Role of the C(6)-Hydroxy Group in Bicyclomycin: Synthesis, Structure, and Chemical, Biochemical, and Biological Properties

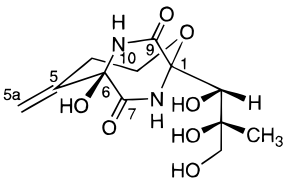
Alejandro Santillán, Jr.,[†] Xiangdong Zhang,[†] Jon Hardesty,[†] William R. Widger,[‡] and Harold Kohn*,[†]

Departments of Chemistry and Biochemical and Biophysical Sciences, University of Houston, Houston, Texas 77204

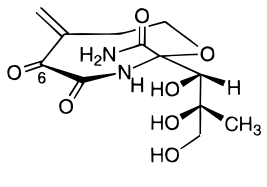
Received December 10, 1997

Bicyclomycin (**1**) is a commercial antibiotic whose primary site of action in *Escherichia coli* is the transcription termination factor rho. A recent structure−activity relationship study of **1** showed that replacing the C(6)-hydroxy group with alkoxy and thioalkoxy substituents led to dramatic losses of inhibitory activity in rho biochemical assays. The origin for this structural specificity has been explored by the synthesis and chemical, biochemical, and biological evaluation of C(6)-amino- (**13**), C(6)-(hydroxylamino)-(**14**), and C(6)-mercaptobicyclomycin (**15**). These compounds, like **1**, are capable of entering into hydrogen bond donor interactions with rho and are capable of undergoing C(6) ring opening to generate α,β-unsaturated carbonyl, imine, or thione systems. The chemical reactivity of **13**−**15** was compared with that of **1**. We observed that **1**, upon treatment with EtSH under moderate and basic conditions, readily underwent C(6) hemiaminal bond cleavage followed by conjugate addition to β-methylene-α-ketoamide **2** to give Michael addition adducts whereas **13**−**15** reacted by initial cleavage of the C(1)−O(2) bond. Biochemical and biological assays of **13**−**15** and related analogues demonstrated that the C(6) hydroxy group in **1** was essential for activity. We found that replacing the C(6)-hydroxy group in **1** with weaker hydrogen bond donors led to low inhibitory activities in the rho-dependent ATPase and transcription termination assays. None of the bicyclomycin derivatives exhibited antibiotic activity against *E. coli* W3350 cells at a 32 mg/mL concentration. The apparent specificity for the C(6)-hydroxy group in **1** suggests that an efficient hydrogen bond donor interaction from the C(6)-hydroxy group to rho or the C(6) hemiaminal bond cleavage to **2** or both is necessary for drug function.

Bicyclomycin (**1**) is a structurally unique antibiotic possessing a diverse spectrum of biological activity.[1−4] Chemical studies have demonstrated that in the presence of nucleophiles, **1** readily undergoes C(6) hemiaminal bond cleavage to give β-methylene-α-ketoamide **2** followed by reaction with the nucleophile to provide a Michael addition product.[5−9] The primary site of action for **1** in *Escherichia coli* is the essential cellular protein transcription termination factor rho.[10] Mechanistic studies have shown that bicyclomycin efficiently inhibits the production of rho-dependent transcripts[11] and inhibits rho poly(C)-stimulated ATP hydrolysis with simple noncompetitive kinetics with respect to ATP.[12]



1



2

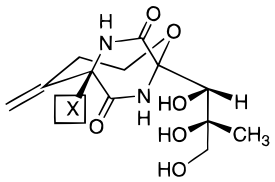
In a recent structure−activity relationship (SAR) study[13] of **1** we reported that replacing the C(6)-hydroxy group with alkoxy and thioalkoxy substituents led to dramatic losses of bicyclomycin inhibitory activity in the in vitro rho-dependent transcription termination[14] and the poly(C)-stimulated ATPase[15] assays and the elimination of antimicrobial activity in a filter disk assay.[16] Our study included the 10 C(6)-substituted bicyclomycins **3**−**12**. Most of these compounds, unlike **1**, can neither undergo C(6)−N(10) cleavage to generate a neutral ring-opened intermediate nor provide a C(6) hydrogen bond to a protein residue surrounding the bicyclomycin binding pocket in rho. Accordingly, we have prepared and evaluated C(6)-amino- (**13**), C(6)-(hydroxylamino)- (**14**), and C(6)-mercaptobicyclomycin (**15**) to determine the importance of C(6) ring-opening transformations and hydrogen bond donor interactions for drug binding. In the work reported herein, we demonstrate that replacement of the C(6)-hydroxy group by amino, hydroxylamino, and thiol units altered the chemical reactivity of bicyclomycin and led to significant losses in biochemical activity.

Results and Discussion

A. Choice of Substrates and Synthesis. The primary compounds selected for study were **13**−**15**. For C(6)-aminobicyclomycin (**13**) and C(6)-(hydroxylamino)-bicyclomycin (**14**) we used methylation to progressively remove potential hydrogen bond donors located at C(6). Accordingly, we prepared **16**, **17**, and **19**−**21**. We did not prepare the *S*-methyl derivative of **15** since we showed that C(6)-*S*-ethylbicyclomycin (**9**) did not inhibit rho-dependent ATPase or transcription termination activities.[13] Added to our list was the C(6)-acetamidobicyclomycin (**18**). This compound was included to

* Author to whom correspondence should be addressed. Phone: 713/743-3240. Fax: 713/743-2709. E-mail: HKohn@uh.edu.
[†] Department of Chemistry.
[‡] Department of Biochemical and Biophysical Sciences.

S0022-2623(97)00838-8 CCC: $15.00
Published on Web 03/06/1998



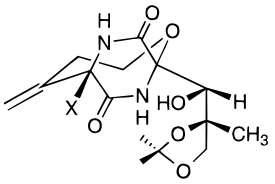
3 X = OCH_3
4 X = OCH_2CH_3
5 X = OCH_2CF_3
6 X = $OCH(CH_3)_2$
7 X = $OC(O)CH_3$
8 X = OC(O)Ph
9 X = SCH_2CH_3
10 X = SPh
11 X = NHPh
12 X = H

13 X = NH_2
14 X = NHOH
15 X = SH

16 X = $N(H)CH_3$
17 X = $N(CH_3)_2$
18 X = $N(H)C(O)CH_3$
19 X = $N(CH_3)OH$
20 X = $N(H)OCH_3$
21 X = $N(CH_3)OCH_3$

provide a modified C(6)-aminobicyclomycin that would not be protonated under the assay conditions (pH 7.9).

Compounds **13**−**17** and **19**−**21** were prepared by a method similar to that described for **3**.[13] Bicyclomycin was first converted to the known bicyclomycin C(2′),C(3′)-acetonide (**22**)[17,18] and then to the tentatively assigned mesylate **23**.[13] For **13**, **16**, and **17**, compound **23** was dissolved in THF and treated with excess amine to give acetonides **24**−**26**. Removing the C(2′),C(3′)-acetonide group in **24**−**26** with trifluoroacetic acid in aqueous methanol yielded **13**, **16**, and **17**, respectively. For compounds **14** and **19**−**21**, the mesylate **23** was dissolved in isopropyl alcohol in the presence of an excess amount of the hydroxylamine and then the "pH" of the solution was adjusted to 5.5. Deprotection of the acetonide group in **27**−**30** with acid gave **14** and **19**−**21**. We also employed isopropyl alcohol as the solvent to prepare **15**. Using thiolacetic acid in place of the hydroxylamine, we obtained **31**. Successive removal of the acetyl and the acetonide groups in **31** with base and acid, respectively, yielded first **32** and then **15**. Synthesis of **18** was accomplished first by acetylation of **24** with acetic anhydride in the presence of Proton Sponge (Aldrich) to give **33** and then by careful deprotection of the acetonide group with trifluoroacetic acid.



22 X = OH
23 X = OSO_2CH_3
24 X = NH_2
25 X = $N(H)CH_3$
26 X = $N(CH_3)_2$
27 X = N(H)OH

28 X = $N(CH_3)OH$
29 X = $N(H)OCH_3$
30 X = $N(CH_3)OCH_3$
31 X = $SC(O)CH_3$
32 X = SH
33 X = $N(H)C(O)CH_3$

B. Spectral Studies. The ^1H and ^{13}C NMR spectral properties for bicyclomycin acetonides **24**−**33** and bicyclomycins **13**−**21** agreed with the proposed structures[19,20] and were consistent with those observed for **3**−**12**.[13] X-ray crystallographic analysis of **24** documented the incorporation of the amino group at C(6) (Figure 1).[21]

C. Chemical Studies. A distinguishing feature of bicyclomycin chemistry is the ease with which **1** undergoes C(6) hemiaminal ring cleavage to give **2** followed by conjugate addition.[6−9] We have shown that at near neutral "pH" values in THF−H_2O mixtures ("pH" 7.4−8.5) **1** reacts with EtSH to give the novel rearranged adduct **34**[6,9] whereas at higher "pH" values ("pH" 10−11) the C(5a)-substituted dihydrobicyclomycin **35** predominated.[9]

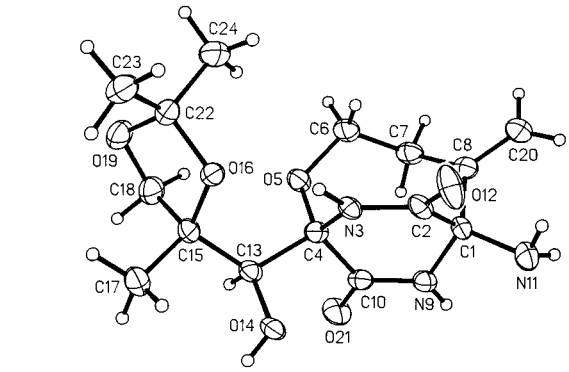
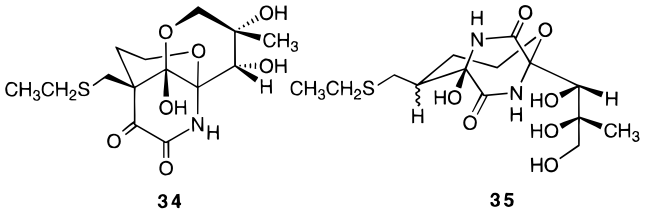


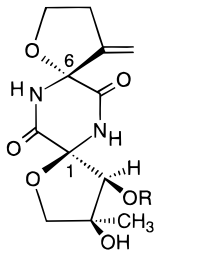
Figure 1. View of **24** showing the atom numbering scheme. Thermal ellipsoids are 40% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter.



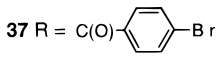
34 **35**

Compounds **13**−**15**, like **1**, each has an ionizable proton attached to the C(6) substituent. These three compounds can potentially undergo C(6)−N(10) bond cleavage to generate an α,β-unsaturated imine or thione system capable of undergoing conjugate addition. In an effort to determine the structural features that govern bicyclomycin reactivity with nucleophiles, we treated these compounds with EtSH. No reaction was observed for C(6)-aminobicyclomycin (**13**) at "pH" 9.0, while at "pH" 10.5, the bis-spiro adduct **36** was generated. NMR analyses indicated the presence of a *single* diastereomer. X-ray crystallographic analysis of the corresponding *p*-bromobenzoate **37** identified **36** as the trans diastereomer having C(1)-*S* and C(6)-*R* stereochemistry.[21] Compound **37** was previously characterized by Maag and co-workers when they determined the absolute stereochemistry of **1**.[22] The EtSH-mediated reactions of C(6)-(hydroxylamino)bicyclomycin (**14**) gave a different product. We observed only **38** at both "pH" 8.8 and 10.0. Once again, NMR analyses indicated that the reaction was stereospecific.[23] Addition of EtSH to a THF−H_2O solution of C(6)-mercaptobicyclomycin (**15**) maintained at "pH" 9.5 gave only **36**, and NMR analyses indicated stereospecificity.

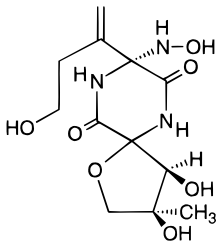
These findings demonstrated the importance of the C(6) substituent in bicyclomycin chemical transformations. Replacement of the C(6)-hydroxy group in **1** by an amino, hydroxylamino, or thiol group prevented EtSH addition reactions to the C(5)−C(5a) olefinic group. In each case, the reaction appears to proceed by initial cleavage of the C(1)−O(2) bond in **39** to give **40** (Scheme 1). Energy minimization studies[24] indicate



36 R = H



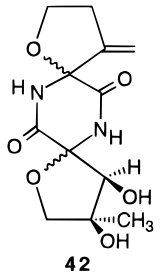
37



38

that **40** (X = OH) undergoes stereospecific cyclization to **41** due in part to favorable hydrogen bonds that exist between the C(1′)-hydroxy and C(9)-carbonyl groups and between the C(2′)-hydroxy and N(8)-imine units. This facial alignment of the C(1) triol group promotes ring closure to give the C(1)-*S*-substituted tetrahydrofuran **41**. Subsequent cyclization of **41** can then give either the C(1)-*S*, C(6)-*R* or the C(1)-*S*, C(6)-*S* bis-spiro tetrahydrofuranyl adducts. Energy minimization studies using molecular mechanics[24] indicate that of these compounds, **36** (C(1)-*S*, C(6)-*R*) has lower energy.

Information concerning the importance of the C(6) substituent in governing bicyclomycin chemistry was also obtained by comparing the stabilities of **1** and **13**−**15** in aqueous solutions (pH 7.4, 37 °C). We found that **1**, **14**, and **15** had approximately the same stabilities in buffered water solutions ($t_{1/2} \approx 28-31$ h), but **13** was appreciably more stable ($t_{1/2} \approx 155$ h). HPLC analyses of the reaction mixtures showed that **1**[13] and **14** produced a series of unidentified, polar adducts, **13** gave diastereomeric **42**,[22,25] and **15** gave **42** along with several additional products. Identification of **42** in the reaction mixtures of **13** and **15** was accomplished by coinjecting (cospotting) authentic samples of **42**[22,25] in the HPLC (TLC) and by running a larger scale reaction for **13** and verifying **42** by NMR spectroscopy. Significantly, neither bicyclomycin nor its reaction products were detected in the HPLC product profiles for **13**−**15**, signifying that these compounds did not hydrolyze to **1**. Comparable results were observed for **3**, **5**, and **9**−**11**.[13]

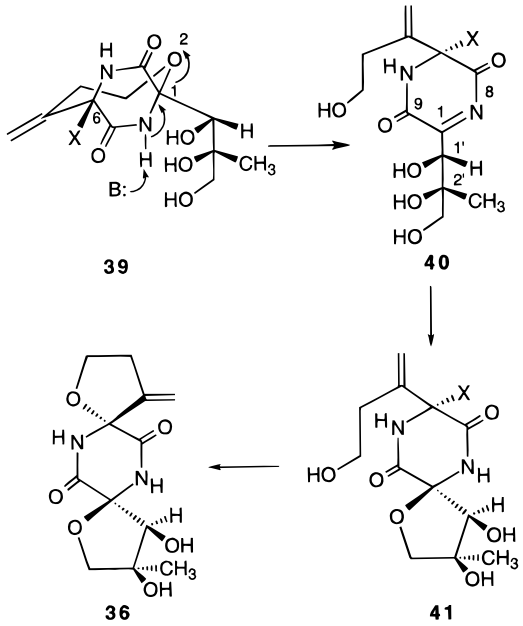


42

Our cumulative studies demonstrated that at moderate "pH" values bicyclomycin underwent predominant C(6) hemiaminal ring opening to generate β-methylene-α-ketoamide **2**. Replacing the C(6)-hydroxy group with nitrogen and sulfur groups permitted C(1)−O(2) and C(6)−X bond cleavage to become competitive with C(6) hemiaminal ring opening.

D. Biochemical and Biological Studies. In our initial SAR study we showed that the C(6)-substituted bicyclomycins **3**−**12** neither appreciably inhibited rho

Scheme 1. Proposed Pathway for Base-Mediated Conversion of C(6)-Substituted Bicyclomycins to **36**

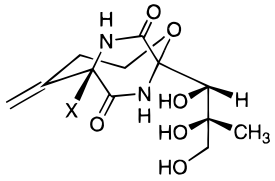


poly(C)-stimulated ATPase activity at a concentration of 400 μM nor did they inhibit transcription termination at a concentration of 100 μM.[13] Comparable findings were observed for the new C(6)-substituted bicyclomycins **13**−**21** (Table 1). In the ATPase assay[15] all I_{50} values for **13**−**21** exceeded 400 μM, with the most active bicyclomycin being **14** followed by **15** and then **13**. Similarly, the I_{50} values for **13**−**21** in the transcription termination assay[14] exceeded 100 μM.

The data in Table 1 demonstrate the exquisite dependency of the C(6)-hydroxy group in bicyclomycin on the inhibition of rho-dependent processes. Our earlier study showed that C(6)-alkoxybicyclomycins **3**−**6** and C(6)-deoxybicyclomycin (**12**) had little inhibitory activity in rho functional assays.[13] This result demonstrated that steric interactions were not solely responsible for the loss of biochemical activities when the C(6)-hydroxy group was replaced. Our original investigation also demonstrated that hydrogen bond *acceptor* interactions from rho to the C(6)-hydroxy group in **1** did not promote drug binding. Compounds **3**−**11** are all capable of accepting a hydrogen bond, but none inhibited rho functional processes. Two other factors were briefly considered. First, hydrogen bond donor interactions between the C(6)-hydroxy group in **1** and the amino acid residue(s) in rho may be necessary for efficient drug binding. Second, bicyclomycin may undergo ring opening to β-methylene-α-ketoamide **2** upon binding to rho. The data for C(6)-substituted bicyclomycins **3**−**12** did not provide information concerning the likelihood of these two factors. Of these compounds, only the bulky C(6)-*N*-phenylbicyclomycin (**11**) was capable of either donating a hydrogen bond or undergoing C(6)−N(10) cleavage to generate a neutral ring-opened intermediate.

Many of the bicyclomycin C(6) substituents in this study were capable of serving as hydrogen bond donors. We expected that the parent compounds, **13**−**15**, would be the most efficient hydrogen bond donors and that successive methylation of the C(6) heteroatom(s) would

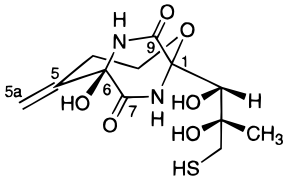
Table 1. Biochemical and Biological Activities of C(6)-Substituted Bicyclomycins



		inhibition of ATPase activity[a]		TT activity[d]		
compd no.	X	I_{50} (μM) (BCM)[b]	400 μM (%) (BCM)[c]	I_{50} (μM)[e]	100 μM (%)[f]	MIC[g] (mg/mL)
1	OH	60	95	~5	100	0.25
13	NH_2	>400 (70)	17 (88)	>100	5	>32 (0.27)
16	$N(H)CH_3$	>400 (70)	3 (88)	>100	2	>32 (0.27)
17	$N(CH_3)_2$	>400 (70)	1 (88)	>100	1	>32 (0.27)
18	$N(H)C(O)CH_3$	>400 (70)	15 (92)	>100	1	>32 (0.31)
14	N(H)OH	>400 (70)	45 (89)	>100	22	>32 (0.35)
19	$N(CH_3)OH$	>400 (70)	16 (89)	>100	0	>32 (0.35)
20	$N(H)OCH_3$	>400 (70)	13 (89)	>100	0	>32 (0.35)
21	$N(CH_3)OCH_3$	>400 (60)	7 (90)	>100	0	>32 (0.35)
15	SH	>400 (70)	28 (93)	>100	8	>32 (0.27)

[a] Activity measured using the ATPase assay (ref 15). [b] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [c] The percent inhibition of ATPase activity at 400 μM. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [d] Activity in the transcription termination assay was determined by the method of T. Platt and co-workers (ref 14). [e] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. [f] The percentage of transcription termination at 100 μM. [g] MIC value is the average minimum inhibitory concentration of the tested compound determined from duplicate tests using a filter disk assay (ref 16). The number in parentheses is the corresponding value obtained from bicyclomycin in a concurrently run experiment.

diminish this interaction. Of these, we noted that **13** is likely to exist as the protonated amine possibly preventing its binding to rho. Correspondingly, nitrogen protonation should not be significant for hydroxylamine **14** (p*K*a alkylhydroxylamines $\approx$ 5−6[26]) at the pH employed in the ATPase and transcription termination assays (pH 7.9). In hydroxylamine **14**, the hydrogen bond could be donated to rho from either the N−H or O−H sites. We found that none of the compounds capable of donating a hydrogen bond to rho from the C(6) site (**13**−**16, 18, 20**) approached the inhibitory activity observed for **1**. However, within this series we observed that **14** and **15** were the most effective inhibitors. Partial inhibition of rho-dependent ATPase and transcription termination were seen at 400 and 100 μM, respectively. The steep drop in inhibitory ATPase activity observed for **15** was reminiscent of the loss in activity observed upon replacement of the C(3′) hydroxy group in **1** with a thiol unit to give **43**.[27] These findings, then, do not permit us to rule out the notion that the C(6)-hydroxy group fosters antibiotic binding to rho by hydrogen bonding to a protein acceptor residue or that this moiety simultaneously serves as a hydrogen bond donor and an acceptor to protein residue(s) that line the bicyclomycin binding pocket.[28]

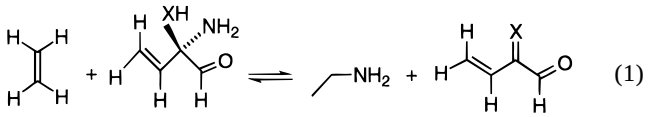


43 (400 μM: 23% ATPase inhibition)

Another factor that may account for the observed specificity of the C(6)-hydroxy group in bicyclomycin is the relative ease with which the parent bicyclomycin underwent C(6)−N(10) bond scission (C(6) hemiaminal ring opening) to give the β-methylene-α-ketoamide **2**. Our chemical studies demonstrated that replacing the C(6)-hydroxy group with a C(6)-amino, -hydroxylamino, or -mercapto substituent led to decreased reactivity with EtSH and to the preferential scission of the C(1)−O(2) and C(6)−XH bonds, compared with cleavage of the C(6)−N(10) bond (Scheme 1). These results suggest that, for bicyclomycin, β-methylene-α-ketoamide **2** may be the preferred species that binds to rho leading to inhibition of rho transcription termination processes. Accordingly, we calculated the thermodynamic characteristics of the model equilibrium processes shown in eq 1, in which **45** and **47** correspond to the parent

Table 2. Calculated $\Delta G°_{rxn}$ and K_{eq} Values for Equilibrium in Eq 1

reaction series	$\Delta G°_{rxn}$ (kcal/mol)	K_{eq}
O	−13.4	6.7×10^9
NH	−10.1	2.5×10^7
S	− 8.0	7.3×10^5



(1)

44 **45** **46** **47**

a X = O
b X = NH
c X = S

a X = O
b X = NH
c X = S

bicyclomycin structure and the ring-opened species, respectively. These calculations, performed at the density functional level, and summarized in Table 2, reveal that in the absence of protein interactions the transformation of **45** to **47** is exothermic for X = O, NH, and S, with **45a** possessing the greatest thermodynamic driving force. The fact that all three reactions are thermodynamically favorable suggests that biochemical

activity in the rho-dependent assays should also be observed for **13** and **15** if the bioactive structure is the ring-opened β-methylene-α-ketoamide **2**. However, comparison of equilibrium constants reveals that the equilibrium lies 270 × further to the right when X = O than when X = NH and ~9200× further to the right than when X = S. Thus the hydroxy-substituted species is expected to undergo ring opening more readily than either the amino- or the thio-substituted compounds. While not indicative of the absolute rate at which ring opening occurs, these values do express the relative thermodynamic preferences for the model reaction, revealing a trend in reactivity that parallels our observation that bicyclomycin undergoes ring opening to the α,β-unsaturated conjugated system **2** followed by C(5)−C(5a) modification by nucleophiles at near neutral to basic pH values, but the C(6)-substituted bicyclomycins **13** and **15** do not.

Our studies indicated that the C(6)-hydroxy group in bicyclomycin was essential for drug function. They also suggested that binding of the antibiotic to rho was fostered either by a hydrogen bond donor interaction from the C(6)-hydroxy group in **1** to the protein or by the conversion of **1** to ring-opened β-methylene-α-ketoamide **2** or by both. Experiments are in progress to study these possibilities.

Bicyclomycins **13**−**21** were all inactive (MIC >32 mg/mL) against W3350 *E. coli* in the filter disk assay[16] (Table 1). This result was consistent with the lack of significant inhibitory activities in the rho poly(C)-stimulated ATPase and transcription termination assays. A similar finding was observed for **3**−**12**.[13]

Conclusions

This report highlights the essential role of the C(6)-hydroxy group in bicyclomycin. Simple replacement of this unit with an amino, hydroxylamino, or mercapto moiety altered the chemical reactivity of the drug analogue leading to the near total elimination of bicyclomycin inhibitory activities in rho functional assays and resulting in the loss of antimicrobial activity against W3350 *E. coli*.

Experimental Section

General Methods. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on 300 and 75 MHz spectrometers, respectively. Low-resolution and high-resolution (CI) mass spectral investigations were run at the University of Texas at Austin by Dr. M. Moini. "pH" measurements of mixed organic-water solutions were determined using electrodes calibrated against aqueous pH 4.0 and 7.0 buffer solutions. The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. Tetrahydrofuran was distilled from Na metal and benzophenone. Thin-layer chromatography was run on general purpose silica gel plates (20 × 20 cm; Aldrich No. Z12272-6).

Rho protein was isolated from *E. coli* AR120 containing the overexpressing plasmid p39-AS[29] following previously published protocols.[29] Rho purity was determined by SDS−PAGE, and concentrations were determined by Lowry protein determination.[30] Procedures identical to those previously described[27] were used in the evaluation of the C(6)-substituted bicyclomycins in the poly(C)-dependent ATPase,[15] rho-dependent transcription termination,[14] and antimicrobial assays.[16] [γ-^{32}P]ATP and [α-^{32}P]UTP (3000 Ci/mmol) were purchased from Dupont-New England Nuclear (Doraville, GA); nucleotides and RNase inhibitor were obtained from Ambion, Inc. (Austin, TX). Polyethylenimine (PEI) thin-layer chromatography plates used for ATPase assays were purchased from J. T. Baker, Inc. (Phillipsburg, NJ).

General Procedure for the Preparation of C(6)-Aminobicyclomycin C(2′),C(3′)-Acetonides 24−26. To an anhydrous THF solution (4 mL) of **22** (1 equiv) and triethylamine (2−3 equiv) was added methanesulfonyl chloride (2-3 equiv). The reaction mixture was stirred at room temperature (15 min), filtered (glass wool), and treated with the desired nucleophile (excess). The solution was then concentrated in vacuo, and the residue was purified by PTLC (15−20% MeOH−$CHCl_3$) to afford the desired product.

By use of this procedure, the following compounds were prepared.

Synthesis of C(6)-Aminobicyclomycin C(2′),C(3′)-Acetonide (24). Using **22** (17 mg, 0.05 mmol), triethylamine (15 mg, 0.15 mmol), methanesulfonyl chloride (17 mg, 0.15 mmol) and ammonia(g) (2 min, excess) gave **24** as a white solid (10 mg, 59%): mp 183−185 °C; R_f 0.49 (10% MeOH−$CHCl_3$); IR (KBr) 3364, 3302, 1702, 1669, 1603, 1467 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.42 (s, 3 H, C$(CH_3)_2$), 1.45 (s, 3 H, C$(CH_3)_2$), 2.56−2.73 (m, 2 H, C(4)H_2), 3.72 (d, J = 8.3 Hz, 1 H, C(3′)*H*H′), 3.84 (ddd, J = 1.7, 6.2, 8.9 Hz, 1 H, C(3)-*H*H′), 3.96 (ddd, J = 1.7, 6.2, 8.9 Hz, 1 H, C(3)H*H*′), 4.16 (s, 1 H, C(1′)H), 4.46 (d, J = 8.3 Hz, 1 H, C(3′)H*H*′), 5.16 (s, 1 H, C(5a)*H*H′), 5.50 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 25.1 (C(2′)*C*H_3), 26.8 (C(*C*$H_3)_2$), 28.2 (C(*C*$H_3)_2$), 37.1 (C(4)), 66.7 (C(3)), 72.3 (C(6)), 72.8 and 73.1 (C(1′), C(3′)), 86.5 (C(2′)), 89.2 (C(1)), 111.6 (*C*$(CH_3)_2$), 116.6 (C(5a)), 151.6 (C(5)), 168.8 and 172.4 (C(7), C(9)) ppm; MS (+CI) 342 [M + 1]$^+$; M_r (+CI) 342.166 20 [M + 1]$^+$ (calcd for $C_{15}H_{24}N_3O_6$ 342.166 51).

Synthesis of C(6)-(*N*-Methylamino)bicyclomycin C(2′),C(3′)-Acetonide (25). Using **22** (32 mg, 0.09 mmol), triethylamine (19 mg, 0.19 mmol), methanesulfonyl chloride (21 mg, 0.19 mmol), and methylamine (29 mg, 0.94 mmol) gave **22** (10 mg, 31% recovery) and **25** as a white solid (14 mg, 42%): mp 158−162 °C; R_f 0.57 (10% MeOH−$CHCl_3$); IR (KBr) 3446, 3311, 1686, 1457 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.37 (s, 3 H, C(2′)-CH_3), 1.43 (s, 3 H, C$(CH_3)_2$), 1.45 (s, 3 H, C$(CH_3)_2$), 2.56 (dd, J = 6.9, 15.3 Hz, 1 H, C(4)*H*H′), 2.63−2.70 (m, 1 H, C(4)H*H*′), 3.72 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.83−3.97 (m, 2 H, C(3)-H_2), 4.15 (s, 1 H, C(1′)H), 4.45 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.13 (s, 1 H, C(5a)*H*H′), 5.50 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3-OD) 25.0 (C(2′)*C*H_3), 26.8 (C(*C*$H_3)_2$), 28.2 (C(*C*$H_3)_2$), 29.9 (NCH_3), 37.4 (C(4)), 67.0 (C(3)), 73.0 and 73.2 (C(1′), C(3′)), 77.1 (C(6)), 86.4 (C(2′)), 89.0 (C(1)), 111.7 (*C*$(CH_3)_2$), 116.5 (C(5a)), 150.6 (C(5)), 169.8 and 171.5 (C(7), C(9)) ppm; MS (+CI) 356 [M + 1]$^+$; M_r (+CI) 356.181 99 [M + 1]$^+$ (calcd for $C_{16}H_{26}N_3O_6$ 356.182 16).

Synthesis of C(6)-(*N*,*N*-Dimethylamino)bicyclomycin C(2′),C(3′)-Acetonide (26). Using **22** (30 mg, 0.09 mmol), triethylamine (18 mg, 0.18 mmol), methanesulfonyl chloride (20 mg, 0.18 mmol), and dimethylamine (40 mg, 0.88 mmol) gave **22** (12 mg, 40% recovery) and **26** as a white solid (11 mg, 34%): mp 98−104 °C; R_f 0.55 (10% MeOH−$CHCl_3$); IR (KBr) 3440, 3326, 1687, 1458 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.40 (s, 3 H, C$(CH_3)_2$), 1.44 (s, 3 H, C$(CH_3)_2$), 2.37 (s, 6 H, N$(CH_3)_2$), 2.50−2.63 (m, 2 H, C(4)H_2), 3.60−3.70 (m, 1 H, C(3)*H*H′), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 4.05−4.11 (m, 1 H, C(3)H*H*′), 4.15 (s, 1 H, C(1′)H), 4.42 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.36 (s, 1 H, C(5a)*H*H′), 5.53 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 25.1 (C(2′)*C*H_3), 26.8 (C(*C*$H_3)_2$), 28.0 (C(*C*$H_3)_2$), 37.0 (C(4)), 39.0 (N$(CH_3)_2$), 68.2 (C(3)), 72.8 and 73.2 (C(1′), C(3′)), 82.4 (C(6)), 86.4 (C(2′)), 88.1 (C(1)), 111.5 (*C*$(CH_3)_2$), 119.9 (C(5a)), 149.6 (C(5)), 170.9 and 171.1 (C(7), C(9)) ppm; the spectral assignments were in agreement with the HMQC experiment; MS (+CI) 370 [M + 1]$^+$; M_r (+CI) 370.197 91 [M + 1]$^+$ (calcd for $C_{17}H_{28}N_3O_6$ 370.197 81).

General Procedure for the Preparation of C(6)-(*N*-Substituted Hydroxylamino)bicyclomycin C(2′),C(3′)-Acetonides 27-30. To an anhydrous THF solution (4 mL) of **22** (1 equiv) and triethylamine (3 equiv) was added methanesulfonyl chloride (3 equiv). The reaction mixture was stirred

at room temperature (15 min), filtered (glass wool), and concentrated in vacuo. The residue was dissolved in isopropyl alcohol (4 mL) and treated with the desired nucleophile (10 equiv). The "pH" of the reaction mixture was adjusted to approximately 5.5 with dilute aqueous NaOH and stirred at room temperature (20 min). The "pH" of the reaction mixture was adjusted to 7.0 with dilute aqueous NaOH and concentrated in vacuo. The residue was suspended in H_2O (8 mL) and extracted with ethyl acetate (4 × 8 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by PTLC (10% MeOH−$CHCl_3$) to afford the desired product.

By use of this procedure, the following compounds were prepared.

Synthesis of C(6)-(Hydroxylamino)bicyclomycin C(2′), C(3′)-Acetonide (27). Using **22** (37 mg, 0.11 mmol), triethylamine (22 mg, 0.22 mmol), methanesulfonyl chloride (25 mg, 0.22 mmol), and NH_2OH·HCl (74 mg, 1.1 mmol) gave **22** (5 mg, 14% recovery) and **27** as a white solid (15 mg, 39%): mp 119−122 °C; R_f 0.47 (10% MeOH−$CHCl_3$); IR (KBr) 3426, 3271, 1690 cm^{-1}; ^1H NMR (CD_3OD) δ 1.37 (s, 3 H, C(2′)CH_3), 1.43 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.49−2.68 (m, 2 H, C(4)H_2), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.86−3.98 (m, 2 H, C(3)*H*H′), 4.13 (s, 1 H, C(1′)H), 4.43 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.11 (s, 1 H, C(5a)*H*H′), 5.32 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.7 (C(2′)CH_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 35.5 (C(4)), 67.1 (C(3)), 73.4 (C(3′)), 73.8 (C(1′)), 78.4 (C(6)), 86.2 (C(2′)), 88.8 (C(1)), 111.7 (C(*C*H_3)$_2$), 116.2 (C(5a)), 147.3 (C(5)), 168.7 and 171.0 (C(7), C(9)) ppm; MS (+CI) 358 [M + 1]$^+$; M_r (+CI) 358.161 87 [M + 1]$^+$ (calcd for $C_{15}H_{24}N_3O_7$ 358.161 43).

Synthesis of C(6)-(*N*-Methylhydroxylamino)bicylomycin C(2′),C(3′)-Acetonide (28). Using **22** (41 mg, 0.12 mmol), triethylamine (24 mg, 0.24 mmol), methanesulfonyl chloride (28 mg, 0.24 mmol), and *N*-methylhydroxylamine hydrochloride (102 mg, 1.2 mmol) gave **22** (8 mg, 20% recovery) and **28** as a white solid (14 mg, 31%): mp 109−113 °C; R_f 0.53 (10% MeOH−$CHCl_3$); IR (KBr) 3291, 1688 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.39 (s, 3 H, C(CH_3)$_2$), 1.43 (s, 3 H, C(CH_3)$_2$), 2.54−2.60 (m, 2 H, C(4)H_2), 2.68 (s, 3 H, NCH_3), 3.64−3.70 (m, 1 H, C(3)*H*H′), 3.73 (d, J = 8.6 Hz, 1 H, C(3′)-*H*H′), 4.05−4.11 (m, 1 H, C(3)H*H*′), 4.17 (s, 1 H, C(1′)H), 4.42 (d, J = 8.6 Hz, 1 H, C(3′)H*H*′), 5.30 (s, 1 H, C(5a)*H*H′), 5.68 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 25.1 (C(2′)CH_3), 26.8 (C(*C*H_3)$_2$), 28.0 (C(*C*H_3)$_2$), 37.0 (C(4)), 41.8 (NCH_3), 67.4 (C(3)), 72.7 and 73.2 (C(1′), C(3′)), 83.0 (C(6)), 86.4 (C(2′)), 88.1 (C(1)), 111.5 (*C*(CH_3)$_2$), 119.0 (C(5a)), 148.7 (C(5)), 170.0 and 170.6 (C(7), C(9)) ppm; MS (+CI) 372 [M + 1]$^+$; M_r (+CI) 372.176 51 [M + 1]$^+$ (calcd for $C_{16}H_{26}N_3O_7$ 372.177 08).

Synthesis of C(6)-(Methoxyamino)bicyclomycin C(2′),C-(3′)-Acetonide (29). Using **22** (35 mg, 0.10 mmol), triethylamine (21 mg, 0.21 mmol), methanesulfonyl chloride (23 mg, 0.21 mmol), and methoxyamine hydrochloride (95 mg, 1.0 mmol) gave **22** (6 mg, 17% recovery) and **29** as a white solid (13 mg, 34%): mp 92−96 °C; R_f 0.60 (10% MeOH−$CHCl_3$); IR (KBr) 3417, 3296, 1692 cm^{-1}; ^1H NMR (CD_3OD) δ 1.36 (s, 3 H, C(2′)CH_3), 1.43 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.51 (dd, J = 7.2, 16.1 Hz, 1 H, C(4)*H*H′), 2.63 (dd, J = 8.4, 16.1 Hz, 1 H, C(4)H*H*′), 3.59 (s, 3 H, OCH_3), 3.72 (d, J = 8.6 Hz, 1 H, C(3′)*H*H′), 3.84−3.99 (m, 2 H, C(3)H_2), 4.12 (s, 1 H, C(1′)H), 4.43 (d, J = 8.6 Hz, 1 H, C(3′)H*H*′), 5.10 (s, 1 H, C(5a)-*H*H′), 5.31 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.6 (C(2′)-*C*H_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 37.7 (C(4)), 63.3 (OCH_3), 67.1 (C(3)), 73.4 (C(3′)), 73.8 (C(1′)), 77.9 (C(6)), 86.3 (C(2′)), 88.8 (C(1)), 111.7 (*C*(CH_3)$_2$), 116.1 (C(5a)), 147.2 (C(5)), 168.6 and 170.7 (C(7), C(9)) ppm; MS (+CI) 372 [M + 1]$^+$; M_r (+CI) 372.175 70 [M + 1]$^+$ (calcd for $C_{16}H_{26}N_3O_7$ 372.177 08).

Synthesis of C(6)-(*N*,*O*-Dimethylhydroxylamino)bicyclomycin C(2′),C(3′)-Acetonide (30). Using **22** (40 mg, 0.12 mmol), triethylamine (24 mg, 0.23 mmol), methanesulfonyl chloride (27 mg, 0.23 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (116 mg, 1.2 mmol) gave **22** (8 mg, 20% recovery) and **30** as a white solid (14 mg, 31%): mp 76−80 °C; R_f 0.58 (10% MeOH−$CHCl_3$); IR (KBr) 3446, 3314, 1693, 1457 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.39 (s, 3 H, C(CH_3)$_2$), 1.43 (s, 3 H, C(CH_3)$_2$), 2.54−2.61 (m, 2 H, C(4)H_2), 2.69 (s, 3 H, NCH_3), 3.53 (s, 3 H, OCH_3), 3.61−3.68 (m, 1 H, C(3)*H*H′), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 4.08−4.14 (m, 1 H, C(3)H*H*′), 4.16 (s, 1 H, C(1′)H), 4.43 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.31 (s, 1 H, C(5a)*H*H′), 5.65 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 25.1 (C(2′)CH_3), 26.8 (C(*C*H_3)$_2$), 28.0 (C(*C*H_3)$_2$), 37.0 (C(4)), 37.2 (NCH_3), 60.3 (OCH_3), 67.8 (C(3)), 72.7 and 73.2 (C(1′), C(3′)), 83.2 (C(6)), 86.4 (C(2′)), 88.0 (C(1)), 111.5 (*C*(CH_3)$_2$), 118.5 (C(5a)), 149.5 (C(5)), 169.3 and 170.5 (C(7), C(9)) ppm; MS (+CI) 386 [M + 1]$^+$; M_r (+CI) 386.192 73 [M + 1]$^+$ (calcd for $C_{17}H_{28}N_3O_7$ 386.192 73).

Synthesis of C(6)-*S*-Acetylbicyclomycin C(2′),C(3′)-Acetonide (31). To an anhydrous THF solution (4 mL) of **22** (56 mg, 0.16 mmol) and triethylamine (33 mg, 0.33 mmol) was added methanesulfonyl chloride (38 mg, 0.33 mmol). The reaction mixture was stirred at room temperature (15 min), filtered (glass wool), and concentrated in vacuo. The residue was dissolved in isopropyl alcohol (4 mL) and treated with thiolacetic acid (125 mg, 1.64 mmol). The "pH" of the reaction mixture was adjusted to approximately 5.5 with dilute aqueous NaOH and stirred at room temperature (20 min). The "pH" of the reaction mixture was adjusted to 7.0 with dilute aqueous NaOH and concentrated in vacuo. The residue was suspended in H_2O (8 mL) and extracted with ethyl acetate (4 × 8 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by PTLC (10% MeOH−$CHCl_3$) to provide **22** (18 mg, 32% recovery) and **31** as a white solid (23 mg, 35%): mp 157−160 °C; R_f 0.67 (10% MeOH−$CHCl_3$); IR (KBr) 3440, 3325, 1699, 1458 cm^{-1}; ^1H NMR (CD_3OD) δ 1.41 (s, 3 H, C(2′)CH_3), 1.44 (s, 6 H, C(CH_3)$_2$), 2.34 (s, 3 H, SC(O)CH_3), 2.61 (dd, J = 6.6, 15.9 Hz, 1 H, C(4)*H*H′), 2.75 (dd, J = 9.0, 15.9 Hz, 1 H, C(4)H*H*′), 3.71−3.81 (m, 1 H, C(3)*H*H′), 3.74 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 4.04 (dd, J = 6.6, 13.2 Hz, 1 H, C(3)H*H*′), 4.09 (s, 1 H, C(1′)H), 4.38 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.23 (s, 1 H, C(5a)*H*H′), 5.38 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)CH_3), 26.8 (C(*C*H_3)$_2$), 27.9 (C(*C*H_3)$_2$), 30.2 (SC(O)*C*H_3), 37.3 (C(4)), 66.6 (C(3)), 73.4 (C(6)), 73.6 (C(3′)), 75.0 (C(1′)), 86.1 (C(2′)), 87.3 (C(1)), 111.6 (*C*(CH_3)$_2$), 118.4 (C(5a)), 148.8 (C(5)), 168.8 and 168.9 (C(7), C(9)), 194.0 (S*C*(O)CH_3) ppm; MS (+CI) 401 [M + 1]$^+$; M_r (+CI) 401.137 11 [M + 1]$^+$ (calcd for $C_{17}H_{25}N_2O_7S$ 401.138 25).

Synthesis of C(6)-Mercaptobicyclomycin C(2′),C(3′)-Acetonide (32). To a solution of **31** (25 mg, 0.06 mmol) in degassed MeOH (3 mL) was added aqueous 0.1 M NaOH (3 mg, 0.06 mmol). The solution was stirred (0 °C, 1 h), neutralized (aqueous dilute HCl), and concentrated in vacuo. The residue was suspended in H_2O (4 mL) and extracted with ethyl acetate (4 × 8 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by PTLC (2 × 5% MeOH−$CHCl_3$) to afford **32** as a white solid (15 mg, 67%): mp 192−196 °C; R_f 0.51 (10% MeOH−$CHCl_3$); IR (KBr) 3446, 3289, 1673, 1650, 1458 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.62 (dd, J = 7.5, 16.1 Hz, 1 H, C(4)*H*H′), 2.76 (dd, J = 8.4, 16.1 Hz, 1 H, C(4)H*H*′), 3.72 (d, J = 8.3 Hz, 1 H, C(3′)*H*H′), 3.76−3.84 (m, 1 H, C(3)*H*H′), 3.92−3.98 (m, 1 H, C(3)H*H*′), 4.16 (s, 1 H, C(1′)H), 4.45 (d, J = 8.3 Hz, 1 H, C(3′)H*H*′), 5.24 (s, 1 H, C(5a)*H*H′), 6.18 (br s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 25.0 (C(2′)CH_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 37.0 (C(4)), 67.4 (C(3)), 71.7 (C(6)), 73.1 (C(1′)), 73.3 (C(3′)), 86.5 (C(2′)), 89.5 (C(1)), 111.7 (*C*(CH_3)$_2$), 121.7 (C(5a)), 152.6 (C(5)), 166.9 and 167.0 (C(7), C(9)) ppm, the structural assignments were in agreement with the HMBC and HMQC experiments; MS (+CI) 359 [M + 1]$^+$; M_r (+CI) 359.128 60 [M + 1]$^+$ (calcd for $C_{15}H_{23}N_2O_6S$ 359.127 68).

Synthesis of C(6)-Acetamidobicyclomycin C(2′),C(3′)-Acetonide (33). To an anhydrous THF (2 mL) solution of **24** (12 mg, 0.04 mmol) and acetic anhydride (18 mg, 0.18 mmol) was added Proton Sponge (11 mg, 0.05 mmol). The solution was stirred at room temperature (24 h) and concentrated in vacuo. The residue was purified by PTLC (10% MeOH−$CHCl_3$) to provide **24** (5 mg, 42% recovery) and **33** (8 mg, 58%)

as a white solid: mp 156−160 °C; R_f 0.52 (10% MeOH−$CHCl_3$); IR (KBr) 3420, 1686, 1541 cm^{-1}; ^1H NMR (CD_3OD) δ 1.40 (s, 6 H, C(2′)CH_3, C$(CH_3)_2$), 1.44 (s, 3 H, C$(CH_3)_2$), 2.06 (s, 3 H, C(O)CH_3), 2.58−2.75 (m, 2 H, C(4)H_2), 3.66−3.75 (m, 1 H, C(3)-*H*H′), 3.73 (d, J = 8.6 Hz, 1 H, C(3′)*H*H′), 4.04−4.09 (m, 1 H, C(3)H*H*′), 4.11 (s, 1 H, C(1′)H), 4.39 (d, J = 8.6 Hz, 1 H, C(3′)-H*H*′), 5.24 (s, 1 H, C(5a)*H*H′), 5.37 (s, 1 H, C(5a)H*H*′); ^1H NMR (THF-d_8) δ 1.34 (s, 3 H, C(2′)CH_3), 1.35 (s, 3 H, C$(CH_3)_2$), 1.40 (s, 3 H, C$(CH_3)_2$), 2.01 (s, 3 H, C(O)CH_3), 2.69 (dd, J = 9.5, 16.1 Hz, 1 H, C(4)*H*H′), 3.66 (d, J = 8.3 Hz, 1 H, C(3′)*H*H′), 3.66−3.73 (m, 1 H, C(3)*H*H′), 4.01 (dd, J = 6.3, 12.5 Hz, 1 H, C(3)H*H*′), 4.12 (d, J = 8.4 Hz, 1 H, C(1′)H), 4.37 (d, J = 8.3 Hz, 1 H, C(3′)H*H*′), 5.04 (s, 1 H, C(5a)*H*H′), 5.18 (s, 1 H, C(5a)-H*H*′), 5.28 (d, J = 8.4 Hz, 1 H, C(1′)OH), 8.07, 8.11, 8.15 (s, 3 H, C(6)NH, N(8)H, N(10)H), the C(4)H*H*′ was not detected and is believed to be beneath the impurity peak at 2.56 ppm; ^{13}C NMR (CD_3OD) 22.8 (C(O)*C*H_3), 24.5 (C(2′)*C*H_3), 26.8 (C(*C*$H_3)_2$), 27.9 (C(*C*$H_3)_2$), 36.7 (C(4)), 66.9 (C(3)), 72.5 (C(6)), 73.4 (C(3′)), 74.3 (C(1′)), 86.2 (C(2′)), 87.4 (C(1)), 111.6 (*C*$(CH_3)_2$), 117.0 (C(5a)), 148.7 (C(5)), 168.7, 169.0 and 173.6 (C(7), C(9), *C*(O)-CH_3)) ppm; MS (+CI) 384 $[M + 1]^+$; M_r (+CI) 384.175 82 $[M + 1]^+$ (calcd for $C_{17}H_{26}N_3O_7$ 384.177 08).

General Procedure for the Preparation of C(6)-Substituted Bicyclomycins 13-21. To a 50% aqueous methanol solution (3 mL) of the C(6)-substituted acetonides was added trifluoroacetic acid (3 drops). The reaction solution was stirred at room temperature (2 h) and concentrated in vacuo. The residue was purified by PTLC (20% MeOH−$CHCl_3$) to provide the desired product.

By use of this procedure, the following compounds were prepared.

Synthesis of C(6)-Aminobicyclomycin (13). Using **24** (7 mg, 0.02 mmol) gave **13** as a white solid (3 mg, 62%): mp 108−111 °C; R_f 0.23 (20% MeOH−$CHCl_3$); IR (KBr) 3420, 3306, 1686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.61−2.67 (m, 2 H, C(4)H_2), 3.50 (d, J = 11.3 Hz, 1 H, C(3′)-*H*H′), 3.66 (d, J = 11.3 Hz, 1 H, C(3′)H*H*′), 3.80 (ddd, J = 2.0, 6.3, 8.0 Hz, 1 H, C(3)*H*H′), 3.95 (ddd, J = 2.0, 6.3, 8.0 Hz, 1 H, C(3)H*H*′), 4.07 (s, 1 H, C(1′)H), 5.16 (s, 1 H, C(5a)*H*H′), 5.46 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.2 (C(2′)*C*H_3), 37.3 (C(4)), 65.7 (C(3)), 68.5 (C(3′)), 72.1 (C(1′)), 72.4 (C(6)), 78.2 (C(2′)), 89.6 (C(1)), 116.7 (C(5a)), 151.9 (C(5)), 169.1 and 172.6 (C(7), C(9)) ppm; MS (+FAB) 302 $[M + 1]^+$; M_r (+FAB) 302.134 32 $[M + 1]^+$ (calcd for $C_{12}H_{20}N_3O_6$ 302.135 21).

Synthesis of C(6)-(*N*-Hydroxylamino)bicyclomycin (14). Using **27** (14 mg, 0.04 mmol) gave **14** as a white solid (8 mg, 61%): mp 120−123 °C; R_f 0.20 (20% MeOH−$CHCl_3$); IR (KBr) 3405, 3226, 1686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)-CH_3), 2.54−2.58 (m, 1 H, C(4)*H*H′), 2.61−2.65 (m, 1 H, C(4)-H*H*′), 3.52 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.86−3.92 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 5.11 (s, 1 H, C(5a)*H*H′), 5.31 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)*C*H_3), 37.9 (C(4)), 66.2 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.2 (C(2′)), 78.5 (C(6)), 89.6 (C(1)), 116.3 (C(5a)), 147.5 (C(5)), 169.9 and 171.2 (C(7), C(9)) ppm; MS (−CI) 317 $[M]^-$; M_r (−CI) 317.121 09 $[M]^-$ (calcd for $C_{12}H_{19}N_3O_7$ 317.122 30).

Synthesis of C(6)-Thiolbicyclomycin (15). Using **32** (20 mg, 0.03 mmol) gave **15** as a white solid (11 mg, 62%): mp 143−147 °C; R_f 0.27 (30% MeOH−$CHCl_3$); IR (KBr) 3390, 1686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.63 (dd, J = 7.0, 16.1 Hz, 1 H, C(4)*H*H′), 2.76 (dd, J = 8.5, 16.1 Hz, 1 H, C(4)H*H*′), 3.53 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.68 (d, J = 11.3 Hz, 1 H, C(3′)H*H*′), 3.81 (dd, J = 8.5, 12.8 Hz, 1 H, C(3)-*H*H′), 3.92 (dd, J = 7.0, 12.8 Hz, 1 H, C(3)H*H*′), 4.09 (s, 1 H, C(1′)H), 5.23 (s, 1 H, C(5a)*H*H′), 6.20 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)*C*H_3), 37.1 (C(4)), 66.6 (C(3)), 68.4 (C(3′)), 71.8 (C(6)), 72.2 (C(1′)), 78.3 (C(2′)), 90.1 (C(1)), 121.9 (C(5a)), 152.8 (C(5)), 177.3 and 177.8 (C(7), C(9)) ppm; MS (+CI) 319 $[M + 1]^+$; M_r (+CI) 319.097 44 $[M + 1]^+$ (calcd for $C_{12}H_{19}N_2O_6S$ 319.096 38).

Synthesis of C(6)-(*N*-Methylamino)bicyclomycin (16). Using **25** (12 mg, 0.03 mmol) gave **16** as a white solid (5 mg, 47%): mp 105−108 °C; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3414, 1686, 1456 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)-CH_3), 2.31 (s, 3 H, NCH_3), 2.56 (dd, J = 6.9, 16.1 Hz, 1 H, C(4)*H*H′), 2.66 (dd, J = 8.5, 16.1 Hz, 1 H, C(4)H*H*′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)-H*H*′), 3.81 (dd, J = 8.5, 12.9 Hz, 1 H, C(3)*H*H′), 3.93 (dd, J = 6.9, 12.9 Hz, 1 H, C(3)H*H*′), 4.08 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)*H*H′), 5.47 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.2 (C(2′)*C*H_3), 29.4 (NCH_3), 37.6 (C(4)), 66.1 (C(3)), 68.5 (C(3′)), 72.1 (C(1′)), 77.2 (C(6)), 78.2 (C(2′)), 89.5 (C(1)), 116.5 (C(5a)), 150.9 (C(5)), 170.1 and 171.7 (C(7), C(9)) ppm; MS (+CI) 316 $[M + 1]^+$; M_r (+CI) 316.151 15 $[M + 1]^+$ (calcd for $C_{13}H_{22}N_3O_6$ 316.150 86).

Synthesis of C(6)-N,N-Dimethylaminobicyclomycin (17). Using **26** (10 mg, 0.03 mmol) gave **17** as a white solid (4 mg, 45%): mp 98−104 °C; R_f 0.54 (20% MeOH−$CHCl_3$); IR (KBr) 3414, 1686, 1460 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.36 (s, 6 H, N$(CH_3)_2$), 2.48−2.63 (m, 2 H, C(4)-H_2), 3.48 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.62 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.62−3.79 (m, 1 H, C(3)*H*H′), 4.00−4.06 (m, 1 H, C(3)H*H*′), 4.07 (s, 1 H, C(1′)H), 5.35 (s, 1 H, C(5a)*H*H′), 5.52 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)*C*H_3), 37.2 (C(4)), 39.0 (N$(CH_3)_2$), 67.0 (C(3)), 68.5 (C(3′)), 71.9 (C(1′)), 78.2 (C(2′)), 82.5 (C(6)), 88.5 (C(1)), 119.7 (C(5a)), 149.6 (C(5)), 170.8 and 171.3 (C(7), C(9)) ppm; MS (+CI) 330 $[M + 1]^+$; M_r (+CI) 330.166 48 $[M + 1]^+$ (calcd for $C_{14}H_{24}N_3O_6$ 330.166 51).

Synthesis of C(6) Acetamidobicyclomycin (18). Using **33** (16 mg, 0.04 mmol) gave an unidentified adduct plus **18** as a white solid (5 mg, 35%): mp 185−187 °C; R_f 0.25 (20% MeOH−$CHCl_3$); IR (KBr) 3406, 3239, 1686, 1520 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.06 (s, 3 H, C(O)-CH_3), 2.58−2.74 (m, 2 H, C(4)H_2), 3.48 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.62 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.70 (dd, J = 8.9, 13.3 Hz, 1 H, C(3)*H*H′), 4.00−4.08 (m, 1 H, C(3)H*H*′), 4.06 (s, 1 H, C(1′)H), 5.21 (s, 1 H, C(5a)*H*H′), 5.32 (s, 1 H, C(5a)-H*H*′); ^{13}C NMR (CD_3OD) 22.9 (C(O)*C*H_3), 24.1 (C(2′)*C*H_3), 36.8 (C(4)), 65.9 (C(3)), 68.5 (C(3′)), 72.1 (C(1′)), 72.7 (C(6)), 78.2 (C(2′)), 88.7 (C(1)), 116.8 (C(5a)), 149.0 (C(5)), 168.9, 169.0 and 173.5 (C(7), C(9), *C*(O)CH_3) ppm; MS (+CI) 344 $[M + 1]^+$; M_r (+CI) 344.146 20 $[M + 1]^+$ (calcd for $C_{14}H_{22}N_3O_7$ 344.145 78).

Synthesis of C(6)-(*N*-Methylhydroxylamino)bicyclomycin (19). Using **28** (14 mg, 0.04 mmol) gave **19** as a white solid (6 mg, 48%): mp 119−123 °C; R_f 0.38 (20% MeOH−$CHCl_3$); IR (KBr) 3408, 3258, 1686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.48−2.63 (m, 2 H, C(4)H_2), 2.67 (s, 3 H, NCH_3), 3.47 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.62 (d, J = 11.3 Hz, 1 H, C(3′)H*H*′), 3.65−3.72 (m, 1 H, C(3)*H*H′), 4.01−4.07 (m, 1 H, C(3)H*H*′), 4.09 (s, 1 H, C(1′)H), 5.28 (s, 1 H, C(5a)-*H*H′), 5.67 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)*C*H_3), 37.2 (C(4)), 41.8 (NCH_3), 66.3 (C(3)), 68.5 (C(3′)), 71.8 (C(1′)), 78.2 (C(2′)), 83.0 (C(6)), 88.6 (C(1)), 118.9 (C(5a)), 148.7 (C(5)), 170.2 (C(7), C(9) overlap) ppm; MS (+CI) 332 $[M + 1]^+$; M_r (+CI) 332.145 74 $[M + 1]^+$ (calcd for $C_{13}H_{22}N_3O_7$ 332.145 78).

Synthesis of C(6)-(Methoxyamino)bicyclomycin (20). Using **29** (12 mg, 0.03 mmol) gave **20** as a white solid (7 mg, 61%): mp 115−121 °C; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3427, 3244, 1686, 1461 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.48−2.59 (m, 1 H, C(4)*H*H′), 2.60−2.68 (m, 1 H, C(4)H*H*′), 3.52 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.57 (s, 3 H, OCH_3), 3.68 (d, J = 11.3 Hz, 1 H, C(3′)H*H*′), 3.86-3.93 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 5.10 (s, 1 H, C(5a)*H*H′), 5.29 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.2 (C(2′)*C*H_3), 37.8 (C(4)), 63.2 (OCH_3), 66.2 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.1 (C(6)), 78.2 (C(2′)), 89.7 (C(1)), 116.1 (C(5a)), 147.5 (C(5)), 168.9 and 170.9 (C(7), C(9)) ppm; MS (+CI) 332 $[M + 1]^+$; M_r (+CI) 332.144 59 $[M + 1]^+$ (calcd for $C_{13}H_{22}N_3O_7$ 332.145 78).

Synthesis of C(6)-(*N*,*O*-Dimethylhydroxylamino)bicyclomycin (21). Using **30** (12 mg, 0.03 mmol) gave **21** as a white solid (5 mg, 46%): mp 124−127 °C; R_f 0.61 (20% MeOH−$CHCl_3$); IR (KBr) 3421, 3269, 1687, 1456 cm^{-1}; ^1H NMR (CD_3-OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.53−2.63 (m, 2 H, C(4)H_2), 2.68 (s, 3 H, NCH_3), 3.46 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.53 (s, 3 H, OCH_3), 3.61 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.62−3.69 (m, 1 H, C(3)*H*H′), 4.03−4.08 (m, 1 H, C(3)H*H*′), 4.08 (s, 1 H,

C(1′)H), 5.30 (s, 1 H, C(5a)*H*H′), 5.65 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 24.1 (C(2′)CH_3), 37.2 (C(4)), 37.3 (NCH_3), 60.2 (OCH_3), 66.7 (C(3)), 68.5 (C(3′)), 71.8 (C(1′)), 78.2 (C(2′)), 83.2 (C(6)), 88.5 (C(1)), 118.2 (C(5a)), 149.5 (C(5)), 169.6 and 170.3 (C(7), C(9)) ppm; MS (+CI) 346 $[M + 1]^+$; M_r (+CI) 346.162 23 $[M + 1]^+$ (calcd for $C_{14}H_{24}N_3O_7$ 346.161 43).

General Procedure for the Reaction of Bicyclomycins 1 and 13−15 with EtSH. To a degassed (Ar, 5 min) THF−H_2O solution (3:1, 4 mL) containing the bicyclomycin (1 equiv) was added EtSH (~16 equiv). The "pH" of the reaction mixture was adjusted with aqueous dilute NaOH, and the solution was maintained at room temperature under an Ar atmosphere. The solvent was removed in vacuo, and the residue was purified by PTLC (20% MeOH−$CHCl_3$) to afford the product(s).

By use of this procedure, the following compounds were prepared.

Reaction of 1 with EtSH at "pH" 9. Using **1** (10 mg, 0.03 mmol) and EtSH (33 mg, 0.53 mmol) resulted in a drop of "pH" to 8.3 during the reaction time (16 h) and provided **34**[6,9] as a white solid (4 mg, 33%): R_f 0.65 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2′)CH_3), 1.24 (t, J = 7.5 Hz, 3 H, SCH_2CH_3), 1.81−1.93 (m, 1 H, C(4)*H*H′), 2.30 (dt, J = 6.5, 14.1 Hz, 1 H, C(4)H*H*′), 2.58 (q, J = 7.5 Hz, 2 H, SCH_2CH_3), 2.90 (d, J = 14.1 Hz, 1 H, C(5a)*H*H′), 3.00 (d, J = 14.1 Hz, 1 H, C(5a)H*H*′), 3.64 (d, J = 12.0 Hz, 1 H, C(3′)*H*H′), 3.68−3.78 (m, 1 H, C(3)*H*H′), 3.90 (s, 1 H, C(1′)H), 4.03 (d, J = 12.0 Hz, 1 H, C(3′)H*H*′), 3.92−4.06 (m, 1 H, C(3)H*H*′).

Reaction of 1 with EtSH at "pH" 10.5. Using **1** (21 mg, 0.07 mmol) and EtSH (69 mg, 1.11 mmol) resulted in a drop of "pH" to 9.9 during the reaction time (14 h) and provided the following compounds.

Compound **35**:[9] yield, 8 mg (33%); R_f 0.45 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.23 (t, J = 7.2 Hz, 3 H, SCH_2CH_3), 1.32 (s, 3 H, C(2′)CH_3), 2.05−2.26 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.42−2.55 (m, 2 H, SCH_2CH_3), 3.15 (d, J = 11.7 Hz, 1 H, C(5a)H*H*′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.73−3.84 (m, 1 H, C(3)-*H*H′), 3.90−4.00 (m, 1 H, C(3)H*H*′), 4.03 (s, 1 H, C(1′)H).

Compound **34**:[6,9] yield, 10 mg (40%); R_f 0.65 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.15 (s, 3 H, C(2′)CH_3), 1.24 (t, J = 7.3 Hz, 3 H, SCH_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*′), 2.58 (q, J = 7.3 Hz, 2 H, SCH_2CH_3), 2.89 (d, J = 14.0 Hz, 1 H, C(5a)*H*H′), 3.00 (d, J = 14.0 Hz, 1 H, C(5a)H*H*′), 3.64 (d, J = 12.2 Hz, 1 H, C(3′)*H*H′), 3.68−3.78 (m, 1 H, C(3)*H*H′), 3.90 (s, 1 H, C(1′)H), 4.03 (d, J = 12.2 Hz, 1 H, C(3′)H*H*′), 3.97−4.03 (m, 1 H, C(3)H*H*′).

Reaction of 13 with EtSH at "pH" 9.5. Using **13** (6 mg, 0.02 mmol) and EtSH (20 mg, 0.32 mmol) resulted in a drop of "pH" to 9.0 during the reaction time (2 d). TLC analysis indicated no reaction. PTLC of the reaction solution led to the recovery of **13** (3 mg, 50% recovery) as a white solid: R_f 0.22 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.61−2.67 (m, 2 H, C(4)H_2), 3.50 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.66 (d, J = 11.3 Hz, 1 H, C(3′)H*H*′), 3.80−3.84 (m, 1 H, C(3)*H*H′), 3.92−3.96 (m, 1 H, C(3)H*H*′), 4.07 (s, 1 H, C(1′)H), 5.16 (s, 1 H, C(5a)*H*H′), 5.46 (s, 1 H, C(5a)H*H*′).

Reaction of 13 with EtSH at "pH" 10.0. Using **13** (6 mg, 0.02 mmol) and EtSH (20 mg, 0.32 mmol) resulted in a drop of "pH" to 9.5 during the reaction time (22 h) and provided **36**[13] (2 mg, 36%) as a white solid: R_f 0.68 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.74−2.82 (m, 2 H, C(4)H_2), 3.82 (d, J = 9.6 Hz, 1 H, C(3′)*H*H′), 3.96 (d, J = 9.6 Hz, 1 H, C(3′)H*H*′), 4.07−4.18 (m, 2 H, C(3)H_2), 4.41 (s, 1 H, C(1′)H), 5.34 (br s, 1 H, C(5a)*H*H′), 5.40 (br s, 1 H, C(5a)-H*H*′).

Reaction of 14 with EtSH at "pH" 8.8. Using **14** (5.0 mg, 0.02 mmol) and EtSH (16 mg, 0.32 mmol) resulted in a drop of the solution "pH" to 8.2 during the reaction (46 h) and provided **38** (2 mg, 40%) as a semisolid: R_f 0.25 (20% MeOH−$CHCl_3$); IR (KBr) 3434, 1681 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.37−2.44 (m, 2 H, C(4)H_2), 3.67 (t, J = 6.9 Hz, 2 H, C(3)H_2), 3.77 (d, J = 9.5 Hz, 1 H, C(3′)*H*H′), 3.90 (d, J = 9.5 Hz, 1 H, C(3′)H*H*′), 4.48 (s, 1 H, C(1′)H), 5.19 (s, 1 H, C(5a)*H*H′), 5.46 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 21.3 (C(2′)CH_3), 35.3 (C(4)), 62.0 (C(3)), 77.6, 78.2 and 78.5 (C(1′), C(2′), C(3′)), 80.0 (C(6)), 90.1 (C(1)), 116.8 (C(5a)), 143.7 (C(5)), 168.6 and 169.3 (C(7), C(9)) ppm; MS (+CI) 318 $[M + 1]^+$; M_r (+CI) 318.132 29 $[M + 1]^+$ (calcd for $C_{12}H_{20}N_3O_7$ 318.130 13).

Reaction of 14 with EtSH at "pH" 10.0. Using **14** (14.0 mg, 0.04 mmol) and EtSH (44 mg, 0.64 mmol) resulted in a drop of the solution "pH" to 9.6 during the reaction (17 h) and provided **38** (3 mg, 21%) as a semisolid: R_f 0.25 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.35−2.47 (m, 2 H, C(4)H_2), 3.67 (t, J = 6.9 Hz, 2 H, C(3)H_2), 3.77 (d, J = 9.5 Hz, 1 H, C(3′)*H*H′), 3.90 (d, J = 9.5 Hz, 1 H, C(3′)-H*H*′), 4.48 (s, 1 H, C(1′)H), 5.19 (s, 1 H, C(5a)*H*H′), 5.46 (s, 1 H, C(5a)H*H*′).

Reaction of 15 with EtSH at "pH" 9.5. Using **15** (8 mg, 0.02 mmol) and EtSH (21 mg, 0.34 mmol) resulted in a drop of "pH" to 9.0 during the reaction time (18 h) and provided **36**[13] (3 mg, 42%) as a white solid: R_f 0.70 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.77−2.87 (m, 2 H, C(4)H_2), 3.82 (d, J = 9.5 Hz, 1 H, C(3′)*H*H′), 3.96 (d, J = 9.5 Hz, 1 H, C(3′)H*H*′), 4.09−4.20 (m, 2 H, C(3)H_2), 4.42 (s, 1 H, C(1′)H), 5.34 (br s, 1 H, C(5a)*H*H′), 5.40 (br s, 1 H, C(5a)-H*H*′).

Synthesis of 37. To an anhydrous dioxane solution (2 mL) of **36** (6 mg, 0.02 mmol), *p*-bromobenzyl chloride (14 mg, 0.06 mmol), and diisopropylethylamine (8 mg, 0.06 mmol) was added a catalytic amount of DMAP. The solution was stirred at room temperature (4 h) and concentrated in vacuo. The residue was purified by PTLC (2 × 20% ethyl acetate−benzene) to provide **36** (3 mg, 50% recovery) and **37**[22] (2 mg, 33%) as a white solid: mp 215−218 °C, R_f 0.60 (10% MeOH−$CHCl_3$); IR (KBr) 3447, 3206, 3113, 1735, 1688, 1590, 1485 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.41 (s, 3 H, C(2′)CH_3), 2.70−2.79 (m, 2 H, C(4)H_2), 3.84 (dd, J = 6.8, 13.7 Hz, 2 H, C(3)H_2), 3.98 (d, J = 9.5 Hz, 1 H, C(3′)*H*H′), 4.08 (d, J = 9.5 Hz, 1 H, C(3′)-H*H*′), 5.29 (br s, 1 H, C(5a)*H*H′), 5.32 (br s, 1 H, C(5a)H*H*′), 5.56 (s, 1 H, C(1′)H), 7.66 (d, J = 8.4 Hz, 2 H, ArH), 8.01 (d, J = 8.4 Hz, 2 H, ArH); ^{13}C NMR (CD_3OD) 21.0 (C(2′)CH_3), 32.7 (C(4)), 68.4 (C(3)), 77.8, 78.7 and 80.2 (C(1′), C(2′), C(3′)), 89.0, 91.0 (C(1), C(6)), 111.4 (C(5a)), 129.5 (Ar, C(4″)), 132.8 (2 Ar, C(2″), 132.9 (2 Ar, C(3″)), 133.1 (Ar, C(1″)), 150.5 (C(5)), 166.2, 168.4 and 169.4 (C(7), C(9), *C*(O)Ar) ppm; MS (+CI) 467 (41), 469 (59) $[M + 1]^+$; M_r (+CI) 467.045 85 $[M + 1]^+$ (calcd for $C_{19}H_{20}{}^{79}BrN_2O_7$ 467.045 39).

X-ray Crystallographic Study of 37.[21] Crystals of **37** belong to the space group P_1 (triclinic) with a = 6.077(1) Å, b = 10.039(2) Å, c = 11.403(2) Å, α = 114.62(1)°; β = 97.05(1)°, γ = 102.22(1)°; V = 600 Å^3, D_{calcd} = 1.62 g cm^{-3}, and Z = 1. Data were collected at −50 °C, and the structure was refined to R_f = 0.034, R_w = 0.025 for 2178 reflections with $I > 3\sigma(I)$.

X-ray Crystallographic Study of 24.[21] Crystals of **24** belong to the space group $P2_12_12_1$ (orthorhombic) with a = 7.155(1) Å, b = 9.104(3) Å, c = 25.169(7) Å; V = 1639 Å^3, D_{calcd} = 1.38 g cm^{-3}, and Z = 4. Data were collected at −50 °C, and the structure was refined to R_f = 0 034, R_w = 0.028 for 1359 reflections with $I > 3\sigma(I)$.

General Procedure for the Determination of the Stability of Bicyclomycin Derivatives. The stability of bicyclomycins (**1**, **13**−**15**) in aqueous buffered solution (40 mM Tris, pH = 7.4) was determined by HPLC. A 2 mg/mL solution of the bicyclomycin derivative was maintained at 37 ± 1 °C. The pH of the reaction mixture was determined before and after the reaction. The pH at the end of the reaction was 7.4 ± 0.1. Linear plots of ln A_0/A (λ = 214 nM) versus time were obtained for an average of 10 points per bicyclomycin derivatives over the course of the reaction. Most of the reactions were monitored for more than two half lives and the identified products were verified by co-injection of authentic samples with the reactions and further verified by TLC analysis. Using a least squared program the half life of the derivative was calculated.

Stability of C(6)-Aminobicyclomycin (13). An aqueous buffer solution (2 mL, 40 mM Tris, pH = 7.4) of **13** (5 mg, 0.02 mmol) was maintained at 37 ± 1 °C (125 h). During the course

of the reaction a change of pH was recorded from 7.4 to 7.7. The reaction was then concentrated in vacuo, and the residue was purified by PTLC (2 × 15% MeOH−$CHCl_3$) to provide **42**[22,25] (6 mg, 63%) as a white solid: R_f 0.56 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H, C(2′)CH_3), 2.72−2.84 (m, 2 H, C(4)H_2), 3.80−3.84 (m, 1 H, C(3′)*H*H′), 3.95−3.97 (m, 1 H, C(3′)H*H*′), 4.05−4.22 (m, 2 H, C(3)H_2), 4.41−4.42 (m, 1 H, C(1′)H), 5.25−5.41 (m, 2 H, C(5a)H_2).

Molecular Orbital Calculations. Density functional calculations were performed with the Gaussian92/DFT[31] program package using the internally stored 6-31G** [32] basis set at the B3LYP[33] level. Geometry optimizations of all species in eq 1 were performed under their respective point groups (D_{2h} for ethylene (**44**), C_s for ethylamine (**46**), and C_1 for **45a**−**c** and **47a**−**c**). The only additional constraints were imposed upon **47a**−**c** in which the newly formed α,β-unsaturated ketone, imine, or thione was held in a cisoid conformation maintaining a 45° dihedral angle between the C═C and C═X bonds, mimicking the lowest energy conformation of β-methylene-α-ketoamide **2** as determined by molecular modelling using PC MODEL.[24] Single-point calculations in which this dihedral was held at 0° and the dihedral angle between the C═O and C═X bonds was widened to 45° resulted in consistent energy changes smaller than 0.5 kcal/mol lending confidence in the energy values obtained from this model compound. All converged geometries were confirmed as minima by performing vibrational frequency calculations. Free energies of all compounds were calculated at STP by applying thermal and entropy corrections obtained from the frequency calculations to the electronic energies. The thermal corrections account for zero-point vibrational energy as well as contributions from the population of various translational, rotational, and vibrational energy levels assuming an ideal gas in the canonical ensemble.

Acknowledgment. We thank the National Institutes of Health (Grant GM37934) and the Robert A. Welch Foundation (Grants E-607, H.K., E-1381, W.R.W.) for their support of this research. We thank Dr. M. Kawamura and the Fujisawa Pharmaceutical Co., Ltd., Japan, for the gift of bicyclomycin, Dr. T. Platt (University of Rochester) for the overproducing strain of rho, Dr. A. Magyar (University of Houston) for his help in the transcription termination assays, and Dr. James Korp (University of Houston) for conducting the X-ray crystallographic analyses of **24** and **37**. We thank Dr. Thomas Albright (University of Houston) for his contributions to the computational studies.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds prepared in this study and X-ray crystallographic information (data collection, final atomic positional parameters, atomic thermal parameters (anisotropic displacement parameters, H atom coordinates and isotropic displacement parameters), bond distances and angles) for **24** and **37** (60 pages). Ordering information is given on any current masthead page.

References

(1) Tanaka, N. *Antibiotics (N.Y.)* **1979**, *5*, 18−25.
(2) (a) Miyoshi, T.; Miyari, N.; Aoki, H.; Kohsaka, M.; Sakai, H.; Imanaka, H. Bicyclomycin, a new antibiotic I. Taxonomy, isolation and characterization. *J. Antibiot.* **1972**, *25*, 569−575. (b) Kamiya, T.; Maeno, S.; Hashimoto, M.; Mine, Y. Bicyclomycin, a new antibiotic. II. Structural elucidation and acyl derivatives. *J. Antibiot.* **1972**, *25*, 576−581. (c) Nishida, M.; Mine, Y.; Matsubara, T. Bicyclomycin, a new antibiotic. III. *In vitro* and *in vivo* antimicrobial activity. *J. Antibiot.* **1972**, *25*, 582−593. (d) Nishida, M.; Mine, Y.; Matsubara, T.; Goto, S.; Kuwahara, S. Bicyclomycin, a new antibiotic. IV. Absorption, excretion and tissue distribution. *J. Antibiot.* **1972**, *25*, 594−601.
(3) (a) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Takana, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K.; Matuski, K.; Hoshino, T. Antibiotic No. 5879, a new water-soluble antibiotic against gram-negative bacteria. *J. Antibiot.* **1972**, *25*, 610−612. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H. Antibiotic 5879 produced by *Streptomyces Aizunesis*, identical with bicyclomycin *J. Antibiot.* **1973**, *26*, 479−484.
(4) Williams, R. M.; Durham, C. A. Bicyclomycin: Synthetic, mechanistic, and biological studies. *Chem. Rev.* **1988**, *88*, 511−540 and references cited therein.
(5) Someya, S.; Iseki, M.; Tanaka, N. Active groups of bicyclomycin and the reaction with thiols. *J. Antibiot.* **1979**, *32*, 402−407.
(6) Abuzar, S.; Kohn, H. Observations on the activation of bicyclomycin. *J. Am. Chem. Soc.* **1988**, *110*, 4089−4090.
(7) Abuzar, S.; Kohn, H. Studies on the reactivity of bicyclomycin with nucleophilic amino acid derivatives. *J. Org. Chem.* **1989**, *54*, 4000−4003.
(8) Abuzar, S.; Kohn, H. Studies on the reactivity of bicyclomycin with amines. *J. Am. Chem. Soc.* **1989**, *111*, 4895−4903.
(9) Abuzar, S.; Kohn, H. Studies on the reactivity of bicyclomycin with thiols. *J. Am. Chem. Soc.* **1990**, *112*, 3114−3121.
(10) Zweifka, A.; Kohn, H.; Widger, W. R. Transcription termination factor rho: The site of bicyclomycin inhibition in *Escherichia coli*. *Biochemistry* **1993**, *32*, 3564−3570.
(11) Magyar, A.; Zhang, X.; Kohn, H.; Widger, W. R. The antibiotic bicyclomycin affects the secondary RNA binding site of *Escherichia coli* transcription termination factor Rho. *J. Biol. Chem.* **1996**, *271*, 25369−25374.
(12) Park, H.-g.; Zhang, X.; Moon, H.-s.; Zwiefka, A.; Cox, K.; Gaskell, S. J.; Widger, W. R.; Kohn, H. Bicyclomycin and dihydrobicyclomycin inhibition kinetics of *Escherichia coli* rho-dependent transcription termination factor ATPase activity. *Arch. Biochem. Biophys.* **1995**, *323*, 447−454.
(13) Santillán, Jr., A.; Park, H.-g.; Zhang, X.; Lee, O.-S.; Widger, W. R.; Kohn, H. Role of the [4.2.2] bicyclic unit in bicyclomycin: Synthesis, structure, chemical, biochemical, and biological properties. *J. Org. Chem.* **1996**, *61*, 7756−7763.
(14) Wu, A. M.; Christie, G. E.; Platt, T. Tandem termination sites in the tryptophan operon of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2913−2917.
(15) Sharp, J. A.; Galloway, J. L.; Platt, T. A kinetic mechanism for the poly(C)-dependent ATPase of the *Escherichia coli* transcription termination protein, rho. *J. Biol. Chem.* **1983**, *258*, 3482−3486.
(16) Ericsson, H. M.; Sherris, J. C. Antibiotic sensitivity testing. *Acta Pathol. Microbiol. Scand.* **1971**, Suppl. 217, 1−90.
(17) Kamiya, T.; Maeno, S.; Kitaura, Y. Belgium patent 847 475.
(18) (a) Zhang, Z.; Kohn, H. The role of the C-1 triol group in bicyclomycin. *J. Chem. Soc., Chem. Commun.* **1994**, 1343−1344. (b) Zhang, Z.; Kohn, H. Chemical, biochemical, and biological studies on select C(1) triol modified bicyclomycins. *J. Am. Chem. Soc.* **1994**, *116*, 9815−9826.
(19) Jackman, L. M.; Sternhall, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969.
(20) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972.
(21) The authors have deposited X-ray crystallographic data, a description of the structure determination, and tables of atomic coordinates and isotropic thermal parameters, bond lengths and angles, anisotropic thermal parameters, and refined and calculated hydrogen atom coordinates with the Cambridge Crystallographic Data Centre. The data can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 lEZ, U.K.
(22) Maag, H.; Blount, J. F.; Coffen, D. L.; Steppe, T. V.; Wong, F. Structure, absolute configuration, and total synthesis of an acid-catalyzed rearrangement product of bicyclomycin. *J. Am. Chem. Soc.* **1978**, *100*, 6786−6788.
(23) Comparable findings were observed for C(6)-*O*-methylhydroxylaminobicyclomycin (**20**); see: Santillán, A., Jr. Ph.D. Thesis, University of Houston, 1997.
(24) Molecular mechanics calculations were performed using the program PC MODEL available from Serena Software, Bloomington, IN.
(25) Kohn, H.; Abuzar, S. Studies on the chemical reactivity of bicyclomycin: Acid hydrolysis. *J. Org. Chem.* **1988**, *53*, 2769−2773.
(26) Smith, P. A. S. *The Chemistry of Open-Chain Organic Nitrogen Compounds*; W. A. Benjamin, Inc.: New York, 1966; Chapter 8, pp 1−118.
(27) Park, H.-g.; Zhang, X.; Widger, W. R.; Kohn, H. Role of the C(1) trial group in bicyclomycin: Synthesis and biochemical and biological properties. *J. Org Chem.* **1996**, *61*, 7750−7755.
(28) For representative examples, see: (a) Wells, T. N. C.; Fersht, A. R. Hydrogen bonding in enzymatic catalysis analysed by protein engineering. *Nature* **1985**, *316*, 656−657. (b) Sams, C. F.; Vyas, N. K.; Quiocho, F. A.; Matthews, K. S. Predicted structure of the sugar-binding site of the *lac* repressor. *Nature* **1984**, *310*, 429−430.

(29) Mott, J. E.; Grant, R. A.; Ho, Y.-S.; Platt, T. Maximizing gene expression from plasmid vectors containing the λ PL promoter: strategies for overproducing transcription termination factor rho. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 88−92.
(30) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Wong, M. W.; Foresman, J. B.; Robb, M. A.; Head-Gordon, M.; Replogle, E. S.; Gomperts, R.; Andres, K. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *Gaussian 92/DFT, Revision G.4*, Gaussian, Inc., Pittsburgh, PA, 1993.
(32) (a) Hehre, W. J.; Ditchfield, R.; Pople, J. A. Self-consistent molecular orbital methods. XII. Further extensions of Gaussian-type basis sets for use in molecular orbital studies of organic molecules. *J. Chem. Phys.* **1972**, *56*, 2257−2261. (b) Francl, M. M.; Pietro, W. J.; Hehre, W. J.; Binkley, J. S.; Gordon, M. S.; Defrees, D. J.; Pople, J. A. Self-consistent molecular orbital methods. XXIII. A polarization-type basis set for second-row elements. *J. Chem. Phys.* **1982**, *77*, 3654−3665.
(33) (a) Becke, A. D. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys. Rev. A* **1988**, *38*, 3098−3100. (b) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B.* **1988**, *37*, 785−789. (c) Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

JM9708386