{13}C NMR (CD_3OD) 23.29 (C(2′)CH_3), 36.83 (C(4)), 66.04 (C(3)), 70.83 (C(3′)), 82.48, 83.13 (C(2′), C(6)), 86.78 (C(1)), 117.05 (C(5a), 149.41 (C(5)), 166.98 (C(9)), 171.87 (C(7)), 204.64 (C(1′)) ppm; MS (+FAB) 301 [M + 1]$^+$; M_r (+CI) 301.101 90 [M + 1]$^+$ (calcd for $C_{12}H_{17}N_2O_7$ 301.103 58).

Preparation of (1′*R*,2′*S*)-Bicyclomycin C(2′), C(3′) Acetonide (11). To a methanolic solution (5 mL) of **10** (30 mg, 0.088 mmol) was sequentially added $CeCl_3 \cdot 7H_2O$ (32.8 mg, 0.088 mmol) and $NaBH_4$ (4 mg, 0.106 mmol) at room temperature under Ar. The solution was stirred at room temperature (1.5 h), then H_2O (1 mL) was added, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of two products. The residue was purified by preparative TLC using 5% methanol–chloroform as the eluent (2×) to give **11** (12.1 mg, 43%) and **9** (9.9 mg, 35%).

Compound 11: mp 161–163 °C; R_f 0.57 (10% CH_3OH–$CHCl_3$); IR (KBr) 3435, 3312, 3217, 2986, 1699 cm^{-1}; ^1H NMR (CD_3OD) δ 1.28 (s, 3 H, C(2′)CH_3), 1.35 (s, 3 H, $C(CH_3)_2$), 1.38 (s, 3 H, $C(CH_3)_2$), 2.56–2.64 (m, 2 H, $C(4)H_2$), 3.67 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 3.80–3.90 (m, 1 H, C(3)HH′), 3.94–4.04 (m, 1 H, C(3)HH′), 4.22 (s, 1 H, C(1′)H), 4.24 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 5.13 (s, 1 H, C(5a)-HH′), 5.55 (s, 1 H, C(5a)HH′); ^{13}C NMR (CD_3OD) 25.57 (C(2′)CH_3), 26.39 ($C(CH_3)_2$), 28.56 ($C(CH_3)_2$), 36.95 (C(4)), 65.83 (C(3)), 73.53 (C(3′)), 75.30 (C(1′)), 83.27 (C(6), C(2′)), 85.81 (C(1)), 111.76 ($C(CH_3)_2$), 116.69 (C(5a)), 149.83 (C(5)), 168.80 (C(9)), 171.56 (C(7)) ppm; MS (+CI) 343 [M + 1]$^+$; M_r (+CI) 343.150 50 [M + 1]$^+$ (calcd for $C_{15}H_{23}N_2O_7$ 343.150 53). The proposed structure was confirmed by X-ray crystallographic analysis.

Compound 9: R_f 0.51 (10% CH_3OH–$CHCl_3$, cospot with an authentic sample of **9**); ^1H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.42 (s, 3 H, $C(CH_3)_2$), 1.44 (s, 3 H, $C(CH_3)_2$), 2.58–2.66 (m, 2 H, $C(4)H_2$), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 3.79–4.01 (m, 2 H, $C(3)H_2$), 4.14

(s, 1 H, C(1′)H), 4.44 (d, J = 8.4 Hz, 1 H, C(3′)HH'), 5.13 (s, 1 H, C(5a)HH′), 5.57 (s, 1 H, C(5a)HH').

Preparation of (1′*R*, 2′*S*)-Bicyclomycin (6). A methanol–water solution (1:1, 5 mL) containing **11** (20 mg, 0.058 mmol) was acidified ("pH" 2.6) with a few drops of aqueous sulfuric acid (0.2 N) and then was heated at 40 °C (2 h). The solution was neutralized with an aqueous saturated $NaHCO_3$ solution. The solvent was removed *in vacuo*. The residue was purified by preparative TLC using 20% methanol–chloroform as the eluent to yield **6** as a solid: (6.2 mg, 35%) along with several unidentified compounds.

Compound 6: mp 170–171 °C dec; R_f 0.28 (20% CH_3OH–$CHCl_3$); IR (KBr) 3428, 1692 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 1.21 (s, 3 H, C(2′)CH_3), 2.58 (dd, J = 7.0, 16.2 Hz, 1 H, C(4)HH′), 2.65 (dd, J = 9.0, 16.2 Hz, 1 H, C(4)HH'), 3.41 (d, J = 10.9 Hz, 1 H, C(3′)HH′), 3.56 (d, J = 10.9 Hz, 1 H, C(3′)HH'), 3.80 (dd, J = 9.0, 13.1 Hz, 1 H, C(3)HH′), 3.97 (dd, J = 7.0, 13.1 Hz, 1 H, C(3)HH'), 4.24 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH'); the assignment was in agreement with the COSY NMR data; ^{13}C NMR (150 MHz, CD_3OD) 22.10 (C(2′)CH_3), 37.01 (C(4)), 65.64 (C(3)), 69.15 (C(3′)), 74.69, 75.05 (C(1′), C(2′)), 83.31 (C(6)), 86.07 (C(1)), 116.71 (C(5a)), 149.52 (C(5)), 169.21 (C(9)), 171.76 (C(7)) ppm; MS (–FAB) 301 [M – 1]$^-$; M_r (–FAB) 301.102 43 [M – 1]$^-$ (calcd for $C_{12}H_{17}N_2O_7$ 301.103 58).

Preparation of Bicyclomycin-3′-*O*-methanesulfonate (12).[18] Bicyclomycin (150 mg, 0.50 mmol) was dissolved in anhydrous pyridine (3.0 mL), and the temperature of the solution was lowered to –10 °C. Methanesulfonyl chloride (100 μL, 1.30 mmol) was added, and the temperature was maintained at 0 °C (2 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was subjected to flash chromatography on silica gel using 10% methanol–chloroform as the eluent. A pale-yellow solid was obtained after drying overnight under vacuum (0.1 Torr) to give 149 mg (78%) of **12**; mp 138–140 ° C (lit.[18] mp 151–153 °C); R_f 0.56 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.40 (s, 3 H, C(2′)CH_3), 2.58–2.68 (m, 2 H, C(4)H_2), 3.08 (s, 3 H, CH_3SO_3), 3.80–3.90 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 4.25 (d, J = 9.9 Hz, 1 H, C(3′)HH′), 4.32 (d, J = 9.9 Hz, 1 H, C(3′)HH'), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 23.67 (C(2′)CH_3), 36.66 (C(4)), 37.30 (CH_3SO_3), 65.73 (C(3)), 71.41 C(1′)), 75.74 (C(3′)), 76.93 (C(2′)), 82.96 (C(6)), 89.71 (C(1)), 116.96 (C(5a)), 149.51 (C(5)), 168.35 (C(9)), 172.48 (C(7)) ppm.

Preparation of Bicyclomycin C(2′), C(3′) Epoxide (13).[18] A solution of **12** (150 mg, 0.39 mmol) and triethylamine (250 μL, 1.80 mmol) in anhydrous methanol (6 mL) was stirred at room termperature (3 h). The solvent was removed *in vacuo*, and the residue was dissolved in a small amount methanol. Preparative TLC using 10% methanol–chloroform as the eluent gave the desired product as a white solid: 57 mg (49%); mp 182–184 °C (lit.[18] mp 190–192 °C); R_f 0.65 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.42 (s, 3 H, C(2′)CH_3), 2.57–2.67 (m, 2 H, C(4)H_2), 2.71 (d, J = 4.5 Hz, 1 H, C(3′)HH′), 3.27 (d, J = 4.5 Hz, 1 H, C(3′)HH'), 3.75–3.85 (m, 2 H, C(3)H_2), 4.31 (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) 21.06 (C(2′)-CH_3), 36.59 (C(4)), 53.84 (C(3′)), 59.74 (C(2′)), 65.94 (C(3)), 71.73 (C(1′)), 83.16 (C(6)), 87.97 (C(1)), 116.94 (C(5a)), 149.41 (C(5)), 164.33 (C(9)), 168.94 (C(7)) ppm.

Reaction of Bicyclomycin C(2′), C(3′) Epoxide (13) with Dilute H_2SO_4 in Tetrahydrofuran–Water. A tetrahydrofuran–water solution (3:1, 5 mL) of **13** (25 mg, 0.088 mmol) was acidified ("pH" 1.8) using dilute H_2SO_4 and then stirred at room temperature (0.5 h). The reaction was neutralized with saturated aqueous $NaHCO_3$ solution, and the solvent was removed *in vacuo*. The residue was purified by preparative TLC (15% methanol–chloroform) as the eluent to give two compounds.

Compound 14: yield, 9 mg (36%); mp 199–201 °C; R_f 0.40 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.50 (s, 3 H, C(2′)CH_3), 2.55–2.66 (m, 2 H, C(4)H_2), 3.69 (d, J =12.6 Hz, 1 H, C(3′)HH′), 3.75–3.85 (m, 1 H, C(3)HH′), 3.98–4.08 (m, 1 H, C(3)HH'), 4.10 (d, J = 12.6 Hz, C(3′)HH'), 4.20 (s, 1 H, C(1′)H), 5.16 (s, 1 H, (5a)HH′), 5.60 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 22.44 (C(2′)CH_3), 36.27 (C(4)), 64.97 (C(3)), 66.41 (C(3′)), 82.27 (C(1′)), 82.94 (C(6)), 86.78 (C(2′)), 91.07 (C(1)), 117.18 (C(5a)), 149.60 (C(5)), 166.30 (C(9)), 175.61 (C(7)) ppm.

Compound 15: yield, 10 mg (40%); mp 127–130 °C (lit.[18] mp 120 °C); R_f 0.60 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.45 (s, 3 H, C(2′)CH_3), 2.58–2.64 (m, 2 H, C(4)H_2), 3.48 (d, J = 12.3 Hz, 1 H, C(3′)HH′), 3.52–3.62 (m, 1 H, C(3)HH′), 3.74 (d, J = 12.3 Hz, 1 H, C(3′)HH'), 3.85 (s, 1 H, C(1′)H), 3.90–4.00 (m, 1 H, C(3)HH'), 5.11 (s, 1 H, C(5a)HH′), 5.55 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 26.73 (C(2′)CH_3), 36.50 (C(4)), 58.41 (C(3′)), 66.36 (C(3)), 75.20 (C(2′)), 81.80 (C(1′)), 84.27 (C(6)), 94.70 (C(1)), 117.07 (C(5a)), 149.59 (C(5)), 167.56 (C(9)), 170.11 (C(7)) ppm.

Preparation of [O(9)–C(2′)] Cyclized Bicyclomycin (14). To a solution of **13** (25 mg, 0.088 mmol) in anhydrous methanol (5 mL) was added one drop of concentrated H_2SO_4 (~0.04 mL). The solution was stirred at room temperature (1 h) and then neutralized with an aqueous saturated $NaHCO_3$ solution. The solvent was removed *in vacuo*, and the residue was triturated with methanol and filtered, and the filtrate was concentrated. Preparative TLC using 20% methanol–chloroform as the eluent gave the product as a colorless solid: 17.5 mg (70%); mp 199–201 °C; R_f 0.42 (20% CH_3OH–$CHCl_3$); IR (KBr) 3428, 2940, 1692 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.50 (s, 3 H, C(2′)CH_3), 2.58–2.64 (m, 2 H, C(4)H_2), 3.69 (d, J = 12.6 Hz, 1 H, C(3′)HH′), 3.75–3.90 (m, 1 H, C(3)HH′), 3.95–4.08 (m, 1 H, C(3)HH'), 4.10 (d, J = 12.6 Hz, C(3′)HH'), 4.19 (s, 1 H, C(1′)H), 5.16 (s, 1 H, C(5a)HH′), 5.60 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 22.52 (C(2′)CH_3), 36.29 (C(4)), 65.01 (C(3)), 66.47 (C(3′)), 82.58 (C(1′)), 83.03 (C(6)), 86.82 (C(2′)), 91.07 (C(1)), 117.18 (C(5a)), 149.63 (C(5)), 166.28 (C(9)), 175.50 (C(7)) ppm; the proposed structural assignment was in agreement with the ^{13}C APT experiment; MS (+FAB) 285 [M + 1]$^+$. The proposed structure was confirmed by X-ray crystallographic analysis.

Preparation of (1′*S*, 2′*R*)-Bicyclomycin (7). To a solution of **14** (20 mg, 0.070 mol) in tetrahydrofuran–water (5 mL, 3:1) was added a few drops of an aqueous 0.2 N H_2SO_4 solution. The solution (~"pH" 1.5) was stirred at room temperature (30 h) and then neutralized with an aqueous $NaHCO_3$ solution. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC using 20% methanol–chloroform as the eluent to give **7** and **16**.

Compound 7: yield, 11 mg (52%); mp 207–211 °C dec; R_f 0.39 (20% CH_3OH–$CHCl_3$); IR (KBr) 3441, 1688 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.32 (s, 3 H, C(2′)CH_3), 2.58–2.65 (m, 2 H, C(4)H_2), 3.32 (d, J = 10.5 Hz, 1 H, C(3′)HH′), 3.65 (d, J = 10.5 Hz, 1 H, C(3′)HH'), 3.78–3.96 (m, 2 H, C(3)H_2), 4.19 (s, 1 H, C(1′)H), 5.14 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 23.56 (C(2′)CH_3), 36.80 (C(4)), 65.40 (C(3)), 69.21 (C(3′)), 71.73 (C(1′)), 77.10 (C(2′)), 83.00 (C(6)), 89.92 (C(1)), 116.88 (C(5a)), 149.57 (C(5)), 168.88 (C(9)), 172.79 (C(7)) ppm; MS (+FAB) 303 [M + 1]$^+$; M_r (+FAB) 303.121 11 [M + 1]$^+$ (calcd for $C_{12}H_{19}N_2O_7$ 303.119 23).

Compound 16: yield, 4 mg (20%); mp 145–147 °C; R_f 0.05 (20% CH_3OH–$CHCl_3$); IR (KBr) 3393, 2947, 1690, 1640 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.26 (s, 3 H, C(2′)CH_3), 2.32–2.46 (m, 1 H, C(4)HH′), 2.82–2.96 (m, 1 H, C(4)HH'), 3.44 (d, J = 12.0 Hz, 1 H, C(3′)HH′), 3.72–3.88 (m, 1 H, C(3)HH′), 3.92 (s, 1 H, C(1′)H), 3.92–4.10 (m, 1 H, C(3)HH'), 3.96 (d, J = 12.0 Hz, 1 H, C(3′)HH'), 5.11 (s, 1 H, C(5a)HH′), 5.45 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 24.36 (C(2′)-CH_3), 38.77 (C(4)), 65.56 (C(3)), 66.82 (C(3′)), 74.97 (C(2′)), 83.01 (C(1′)), 88.01 (C(1)), 97.88 (C(6)), 116.77 (C(5a)), 149.52 (C(5)), 171.85, 173.31 (C(7), C(9)) ppm; the proposed assignments were supported by the HMBC NMR experiment; MS (–FAB) 302 [M – 1]$^-$; M_r (–FAB) 302.091 00 [M – 1]$^-$ (calcd for $C_{12}H_{16}NO_8$ 302.087 59).

Preparation of (1′*S*, 2′*R*)-Bicyclomycin Acetonides 17 and 18. A solution of **7** (24 mg, 0.079 mmol), 2,2-dimethoxypropane (1.00 mL, 8.13 mmol), and a few crystals of *p*-toluenesulfonic acid in dry dimethylformamide (1.0 mL) was heated at 80 °C (2 h) under Ar. The solvent was removed *in vacuo*, and the residue was taken up in methanol. Preparative TLC using 10% methanol–chloroform as the eluent gave two products.

Compound 17: yield, 8 mg (29%); mp 194–197 °C; R_f 0.40 (10% CH_3OH–$CHCl_3$); IR (KBr) 1705, 1676 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.41 (s, 3 H, C(2′)CH_3), 1.44 (s, 6 H, C(CH_3)$_2$), 2.56–2.65 (m, 2 H, C(4)H_2), 3.67 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 3.78–3.96 (m, 2 H, C(3)H_2), 4.12 (s, 1 H, C(1′)H), 4.25 (d, J = 8.4 Hz, 1 H, C(3′)HH'), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 25.83 (C(2′)CH_3), 26.25 (C(CH_3)$_2$)), 27.83 (C(CH_3)$_2$), 36.78 (C(4)), 65.64 (C(3)), 71.46 (C(1′)), 74.58 (C(3′)), 83.15 (C(6)), 86.08 (C(2′)), 89.90 (C(1)), 111.99 (C(CH$_3$)$_2$), 116.85 (C(5a)), 149.60 (C(5)), 168.47 (C(9)), 172.70 (C(7)) ppm; MS (+FAB) 342 [M]$^+$; M_r (+FAB) 342.143 33 [M]$^+$ (calcd for $C_{15}H_{22}N_2O_7$ 342.142 70).

Compound 18: yield, 7 mg (26%): mp 189–193 °C; R_f 0.49 (10% CH_3OH–$CHCl_3$); IR (Nujol) 1692 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.22 (s, 3 H, C(2′)CH_3), 1.32 (s, 3 H, C(CH_3)$_2$), 1.42 (s, 3 H, C(CH_3)$_2$), 2.58–2.65 (m, 2 H, C(4)H_2), 3.37 (d, J = 12.3 Hz, 1 H, C(3′)HH′), 3.80 (d, J = 12.3 Hz, 1 H, C(3′)HH'), 3.83–3.96 (m, 2 H, C(3)H_2), 4.38 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH'); ^{13}C NMR (CD_3OD) 19.05 (C(2′)CH_3), 23.02 (C(CH_3)$_2$), 28.67 (C(CH_3)$_2$),

36.79 (C(4)), 65.57 (C(3)), 70.58, 71.91, 73.89 (C(1'), C(2'), C(3')), 83.04 (C(6)), 88.89 (C(1)), 100.83 (*C*$(CH_3)_2$), 116.80 (C(5a)), 149.64 (C(5)), 168.04 (C(9)), 172.31 (C(7)) ppm; MS (+CI) 343 $[M + 1]^+$; M_r (+FAB) 343.149 75 $[M + 1]^+$ (calcd for $C_{15}H_{23}N_2O_7$ 343.150 52). The proposed structure was confirmed by X-ray crystallographic analysis.

Reaction of (1'*R*, 2'*S*)-Bicyclomycin (6) with Ethanethiol. A tetrahydrofuran–water solution (3:1, 1.5 mL, "pH" 9.1 adjusted with aqueous 0.1 N NaOH) of **6** (5 mg, 0.017 mmol) was degassed with Ar and capped, and then ethanethiol (30 μL, 0.39 mmol) was added. The solution was stirred at room temperature (20 h) during which time the "pH" decreased to 8.2. The solvent was removed *in vacuo*, and the residue was dissolved in methanol and purified by preparative TLC using 15% methanol–chloroform as the eluent to give the following compounds.

Compound 27: yield, 1.5 mg (26%); mp 115–116 °C dec; R_f 0.60 (15% CH_3OH–$CHCl_3$); IR (KBr) 3389, 2940, 1696 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 1.15 (s, 3 H, C(2')CH_3), 1.22 (t, *J* = 7.4 Hz, 3 H, CH_2CH_3), 1.94 (br d, *J* = 13.9 Hz, 1 H, C(4)H*H'*), 2.32 (dt, *J* = 6.5, 13.9 Hz, 1 H, C(4)H*H'*), 2.54 (br q, *J* = 7.4 Hz, 2 H, CH_2CH_3), 2.77 (d, *J* = 13.9 Hz, 1 H, C(5a)H*H'*), 3.11 (d, *J* = 13.9 Hz, 1 H, C(5a)H*H'*), 3.44 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*), 3.54 (s, 1 H, C(1')H), 3.76 (br t, *J* = 13.9 Hz, 1 H, C(3)H*H'*), 4.01 (dd, *J* = 6.5, 13.9 Hz, 1 H, C(3)H*H'*), 4.13 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*); ^{13}C NMR (150 MHz, CD_3OD) 15.17 (CH_2CH_3), 21.63 (C(2')CH_3), 29.75 (CH_2CH_3), 31.54 (C(5a)), 32.34 (C(4)), 57.27 (C(5)), 59.56 (C(3)), 66.59, 71.94, 75.20 (C(1'), C(2'), C(3')), 83.46 (C(1)), 96.10 (C(9)), 161.14 (C(7)), 195.38 (C(6)) ppm; MS (+FAB) 348 $[M + 1]^+$; M_r (+FAB) 348.112 06 $[M + 1]^+$ (calcd for $C_{14}H_{22}NO_7S$ 348.111 70).

Compound 28: yield, 2.6 mg (43%); R_f 0.40 (15% CH_3OH–$CHCl_3$); IR (KBr) 3372, 2972, 1686, 1559 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 1.20–1.24 (m, 3 H, CH_2CH_3), 1.27 (s, 3 H, C(2')CH_3), 1.80–1.90 (m, 1 H, C(4)H*H'*), 2.22–2.35 (m, 1 H, C(4)H*H'*), 2.45–2.54 (m, 4 H, CH_2CH_3, C(5)H, C(5a)H*H'*), 2.75–2.95 (m, 1 H, C(5a)H*H'*), 3.70–3.73 (m, 1 H, C(3')H*H'*), 3.80–3.83 (m, 1 H, C(3')H*H'*), 3.90–3.93 (m, 1 H, C(3)H*H'*), 4.08 (br s, 1 H, C(1')H), 4.03–4.10 (m, 1 H, C(3)H*H'*); ^{13}C NMR (150 MHz, CD_3OD) 15.12, 15.15 (CH_2CH_3), 19.20 (C(2')CH_3), 26.78, 26.88 (CH_2CH_3), 31.46, 32.37, 31.25, 32.30 (C(4), C(5a)), 68.21, 68.46 (C(3)), 77.99, 78.18 (C(3')), 80.01, 80.25 (C(2')), 83.46 (C(1')), 91.71 (C(1)), 102.69, 103.10 (C(6)), 173.38, 173.84 (C(9)), 175.36 (C(7)) ppm; the C(5) signal is believed to be beneath the solvent peaks and was confirmed using the HMBC NMR experiment; the proposed structural assignment was consistent with HMBC, HMQC, and COSY NMR spectral data; MS (+FAB) 365 $[M + 1]^+$; M_r (+FAB)365.138 69 $[M + 1]^+$ (calcd for $C_{14}H_{25}N_2O_7S$ 365.138 25).

Reaction of (1'*S*, 2'*R*)-Bicyclomycin (7) with Ethanethiol. A tetrahydrofuran–water solution (3:1, 1.0 mL, "pH" 9.4, adjusted with aqueous 0.1 N NaOH) of **7** (5 mg, 0.017 mmol) was degassed with Ar and capped, and then ethanethiol (50 μL, 0.65 mmol) was added. The solution was stirred at room temperature (48 h) during which time the "pH" decreased to 7.9. The solvent was removed *in vacuo*, and the residue was dissolved in methanol. Preparative TLC using 15% methanol–chloroform as the eluent gave the following compounds.

Compound 29: yield, 1.5 mg (26%); mp 137–139 °C dec; R_f 0.40 (15% CH_3OH–$CHCl_3$); IR (KBr) 3374, 3283, 2930, 1692 cm^{-1}; ^1H NMR (CD_3OD) δ 1.24 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.42 (s, 3 H, C(2')CH_3), 1.82–1.92 (m, 1 H, C(4)H*H'*), 2.20 (dt, *J* = 6.6, 14.1 Hz, 1 H, C(4)H*H'*), 2.57 (q, *J* = 7.2 Hz, 2 H, CH_2CH_3), 2.89 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 2.97 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.49 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 3.60–3.70 (m, 1 H, C(3)H*H'*), 3.85–3.92 (m, 1 H, C(3)H*H'*), 3.95 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 4.09 (s, 1 H, C(1')H); ^{13}C NMR (CD_3OD) 15.05 (CH_2CH_3), 21.23 (C(2')CH_3), 29.59 (CH_2CH_3), 32.49 (C(5a)), 33.25 (C(4)), 56.70 (C(5)), 58.40 (C(3)), 70.01, 72.01, 75.54 (C(1'), C(2'), C(3')), 85.13 (C(1)), 95.76 (C(9)), 160.24 (C(7)), 195.64 (C(6)) ppm; MS (+FAB) 348 $[M + 1]^+$; M_r (+FAB) 347.104 08 $[M]^+$ (calcd for $C_{14}H_{21}NO_7S$ 347.103 87).

Compound 30: yield, 3.0 mg (52%); R_f 0.26 (15% CH_3OH–$CHCl_3$); ^1H NMR (CD_3OD) (consists of two diastereomers) δ 1.20–1.28 (m, 3 H, CH_2CH_3), 1.32, 1.33 (2s, 3 H, C(2')CH_3), 2.00–2.50 (m, 4 H, C(4)-H_2, C(5a)H*H'*, C(5)H), 2.40–2.60 (m, 2 H, CH_2CH_3), 3.15 (d, *J* = 12.3 Hz, 1 H, C(5a)H*H'*), 3.34 (d, *J* = 10.8 Hz, 1 H, C(3')H*H'*), 3.62 (d, *J* = 10.8 Hz, 1 H, C(3')H*H'*), 3.68–4.10 (m, 2 H, C(3)H_2), 4.14 (s, 1 H, C(1')H); the proposed structural assignments were in agreement with the COSY NMR spectral data; ^{13}C NMR (CD_3OD) 15.14 (CH_2CH_3), 23.56 (C(2')CH_3), 26.85 (CH_2CH_3), 30.01, 31.33 (C(4), C(5a)), 52.46 (C(5)), 61.87 (C(3)), 69.25 (C(3')), 71.96 (C(1')), 77.06 (C(2')), 83.67, 83.76 (C(6)), 89.77 (C(1)), 168.92 (C(9)), 172.32, 173.22 (C(7)) ppm; MS (+CI) 365 $[M + 1]^+$; M_r (+FAB) 365.137 87 $[M + 1]^+$ (calcd for $C_{14}H_{25}N_2O_7S$ 365.138 25).

Reaction of C(1') Ketobicyclomycin (8) with Ethanethiol. A tetrahydrofuran–water solution (3:1, 5 mL, "pH" 9.6) of **8** (15 mg, 0.05 mmol) was degassed with Ar (3 min) and capped, and then ethanethiol (50 μL, 0.65 mmol) was added. The solution was stirred at room temperature (24 h) during which time the "pH" decreased to 8.9. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC using 30% methanol–chloroform as the eluent to give **33** as a solid: 14 mg (77%); mp 165–168 °C; R_f 0.18 (30% CH_3OH–$CHCl_3$); IR (KBr) 3393, 2969, 2930, 1688, 1645 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 1.24 (t, *J* = 7.3 Hz, 3 H, CH_2CH_3), 1.39 (s, 3 H, C(2')CH_3), 1.56–1.66 (m, 1 H, C(4)H*H'*), 2.32–2.42 (m, 1 H, C(4)H*H'*), 2.48 (q, *J* = 7.3 Hz, 1 H, CH*H'*CH_3), 2.50 (q, *J* = 7.3 Hz, 1 H, CH*H'*CH_3), 2.73 (d, *J* = 13.0 Hz, 1 H, C(5a)H*H'*), 3.24 (d, *J* = 13.0 Hz, 1 H, C(5a) H*H'*), 3.56 (d, *J* = 12.1 Hz, 1 H, C(3')H*H'*), 3.71 (dt, *J* = 4.6, 11.7 Hz, 1 H, C(3)H*H'*), 3.75–3.85 (m, 1 H, C(3)H*H'*), 3.98 (d, *J* = 12.1 Hz, 1 H, C(3')H*H'*); ^{13}C NMR (150 MHz, CD_3OD) 15.09 (CH_2CH_3), 20.11 (C(2')CH_3), 25.64 (C(4)), 29.63 (CH_2CH_3), 38.87 (C(5a)), 58.63 (C(3)), 68.14 (C(3')), 81.92 (C(1')), 83.40 (C(1)), 83.88 (C(2')), 100.39 (C(6)), 172.25, 173.77 (C(7), C(9)) ppm; the C(5) signal was undetected and is believed to be beneath the solvent peak; ^{13}C NMR (DMSO-d_6) 14.73 (CH_2CH_3), 19.37 (C(2')CH_3), 24.30 (C(4)), 28.06 (CH_2CH_3), 37.54 (C(5a)), 46.68 (C(5)), 56.30 (C(3)), 66.33 (C(3')), 79.88 (C(1')), 81.33 (C(1)), 81.91 (C(2')), 98.35 (C(6)), 169.82, 170.23 (C(7), C(9)) ppm; the proposed structural assignment was in agreement with the COSY, HMQC, HMBC NMR spectral data; MS (+FAB) 364 $[M + 1]^+$; M_r (+FAB) 364.106 27 $[M + 1]^+$ (calcd for $C_{14}H_{22}NO_8S$ 364.106 61).

The structure was further verified by X-ray crystallographic study of the tetra-*n*-butylammonium salt of **33**. To a methanolic solution of **33** was added 1 equiv of tetra-*n*-butylammonium hydroxide (1 M in CH_3OH, Aldrich), followed by the addition of diethyl ether. The precipitate was filtered, collected, and dissolved in H_2O. A colorless crystal was obtained after 4 days.

Reaction of Compound 10 with Ethanethiol. A tetrahydrofuran–water solution (3:1, 3 mL, "pH" 10.5 adjusted with aqueous 0.1 N NaOH) of **10** (20 mg, 0.059 mmol) was degassed with Ar and capped, and then ethanethiol (100 μL, 1.31 mmol) was added. The solution was stirred at room temperature (24 h) during which time the "pH" decreased to 9.2. The solvent was evaporated. TLC analysis indicated the presence of compound **37** and another minor unidentified product. The residue was taken up in ethyl acetate and purified by preparative TLC using ethyl acetate–chloroform (2:1) as the eluent to give compound **37**: yield, 7.6 mg (32%); mp 109–111 °C dec; R_f 0.70 (EtOAc); IR (KBr) 3478, 3328, 3252, 2986, 1693 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 3 H, C$(CH_3)_2$), 1.29 (t, *J* = 7.5 Hz, 3 H, CH_2CH_3), 1.36 (s, 3 H, C$(CH_3)_2$), 1.35–1.42 (m, 1 H, C(4)H*H'*), 1.44 (s, 3 H, C(2')CH_3), 2.53–2.72 (m, 2 H, CH_2CH_3), 3.15 (dt, *J* = 6.5, 13.5 Hz, 1 H, C(4)H*H'*), 3.22 (d, *J* = 13.8 Hz, 1 H, C(5a)H*H'*), 3.39 (d, *J* = 13.8 Hz, 1 H, C(5a)H*H'*), 3.71 (dt, *J* = 3.1, 13.5 Hz, 1 H, C(3)H*H'*), 3.76 (d, *J* = 8.4 Hz, 1 H, C(3')H*H'*), 4.00 (dd, *J* = 6.5, 13.5 Hz, 1 H, C(3)H*H'*), 4.28 (d, *J* = 8.4 Hz, 1 H, C(3')H*H'*), 5.17 (s, 1 H, C(1')OH), 5.78 (s, 1 H, N(10)H*H'*), 6.85 (s, 1 H, N(10)-H*H'*), 7.57 (s, 1 H, N(8)H); ^{13}C NMR ($CDCl_3$) 14.47 (CH_2CH_3), 24.70 (C(2')CH_3), 25.36 (C$(CH_3)_2$), 28.49 (C$(CH_3)_2$), 29.16 (CH_2CH_3), 33.11 (C(4)), 37.33 (C(5a)), 53.60 (C(5)), 57.88 (C(3)), 70.27 (C(3')), 80.16 (C(1')), 84.84 (C(1)), 86.06 (C(2')), 110.30 (*C*$(CH_3)_2$), 155.91 (C(7)), 167.99 (C(9)), 194.42 (C(6)) ppm; the proposed structural assignment was in agreement with the HMBC, HMQC, and COSY NMR spectral data; MS (+FAB) 403 $[M + 1]^+$; M_r (+FAB) 402.145 10 $[M]^+$ (calcd for $C_{17}H_{26}N_2O_7S$ 402.146 07).

Reaction of Bicyclomycin (1) with Ethanethiol. A tetrahydrofuran–water solution (3:1, 5 mL, "pH" 9.0 adjusted with aqueous ~0.1 N NaOH) containing bicyclomycin (15 mg, 0.050 mmol) and ethanethiol (40 μL, 0.54 mmol) was stirred at room temperature (24 h). During the course of reaction, the "pH" of the solution dropped to 8.1. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC using 20% methanol–chloroform to give **26** as the major product: yield, 7.0 mg (46%); mp 214–217 °C (lit.[6,7] mp 216–218 °C); R_f 0.65 (15% CH_3OH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2')CH_3), 1.24 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.80–1.94 (m, 1 H, C(4)H*H'*), 2.32 (dt, *J* = 6.6, 14.1 Hz, 1 H, C(4)H*H'*), 2.87 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.04 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.59 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*), 3.68–3.78 (m, 1 H, C(3)H*H'*), 3.92 (s, 1 H, C(1')H), 4.04 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*), 3.90–4.02 (m, 1 H, C(3)H*H'*); ^{13}C NMR (CD_3OD) 15.14 (CH_2CH_3), 21.26 (C(2')CH_3), 29.63 (CH_2CH_3), 32.42 (C(5a)), 33.14 (C(4)), 56.70 (C(5)), 58.71 (C(3)), 70.88, 71.93,

72.39 (C(1'), C(2'), C(3')), 85.33 (C(1)), 96.10 (C(9)), 160.04 (C(7)), 195.23 (C(6)) ppm.

General Procedure for Competition Experiments of Bicyclomycin Derivatives with Ethanethiol in Tetrahydrofuran–Water Mixtures. Each reaction was performed in buffered tetrahydrofuran–water mixtures (3:1, 0.5 mL, 0.1 M Tris·HCl) unless otherwise specified containing equimolar amounts of the bicyclomycin substrates and ethanethiol (~4 equiv). The solution was degassed with Ar prior to the addition of ethanethiol, capped, and stirred at room temperature. The reaction was analyzed by TLC, and all compounds were verified by cospotting the reaction mixture with authentic samples. The solvents were removed *in vacuo*, and the residue was dissolved in methanol unless otherwise specified and then purified by preparative TLC. The identities of the reaction products were confirmed by ^{1}H NMR analyses. The molar ratio of the starting reaction mixtures and products were measured by ^{1}H NMR spectroscopy on a QE-300 NMR spectrometer by integration using the parameters of P2 = 4 μs, D5 = 3 s.

Reaction of (1'*R*, 2'*S*)-Bicyclomycin (6) vs (1'*S*, 2'*S*)-Bicyclomycin (1). Treatment of **6** (2.5 mg, 0.0086 mmol) and **1** (2.0 mg, 0.0066 mmol) with ethanethiol (3 μL, 0.043 mmol) in buffered tetrahydrofuran–water ("pH" 8.1) followed by preparative TLC (20% methanol–chloroform) gave two fractions. The first fraction (1.0 mg) consisted of **27** and **26** in a 1.0:1.3 molar ratio. The second fraction (1.5 mg) contained **28**, **1**, and **6** in a 0.9:1.0: < 0.1 molar ratio. The identities of **28**, **27**, and **26** in the two fractions were verified by ^{1}H NMR spectroscopy by the addition of an authentic sample of each compound to the NMR solution of the mixtures to observe a selective increase in the peaks corresponding to the added compound.

Fraction I: R_f 0.55 (20% CH_3OH–$CHCl_3$). Compound 27: ^{1}H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2')CH_3), 1.22 (t, *J* = 7.3 Hz, 3 H, CH_2CH_3), 1.80–1.90 (m, 1 H, C(4)*H*H'), 2.32 (dt, *J* = 6.6, 14.0 Hz, 1 H, C(4)H*H'*), 2.54 (br q, *J* = 7.3 Hz, 2 H, CH_2CH_3), 2.77 (d, *J* = 14.1 Hz, 1 H, C(5a)*H*H'), 3.11 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.45 (d, *J* = 12.2 Hz, 1 H, C(3')*H*H'), 3.57 (s, 1 H, C(1')H), 3.78–4.01 (m, 2 H, C(3)H_2), 4.14 (d, *J* = 12.2 Hz, 1 H, C(3')H*H'*).

Compound 26: ^{1}H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2')CH_3), 1.24 (m, 3 H, CH_2CH_3), 1.80–1.90 (m, 1 H, C(4)*H*H'), 2.32 (dt, *J* = 6.6, 14.0 Hz, 1 H, C(4)H*H'*), 2.87 (d, *J* = 14.1 Hz, 1 H, C(5a)*H*H'), 3.04 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.59 (d, *J* = 12.3 Hz, 1 H, C(3')*H*H'), 3.65–3.80 (m, 1 H, C(3)*H*H'), 3.90–4.02 (m, 1 H, C(3)H*H'*), 3.92 (s, 1 H, C(1')H), 4.04 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*).

Fraction II: R_f 0.34–0.40 (20% CH_3OH–$CHCl_3$). Compound 28: ^{1}H NMR (CD_3OD) δ 1.20–1.24 (m, 3 H, CH_2CH_3), 1.26 (s, 3 H, C(2')CH_3), 1.80–1.90 (m, 1 H, C(4)*H*H'), 2.22–2.35 (m, 1 H, C(4)H*H'*), 2.45–2.55 (m, 4 H, CH_2CH_3, C(5)H, C(5a)*H*H'), 2.75–2.95 (m, 1 H, C(5a)H*H'*), 3.70–3.73 (m, 1 H, C(3')*H*H'), 3.80–3.83 (m, 1 H, C(3')H*H'*), 3.90–3.93 (m, 1 H, C(3)*H*H'), 4.08 (br s, 1 H, C(1')H), 4.02–4.10 (m, 1 H, C(3)H*H'*).

Compound 1: ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2')CH_3), 2.58–2.65 (m, 2 H, C(4)H_2), 3.51 (d, *J* = 11.4 Hz, 1 H, C(3')*H*H'), 3.66 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 3.74–3.95 (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 6: ^{1}H NMR (CD_3OD) δ 1.21 (s, 3 H, C(2')CH_3), 4.24 (s, 1 H, C(1')H); the remaining peaks were not clearly discerned and overlapped with the other signals.

Reaction of Bicyclomycin (1) vs C(1') Ketobicyclomycin (8). Use of **1** (3.0 mg, 0.0099 mmol), **8** (2.8 mg, 0.0093 mmol), and ethanethiol (3 μL, 0.043 mmol) in tetrahydrofuran–water (0.5 mL, "pH" initial 8.5 (adjusted with aqueous 0.1 N NaOH); "pH" final: 8.0), followed by preparative TLC (20% CH_3OH–$CHCl_3$) led to the isolation of **33**, **1**, and **26**. The identity of each compound was further proved by ^{1}H NMR spectroscopy and cospotting on TLC with an authentic sample.

Compound 26: yield, 1.2 mg (34%); R_f 0.77 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2')CH_3), 1.25 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.91 (br d, *J* = 14.0 Hz, 1 H, C(4)*H*H'), 2.30 (dt, *J* = 6.3 Hz, 14.0 Hz, 1 H, C(4)H*H'*), 2.59 (q, *J* = 7.2 Hz, 2 H, CH_2CH_3), 2.90 (d, *J* = 14.1 Hz, 1 H, C(5a)*H*H'), 3.05 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.58 (d, *J* = 12.0 Hz, 1 H, C(3')*H*H'), 3.72 (dt, *J* = 2.1, 14.0 Hz, 1 H, C(3)*H*H'), 3.92 (s, 1 H, C(1')H), 4.00 (dd, *J* = 6.3, 14.0 Hz, 1 H, C(3)H*H'*), 4.04 (d, *J* = 12.0 Hz, 1 H, C(3')H*H'*).

Compound 1: yield, 2.0 mg (66%); R_f 0.40 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2')CH_3), 2.56–2.65 (m, 2 H, C(4)H_2), 3.49 (d, *J* = 11.4 Hz, 1 H, C(3')*H*H'), 3.65 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 3.72–3.97 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1')H), 5.13 (s, 1 H, C(5a)*H*H'), 5.56 (s, 1 H, C(5a)H*H'*).

Compound 33: yield, 3.0 mg (88%); R_f 0.12 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.24 (t, *J* = 7.4 Hz, 3 H, CH_2CH_3), 1.39 (s, 3 H, C(2')CH_3), 1.55–1.68 (m, 1 H, C(4)*H*H'), 2.35–2.45 (m, 1 H, C(4)H*H'*), 2.49 (br q, *J* = 7.4 Hz, 2 H, CH_2CH_3), 2.73 (d, *J* = 12.9 Hz, 1 H, C(5a)*H*H'), 3.24 (d, *J* = 12.9 Hz, 1 H, C(5a)H*H'*), 3.56 (d, *J* = 12.4 Hz, 1 H, C(3')*H*H'), 3.65–3.85 (m, 2 H, C(3)H_2), 3.98 (d, *J* = 12.4 Hz, 1 H, C(3')H*H'*).

Reaction of Bicyclomycin (1) vs C(1') Ketobicyclomycin Acetonide (10). Use of **1** (4.0 mg, 0.013 mmol), **10** (4.9 mg, 0.015 mmol), and ethanethiol (4 μL, 0.056 mmol) in buffered tetrahydrofuran–water ("pH" 8.2), followed by two successive preparative TLCs using first ethyl acetate, and then 20% methanol–chloroform as the eluent led to the isolation of **1**, **26**, and **37**.

Compound 1: yield, 2.5 mg (63%); R_f 0.40 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2')CH_3), 2.60–2.68 (m, 2 H, C(4)H_2), 3.50 (d, *J* = 11.4 Hz, 1 H, C(3')*H*H'), 3.66 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 3.72–3.96 (m, 2 H, C(3)H_2), 4.07 (s, 1 H, C(1')H), 5.12 (s, 1 H, C(5a)*H*H'), 5.55 (s, 1 H, C(5a)H*H'*).

Compound 26: yield, 1.5 mg (35%); R_f 0.80 (20% CH_3OH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2')CH_3), 1.24 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.91 (br d, *J* = 14.0 Hz, 1 H, C(4)*H*H'), 2.30 (dt, *J* = 6.3, 14.0 Hz, 1 H, C(4)H*H'*), 2.58 (q, *J* = 7.2 Hz, 2 H, CH_2CH_3), 2.88 (d, *J* = 13.8 Hz, 1 H, C(5a)*H*H'), 3.03 (d, *J* = 13.8 Hz, 1 H, C(5a)H*H'*), 3.61 (d, *J* = 12.0 Hz, 1 H, C(3')*H*H'), 3.72 (br t, *J* = 14.0 Hz, 1 H, C(3)*H*H'), 3.91 (s, 1 H, C(1')H), 4.00 (dd, *J* = 6.3, 14.0 Hz, 1 H, C(3)H*H'*), 4.04 (d, *J* = 12.0 Hz, 1 H, C(3')H*H'*).

Compound 37: yield, 2.5 mg (43%); R_f 0.70 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H, C(CH_3)$_2$), 1.29 (t, *J* = 7.5 Hz, 3 H, CH_2CH_3), 1.36 (s, 3 H, C(CH_3)$_2$), 1.38–1.42 (m, 1 H, C(4)*H*H'), 1.44 (s, 3 H, C(2')CH_3), 2.53–2.72 (m, 2 H, CH_2CH_3), 3.15 (dt, *J* = 6.5, 13.8 Hz, 1 H, C(4)H*H'*), 3.22 (d, *J* = 13.8 Hz, 1 H, C(5a)*H*H'), 3.39 (d, *J* = 13.8 Hz, 1 H, C(5a)H*H'*), 3.71 (dt, *J* = 3.1, 13.8 Hz, 1 H, C(3)*H*H'), 3.76 (d, *J* = 8.4 Hz, 1 H, C(3')*H*H'), 4.00 (dd, *J* = 6.5, 13.8 Hz, 1 H, C(3)H*H'*), 4.28 (d, *J* = 8.4 Hz, 1 H, C(3')H*H'*), 5.17 (s, 1 H, C(1')OH), 5.78 (s, 1 H, N(10)*H*H'), 6.85 (s, 1 H, N(10)H*H'*), 7.57 (s, 1 H, N(8)H).

Reaction of Bicyclomycin (1) vs Bicyclomycin C(2'), C(3') Acetonide (9). Treatment of **1** (3.0 mg, 0.010 mmol) and **9** (3.4 mg, 0.010 mmol) with ethanethiol (4 μL, 0.056 mmol) in buffered tetrahydrofuran–water (3:1, "pH" 8.4) followed by preparative TLC using 20% methanol–chloroform as the eluent gave two fractions. The first fraction (4.5 mg) contained unreacted **9**, **38**, **26** in a 1.4:1.5:1.0 molar ratio. The second fraction (1.5 mg) contained unreacted **1** and **32** in a 5.1:1.0 molar ratio.

Fraction I: R_f 0.60–0.75 (20% CH_3OH–$CHCl_3$). Compound 9: ^{1}H NMR (CD_3OD) δ 1.42 (s, 3 H, C(2')CH_3), 1.46 (s, 3 H, C(CH_3)$_2$), 1.48 (s, 3 H, C(CH_3)$_2$), 2.61–2.67 (m, 2 H, C(4)H_2), 3.76 (d, *J* = 8.4 Hz, 1 H, C(3')*H*H'), 3.80–4.04 (m, 2 H, C(3)H_2), 4.19 (s, 1 H, C(1')H), 4.49 (d, *J* = 8.4 Hz, 1 H, C(3')H*H'*), 5.18 (s, 1 H, C(5a)*H*H'), 5.62 (s, 1 H, C(5a)H*H'*).

Compound 38: ^{1}H NMR (CD_3OD) δ 1.26 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.36 (s, 3 H, C(2')CH_3), 1.45 (s, 6 H, C(CH_3)$_2$), 1.88–2.45 (m, 4 H, C(4)H_2, C(5a)*H*H', C(5)H), 2.45–2.65 (m, 2 H, CH_2CH_3), 3.06–3.16 (m, 1 H, C(5a)H*H'*), 3.71 (d, *J* = 8.1 Hz, 1 H, C(3')*H*H'), 3.75–4.09 (m, 2 H, C(3)H_2), 4.10 (s, 1 H, C(1')H), 4.45 (d, *J* = 8.1 Hz, 1 H, C(3')H*H'*).

Compound 26: ^{1}H NMR (CD_3OD) δ 1.14 (s, 3 H, C(2')CH_3), 1.24 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 1.80–1.94 (m, 1 H, C(4)*H*H'), 2.30–2.35 (m, 1 H, C(4)H*H'*), 2.50–2.63 (m, 2 H, CH_2CH_3), 2.87 (d, *J* = 14.1 Hz, 1 H, C(5a)*H*H'), 3.04 (d, *J* = 14.1 Hz, 1 H, C(5a)H*H'*), 3.59 (d, *J* = 12.3 Hz, 1 H, C(3')*H*H'), 3.68–4.02 (m, 2 H, C(3)H_2), 3.92 (s, 1 H, C(1')H), 4.04 (d, *J* = 12.3 Hz, 1 H, C(3')H*H'*).

Fraction II: R_f 0.30–0.35 (20% CH_3OH–$CHCl_3$). Compound 1: ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2')CH_3), 2.62–2.68 (m, 2 H, C(4)H_2), 3.49 (d, *J* = 11.4 Hz, 1 H, C(3')*H*H'), 3.65 (d, *J* = 11.4 Hz, 1 H, C(3')H*H'*), 3.75–3.95 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1')H), 5.16 (s, 1 H, C(5a)*H*H'), 5.57 (s, 1 H, C(5a)H*H'*).

Compound 32: ^{1}H NMR (CD_3OD) δ 1.20–1.30 (m, 3 H, CH_2CH_3), 1.32 (s, 3 H, C(2')CH_3), 2.47–2.65 (m, 2 H, CH_2CH_3), 4.03 (s, 1 H, C(1')H); the remaining peaks were not clearly discerned and overlapped with the other signals.

Reaction of (1'*S*, 2'*R*)-Bicyclomycin (7) vs (1'*S*,2'*S*)-Bicyclomycin (1). Use of **7** (2.0 mg, 0.0066 mmol), **1** (2.5 mg, 0.0083 mmol), and ethanethiol (3 μL, 0.043 mmol) in buffered tetrahydrofuran–water (3:1 "pH" 8.2) followed by preparative TLC using 15% methanol–chloroform as the eluent led to the isolation of two fractions. The first fraction consisted of **26** and **29** (1.8 mg) in a >10:1 molar ratio. The second fraction consisted of **1**, **7**, and **30** (2.2 mg, **1**:**7**:**30**, 0.7:1.0:0.5).

Fraction I: R_f 0.50–0.60 (15% CH_3OH–$CHCl_3$). Compound 26: ^1H NMR δ 1.14 (s, 3 H, C(2′)CH_3), 1.24 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.85–1.96 (m, 1 H, C(4)HH′), 2.23–2.40 (m, 1 H, C(4)HH′), 2.50–2.63 (m, 2 H, CH_2CH_3), 2.87 (d, J = 14.1 Hz, 1 H, C(5a)HH′), 3.04 (d, J = 14.1 Hz, 1 H, C(5a)HH′), 3.59 (d, J = 12.3 Hz, 1 H, C(3′)HH′), 3.65–3.80 (m, 1 H, C(3)HH′), 3.90–4.02 (m, 1 H, C(3)HH′), 3.92 (s, 1 H, C(1′)H), 4.04 (d, J = 12.3 Hz, 1 H, C(3′)HH′).

Compound 29: ^1H NMR (CD_3OD) δ 1.40 (s, 3 H, C(2′)CH_3); the remaining peaks were not clearly discerned and overlapped with the signals of compound **26**.

Fraction II: R_f 0.30 (15% CH_3OH–$CHCl_3$). Compound 1: ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 4.07 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH′); the remaining peaks overlapped with the signals for the other compounds in the mixture.

Compound 7: ^1H NMR (CD_3OD) δ 1.32 (s, 3 H, C(2′)CH_3), 4.19 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)HH′), 5.56 (s, 1 H, C(5a)HH′); the remaining peaks overlapped with the signals for the other compounds in the mixture.

Compound 30: ^1H NMR (CD_3OD) δ 1.20–1.28 (m, 3 H, CH_2CH_3), 1.31, 1.33 (2 s, 3 H, C(2′)CH_3), 3.15 (d, J = 12.3 Hz, 1 H, C(5a)HH′), 4.13 (s, 1 H, C(1′)H); the remaining peaks overlapped with the signals for the other compounds in the mixture.

Reaction of C(1′) Ketobicyclomycin (8) vs C(1′) Ketobicyclomycin C(2′) C(3′) Acetonide (10). Treatment of **8** (3.0 mg, 0.010 mmol) and **10** (3.4 mg, 0.010 mmol) with ethanethiol (3.0 μL, 0.043 mmol) in buffered tetrahydrofuran–water (3:1, "pH" 8.2) followed by two successive preparative TLCs using first ethyl acetate and then 20% methanol–chloroform as the eluents led to the isolation of three fractions. The first fraction consisted of **37** (1.0 mg). The second fraction contained a mixture of **10** and **8** (2.8 mg) in a 5:1 molar ratio. The third fraction consisted of **33** (2.5 mg).

Fraction I: R_f 0.70 (ethyl acetate). Compound 37: ^1H NMR ($CDCl_3$) δ 1.26 (s, 3 H, C(CH_3)$_2$), 1.29 (t, J = 7.5 Hz, CH_2CH_3), 1.36 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(2′)CH_3), 1.35–1.40 (m, 1 H, C(4)HH′), 2.53–2.72 (m, 2 H, CH_2CH_3), 3.10–3.20 (m, 1 H, C(4)HH′), 3.22 (d, J = 13.8 Hz, 1 H, C(5a)HH′), 3.39 (d, J = 13.8 Hz, 1 H, C(5a)HH′), 3.71 (dt, J = 3.1, 13.8 Hz, 1 H, C(3)HH′), 3.76 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 4.00 (dd, J = 6.5, 13.8 Hz, 1 H, C(3)HH′), 4.28 (d, J = 8.4 Hz, 1 H, C(3′)HH′), 5.17 (s, 1 H, C(1′)OH), 5.78 (s, 1 H, N(10)HH′), 6.85 (s, 1 H, N(10)HH′), 7.57 (s, 1 H, N(8)H).

Fraction II: R_f 0.30–0.55 (20% methanol–chloroform). Compound 10: ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(CH_3)$_2$), 1.40 (s, 3 H, C(CH_3)$_2$), 1.40 (s, 3 H, C(CH_3)$_2$), 1.59 (s, 3 H, C(2′)CH_3), 2.62–2.69 (m, 2 H, C(4)H_2), 3.80–3.88 (m, 1 H, C(3)HH′), 3.92 (d, J = 8.4 Hz, 1 H, C(3′)-HH′), 3.98–4.12 (m, 1 H, C(3)HH′), 4.15 (d, J = 8.4 Hz, 1 H, C(3′)-HH′), 5.14 (s, 1 H, C(5a)HH′), 5.57 (s, 1 H, C(5a)HH′).

Compound 8: ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2′)CH_3), 3.45 (d, J = 10.5 Hz, 1 H, C(3′)HH′), 3.74 (d, J = 10.5 Hz, 1 H, C(3′)HH′); the remaining peaks were not clearly discerned and overlapped with other signals.

Fraction III: R_f 0.05–0.15 (20% methanol–chloroform). Compound 33: ^1H NMR (CD_3OD) δ 1.24 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.39 (s, 3 H, C(2′)CH_3), 1.56–1.66 (m, 1 H, C(4)HH′), 2.32–2.42 (m, 1 H, C(4)HH′), 2.48 (q, J = 7.2 Hz, CHH′CH_3), 2.50 (q, J = 7.2 Hz, 1 H, CHH′CH_3), 2.73 (d, J = 13.2 Hz, 1 H, C(5a)HH′), 3.24 (d, J = 13.2 Hz, 1 H, C(5a)HH′), 3.56 (d, J = 12.3 Hz, 1 H, C(3′)HH′), 3.68–3.85 (m, 2 H, C(3)H_2), 3.98 (d, J = 12.3 Hz, 1 H, C(3′)HH′).

General Procedure for the Determination of the Stability of Bicyclomycin Substrates. The stability of each bicyclomycin (i.e., **1** and **6–8**) in dimethylformamide-d_7 (0.5 mL), 0.1 M K_2DPO_4–D_2O (0.5 mL, "pD" 9.3), 0.1 M KD_2PO_4–K_2DPO_4–D_2O (0.5 mL, "pD" 7.4), and 0.1 M KD_2PO_4–D_2O (0.5 mL, "pD" 5.6) was monitored by ^1H NMR spectroscopy (General Electric QE-300 NMR spectrometer) using 1 mg of the substrate. The reaction temperature was maintained at 21 ± 1 °C. The pD of the solutions were determined both before and after the reaction. The pD of the solutions were calculated from the observed pH meter readings by the relationship pD = pH meter reading + 0.4.[25] The NMR parameters were P2 = 6 μs, D5 = 3 s. Each transformation was monitored for at least 2 half-lives, during which time at least six measurements were made. The relative amounts of bicyclomycin substrate and product at each time point were calculated from the ^1H NMR spectra by comparing the integrated area for the C(2′)CH_3 hydrogens in the initial substrate versus the C(2′)CH_3 hydrogens in the product(s). Standard data plots of ln a_0/a versus time, where a_0 is the initial amount of the bicyclomycin substrate and a is the remaining amount of the starting material yielded linear slopes (Figure 3) from which the psuedo first-order rate constants (k_1) and half-lives were calculated (Table 1).

Stability of (1′*R*,2′*S*)-Bicyclomycin (6) in Dimethylformamide-d_7. Dissolution of **6** in dimethylformamide-d_7 (4 h) led to the tentative identification of **39** as the major product. Key spectral data: ^1H NMR (600 MHz, DMF-d_7) δ 1.22, 1.23, 1.25, 1.28 (4s), 2.25–2.75 (m), 3.42–4.12 (m), 4.37 (s), 5.09, 5.11 (2s), 5.52 (s), 6.20, 6.61 (2s); additional unassigned peaks were observed at δ 5.22, 5.30 (2s), 5.50 (s), 7.01–7.62 (m), 7.80 (s), 8.40, 8.43 (2s), 8.58 (s); ^{13}C NMR (150 MHz, DMF-d_7) 18.91, 19.83, 31.99, 68.45, 77.34, 77.70, 78.75, 78.98, 79.54, 79.99, 83.30, 83.46, 91.02, 91.76, 92.56, 113.98, 114.47, 145.18, 169.90, 172.94, 173.94, 175.60 ppm; additional unassigned signals were observed at 60.52, 61.71, 95.68, 102.07, 109.07, 133.85 ppm; MS (+FAB) 285 [M + 1]$^+$; M_r (+CI) 285.109 26 [M + 1]$^+$ (calcd for $C_{12}H_{17}N_2O_6$ 285.108 66).

Reaction of (1′*R*, 2′*S*)-Bicyclomycin (6) vs (1′*S*, 2′*S*)-Bicyclomycin (1) with Aniline at "pH" 5.5. Treatment of **6** (2.0 mg, 0.0066 mmol) and **1** (1.7 mg, 0.0056 mmol) with aniline (3.0 μL, 0.033 mmol) in tetrahydrofuran–water (3:1, 0.5 mL, 0.1 N bis·Tris·HCl, "pH" 5.5) followed by preparative TLC (15% methanol–chloroform, three elutions) led to the isolation of two fractions. The first fraction contained **1** (1.0 mg, 0.0033 mmol), and the second fraction contained **40** (1.0 mg, 0.0025 mmol).

Compound 1: R_f 0.30 (15% CH_3OH–$CHCl_3$, three elutions); ^1H NMR δ 1.33 (s, 3 H, C(2′)CH_3), 2.58–2.65 (m, 2 H, C(4)H_2), 3.48 (d, J = 11.4 Hz, 1 H, C(3′)HH′), 3.66 (d, J = 11.4 Hz, 1 H, C(3′)HH′), 3.68–4.00 (m, 2 H, C(3)H_2), 4.07 (s, 1 H, C(1′)H), 5.12 (s, 1 H, C(5a)HH′), 5.55 (s, 1 H, C(5a)HH′).

Compound 40: R_f 0.38 (15% CH_3OH–$CHCl_3$, three elutions); ^1H NMR (CD_3OD) δ 1.25 (s, 3 H, C(2′)CH_3), 1.60–1.80 (m, 1 H, C(4)-HH′), 1.80–2.00 (m, 1 H, C(5)H), 2.08–2.32 (m, 1 H, C(4)HH′), 2.52–2.70 (m, 1 H, C(5a)HH′), 3.10–3.30 (m, 1 H, C(5a)HH′), 3.62–3.68 (m, 1 H, C(3′)HH′), 3.80–3.85 (m, 1 H, C(3′)HH′), 3.90–4.20 (m, 2 H, C(3)H_2), 3.94 (br s, 1 H, C(1′)H), 6.58–6.68 (m, 3 H, C_6H_5), 7.01–7.20 (m, 2 H, C_6H_5).

Reaction of (1′*R*,2′*S*)-Bicyclomycin (6) with Aniline. A tetrahydrofuran–water solution (3:1, 2 mL, "pH" 10.7 adjusted with aqueous 0.1 N NaOH) of **6** (10 mg, 0.034 mmol) and aniline (50 μL, 0.55 mmol) was stirred at room temperature under Ar (24 h). The solvent was removed *in vacuo*, and the residue was dissolved in a small amount of methanol. TLC analysis indicated the presence of **40**. Preparative TLC gave the product as a white solid: yield, 8 mg (76%); R_f (20% CH_3OH–$CHCl_3$); IR (KBr) 3378, 2936, 1684, 1603, 1503 cm^{-1}; ^1H NMR (CD_3OD) δ 1.25 (s, 3 H, C(2′)CH_3), 1.60–1.80 (m, 1 H, C(4)HH′), 1.82–1.96 (m, 1 H, C(5)H), 2.10–2.32 (m, 1 H, C(4)HH′), 2.50–2.68 (m, 1 H, C(5a)HH′), 3.10–3.28 (m, 1 H, C(5a)HH′), 3.67 (d, J = 8.7 Hz, 1 H, C(3′)HH′), 3.80 (d, J = 8.7 Hz, 1 H, C(3′)HH′), 3.90–4.01 (m, 1 H, C(3)HH′), 3.94 (br s, 1 H, C(1′)H), 4.03–4.18 (m, 1 H, C(3)HH′), 6.58–6.68 (m, 3 H, C_6H_5), 7.01–7.20 (m, 2 H, C_6H_5); the proposed structural assignment was in agreement with the COSY NMR experiment; ^{13}C NMR (CD_3-OD) 19.20 (C(2′)CH_3), 28.89, 30.45, 31.62, 31.76 (C(4)), 44.24, 44.51 (C(5a)), 47.45, 47.67 (C(5)), 68.25, 68.74, 69.18 (C(3)), 77.50, 77.99 (C(3′)), 80.01, 80.86 (C(2′)), 83.51 (C(1′)), 91.77, 92.41, 95.64 (C(1)), 104.16, 104.73, 106.12 (C(6)), 114.26, 114.41 (C_6H_5), 118.01 (C_6H_5), 129.95 (C_6H_5), 149.81, 150.14, 150.21 (C_6H_5), 173.31, 175.34 (C(7), C(9)) ppm; MS(+FAB) 395 [M]$^+$; M_r (+FAB) 395.171 05 [M]$^+$ (calcd for $C_{18}H_{25}N_3O_7$ 395.169 25).

Crystallographic Procedure for Compound 14. A clear, colorless block having approximate dimensions 0.70 × 0.60 × 0.50 mm was cut from a much larger wedge and 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 2. The Laue symmetry was determined to be $2/m$, and from the systematic absences noted the space group was shown to be either $P2_1$ or $P2_1/m$. Intensities were measured using the ω scan technique, with the scan rate depending on the count obtained in rapid pre-scans 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 very small absorption coefficient.

Since the compound was known to be chiral, space group $P2_1$ was assumed from the outset. The structure was solved by use of the SHELXTL direct methods program, which revealed the positions of most of the non-hydrogen atoms comprising the two independent molecules in the asymmetric unit. Remaining non-hydrogen atoms were located in

Table 2. Data Collection and Processing Parameters for Compounds **14** and **33**

	14	**33**
space group	$P2_1$ (monoclinic)	$P2_12_12_1$ (orthorhombic)
cell constants	a = 10.228 (3) Å	a = 10.803 (2) Å
	b = 12.349 (4)	b = 13.065 (2)
	c = 11.564 (2)	c = 23.497 (5)
	β = 112.00 (2)°	
	V = 1354 Å^3	V = 3316 Å^3
molecular formula	$C_{12}H_{16}N_2O_6 \cdot {}^1/_2H_2O$	$C_{16}H_{36}N^+ \cdot C_{14}H_{20}NO_8S^-$
formula weight	293.31	604.94
formula units per cell	Z = 4	Z = 4
density	ρ = 1.44 g-cm^{-3}	ρ = 1.21 g-cm^3
absorption coeff	μ = 1.10 cm^{-1}	μ = 1.38 cm^{-1}
temp	T = 22 °C	T = −50 °C
radiation (Mo Kα)	λ = 0.710 73 Å	λ = 0.710 73 Å
collection range	$4° \le 2\theta \le 55°$	$4° \le 2\theta \le 52°$
scan width	$\Delta\theta$ = 1.20 + (Kα_2 − Kα_1)°	$\Delta\theta$ = 1.25 + (Kα_2 − Kα_1)°
scan speed range	2.5–15.0°-min^{-1}	1.5–15.0°-min^{-1}
total data collected	3255	3672
independent data, $I > 3\sigma(I)$	2881	2722
total variables	402	384
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.042	0.039
$R_w = [\Sigma w(\|F_o\| - \|F_c\|)^2/\Sigma w\|F_o\|^2]^{1/2}$	0.033	0.030
weights	$w = \sigma(F)^{-2}$	$w = \sigma(F)^{-2}$
extinction coeff	x = 0.001 67	

subsequent difference Fourier syntheses. The usual sequence of isotropic and anisotropic refinement was followed after which all hydrogens attached to carbon were entered in ideal calculated positions and constrained to riding motion. The non-water hydrogens attached to O and N were allowed to refine freely. A single isotropic temperature factor was varied for all of the non-water hydrogens. The water molecule was treated as an ideal rigid body and allowed to rotate freely.

One of the two independent molecules was found to be partially disordered at the C3–C4 ethylene moiety, such that the torsion angle O2–C3–C4–C5 is sometimes positive and sometimes negative. Based on analysis of the isotropic temperature factors involved, the model used was 60:40 for C3:C3′. The sample crystal was of extremely high quality, and the diffraction data showed the classic symptoms of extinction, so an empirical isotropic extinction parameter was also refined in the final cycles of least squares.

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(1). After all shift/esd ratios were less than 0.2 convergence was reached at the agreement factors listed in Table 2. 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 Å^3. All calculations were made using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs.

Crystallographic Procedure for Tetra-*n*-butylammonium Salt of Compound 33. A colorless multifaceted plate having approximate dimensions 0.60 × 0.50 × 0.20 mm was mounted in a random orientation on a Nicolet R3m/V automatic diffractometer. The sample was placed in a stream of dry nitrogen 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 are listed in Table 2. 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 pre-scans 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 most of the non-hydrogen atoms in the compound. Remaining atoms were located in subsequent difference Fourier syntheses. The usual sequence of isotropic and anisotropic refinement was followed, after which all hydrogens attached to carbon were entered in ideal calculated positions and constrained to riding motion, with a single variable isotropic temperature factor for those in the cation and separate one for those in the anion. The hydrogens attached to N and O were located in difference maps and allowed to refine independently. The absolute configuration was determined experimentally by refinement of a coefficient multiplying $\Delta f''$. The results of this analysis indicate that there is a very strong probability that the absolute configuration listed in the tables and shown in the figure is correct. After all shift/esd ratios were less than 0.1 convergence was reached at the agreement factors listed in Table 2. 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 0.25 e/Å^3. All calculations were made using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs.

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Supplementary Material Available: Crystallographic procedures for compounds **10**, **11**, and **18**, ORTEP drawing for compounds **10** (Figure 5), **11** (Figure 6), and **18** (Figure 7) and a complete listing of data collection and processing parameters, atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, hydrogen-bonding parameters for compounds **10**, **11**, **14**, **18** and **33** (30 pages); tables of observed and calculated structure factors for compounds **10**, **11**, **14**, **18**, and **33** (52 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.