Studies on the Reactivity of Bicyclomycin with Thiols

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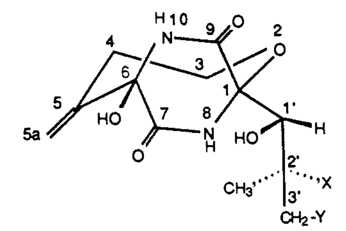
Abstract: The chemical reactivity of the clinically important antibiotic bicyclomycin (**1**) with thiols has been investigated. Emphasis has been placed on discerning the product profiles obtained with cysteine derivatives in light of earlier projections on the likely in vivo biological nucleophile in bicyclomycin-mediated transformations. Three different types of products were observed as a function of "pH". At near neutral "pH" values, the novel piperidinedione adducts **16** were generated stereospecifically and efficiently. Correspondingly, under moderately basic conditions ("pH" 10.2–10.5), the direct C(5a)-substituted compounds **14** were observed as the predominant products, while at higher pH values (pH 12.5) the ring-opened adducts **13** were isolated. New information concerning the importance of the C(1)-triol group in the C(5)-C(5a) functionalization process was also secured by comparing the reactivity of the two bicyclomycin derivatives: 3′-*S*-ethylbicyclomycin (**2**) and bicyclomycin-2′,3′-acetonide (**4**) versus **1**. Both **2** and **4** did not react with thiols at room temperature at near neutral "pH". Elevation of the "pH" to 10.2 led to the formation of the corresponding C(5a)-direct-substitution adducts. In no cases were piperidinedione products (i.e., **16**) observed. Correspondingly, dissolution of **1**, **2**, and **4**, respectively, in ^{18}O-enriched water–tetrahydrofuran mixtures ("pH" 7.8 and 10.2) in the absence of thiols led to substantial amounts of ^{18}O incorporation in recovered samples of **1**, but not **2** and **4**. Analysis of the composite data permitted an unifying mechanism to be proposed for thiolate-induced bicyclomycin processes. Furthermore, the role(s) of the C(1)-triol substituent in these transformations is discussed.

Bicyclomycin (**1**) is a clinically useful antibiotic possessing a diverse spectrum of biological activity.[1-4] Early biochemical studies indicated that **1** expresses its activity by disrupting bacterial cell wall growth. Key findings included the following: (1) the observation that **1** induces pronounced morphological changes in *Escherichia coli*;[5] (2) the discovery that bicyclomycin inhibits in vivo bacterial RNA and protein synthesis but has no effect on DNA or lipid synthesis;[6] (3) the demonstration that **1** forms irreversible, covalent 1:1 complexes with seven select inner-membrane proteins present in the Sarkosyl-soluble fraction of *Escherichia coli* (ATCC 27166)[7]; and (4) the observation that thiols inhibit drug binding to the inner-membrane proteins.[7] These studies, coupled with others,[8,9] served as the basis for several hypotheses pertaining to the mechanism of the drug-binding process.[1,10-12] Although considerable controversy exists concerning this event, most proposals suggest that bicyclomycin reacts with nucleophiles (i.e., a protein sulfhydryl group) necessary for the remodeling of the peptidoglycan assembly within the bacterial cell wall and that drug binding occurs at the *exo*-methylene group in **1**.

In this paper, a detailed account of the reaction of bicyclomycin with sulfur-containing nucleophiles is provided.[13] Emphasis has been placed on discerning the reactivity of **1** with functionalized cysteine derivatives in light of the earlier projections of the likely candidates for the biological nucleophile(s) in the bicyclomycin-binding process. New information is provided on the importance



1 X = OH, Y = OH

2 X = OH, Y = SCH_2CH_3

3 X = OH, Y = $OC(O)NHCH_2CH_3$

4 X,Y = $OC(CH_3)_2O$

5 X,Y = O

of both "pH" and the C(1)-triol group in facilitating drug activation as well as determining the product composition in these transformations.

Results

a. Choice of Reagents and Conditions. Bicyclomycin (**1**) served as the parent compound in this study. In addition, the C(1)-triol group was selectively modified to discern the importance of this moiety in these reactions. Both the C(3′)-*S*-ethyl **2** and C-(3′)-*O*-ethylcarbamoyl **3**[14] derivatives were prepared to evaluate the effect of the primary hydroxyl group in **1** while acetonide **4**[15] was synthesized to ascertain the role of the 2′- and 3′-hydroxyl groups. Synthesis of **2** was readily accomplished by initial preparation of epoxide **5**,[14] followed by treatment with ethanethiol (**6**) in the presence of triethylamine. The primary sulfur-containing reagents chosen for evaluation with **1–4** were ethanethiol (**6**) and *N*-acetyl-L-cysteine *N′*-methylamide (**7**).[12,16] At select "pH" values, reactions were also conducted with methanethiol (**8**), benzyl mercaptan (**9**), ethyl 2-mercaptoacetate (**10**), L-cysteine methyl ester (**11**), and *N*-acetyl-L-cysteine methyl ester (**12**).[12,17] The

RSH	$HSCH_2CH(NHR)(COR')$
6: R = CH_2CH_3	**7**: R = $C(O)CH_3$, R′ = $NHCH_3$
8: R = CH_3	**11**: R = H, R′ = OCH_3
9: R = $CH_2C_6H_5$	**12**: R = $C(O)CH_3$, R′ = OCH_3
10: R = $CH_2C(O)OCH_2CH_3$	

reactions were run in water, tetrahydrofuran–water, or metha-

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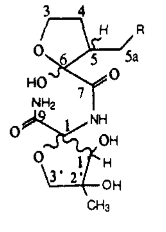
nol-water mixtures. Three approximate "pH" values ("pH" 7.4–8.2, 10.2–10.5, 12.5) spanning the neutral and basic regions were selected for study.

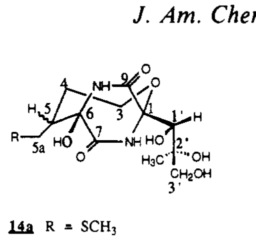
b. Reactivity of 1 with Thiols at pH 12.5. Treatment of aqueous solutions of **1** with ethanethiol (**6**), *N*-acetyl-L-cysteine *N'*-methylamide (**7**), and methanethiol (**8**) at pH 12.5 led to the formation of the C(5a)-substituted ring-opened adducts **13a–c**, respectively. In the case of the ethanethiol (**6**) and methanethiol (**8**) reactions, the resulting diastereomeric mixtures **13a** and **13c**, respectively, were not resolved by PTLC. ^{13}C NMR analyses of these products indicated, however in each case that two major adducts were generated in an approximate 1.4:1 ratio.[13] Fortunately, repeated PTLC chromatography of the cysteine **7**-mediated reaction permitted the isolation of **13b-1** and **13b-2**. No attempt was made to discern the precise stereochemical identity of these two isomers.



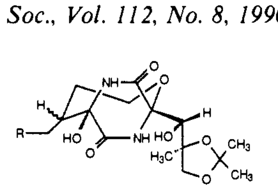
13a R = SCH_2CH_3
b R = $SCH_2CH(C(O)NHCH_3)(NHC(O)CH_3)$
c R = SCH_3
d R = H

Several distinctive features in the ^{1}H and ^{13}C NMR spectra of **13a–c** proved helpful in characterizing the structure of these products. Two multiplets were observed in the ^{1}H NMR spectra of **13** and have been assigned to the C(5a)-methylene hydrogens on the basis of their chemical shift value[18] and the connectivity patterns discerned in the corresponding COSY spectrum. In the ^{13}C NMR spectra for **13** large downfield shifts were noted for the C(1') (~7 ppm), C(3') (~9 ppm), and C(6) (~20 ppm) resonances as compared to **1**.[19] Moreover, the chemical shift value (101.86–103.50 ppm) observed for the C(6) peak was in agreement with the proposed formation of a hemiacetal moiety at this site.[20] Additional data in support of the structural assignments for **13a** and **13c** was provided by the COSY, proton double quantum coherence,[21] heteronuclear chemical shift correlation,[22] and long-range heteronuclear multiple quantum chemical shift correlation (HMBC)[23] NMR studies. In particular, several long-range proton–carbon connectivities (i.e., C(6)O*H*–*C*(7), C(6)O-*H*–*C*(5), C(3')*H*–*C*(1)) were observed in the HMBC experiment consistent with the proposed molecular framework depicted for **13**.[13]

The assignment of the methanethiolate adduct obtained from the pH 12.5 experiment as the ring-opened product **13c** constitutes a structural revision from the original assigned product. Earlier investigations had proposed the isomeric structure **14a** for this compound.[8] Additional support for the revision was secured by the preparation of an authentic sample of **14a**. Treatment of acetonide **4** with **8** in a 1:1 tetrahydrofuran-water mixture ("pH" 12.5) gave a 2.5:1 diastereomeric mixture of the C(5a)-sulfide **15a**. Removal of the acetonide linkage with aqueous 50% acetic acid furnished a 1.9:1 diastereomeric mixture of **14a**. In agreement with the proposed structure, the ^{13}C NMR spectrum for **14a**



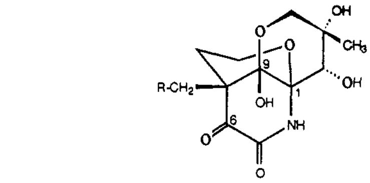
14a R = SCH_3
b R = SCH_2CH_3
c R = $SCH_2CH(C(O)NHCH_3)(NHC(O)CH_3)$
d R = $SCH_2CH(C(O)OCH_3)(NH_2)$
e R = H



15a R = SCH_3
b R = $SCH_2C_6H_5$
c R = $SCH_2CH(C(O)NHCH_3)(NHC(O)CH_3)$

closely matched that for **1**[19] except for the C(5) and C(5a) resonances.

c. Reactivity of 1 with Thiols at "pH" 10.2–10.5. Reduction of the "pH" of the ethanethiol (**6**) and *N*-acetyl-L-cysteine *N'*-methylamide (**7**) mediated reactions led to dramatic changes in the observed product profiles. Ring-opened products **13a** and **13b** were not observed. Instead the ethanethiol (**6**) reaction in tetrahydrofuran-water (3:1) mixtures ("pH" 10.2) gave the direct C(5a)-substituted product **14b** along with piperidinedione **16a**.[11]



16a R = SCH_2CH_3
b R = $SCH_2CH(C(O)NHCH_3)(NHC(O)CH_3)$
c R = $SCH_2C_6H_5$
d R = $SCH_2CH(C(O)OCH_3)(NHC(O)CH_3)$
e R = $SCH_2C(O)OCH_2CH_3$

Analysis of the ^{13}C NMR spectra for both **14b** and **16a** indicated that each product was generated stereospecifically. A slightly different result was observed by using **7** in a 9:1 methanol-water mixture ("pH" 10.4). In this case, the reaction proceeded to give only **14c** as a 3.7:1 mixture of diastereomers (^{13}C NMR analysis). A comparable result (**1** + **11** → **14d**) was obtained by using L-cysteine methyl ester (**11**) in a tetrahydrofuran-water (3:1) mixture.

The C(5a)-substituted compounds **14** were readily assigned on the basis of the close correspondence of the NMR chemical shift values observed for these products versus bicyclomycin itself.[19] The only significant differences between **14** and **1** were those resonances associated with the C(5)–C(5a) *exo*-methylene unit and were in agreement with the proposed C(5a)-sulfur substitution products (Tables I and II).

d. Reactivity of 1 with Thiols at "pH" 7.4–8.2. The thiol-mediated reactions conducted at near neutral "pH" values ("pH" 7.4–8.2) commanded the most attention. Treatment of **1** with **6**, **7**, **9**, and **12** in either tetrahydrofuran-water (3:1) or methanol-water (9:1) mixtures led to the formation of the novel C-(5a)-substituted piperidinediones **16a–d**, respectively.[11,12] Each of these reactions proceeded *stereospecifically* to give a single product (^{13}C NMR analyses). A comparable result was also initially observed for the reaction of **1** with ethyl 2-mercaptoacetate (**10**). ^{1}H NMR analysis of a freshly prepared methanol-d_4 solution of the reaction product indicated the formation of **16e**. However, upon standing (1 day) **16e** was cleanly converted to the C(6)-modified product **17**.

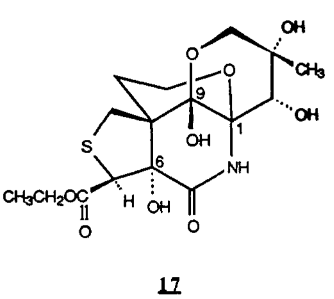


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Table I. Characteristic ^{1}H NMR Data for C(5a)-Substituted Bicyclomycin Derivatives **14**, **15**, **18**, and **19**[a]

compd	C(3)*H*H′	C(3)H*H*′	C(4)HH′	C(5)H	C(5a)*H*H′	C(5a)H*H*′	C(1′)H	C(2′)CH_3	C(3′)*H*H′	C(3′)H*H*′
14a[b]	3.72–4.02 (m)	3.72–4.02 (m)	2.02–2.42 (m)	2.02–2.42 (m)	2.02–2.42 (m)	3.02–3.15 (m)	4.03 (s)	1.32 (s)	3.52 (t, J = 12.00 Hz)	3.66 (d, J = 12.00 Hz)
14b[b]	3.76–3.80 (m)	3.95–4.00 (m)	2.06–2.26 (m)	2.06–2.26 (m)	2.06–2.26 (m)	3.15 (d, J = 11.60 Hz)	4.03 (s)	1.32 (s)	3.51 (d, J = 11.38 Hz)	3.66 (d, J = 11.38 Hz)
14c[b]	3.72–4.01 (m)	3.72–4.01 (m)	1.80–2.50 (m)	1.80–2.50 (m)	1.80–2.50 (m)	3.15 (d, J = 12.62 Hz)	4.03, 4.04 (s)	1.34 (s)	3.51 (d, J = 11.23 Hz)	3.66 (d, J = 11.23 Hz)
14d	3.64–4.01 (m)	3.64–4.01 (m)	1.88–2.43 (m)	1.88–2.43 (m)	1.88–2.43 (m)	3.15 (dd, J = 6.70, 12.25 Hz)	4.03, 4.04 (s)	1.32 (s)	3.53 (app t, J = 10.81 Hz)	3.64–4.01 (m)
14e	3.78 (dd, J = 8.35, 13.69 Hz)	4.03 (dd, J = 8.35, 13.69 Hz)	1.60–1.72 (m) 1.87–2.08 (m)	2.13–2.22 (m)	–[c]	–[c]	4.02 (s)	1.33 (s)	3.53 (d, J = 11.40 Hz)	3.68 (d, J = 11.40 Hz)
15a	3.70–4.07 (m)	3.70–4.07 (m)	1.88–2.45 (m)	1.88–2.45 (m)	1.88–2.45 (m)	3.02–3.16 (m)	4.09 (s)	1.35 (s)	3.70 (d, J = 8.18 Hz)	4.45 (d, J = 8.18 Hz)
15b	3.65–3.71 (m)	3.76–3.89 (m)	1.85–2.31 (m)	1.85–2.31 (m)	1.85–2.31 (m)	3.11–3.17 (m)	4.07, 4.08 (s)	1.35 (s)	3.65–3.71 (m)	4.41–4.46 (m)
15c	3.76–4.04 (m)	3.76–4.04 (m)	1.86–1.92 (m), 2.14–2.26 (m)	2.14–2.26 (m)	2.14–2.26 (m)	3.15–3.21 (m)	4.09 (s)	1.35 (s)	3.70 (d, J = 8.34 Hz)	4.43–4.48 (m)
18	3.72–4.03 (m)	3.72–4.03 (m)	1.88–2.44 (m)	1.88–2.44 (m)	1.88–2.44 (m)	3.16 (d, J = 12.65 Hz)	4.18, 4.20 (s)	1.36 (s)	2.68–2.95 (m)	3.02, 3.06 (2d, J = 13.42 Hz)
19	3.60–4.40 (m)	3.60–4.40 (m)	1.70–2.55 (m)	1.70–2.55 (m)	1.70–2.55 (m)	3.04–3.24 (m)	3.60–4.40[d] (m)	1.33 (s)	3.60–4.40 (m)	3.60–4.40 (m)

[a] 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 constants(s) in hertz. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD. [b] The ^{1}H NMR assignments were verified from the corresponding COSY spectrum. [c] The C(5a)-proton signals appeared as a three proton doublet (J = 7.0 Hz) at δ 1.06. [d] This peak overlapped with nearby signals.

Table II. Characteristic ^{13}C NMR Data for C(5a)-Substituted Bicyclomycin Derivatives **14**, **15**, **18**, and **19**[a]

compd	C(1)	C(3)	C(4)	C(5)	C(5a)	C(6)	C(1′)	C(2′)	C(2′)CH_3	C(3′)
14a	89.35	62.17 63.36	33.18[b] 34.23	52.10	30.06[b] 30.65	83.71	72.62 72.73	78.03	24.20	68.55
14b	89.34	62.02	29.95[b]	52.48	31.32[b]	83.72	72.25	78.12	24.19	68.51
14c	89.33	62.01 63.26	30.05[b]	52.52	32.11[b]	83.61	72.32	78.09 78.73	24.20	68.51 69.00
14d	89.35 89.58	62.03 63.22	29.94[b] 30.52	52.55[c] 52.70	31.30[b] 32.21	83.44 83.57	72.15 72.23	78.13	24.18	68.49
14e[d]	87.68	60.58	33.16	45.01	15.20	82.70	70.62	76.89	23.80	66.63
15a	88.55	63.60	32.79[b] 34.57	52.20	29.98[b] 30.54	83.75	73.34[c]	86.22	24.68	73.90[c]
15b	88.71	63.38 63.52	29.88[b] 30.32	52.35	31.61[b]	83.74	73.23[c] 73.33	86.34	24.84	73.49[c]
15c	88.66	63.62	30.14[b] 30.60	52.68	31.40[b] 32.51	83.66	73.29[c]	86.31	24.78	73.57[c]
18	89.87	62.17 63.19	30.23[b] 30.73	52.58	31.69[b] 32.20	83.47 83.57	72.26	79.01	26.09[e]	43.04
19	89.48	61.83 62.13	30.06[b] 30.17	52.29	31.56[b]	83.69	71.79	77.06	24.29 24.39	70.63

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. All spectra were obtained at 75.5 MHz. The solvent used was CD_3OD unless otherwise indicated. [b] These peaks may be interchanged. [c] These peaks may be interchanged. [d] The solvent used was DMSO-d_6. [e] These peaks may be interchanged with other nearby signals which have been attributed to the C(5a)-substituent.

The key spectral properties associated with **16** have been previously described, including the X-ray crystallographic structure of **16a**.[11,12] In particular, characteristic signals were noted for the C(6) and C(9) resonances between 194.15–195.27 and 95.79–99.16 ppm, respectively. Information in support of the proposed structural assignment **17** was secured from several sources. Rearrangement of **16e** → **17** was associated with the disappearance of the signal at δ 3.30–3.34 in the ^{1}H NMR spectrum for the thiol methylene protons ($SCH_2C(O)OCH_2CH_3$) and the appearance of a new peak between 84.56–86.20 ppm in the ^{13}C NMR spectrum for the C(6) carbon. Definitive proof for the proposed structure was provided by the X-ray crystallographic analysis of **17** (Figure 1).

e. Reactivity of Modified Bicyclomycin Derivatives 2–4 with Thiols. In an effort to discern the factors which govern the pronounced structural patterns in the product profiles for these transformations as a function of "pH", selectively modified derivatives of **1** were prepared and then treated with thiols at various "pH" values. Addition of N-acetyl-L-cysteine N'-methylamide (**7**) to a tetrahydrofuran–water (3:1) mixture of 3′-S-ethylbicyclomycin (**2**) at "pH" 7.8 led to no noticeable consumption of **2** after 48 h (TLC analysis). Increasing the "pH" to 10.2 led to the formation of **18** as the only major product in a 1.7:1 diastereomeric ratio. These results were mirrored with use of 3′-O-(ethylcarbamoyl)bicyclomycin (**3**). At near neutral "pH" (tetrahydrofuran–water (3:1), "pH" 7.8) no reaction was observed upon treatment of **3** with ethyl mercaptan (**6**). Replacement of **6** with the less volatile thiol benzyl mercaptan (**9**) and elevation of the temperature to 45 °C permitted the conversion of **3** to a

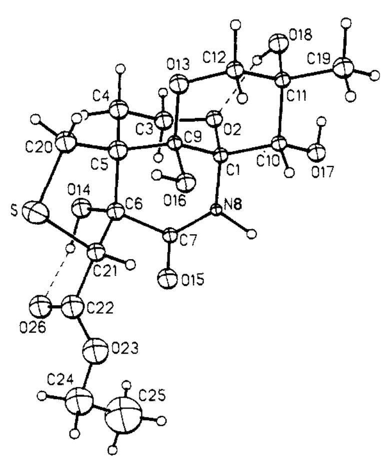
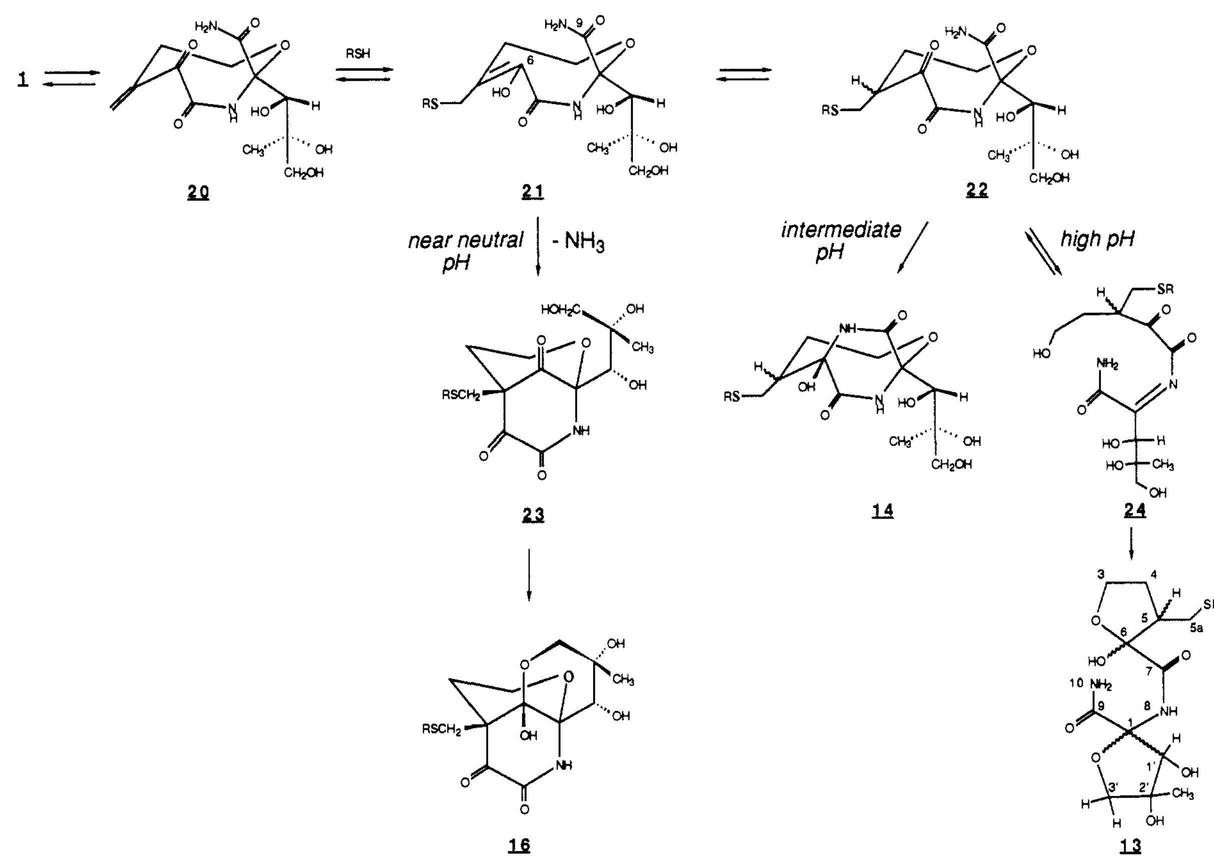
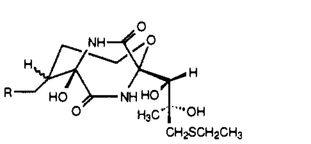


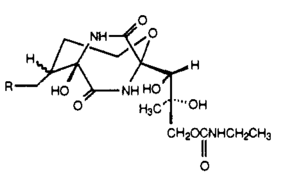
Figure 1. View of the molecule with atom labeling scheme. The nonhydrogen atoms are shown as isotropic spheres (20% equiprobability envelopes), and hydrogens, as spheres of arbitrary diameter.

Scheme I. Proposed Pathway for Bicyclomycin Transformations with Sulfur Nucleophiles



1.6:1 diastereomeric mixture of the C(5a)-substituted products **19** (^{13}C NMR analysis).





18 R = $SCH_2CH(C(O)NHCH_3)(NHC(O)CH_3)$ **19** R = $SCH_2C_6H_5$

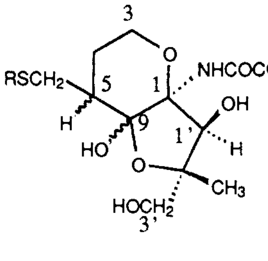
The results secured in the 3′-monofunctionalized bicyclomycin derivative (i.e., **2** and **3**) studies were reinforced by using 2′,3′-acetonide **4**. No reaction was noted upon treatment of **4** with **6**, **7**, or **9** at near neutral "pH" in either tetrahydrofuran–water (3:1) or methanol–water (9:1) mixtures. Elevation of the temperature of the benzyl mercaptan (**9**) reaction led to the slow (40 h) production of the C(5a)-substituted product **15b**. Adjustment of the "pH" of these reactions to 10.2 permitted the conversion of **4** with **7** to **15c** (16 h). ^{13}C NMR analysis indicated that two diastereomers were generated in an approximate 1.9:1 ratio. Further increases in the "pH" of the reaction medium to 12.5 increased the rate of formation of **15c** (25 min), but did not alter the initial product composition. Upon standing at this "pH" value, **15c** was converted to several unidentified, more polar adducts (TLC analysis). A comparable product (**15a**) was obtained upon treatment of acetonide **4** with sodium methanethiolate at "pH" 12.5 (see: Reactivity of **1** with Thiols at pH 12.5).

f. Competition Studies of Bicyclomycin (1) and Modified Bicyclomycin Derivatives (2, 4) with *N*-Acetyl-L-cysteine *N*′-Methylamide (7). The influence of the C(1)-triol group on the drug modification process was gauged by treating binary mixtures of either **1** and **2** or **1** and **4** with 1 equiv of thiol **7**. TLC analysis of the reaction mixtures generated from both sets of experiments conducted at "pH" 7.4 (tetrahydrofuran–water, 3:1) indicated that the sole product was **16b** along with unreacted modified bicyclomycin (**2** or **4**) and a trace of **1**. Elevation of the "pH" of the reaction decreased the selectivity of the reaction. At "pH" 10.2 (tetrahydrofuran–water, 3:1), TLC analysis of the competition study of **1** and **2** with **7** demonstrated that **1** was consumed to a greater extent than **2** and the presence of **14c** and **16b** as well as **18** along with unreacted starting material (**1** and **2**). Repetition of this experiment using **1** and **4** with **7** indicated that both **1** and **4** were partially consumed to give **14c**, **16b**, and **15c**. TLC analysis did not permit us to assess the relative amounts of each of these products. A comparable result was observed at "pH" 12.5 (tetrahydrofuran–water, 1:1) for the competition study of **1** and **4** with **7**. Compounds **13b** and **15c** were both detected in the product mixture (TLC analysis).

Discussion

Despite the marked structural variation in product types observed in this study, the thiolate-mediated bicyclomycin processes appear to stem from a common reaction pathway (Scheme I). Initial ring opening of the C(6)-hemiaminal bond gives enone **20**. Subsequent conjugate addition of the sulfur-containing nucleophile generates **21** or the corresponding enolate anion. At near neutral "pH" values ("pH" 7.4–8.2) an intramolecular mixed Claisen condensation proceeds to give **23** and ammonia. Cyclization of **23** in the final step yields the observed hemiketal **16**.[11,12] Correspondingly, at intermediate "pH" values (i.e., "pH" 10.2–10.5), protonation of the enol system at the C(5)-carbon, followed by reclosure of the ring to generate piperazinedione **14** becomes the dominant process. Finally, at pH 12.5, formation of **22** precedes ring cleavage of the remaining C(1)-aminal bond to give **24**. Subsequent ring closure furnishes the bis(tetrahydrofuranyl) derivatives **13**.[13] In the case of the ethyl 2-mercaptoacetate (**10**) mediated reaction at "pH" 8.2, generation of **16e** is followed by a second intramolecular Claisen condensation to give **17**. The susceptibility of the pyruvamide carbonyl system in **16** to undergo nucleophilic substitution processes has been previously described.[12] Treatment of the C(5a)-monofunctionalized adducts **16a**, **16b**, and **16d** with select primary amines ($R'NH_2$) led to the cleavage of the piperidine ring system and the formation of the bis-alkylated product **25**.

The proposed scenario outlined in Scheme I is in agreement with the mechanism previously postulated by us for the reaction of bicyclomycin with amines.[24] In an effort to discern the origin



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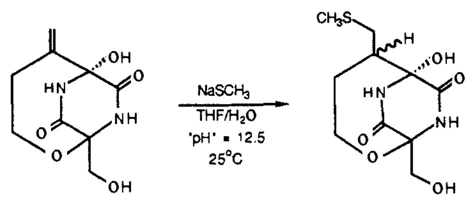
of the ring-cleaved products **13**, dihydrobicyclomycin[3b] (**14e**) was prepared. Compound **14e** can be viewed as the simplest possible C(5a)-substituted bicyclomycin derivative. Dissolution of **14e** in aqueous base (pH 12.5) for 45 min gave **13d** as the only isolable product. This transformation suggests that the ring-cleaved products **13** in the thiol-mediated reactions are generated after initial formation of **14**, or directly from intermediate **22**. The conversion of **14e** to **13d** differs from the observation reported by Williams and co-workers concerning the reactivity of **27**, a C-(5a)-substituted bicyclomycin derivative lacking the C(2′)- and C(3′)-hydroxyl groups.[25] Detailed analysis of the product profile



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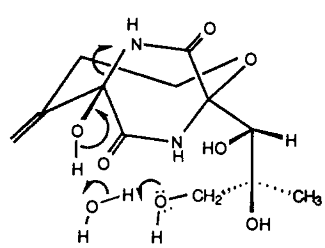
obtained after incubating **27** in deuterium oxide (pD 12.5) led the Colorado State University workers to conclude that **27** was incapable of undergoing further ring-opening reactions. Support for the proposed pathway for the generation of **13** was also derived from the reaction of acetonide **4** with **7** and **8** at "pH" 12.5. Blockage of the 2′,3′-diol groups prevented the formation of a bis(tetrahydrofuranyl) derivative (i.e., **13**). In this case, only the direct C(5a)-substitution product **15** was observed.

Additional information concerning the role of the C(1)-triol group in both the bicyclomycin-activation and the bicyclomycin-modification processes was secured by examining the reactivity of the modified derivatives **2** and **4** versus **1**. Several important observations were noted. First, at room temperature, no significant reaction of either **2** or **4** with **7** was observed at "pH" 8.2. Second, removal of either the C(3′)-hydroxy group or the C(2′),C(3′)-diol moiety prevented the formation of bicyclomycin-derived piperidinedione-type adducts (i.e., **16**). Third, drug modification at C(5a) at "pH" 10.2 (3:1 tetrahydrofuran-water) proceeded more rapidly with **1** and **4** than with **2**. *These combined results suggest that the C(1)-triol moiety plays an important role in the ring opening of the distal C(6)-hemiaminal group.* In an effort to document this conjecture, compounds **1**, **2**, and **4** were incubated (16–24 h) with ^{18}O-enriched water (98% $^{18}OH_2$) at "pH" 7.8 and 10.2 in the absence of thiols. In each experiment, the starting material was recovered, purified, and analyzed by mass spectrometry (FAB). Appreciable amounts (20–30%) of ^{18}O incorporation were detected in recovered bicyclomycin from the "pH" 7.8 and 10.2 experiments, while no ^{18}O incorporation was detected in the analogous reactions performed with bicyclomycin derivatives **2** and **4**. This data suggested that the unmodified triol group in **1** facilitated the ring-opening of the C(6)-hemiaminal bond to give enone **20**, which then underwent hydration and oxygen exchange under the employed conditions.[26]

What, then, is the role of the C(1)-triol group in these transformations? Our results indicate that alteration of the triol group significantly effected the C(5)–C(5a) drug functionalization processes which proceeded at near neutral "pH" values. Several potential explanations can be offered to account for this finding. The first suggests that the triol group facilitated both the ring-opening and the product-determining steps (i.e., Scheme I, **1** → **20**, **21** → **23**) by donating a hydrogen bond to the C(9)–N-(10)-amide group. Williams and co-workers have previously argued that intramolecular proton transfer or hydrogen bonding from the *C(1′)-hydroxy group* is a necessary requirement for functionalization of the exo-methylene group in model bicyclomycin **26** at *pH 12.5*.[1,25] A similar phenomenon may be occurring in the present study, contributing to the enhanced reactivity of **1** versus **2** and **4** *at near neutral "pH" values*. Unfortunately, the precise identity of the hydrogen bond cannot be made. We suspect that modification of either the C(3′)-hydroxyl group (i.e., **2**) or the C(2′),C(3′)-diol moiety (i.e., **4**) will adversely effect the ability of the entire triol substituent to hydrogen bond to the C(9)–N(10)-amide group. Alternatively, C(6)-hemiaminal bond cleavage may be initiated by a general-base-catalyzed process involving the C(3′)-hydroxyl oxygen atom (i.e., **28**). In this pathway, additional hydrogen-bonding interactions with the remaining triol hydroxyl groups with the C(9)–N(10) amide bond system may occur, further facilitating drug modification. In agreement with this proposal the decreased chemical reactivity of **2** versus **1** at near neutral "pH" values can be attributed (in part) to the decreased ability of the C(3′)-sulfide group to hydrogen bond to an intervening water molecule versus the C-(3′)-hydroxy moiety[27] in **1**, thereby decreasing the likelihood of C(6)-hemiaminal bond cleavage. Although both mechanistic scenarios are compatible with the observed data, the structural complexity of **1** demands that additional information be secured to define the factors which control the bicyclomycin-functionalization process.



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Conclusions

This study has provided an intriguing view of the finely tuned reactivity of bicyclomycin and the factors which dictate the drug modification process at the *exo*-methylene group with thiols. These reactions are considered to be among the most important for the antibiotic in light of earlier projections for the likely biological receptor in the drug-binding process. In all cases, bicyclomycin modification proceeded at C(5a). The precise product generated was dependent upon the "pH" of the reaction medium and the substitution pattern at the C(1)-triol group. A common intermediate has been invoked to explain the results. Several potential explanations have been offered to account for the observed variations in this study.

Experimental Section

General Methods. Infrared spectra (IR) were run on either a Perkin-Elmer 283 or Perkin-Elmer 1330 infrared spectrophotometer and calibrated against the 1601-cm^{-1} band of polystyrene. 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 FAB mass spectral

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

(25) (a) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 6419. (b) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *Ibid.* **1987**, *109*, 4028.

(26) A similar experiment was conducted by Williams and co-workers with **26** at "pH" 12.5.[25] In this case, ^{18}O incorporation was observed.

(27) For a discussion of the hydrogen-bonding properties of alcohols and sulfides, see: Jorgensen, W. J. *J. Phys. Chem.* **1986**, *90*, 6379.

investigalions were conducled al lhe University of Texas at Austin on a Finnegan TSQ-70 instrument by Dr. David Laude and Mr. Jerry Hogan. The FAB mass spectra at the Baylor College of Medicine were performed on a VG ZAB-SEQ and VG JS250 instruments by Dr. Simon Gaskell and Mr. Ralph Orkiszewski, while the El mass speclral studies were conducted on a VG JS250 inslrument. pH measurements were determined on a Radiometer pHM26 meter using a Radiometer G202 glass eleclrode.

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521) or silica gel GHLF (20 × 20 cm; Analtech No. 11187).

Reaction of Bicyclomycin (1) and Potassium Ethanethiolate at pH 12.5. An aqueous solution (5 mL. pH 12.5) of **1** (50 mg, 0.165 mmol) and potassium ethanethiolate (30 mg, 0.30 mmol) was stirred at room temperature (45 min). The solvent was removed in vacuo. The residue was dissolved in methanol (2 mL), and the insoluble material was filtered. The filtrate was concentrated and was purified by PTLC using 10% methanol–chloroform as the eluent (two developments) to yield **13a** (10 mg, 17%) as a semisolid: R_f 0.50 (15% methanol–chloroform); IR (KBr) 1670 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.22 (t, J = 7.30 Hz, 3 H, SCH$_2$C*H*$_3$), 1.32 (s, 3 H, C(2')CH$_3$), 1.76–1.96 (m, 1 H, C(4)*H*H'), 2.18–2.32 (m, C(4)H*H'*), 2.35–2.60 (m, 4 H, C(5)H, SC*H*$_2$CH$_3$, C(5a)*H*H'), 2.85–2.98 (m, 1 H, C(5a)H*H'*). 3.82–3.98 (m, 4 H, C(1')H, C(3')HH', C(3)*H*H'), 4.03–4.12 (m, 1 H, C(3)H*H'*). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (DMSO-d_6) 14.63, 14.72 (SCH$_2$*C*H$_3$), 23.56, 23.64 (C(2')*C*H$_3$), 25.14 (S*C*H$_2$CH$_3$), 29.52, 29.73 (C(5a) or C(4)), 29.86, 30.15 (C(4) or C(5a)), 46.52, 46.63 (C(5)), 66.75, 66.95 (C(3)), 74.77, 74.83 (C(2')), 76.04, 76.14 (C(3')), 78.85, 78.98 (C(1')). 88.07, 88.21 (C(1)), 101.86, 102.01 (C(6)), 169.39, 169.51 (C(7)), 171.84 (C(9)) ppm. ^{13}C NMR analysis indicated that the product existed as a 1.4:1 diastereomeric mixture. The ^{13}C NMR assignments were confirmed by the APT experiment. MS (+FAB) 387 [M + Na]$^+$.

Reaction of Bicyclomycin (1) with *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at pH 12.5. Cysteine **7** (50 mg, 0.284 mmol) and **1** (50 mg, 0.165 mmol) were dissolved in water (2.5 mL), and the pH of the solution was adjusted to 12.5. The solution was then degassed with argon (15 min) and capped, and the solution was then stirred at room temperature (45 min). The solvenl was removed in vacuo, and the residue was purified by PTLC using 25% methanol–chloroform as the eluent to give a binary mixture (R_f 0.28, 0.32; 20% methanol–chloroform); yield 13 mg (16%) as a semisolid. The mixture was further purified by PTLC using 20% methanol–chloroform (four developments) to give the following compounds.

Compound **13b-1**: yield 3.5 mg (4%) as a semisolid; R_f 0.32 (20% methanol–chloroform); IR (KBr) 1690, 1670 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.34 (s, 3 H, C(2')CH$_3$), 1.79–1.93 (m, 1 H, C(4)*H*H'), 1.99 (s, 3 H, NHCOC*H*$_3$), 2.21–2.50 (m, 3 H, C(4)H*H'*, C(5)H, C(5a)*H*H'), 2.66 (dd, J = 9.07, 13.93 Hz, 1 H, SC*H*H'CH(NHCOCH$_3$) (CONHCH$_3$)), 2.74 (s, 3 H, CONHC*H*$_3$), 2.89 (dd, J = 5.50, 13.93 Hz, 1 H, SCH*H'CH*(NHCOCH$_3$)(CONHCH$_3$)), 3.00–3.04 (m, 1 H, C(5a)H*H'*), 3.82–3.98 (m, 4 H, C(3')HH', C(1')H, C(3)*H*H'), 4.06–4.12 (m, 1 H, C(3)H*H'*), 4.51 (dd, J = 5.50, 9.07 Hz, 1 H, SCH$_2$C*H*(CONHCH$_3$)-(NHCOCH$_3$)); ^{13}C NMR (CD$_3$OD) 22.55 (NHCO*C*H$_3$), 23.29 (C-(2')*C*H$_3$), 26.39 (CONH*C*H$_3$), 31.25, 31.42 (C(4), C(5a)), 34.67 (S*C*-H$_2$CH(CONHCH$_3$)(NHCOCH$_3$)), 53.62 (SCH$_2$*C*H(CONHCH$_3$)(NH-COCH$_3$)), 69.00 (C(3)), 77.11, 78.12, 80.60 (C(1'), C(2'), C(3')), 89.69 (C(1)), 103.50 (C(6)), 173.39, 173.59 (CO) ppm. The remaining two carbonyl peaks were not detected, and the C(5) resonance is believed to be beneath the solvenl peak. M_r (+FAB) 479.18160 [M + 1]$^+$ (calcd for $C_{18}H_{31}N_4O_9S$, 479.18118).

Compound **13b-2**: yield 3 mg (4%) as a semisolid; R_f 0.28 (20% methanol–chloroform); IR (KBr) 1690, 1660 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.34 (s, 3 H, C(2')CH$_3$), 1.80–1.95 (m, 1 H, C(4)*H*H'), 2.00 (s, 3 H, NHCOC*H*$_3$), 2.20–2.55 (m, 3 H, C(4)H*H'*, C(5)H, C(5a)*H*H'), 2.73 (s, 3 H, CONHC*H*$_3$), 2.78–2.94 (m, 3 H, C(5a)H*H'*, SC*H*$_2$CH-(CONHCH$_3$)(NHCOCH$_3$)), 3.85–3.99 (m, 4 H, C(3')HH', C(1')H, C(3)*H*H'), 4.03–4.12 (m, 1 H, C(3)H*H'*), 4.47 (dd, J = 5.91, 8.12 Hz, 1 H, SCH$_2$C*H*(CONHCH$_3$)(NHCOCH$_3$)); ^{13}C NMR (CD$_3$OD) 22.64 (NHCO*C*H$_3$), 23.31 (C(2')*C*H$_3$), 26.42 (CONH*C*H$_3$), 31.27, 32.63 (C-(4), C(5a)), 34.99 (S*C*H$_2$CH(CONHCH$_3$)(NHCOCH$_3$)), 55.24 (SC-H$_2$*C*H(CONHCH$_3$)(NHCOCH$_3$)), 68.85 (C(3)), 77.07, 78.11, 80.66 (C(1'), C(2'), C(3')), 89.72 (C(1)), 103.24 (C(6)), 173.36, 175.36 (CO) ppm. The remaining two carbonyl peaks were not detected, and the C(5) resonance is believed to be beneath the solvent peak. M_r (+FAB) 479.18157 [M + 1]$^+$ (calcd for $C_{18}H_{31}N_4O_9S$, 479.18118).

Reaction of Bicyclomycin (1) with Sodium Methanethiolate at pH 12.5. An aqueous solution (5 mL, pH 12.5) of **1** (50 mg, 0.165 mmol) and sodium melhanethiolate (28 mg, 0.4 mmol) was stirred at room temperature (40 min). The solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform as the eluent (lwo developments) to yield **13c** (8 mg, 14%) as a semisolid: R_f 0.50 (15% methanol–chloroform); IR (KBr) 1670 cm^{-1}; ^{1}H NMR (C-D$_3$OD) δ 1.33 (s, 3 H, C(2')CH$_3$), 1.75–1.96 (m, 1 H, C(4)*H*H'), 2.07 (s, 3 H, SCH$_3$), 2.22–2.35 (m, 1 H, C(4)H*H'*), 2.40–2.56 (m, 2 H, C(5)H, C(5a)*H*H'), 2.80–2.95 (m, 1 H, C(5a)H*H'*), 3.83–4.00 (m, 4 H, C(1')H, C(3')HH', C(3)*H*H'), 4.03–4.14 (m, 1 H, C(3)H*H'*). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 15.38, 15.66 (SCH$_3$), 23.41, 23.45 (C(2')-*C*H$_3$), 31.28, 31.37 (C(5a)), 34.21, 34.29 (C(4)), 48.05 (C(5)), 68.76, 68.81 (C(3)), 76.88, 76.97 (C(2')), 78.04, 78.09 (C(3')), 80.63 (C(1')), 89.73, 89.81 (C(1)), 103.40 (C(6)), 173.13 (C(7)), 175.37 (C(9)) ppm. ^{13}C NMR analysis indicated that the product existed as a 1.4:1 diastereomeric mixture. MS (+FAB) 351 [M + 1]$^+$.

Reaction of Bicyclomycin (1) with Ethanethiol (6) at "pH" 10.2. A solution of **1** (40 mg, 0.132 mmol) and **6** (0.16 mL, 2.16 mmol) in tetrahydrofuran–waler (3:1, 5 mL, "pH" 10.2) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was purified by PTLC using 20% methanol–chloroform to give the following compounds.

Compound **16a**: yield 12 mg (26%); mp 216–218 °C (lit.[11,12] mp 216–218 °C).

Compound **14b**: yield 8 mg (19%) as a semisolid; R_f 0.52 (15% methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.23 (t, J = 7.38 Hz, 3 H, SCH$_2$C*H*$_3$), 1.32 (s, 3 H, C(2')CH$_3$), 2.06–2.26 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 2.46–2.54 (m, 2 H, SC*H*$_2$CH$_3$), 3.15 (d, J = 11.60 Hz, 1 H, C(5a)H*H'*), 3.51 (d, J = 11.38 Hz, 1 H, C(3')*H*H'), 3.66 (d, J = 11.38 Hz, 1 H, C(3')H*H'*), 3.76–3.80 (m, 1 H, C(3)*H*H'), 3.95–4.00 (m, 1 H, C(3)H*H'*), 4.03 (s, 1 H, C(1')H). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 15.14 (SCH$_2$*C*H$_3$), 24.19 (C(2')*C*H$_3$), 26.85 (S*C*H$_2$CH$_3$), 29.95, 31.32 (C(4), C(5a)), 52.48 (C(5)), 62.02 (C(3)), 68.51 (C(3')), 72.25 (C(1')), 78.12 (C(2')), 83.72 (C(6)), 89.34 (C(1)), 168.84, 172.11 (C(7), C(9)) ppm; M_r (+FAB) 387.1367 [M + Na]$^+$ (calcd for $C_{14}H_{24}N_2O_7SNa$ 387.1202).

Reaction of Bicyclomycin (1) with *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 10.4. A solution of **1** (30 mg, 0.10 mmol) and **7** (25 mg, 0.14 mmol) in a methanol–water mixture (9:1, 3.5 mL, "pH" 10.4) was degassed with argon (15 min), capped, and then stirred at room temperature (2 days). The solvent was removed in vacuo, and the residue was purified by PTLC using 15% methanol–chloroform (two developments) as the eluent to give **14c**: yield 5 mg (11%) as a semisolid; R_f 0.35 (20% methanol–chloroform); IR (KBr) 1690, 1670 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.34 (s, 3 H, C(2')CH$_3$), 1.80–2.50 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 1.99 (s, 3 H, NHCOC*H*$_3$), 2.65–2.80 (m, 1 H, SC*H*H'CH(CONHCH$_3$) (NHCOCH$_3$)), 2.73 (s, 3 H, CONHC*H*$_3$), 2.92 (dd, J = 5.13, 13.92 Hz, 1 H, SCH*H'*CH(CONHCH$_3$)-(NHCONHCH$_3$)), 3.15 (d, J = 12.62 Hz, 1 H, C(5a)H*H'*), 3.51 (d, J = 11.23 Hz, 1 H, C(3')*H*H'), 3.66 (d, J = 11.23 Hz, 1 H, C(3')H*H'*), 3.72–4.01 (m, 2 H, C(3)HH'), 4.03, 4.04 (2s, 1 H, C(1')H), 4.46 (dd, J = 5.13, 8.61 Hz, 1 H, SCH$_2$C*H*(CONHCH$_3$)(NHCOCH$_3$)). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 22.54 (NHCO*C*H$_3$), 24.20 (C(2')*C*H$_3$), 26.41 (CONH*C*H$_3$), 30.05, 32.11 (C(4), C(5a)), 35.11 (S*C*H$_2$CH(CO-NHCH$_3$)(NHCOCH$_3$)), 52.52 (C(5)), 54.39, 54.88 (SCH$_2$*C*H(CONH-CH$_3$)(NHCOCH$_3$)), 62.01, 63.26 (C(3)), 68.51 (C(3')), 72.32 (C(1')), 78.09, 78.73 (C(2')), 83.61 (C(6)), 89.33 (C(1)), 168.81, 171.94 (C(7), C(9)), 173.31, 173.36 (*C*ONHCH$_3$, NH*C*OCH$_3$) ppm. ^{13}C NMR analysis indicated that the product existed as a 3.7:1 diastereomeric mixture. MS (+FAB) 479 [M + 1]$^+$.

Reaction of Bicyclomycin (1) with L-Cysteine Methyl Ester (11) at "pH" 10.5. Cysteine **11** (40 mg, 0.233 mmol) and **1** (60 mg, 0.198 mmol) were dissolved in tetrahydrofuran–water (3:1, 8 mL, "pH" 10.5), degassed with argon (15 min), and then capped. The solution was stirred at room temperature (24 h) and the solvent was removed in vacuo. The crude material was purified by PTLC using 20% methanol–chloroform as the eluent to give the product and unreacted **1** (28 mg). This mixture was further purified by PTLC using 20% methanol–chloroform (two developments) lo give **14d**: yield 17 mg (20%); mp 123–125 °C; R_f 0.35 (20% methanol–chloroform); IR (KBr) 1720, 1670 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.32 (s, 3 H, C(2')CH$_3$), 1.88–2.43 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 2.69–2.95 (m, 2 H, SC*H*$_2$CH(NH$_2$)(COOCH$_3$)), 3.15 (dd, J = 6.70, 12.25 Hz, 1 H, C(5a)H*H'*), 3.53 (app t, J = 10.81 Hz, 1 H, C(3')*H*H'), 3.64–4.01 (m, 4 H, C(3')H*H'*, C(3)HH', SCH$_2$C*H*(NH$_2$)(COOCH$_3$)), 3.74 (s, 3 H, COOCH$_3$), 4.03, 4.04 (2s, 1 H, C(1')H); ^{13}C NMR (CD$_3$OD) 24.18 (C(2')*C*H$_3$), 29.94, 30.52, 31.30, 32.21 (C(4), C(5a)), 37.68, 37.84 (S*C*H$_2$CH(NH$_2$)(COOCH$_3$), 50.38. 52.55, 52.70, 54.68, 54.94 (C(5), SCH$_2$*C*H(NH$_2$)(COO*C*H$_3$)), 62.03, 63.22 (C(3)), 68.49 (C(3')), 72.15, 72.23 (C(1')), 78.13 (C(2')), 83.44, 83.57 (C(6)), 89.35, 89.58 (C(1)), 167.63, 168.74, 171.93, 174.00 (C(7), C(9)), 175.36, 175.49 (*C*OOCH$_3$) ppm. The ^{13}C NMR spectrum indicated that the product existed as a 1:1 diastereomeric mixture. MS (+FAB) 438 [M + 1]$^+$; M_r (+FAB) 438.1576 [M + 1]$^+$ (calcd for $C_{16}H_{28}N_3O_9S$ 438.1546).

Reaction of Bicyclomycin (1) with Benzyl Mercaptan (9) at "pH" 7.7. A solution of **1** (25 mg, 0.083 mmol) and **9** (0.1 mL, 0.85 mmol) in a tetrahydrofuran-water mixture (3:1, 3 mL, "pH" 7.7) was stirred at room temperature (18 h). The solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform as the eluent to give compound **16c**: yield 18 mg (53%); mp 108–110 °C; R_f 0.75 (10% methanol–chloroform); IR (KBr) 1730, 1680 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.13 (s, 3 H, C(2')CH$_3$), 1.83–1.88 (m, 1 H, C(4)*H*H'), 2.17–2.27 (m, 1 H, C(4)H*H'*), 2.80 ($^1/_2$ ABq, J = 13.98 Hz, 1 H, C(5a)*H*H'), 2.93 ($^1/_2$ ABq, J = 13.98 Hz, 1 H, C(5a)H*H'*), 3.59 (d, J = 11.85 Hz, 1 H, C(3')*H*H'), 3.70–3.80 (m, 3 H, C(3)*H*H', S*CH$_2$*C$_6$H$_5$), 3.88 (s, 1 H, C(1')H), 3.96 (d, J = 11.85 Hz, 1 H, C(3')H*H'*), 3.92–4.05 (m, 1 H, C(3)H*H'*), 7.20–7.35 (m, 5 H, SCH$_2$C$_6$*H$_5$*). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$COCD$_3$) 21.10 (C(2')*C*H$_3$), 31.59, 32.78 (C(4), C(5a)), 38.85 (S*C*H$_2$C$_6$H$_5$), 56.27 (C(5)), 58.09 (C(3)), 70.67, 71.44, 71.49 (C(1'), C(2'), C(3')), 84.68 (C(1)), 96.01 (C(9)), 127.86, 129.27, 129.93, 139.21 (*C$_6$*H$_5$), 157.90 (C(7)), 194.15 (C(6)) ppm; MS (CI) 410 [M + 1]$^+$; M_r (EI) 409.11934 [M]$^+$ (calcd for $C_{19}H_{23}NO_7S$ 409.11894).

Reaction of Bicyclomycin (1) with Ethyl 2-Mercaptoacetate (10) at "pH" 8.2. To a solution of **1** (50 mg, 0.165 mmol) in tetrahydrofuran-water mixture (3:1, 6 mL), was added **10** (0.036 mL, 0.328 mmol) and the "pH" of the solution was raised to 8.2 with an aqueous 0.5 N NaOH solution. The reaction mixture was stirred at room temperature (48 h). The solvent was removed in vacuo, and the residue was purified by PTLC using 15% methanol–chloroform as the eluent to give compound **16e**: yield 8.0 mg (12%); R_f 0.75 (15% methanol–chloroform); ^{1}H NMR (CD$_3$OD) δ 1.15 (s, 3 H, C(2')CH$_3$), 1.28 (t, J = 7.05 Hz, 3 H, OCH$_2$C*H$_3$*), 1.95 (dd, J = 2.40, 13.91 Hz, 1 H, C(4)*H*H'), 2.36 (app dt, J = 6.52, 13.91 Hz, 1 H, C(4)H*H'*), 2.97 (d, J = 13.94 Hz, 1 H, C(5a)*H*H'), 3.16 (d, J = 13.94 Hz, 1 H, C(5a)H*H'*), 3.30–3.34 (m, SC*H$_2$*COOCH$_2$CH$_3$, CD$_3$OD), 3.63 (d, J = 12.32 Hz, 1 H, C(3')*H*H'), 3.69–3.79 (m, 1 H, C(3)*H*H'), 3.92 (s, 1 H, C(1')H), 4.00–4.05 (m, 1 H, C(3)H*H'*), 4.01 (d, J = 12.32 Hz, 1 H, C(3')H*H'*), 4.17 (q, J = 7.05 Hz, 2 H, OC*H$_2$*CH$_3$).

Upon standing (1 day), compound **16e** rearranged to compound **17**: mp 272 °C; R_f 0.77 (15% methanol–chloroform); IR (KBr) 1710, 1660 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.12 (s, 3 H, C(2')CH$_3$), 1.26 (t, J = 7.12 Hz, 3 H, OCH$_2$C*H$_3$*), 2.05 (dd, J = 2.39, 12.65 Hz, 1 H, C(4)*H*H'), 2.18 (app dt, J = 5.82, 12.65 Hz, 1 H, C(4)H*H'*), 2.75 (d, J = 11.62 Hz, 1 H, C(5a)*H*H'), 3.47 (d, J = 11.62 Hz, 1 H, C(5a)H*H'*), 3.59 (d, J = 12.29 Hz, 1 H, C(3')*H*H'), 3.74 (app dt, J = 2.39, 12.65 Hz, 1 H, C(3)*H*H'), 3.83–3.89 (m, 1 H, C(3)H*H'*), 3.87 (s, 1 H, C(1')H), 4.03 (d, J = 12.29 Hz, 1 H, C(3')H*H'*), 4.21, 4.22 (2q, J = 7.12 Hz, 2 H, OC*H$_2$*CH$_3$). The ethyl 2-mercaptoacetate methine hydrogen is believed to be beneath the CD$_3$OD peak. ^{13}C NMR (CD$_3$OD) 14.24 (OCH$_2$*C*H$_3$), 21.26 (C(2')*C*H$_3$), 31.00 (C(4)), 35.90 (C(5a)), 54.35 (S*C*HCOOCH$_2$CH$_3$), 57.96 (C(5)), 60.27 (C(3)), 63.05 (SCHCOO*C*H$_2$CH$_3$), 71.10, 71.62, 72.63 (C(1'), C(2'), C(3')), 84.56, 86.20 (C(1), C(6)), 95.20 (C(9)), 173.89, 174.61 (C(7), *C*OOCH$_2$CH$_3$) ppm. The ^{13}C NMR assignments were confirmed by the APT experiment. MS (+FAB) 406 [M + 1]$^+$.

Reaction of Epoxide 5 with Ethanethiol (6). Preparation of 3'-S-Ethylbicyclomycin (2). To a solution of epoxide **5** (30 mg, 0.105 mmol) and triethylamine (5 μL, 0.036 mmol) in methanol (5 mL) was added **6** (40 μL, 0.541 mmol). The reaction mixture was stirred at room temperature (24 h), and then the solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform to give compound **2** as a semisolid: yield 10 mg (27%); R_f 0.40 (10% methanol–chloroform); IR (KBr) 1670 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.21 (t, J = 7.35 Hz, 3 H, SCH$_2$C*H$_3$*), 1.38 (s, 3 H, C(2')CH$_3$), 2.54–2.62 (m, 4 H, C(4)HH', SC*H$_2$*CH$_3$), 2.75 (d, J = 13.39 Hz, 1 H, C(3')*H*H'), 3.00 (d, J = 13.39 Hz, 1 H, C(3')H*H'*), 3.77–3.92 (m, 2 H, C(3)HH'), 4.23 (s, 1 H, C(1')H), 5.13 (br s, 1 H, C(5a)*H*H'), 5.56 (d, J = 1.11 Hz, 1 H, C(5a)H*H'*); ^{13}C NMR (CD$_3$OD) 15.34 (SCH$_2$*C*H$_3$), 26.01 (C(2')*C*H$_3$), 28.97 (S*C*H$_2$CH$_3$), 36.77 (C(4)), 43.07 (C(3')), 65.55 (C(3)), 72.29 (C(1')), 79.00 (C(2')), 82.92 (C(6)), 90.05 (C(1)), 116.75 (C(5a)), 149.62 (C(5)), 168.68 (C(7)), 172.53 (C(9)) ppm; MS (-FAB) 346 [M]$^-$.

Reaction of 3'-S-Ethylbicyclomycin (2) with N-Acetyl-L-cysteine N'-Methylamide (7) at "pH" 10.2. A solution of **2** (8.5 mg, 0.025 mmol) and **7** (6.5 mg, 0.037 mmol) in tetrahydrofuran-water (3:1, 2 mL) was degassed with argon and stirred at room temperature (24 h) at "pH" 10.2. The solvent was removed in vacuo, and the residue was purified by PTLC using 15% methanol–chloroform to give **18**: yield 3 mg (23%) as a semisolid; R_f 0.35 (15% methanol–chloroform); IR (KBr) 1690, 1660 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.21 (t, J = 7.37 Hz, 3 H, SCH$_2$C*H$_3$*), 1.36 (s, 3 H, C(2')CH$_3$), 1.88–2.44 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 1.99 (s, 3 H, COCH$_3$), 2.57 (q, J = 7.37 Hz, 2 H, SC*H$_2$*CH$_3$), 2.68–2.95 (m, 3 H, SC*H$_2$*CH, C(3')*H*H'), 2.73 (s, 3 H, NHC*H$_3$*), 3.02, 3.06 (2d, J = 13.42 Hz, 1 H, C(3')H*H'*), 3.16 (d, J = 12.65 Hz, 1 H, C(5a)H*H'*), 3.72–4.03 (m, 2 H, C(3)HH'), 4.18, 4.20 (2s, 1 H, C(1')H), 4.46 (dd, J = 5.16, 8.55 Hz, 1 H, SCH$_2$C*H*). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 15.35 (SCH$_2$*C*H$_3$), 22.53 (CO*C*H$_3$), 26.09, 26.41 (NHCH$_3$, C(2')*C*H$_3$), 28.97 (S*C*H$_2$CH$_3$), 30.23, 30.73 (C(4)), 31.69, 32.20 (C(5a)), 35.12, 35.44 (S*C*H$_2$CH), 43.04 (C(3')), 52.58 (C(5)), 54.39, 54.91 (SCH$_2$*C*H), 62.17, 63.19 (C(3)), 72.26 (C(1')), 79.01 (C(2')), 83.47, 83.57 (C(6)), 89.87 (C(1)), 173.35, 173.40 (*C*OCH$_3$, *C*ONHCH$_3$) ppm. The C(7) and C(9) carbon resonances were not detected. The ^{13}C NMR spectrum indicated that the product existed as a 1.7:1 diastereomeric mixture. MS (+FAB) 523 [M + 1]$^+$; M_r (+FAB) 523.18962 [M + 1]$^+$ (calcd for $C_{20}H_{35}N_4O_8S_2$ 523.18963).

Reaction of 3'-O-(Ethylcarbamoyl)bicyclomycin (3) with Benzyl Mercaptan (9) at "pH" 7.4. Mercaptan **9** (0.03 mL, 0.255 mmol) was added to a solution of **3** (25 mg, 0.067 mmol) in Tris buffer (0.1 M, 9:1 methanol-water, 8 mL, "pH" 7.3), and the reaction mixture was stirred at 45 °C (40 h). The solvent was removed in vacuo, and the residue was purified by PTLC using 20% methanol–chloroform as the eluent to give **19**: yield 3.5 mg (11%) as a semisolid; R_f 0.55 (10% methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.10 (t, J = 6.46 Hz, 3 H, NCH$_2$C*H$_3$*), 1.33 (s, 3 H, C(2')CH$_3$), 1.70–2.55 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 3.04–3.24 (m, 3 H, NC*H$_2$*CH$_3$, C(5a)H*H'*), 3.60–4.40 (m, 7 H, C(3)HH', C(3')HH', C(1')H, SC*H$_2$*C$_6$H$_5$), 7.20–7.30 (m, 5 H, SCH$_2$C$_6$*H$_5$*); ^{13}C NMR (CD$_3$OD) 15.27, 15.35 (NCH$_2$*C*H$_3$), 24.29, 24.39 (C(2')*C*H$_3$), 30.06, 30.17, 31.56 (C(4), C(5a)), 36.58, 37.38 (N*C*H$_2$CH$_3$, S*C*H$_2$C$_6$H$_5$), 52.29 (C(5)), 61.83, 62.13 (C(3)), 70.63 (C(3')), 71.79 (C(1')), 77.06 (C(2')), 83.69 (C(6)), 89.48 (C(1)), 127.92, 129.42, 130.05, 140.07 (SCH$_2$*C$_6$*H$_5$) ppm. The carbonyl peaks were not detected. ^{13}C NMR analysis indicated that the product existed as a 1.6:1 diastereomeric mixture. MS (+FAB) 498 [M + 1]$^+$; M_r (+FAB) 498.19149 [M + 1]$^+$ (calcd for $C_{22}H_{32}N_3O_8S$ 498.19101).

Reaction of Bicyclomycin-2',3'-acetonide (4) with Sodium Methanethiolate at "pH" 12.5. A tetrahydrofuran-water (1:1) mixture (1 mL) ("pH" 12.5) of **4** (15 mg, 0.044 mmol) and sodium methanethiolate (3.5 mg, 0.05 mmol) was stirred at room temperature (30 min). The solvent was removed in vacuo, and the residue was purified by PTLC (silica gel) using 10% methanol–chloroform as the eluent to yield **15a** (9 mg, 53%): mp 122–125 °C; R_f 0.60 (10% methanol–chloroform); IR (KBr) 1690 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.35 (s, 3 H, C(2')CH$_3$), 1.44 (br s, 6 H, C(CH$_3$)$_2$), 1.88–2.45 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 2.05 (s, 3 H, SCH$_3$), 3.02–3.16 (m, 1 H, C(5a)H*H'*), 3.70 (d, J = 8.18 Hz, 1 H, C(3')*H*H'), 3.75–4.07 (m, 2 H, C(3)HH'), 4.09 (s, 1 H, C(1')H), 4.45 (2d, J = 8.18 Hz, 1 H, C(3')H*H'*). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 15.65 (SCH$_3$), 24.68 (C(2')*C*H$_3$), 26.85, 28.13 (C(*C*H$_3$)$_2$), 29.98, 30.54, 32.79, 34.57 (C(4), C(5a)), 52.20 (C(5)), 63.60 (C(3)), 73.34, 73.90 (C(1'), C(3')), 83.75 (C(6)), 86.22 (C(2')), 88.55 (C(1)), 111.68 (*C*(CH$_3$)$_2$), 168.34 (C(7)), 171.49 (C(9)) ppm. The ^{13}C NMR assignments were confirmed by the APT experiment. MS (+FAB) 391 [M + 1]$^+$.

Hydrolysis of S-Methylbicyclomycin-2',3'-acetonide (15a). A solution of **15a** (9.5 mg, 0.024 mmol) in 50% aqueous acetic acid (1 mL) was heated at 60 °C (30 min). The solvent was removed in vacuo, and the residue was purified by PTLC using 15% methanol–chloroform to yield **14a** (5 mg, 59%) as a semisolid: R_f 0.40 (10% methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.32 (s, 3 H, C(2')CH$_3$), 2.05 (s, 3 H, SCH$_3$), 2.02–2.42 (m, 4 H, C(4)HH', C(5)H, C(5a)*H*H'), 3.02–3.15 (m, 1 H, C(5a)H*H'*), 3.52 (t, J = 12.00 Hz, 1 H, C(3')*H*H'), 3.66 (d, J = 12.00 Hz, 1 H, C(3')H*H'*), 3.72–4.02 (m, 2 H, C(3)HH'), 4.03 (s, 1 H, C(1')H). The ^{1}H NMR assignments were confirmed by the corresponding COSY experiment. ^{13}C NMR (CD$_3$OD) 15.63, 15.79 (SCH$_3$), 24.20 (C(2')*C*H$_3$), 30.06, 30.65 (C(5a)), 33.18, 34.23 (C(4)), 52.10 (C(5)), 62.17, 63.36 (C(3)), 68.55 (C(3')), 72.62, 72.73 (C(1')), 78.03 (C(2')), 83.71 (C(6)), 89.35 (C(1)), 168.71, 172.00 (C(7), C(9)) ppm. ^{13}C NMR analysis indicated that the product existed as a 1.9:1 diastereomeric mixture. MS (+FAB) 351 [M + 1]$^+$.

Reaction of Bicyclomycin-2',3'-acetonide (4) with Benzyl Mercaptan (9) at "pH" 7.4. To a solution of **4** (10 mg, 0.029 mmol) in a Tris buffer (0.1 M, 9:1 methanol-water, 3 mL, "pH" 7.4) was added **9** (0.01 mL, 0.085 mmol), and the reaction mixture was stirred at 45 °C (40 h). The solvent was removed in vacuo and the residue was purified by PTLC using 10% methanol–chloroform as the eluent to give compound **15b**: yield 4.0 mg (29%) as a semisolid; R_f 0.60 (10 % methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.35, 1.41, 1.44, 1.45 (4s, 9 H, C(2')CH_3, $C(CH_3)_2$), 1.85–2.31 (m, 4 H, C(4)HH', C(5)H, C-(5a)*H*H'), 3.11–3.17 (m, 1 H, C(5a)H*H'*), 3.65–3.71 (m, 2 H, C(3)*H*H', C(3')*H*H'), 3.70 (s, 2 H, $SCH_2C_6H_5$), 3.76–3.89 (m, 1 H, C(3)H*H'*), 4.07, 4.08 (2s, 1 H, C(1')H), 4.41–4.46 (m, 1 H, C(3')H*H'*), 7.18–7.35 (m, 5 H, C_6H_5); ^{13}C NMR (CD_3OD) 24.84 (C(2')CH_3), 26.89, 28.21 ($C(CH_3)_2$), 29.88, 30.32, 31.61 (C(4), C(5a)), 37.36 ($SCH_2C_6H_5$), 52.35 (C(5)), 63.38, 63.52 (C(3)), 73.23, 73.33, 73.49 (C(1'), C(3')), 83.74 (C(6)), 86.34 (C(2')), 88.71 (C(1)), 111.64 ($C(CH_3)_2$), 127.93, 129.42, 130.07, 140.05 ($SCH_2C_6H_5$), 168.33 (C(7)), 171.53 (C(9)) ppm. ^{13}C NMR analysis indicated that the product existed as a 1.6:1 diastereomeric mixture. MS (+FAB) 467 [M + 1]$^+$; M_r (+FAB) 467.18578 [M + 1]$^+$ (calcd for $C_{22}H_{31}N_2O_7S$ 467.18520).

Reaction of Bicyclomycin-2',3'-acetonide (4) and *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 10.2. A solution of acetonide **4** (9 mg, 0.026 mmol) and **7** (6 mg, 0.034 mmol) in tetrahydrofuran–water (3:1, 2.0 mL) was degassed with argon and stirred at room temperature (16 h) at "pH" 10.2. The solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform to give **15c** as a semisolid: yield 8 mg (59%); R_f 0.50 (10% methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2')CH_3), 1.44 (s, 6 H, $C(CH_3)_2$), 1.86–1.92 (m, 1 H, C(4)*H*H'), 1.99 (s, 3 H, $COCH_3$), 2.14–2.26 (m, 3 H, C(4)H*H'*, C(5)H, C(5a)*H*H'), 2.65–2.78 (m, 1 H, SC*H*H'CH), 2.72, 2.73 (2s, 3 H, CONHCH_3), 2.81–2.96 (m, 1 H, SCH*H'*CH), 3.15–3.21 (m, 1 H, C (5a)H*H'*), 3.70 (d, J = 8.34 Hz, 1 H, C(3')*H*H'), 3.78–4.04 (m, 2 H, C(3)HH'), 4.09 (s, 1 H, C(1')H), 4.43–4.48 (m, 2 H, C(3')H*H'*, SCH_2CH); ^{13}C NMR (CD_3OD) 22.54 ($COCH_3$), 24.78 (C(2')CH_3), 26.43, 26.87, 28.20 (CONHCH_3, C-$(CH_3)_2$), 30.14, 30.60, 31.40, 32.51 (C(4), C(5a)), 35.05, 35.43 (SC-H_2CH), 50.43, 52.68 (C(5)), 54.45, 54.90 (SCH_2CH), 63.62 (C(3)), 73.29, 73.57 (C(3'), C(1')), 83.66 (C(6)), 86.31 (C(2')), 88.66 (C(1)), 111.69 ($C(CH_3)_2$), 168.19 (C(7)), 171.50 (C(9)), 173.22, 173.30, 173.37 ($CONHCH_3$, $NHCOCH_3$) ppm; MS (−FAB) 518 [M]$^-$.

Reaction of Bicyclomycin-2',3'-acetonide (4) and *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 12.5. A solution of acetonide **4** (5 mg, 0.014 mmol) and **7** (3 mg, 0.017 mmol) in tetrahydrofuran–water (1:1, 1 mL) was degassed with argon and stirred at room temperature (30 min) at "pH" 12.5. The solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform to give **15c** as a semisolid: yield 2 mg (26%); R_f 0.50 (10% methanol–chloroform); IR (KBr) 1680 cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2')CH_3), 1.44 (s, 6 H, $C(CH_3)_2$), 1.86–1.92 (m, 1 H, C(4)*H*H'), 1.99 (s, 3 H, $COCH_3$), 2.14–2.26 (m, 3 H, C(4)H*H'*, C(5)H, C(5a)*H*H'), 2.65–2.78 (m, 1 H, SC*H*H'CH), 2.73 (s, 3 H, NHCH_3), 2.81–2.96 (m, 1 H, SCH*H'*CH), 3.15–3.21 (m, 1 H, C(5a)H*H'*), 3.70 (d, J = 8.10 Hz, 1 H, C(3')*H*H'), 3.78–4.04 (m, 2 H, C(3)HH'), 4.08 (s, 1 H, C(1')H), 4.43–4.48 (m, 2 H, C(3')H*H'*, SCH_2CH).

Reaction of Dihydrobicyclomycin (14e) at pH 12.5. A solution of **14e**[3b] (25 mg, 0.082 mmol) in water (3 mL, pH 12.5) was stirred at room temperature (45 min). The reaction mixture was neutralized with aqueous 1 N HCl and the solvent was removed in vacuo. The residue was purified by PTLC using 15% methanol–chloroform to give compound **13d**: yield 9 mg (36%) as a semisolid; R_f 0.40 (15% methanol–chloroform); IR (KBr) 1670 cm^{-1}; ^1H NMR (CD_3OD) δ 1.02–1.16 (m, 3 H, C(5a)H_3), 1.32 (s, 3 H, C(2')CH_3), 1.70–1.85 (m, 1 H, C(4)*H*H'), 1.95–2.15 (m, 1 H, C(4)H*H'*), 2.20–2.38 (m, 1 H, C(5)H), 3.83–3.95 (m, 4 H, C(3')HH', C(1')H, C(3)*H*H'), 4.01–4.06 (m, 1 H, C(3)H*H'*); ^{13}C NMR (CD_3OD) 13.13, 13.27, 15.57, 15.63 (C(5a)), 21.10, 23.43 (C(2')CH_3), 32.94, 33.23, 33.46 (C(4)), 42.77, 42.95 (C(5)), 68.30, 68.34, 68.45, 68.52 (C(3)), 76.95, 77.96, 78.72, 80.61 (C(1'), C(2'), C(3')), 89.70 (C(1)), 106.36 (C(6)), 175.47 (C(7) or C(9)) ppm. The remaining carbonyl carbon could not be detected. MS (+FAB) 305 [M + 1]$^+$; M_r (+FAB) 305.13362 [M + 1]$^+$ (calcd for $C_{12}H_{21}N_2O_7$ 305.13488).

Reaction of Bicyclomycin (1), 3'-*S*-Ethylbicyclomycin (2), and *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 10.2. A solution of **1** (1.75 mg, 0.005 mmol), **2** (2 mg, 0.005 mmol), and **7** (1 mg, 0.005 mmol) in tetrahydrofuran–water (3:1, 0.5 mL) was degassed with the use of argon and was stirred at room temperature at "pH" 10.2 (18 h). The solvent was removed in vacuo, and the residue was analyzed by TLC (20% methanol–chloroform). Unreacted **2** (R_f 0.80, major) and **1** (R_f 0.45, minor) were detected along with **14c** (R_f 0.35, minor), **16b** (R_f 0.60, major), and **18** (R_f 0.50, minor). The identity of each product was confirmed by cospotting the reaction mixture with an authentic sample.

Reaction of Bicyclomycin (1), Bicyclomycin-2',3'-acetonide (4) and *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 10.5. A solution of **1** (8.83 mg, 0.029 mmol), **4** (10 mg, 0.029 mmol), and **7** (5.2 mg, 0.029 mmol) in tetrahydrofuran–water (3:1, 3.0 mL) was degassed with the use of argon and was stirred at room temperature at "pH" 10.5 (18 h). The solvent was removed in vacuo, and the residue was analyzed by TLC (10% methanol–chloroform). Unreacted **1** (R_f 0.35) and **4** (R_f 0.60) were detected along with **14c** (R_f 0.30), **16b** (R_f 0.40), and **15c** (R_f 0.45). The identity of each product was confirmed by cospotting the reaction mixture with an authentic sample.

Reaction of Bicyclomycin (1), Bicyclomycin-2',3'-acetonide (4), and *N*-Acetyl-L-cysteine *N'*-Methylamide (7) at "pH" 12.5. A solution of **1** (5 mg, 0.016 mmol), **4** (5.6 mg, 0.016 mmol), and **7** (2.9 mg, 0.016 mmol) in tetrahydrofuran–water (1:1, 2 mL) was degassed with argon and stirred at room temperature (30 min) at "pH" 12.5. The solvent was removed in vacuo, and the residue was analyzed by TLC (20% methanol–chloroform). Unreacted **4** (R_f 0.75) was detected along with **13b** (R_f 0.30) and **15c** (R_f 0.60). The identity of each product was confirmed by cospotting the reaction mixture with an authentic sample.

General Procedure for the ^{18}O-Incorporation Studies of Compounds 1, 2, and 4 at "pH" 7.8 and 10.2. Solutions of **1**, **2**, or **4** (5 mg) in tetrahydrofuran–^{18}O-enriched water (98% $^{18}OH_2$) (3:1, 0.8 mL) were stirred at room temperture at either "pH" 7.8 or 10.2 (16–24 h). The solvent was removed in vacuo, and the residue was purified by PTLC using 10% methanol–chloroform, and the desired compound (**1**, **2**, or **4**) was analyzed by mass spectrometry (−FAB). Comparison of the relative intensities of the m/z 303 [M − H + 2]$^-$ and m/z 304 [M + 2]$^-$ ions for recovered **1** versus that of authentic, unreacted bicyclomycin indicated that 20–30% ^{18}O incorporation had occurred in both the "pH" 7.8 and 10.2 experiments. Correspondingly, no detectable ^{18}O incorporation was observed in reactions performed with either **2** or **4** at "pH" 7.8 and 10.2.

X-ray Analysis of 17. Single crystals of **17** suitable for X-ray analysis was obtained from methanol. The data crystal (approximate dimensions: 0.50 × 0.08 × 0.07 mm) was mounted on a glass fiber 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 (Supplementary Material). The structure was solved by use of the SHELXTL direct methods program, which revealed the positions of most of the non-hydrogen atoms. The remaining atoms were located in subsequent difference Fourier syntheses. All hydrogens attached to carbons were added in ideal calculated positions and constrained to riding motion, while the other hydrogens were found in difference maps and allowed to refine. Mild distance constraints had to be applied to H17, and H16 had to be fixed in order to prevent them from moving to unreasonable H–O–C angles. Due to the small amount of observed data which could be obtained from this sample, only the sulfur atom was refined anisotropically. All other atoms were refined isotropically, with a single variable isotropic thermal parameter for all of the hydrogens. No attempt was made to determine the absolute configuration experimentally, rather the configuration was arbitrarily adjusted so as to match that of the known starting material.[28] After all shift–esd ratios were less than 0.7 (except those involving hydrogens), convergence was reached at the agreement factors listed in Table 3. No unusually high correlations were noted between any of the non-hydrogen variables in the last cycle of full-matrix least squares refinement, and the final difference density map showed a maximum peak of about 0.4 e/Å^3. All calculations were made by use of Nicolet's SHELXTL PLUS (1987) series of crystallographic programs.

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Supplementary Material Available: Tables 3–8 giving a complete listing of data collection and processing parameters, atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, and hydrogen-bonding parameters (5 pages); observed and calculated structure factors for compound **17** (3 pages). Ordering information is given on any current masthead page.

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