Role of the C(1) Triol Group in Bicyclomycin: Synthesis and Biochemical and Biological Properties

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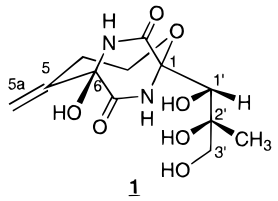
Bicyclomycin (**1**) is a commercial antibiotic whose primary site of action in *Escherichia coli* is the essential cellular protein transcription termination factor rho. The bicyclomycin binding domain in rho is unknown; however, enzyme irreversible inactivators that modify rho upon activation may identify the site. In this study, we investigated the importance for rho binding of the C(1) triol group in **1**. Twelve bicyclomycin derivatives were prepared, and the C(1) triol group was modified at the C(1′), the C(2′), and the C(3′) sites. The compounds were evaluated by rho-dependent ATPase and transcription termination assays and their antimicrobial activities assessed using a filter disc assay. Bicyclomycin inhibited both rho-dependent ATPase (I_{50} = 60 μM) and rho-dependent transcription termination ($I_{50} \sim 5$ μM) processes and had a minimum inhibitory concentration value of 0.25 mg/mL against *E. coli* W3350 cells. None of the 12 C(1) triol bicyclomycin derivatives significantly inhibited rho-dependent ATPase ($I_{50} > 400$ μM) and transcription termination ($I_{50} >$ 100 μM) activities or exhibited antibiotic activity at a 32 mg/mL concentration. These results indicated that there was a strong molecular complement between the C(1) triol group and its rho binding site. We concluded that the C(1) triol group in **1** is a critical structural element necessary for drug binding to rho and that an enzyme irreversible inactivating unit placed at this site would prohibit the bicyclomycin derivative from efficiently binding to rho.

The commercially available bicyclomycin (**1**) is a structurally unique peptide antibiotic that possesses a broad spectrum of antimicrobial activity against Gram-negative bacteria.[1−4] Chemical studies showed that **1** reacts with sulfur and nitrogen nucleophiles at the C(5)−C(5a) exomethylene group.[5−9] These findings have suggested that bicyclomycin functions by modifying select protein amino acid residues.[5,7]



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We have established the primary site of bicyclomycin action in *Escherichia coli* as the rho transcription termination factor.[10] Recent studies demonstrated that **1** inhibited rho-dependent transcription termination processes[11] and that bicyclomycin was a reversible, noncompetitive inhibitor of ATP in the rho-dependent ATPase kinetic assay.[12]

Little is known about the bicyclomycin binding domain in rho and whether *in vivo* bicyclomycin function requires covalent modification of rho. Prolonged incubation (37 °C, 8−48 h) of bicyclomycin with rho without RNA gave rho−mono-, di-, tri-, and tetrabicyclomycin-substituted adducts with diminished transcription termination activities.[13] Identifying these modification sites is an important step in determining the binding pocket provided that drug adduction proceeds at the primary binding site(s). We expect that bicyclomycin mimics or probes (enzyme irreversible inactivators[14]) that efficiently bind to rho and then selectively covalently modify nearby amino acid residues upon treatment (e.g., photochemical, reductive amination) will provide information concerning the amino acid residues that comprise the binding pocket. In order to design these probes, we have determined the structural units in **1** essential for rho binding. Bicyclomycin was divided into three sectors: C(1) triol group; [4.2.2] bicyclic unit; and C(5)−C(5a) exomethylene moiety (Figure 1). Each unit was structurally modified, and the

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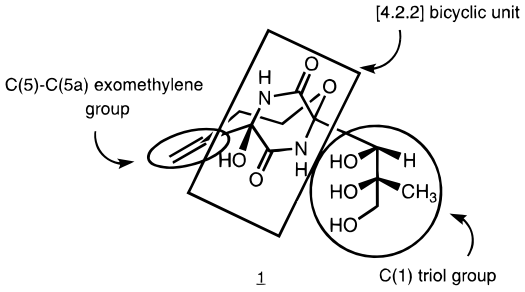

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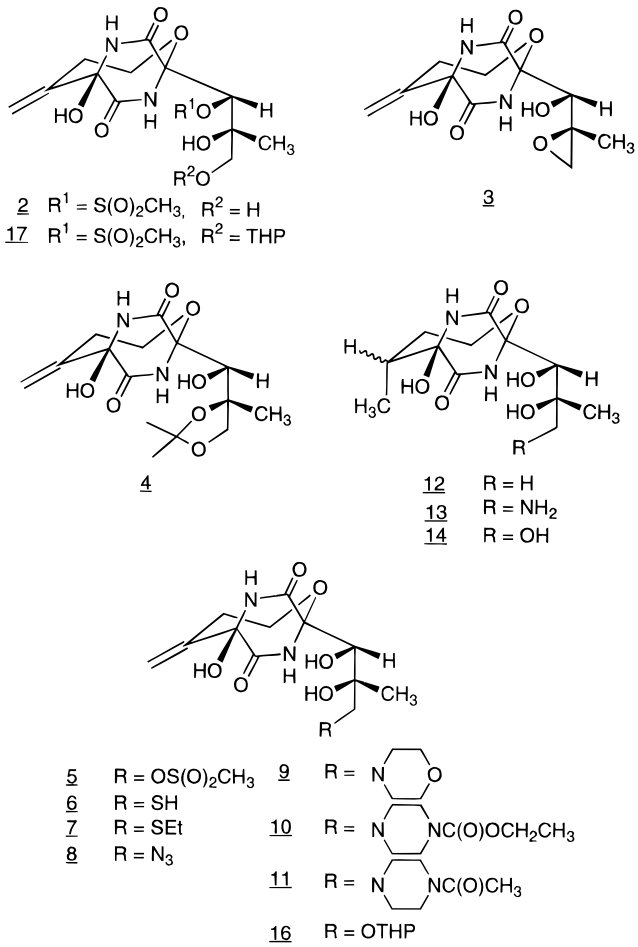
Figure 1. Three Bicyclomycin Structural Components Used in the SAR Study

effect on bicyclomycin function was assessed using biochemical and biological assays. In this paper we report that the C(1) triol group plays a critical role in the binding process to rho. In the following papers, we report the structure−activity relationship (SAR) for the [4.2.2] bicyclic unit and the C(5)−C(5a) exomethylene group in **1**.

Results

A. Selection of Compounds and Criteria for Bicyclomycin Analogue Binding to Rho. Ten bicyclomycin derivatives were prepared to determine the effect of C(1) triol modification on biochemical activity. We made substitutions that potentially altered the hydrogen-bonding interactions of the triol group with the receptor site or sterically impeded drug binding to rho. These compounds were the C(1′)-modified bicyclomycin **2**, the two C(2′),C(3′)-disubstituted bicyclomycins **3**[15] and **4**,[16,17] and the seven C(3′)-modified bicyclomycin adducts **5**−**11**.[9,15,18,19] The dihydrobicyclomycin derivatives **12** and **13** were also evaluated in our study. The synthetic procedures utilized for **12** and **13** did not permit the preparation of the corresponding C(5)−C(5a) unsaturated derivatives. Accordingly, the effects of the C(1) triol modifications in **12** and **13** on rho binding were assessed by comparing their biochemical activities with that of dihydrobicyclomycin (**14**).[2b] Compound **14** displayed rho transcription termination[11] and ATPase inhibitory properties similar to those of **1**.[12]

Two assays were used to assess whether bicyclomycin C(1) triol modification affected the bicyclomycin analogue binding to rho. These results indicated the bicyclomycin adduct's ability to inhibit rho-dependent ATPase activity[20] and rho-dependent transcription termination.[21] We also determined the antibiotic activity of each bicyclomycin analogue.[22] In our SAR study, we assumed that bicyclomycin derivatives that had activity comparable to bicyclomycin in ATPase and transcription termination assays bound to the bicyclomycin−rho binding site. If the bicyclomycin derivatives and bicyclomycin activity



was not comparable, the derivatives did not bind to the active site. Structural modifications that fell into this latter category were assumed to be poor choices for incorporation in bicyclomycin affinity probes. Although the antimicrobial assay results were of interest, they were not used in our design of biomechanistic probes since we could not readily determine the transport, metabolic, and efflux properties of the bicyclomycin derivatives. A comprehensive SAR study based on antibiotic activity has been reported.[15]

B. Syntheses. We used published procedures for the syntheses of **3**−**5**,[15,16,19] **7**,[9] and **9**−**11**.[18] C(3′)-Deoxybicyclomycin C(3′)-thiol[15] (**6**) was produced in 52% yield, along with the corresponding disulfide **15** (36%), upon treatment of a methanolic solution of bicyclomycin C(3′)-*O*-methanesulfonate[15,19] (**5**) with NaSH. Similarly, when NaN_3 was added to a 50% aqueous methanolic solution containing **5**, NH_4Cl, and $LiClO_4$, it gave **8** (44%).[23] Bicyclomycin C(1′)-*O*-methanesulfonate (**2**) was synthesized from **1** in three steps (41% overall yield). Bicyclomycin was first converted to the C(3′)-tetrahydropyranyl ether **16**[15] and then treated with methanesulfonyl chloride in pyridine to give **17**. Deprotection of crude **17** with *p*-toluenesulfonic acid gave **2**. Dihydrobicyclomycin analogues **12** and **13** were prepared by catalytic hydrogenation of **3** and **8**, respectively. Compound **12** showed two sets of ^{13}C NMR signals each for the C(4), C(5), and C(5a) resonances in an approximate 6:1 ratio. This indicates that catalytic reduction had led to a diastereomeric mixture. C(3′)-Deoxy C(5),C(5′)-dihydrobicyclomycin C(3′)-amine **13** was stable in aqueous and methanolic solutions

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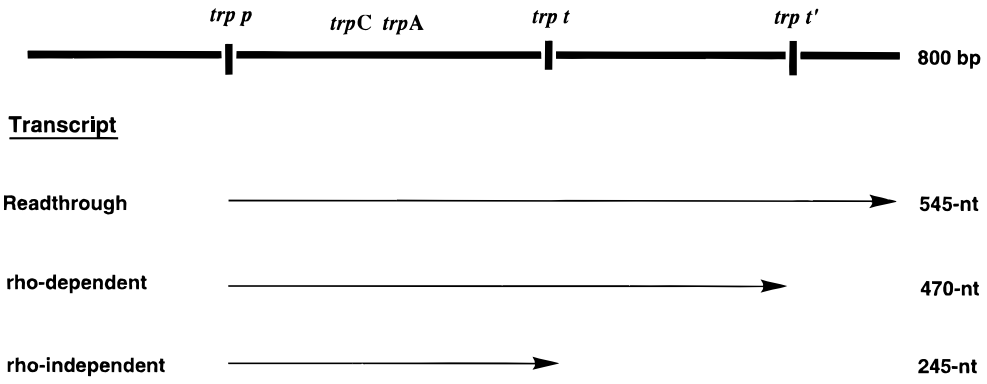
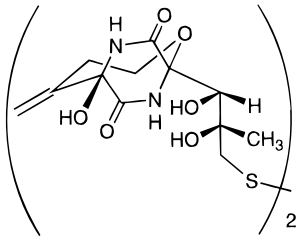


Figure 2.

at room temperature for short periods of times (<4 h). Maintenance of these solutions for longer times (18 h) led to the formation of a new compound whose structure has not been determined (NMR analyses).



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C. Biochemical and Biological Properties. The C(1) triol-modified bicyclomycin and dihydrobicyclomycin derivatives were evaluated to determine the effect of the modification on rho binding. The rho-dependent ATPase assay was the primary measure.[20] This assay measured the ability of the bicyclomycin analogue to inhibit rho-dependent hydrolysis of ATP to ADP and inorganic phosphate (P_i). This activity is essential for rho-dependent termination of RNA transcripts. We have shown that both **1** and **14** were reversible, noncompetitive inhibitors of ATP.[12] Accordingly, we determined the initial rates of rho-dependent [γ-^{32}P]ATP hydrolysis as a function of bicyclomycin analogue concentration (0−400 μM). The I_{50} value corresponded to the bicyclomycin analogue concentration that provided 50% inhibition of ATP hydrolysis. For bicyclomycin (**1**) and dihydrobicyclomycin (**14**), the I_{50} values were 60 and 120 μM, respectively.[12]

In most cases, we also used the rho-dependent transcription termination assay[21] to monitor the bicyclomycin analogue binding to rho. This test measured the efficiency with which the bicyclomycin analogue inhibited rho-dependent RNA transcriptional processes.[11] The assay employed a DNA template containing a transcription initiation site (*trp p*) and downstream rho-independent transcription termination (*trp t*) and rho-dependent transcription termination (*trp t′*) sites (Figure 2). In the presence of rho only, the *trp t* and *trp t′* transcripts are observed when RNA polymerase and the RNA nucleotides (ATP, CTP, GTP, UTP, [α-^{32}P]UTP) were added. The ability of the bicyclomycin derivative to inhibit rho-dependent transcriptional termination processes is then determined by quantifying the relative amounts of radiolabeled RNA that corresponded to the *trp t′* (470 nucleotides) and the terminal readthrough transcript (545 nucleotides), as a function of bicyclomycin analogue concentration. The I_{50} value for the rho inhibitor corresponded to the concentration of bicyclomycin derivative that gave 50% of the transcripts corresponding to the rho-dependent termination (*trp t′*) site, excluding the *trp t* transcript (245 nucleotides). Under these conditions, bicyclomycin prevented *trp t′* production fully at 20 μM and had an approximate I_{50} value of 5 μM.[11] Dihydrobicyclomycin (**14**), a bicyclomycin derivative with lower inhibitory properties in the ATPase assay, inhibited *trp t′* transcripts formation at 100 μM and had an approximate I_{50} value of 20 μM.[11] Due to the expense of this assay, we adopted a four-tier protocol for general screening. New bicyclomycin analogues were first tested at 100 μM. The compounds that completely prevented *trp t′* synthesis were evaluated at 40 μM. If full rho inhibition was observed at this lower dose, the compound was screened at 10 μM. If promising activity was observed at 10 μM, the compound was evaluated at four or five different concentrations and the I_{50} value estimated.

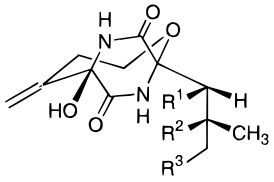
The final method used to evaluate bicyclomycin analogues **3**−**12** and **14** was an antimicrobiological assay. We employed a filter disc assay,[22] in which bicyclomycin or the bicyclomycin analogue was applied to a disc centered on an agar plate with W3350 *E. coli* and then incubated. Determination of the zone of inhibited growth (cm^3) versus the amount of the bicyclomycin analogue permitted the calculation of the minimum inhibitory concentration (MIC) value. We observed that the MIC values for **1**[18] and **14** were 0.25 and 1 mg/mL, respectively.

The biochemical and biological data obtained for compounds **2**−**13** along with those for bicyclomycin (**1**) and dihydrobicyclomycin (**14**) are listed in Table 1. None of the C(1) triol-modified compounds efficiently inhibited rho ATPase activity. Use of a 400 μM solution of **2**−**13** led to less than a 23% inhibition of rho-dependent ATPase activity. Evaluation of compounds **3**−**9** and **12** in the transcription termination assay gave comparable findings (Figure 3). No appreciable inhibition of rho-dependent transcription termination was observed for these bicyclomycin analogues at 100 μM concentration levels. Consistent with these biochemical results, compounds **2**−**12** displayed no significant antimicrobial activities in the filter disc assay. The instability of **13** in aqueous solutions for extended periods of time did not permit us to reliably determine the antimicrobial activity of this compound using this assay.

Discussion

Modification of C(1) triol group of bicyclomycin and dihydrobicyclomycin led to a pronounced loss in the ability to inhibit rho-dependent ATPase and transcription

Table 1. Biochemical and Biological Activities of C(1) Triol Modified Bicyclomycin (1−11) and Dihydrobicyclomycin (12−14) Derivatives



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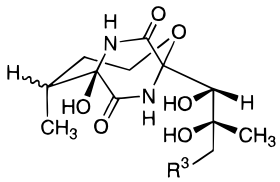


12-14

				inhibition of ATPase activity[a]		TT activity[d]		
compd	R^1	R^2	R^3	I_{50} (μM) (BCM)[b]	400 μM (%) (BCM)[c]	I_{50} (μM)[e]	100 μM (%)[f]	MIC[g] (mg/mL)
1	OH	OH	OH	60	95	~5	100	0.25
2	OMs	OH	OH	>400 (60)	3 (93)	h	h	>32 (0.25)
3	OH	$R^2,R^3 = O$		>400 (60)	6 (93)	>100	24	>32 (0.25)
4	OH	$R^2,R^3 = OC(CH_3)_2O$		>400 (60)	6 (93)	>100	0	>32 (0.25)
5	OH	OH	OMs	>400 (60)	8 (94)	>100	3	>32 (0.34)
6	OH	OH	SH	>400 (60)	23 (94)	>100	0	>32 (0.34)
7	OH	OH	SEt	>400 (60)	2 (93)	>100	0	>32 (0.25)
8	OH	OH	N_3	>400 (60)	6 (94)	>100	0	>32 (0.34)
9	OH	OH	*N*-morpholine	>400 (40)	19 (96)	>100	0	>32 (0.45)
10	OH	OH	*N*-(*N*′-carbethoxy)piperidine	>400 (40)	9 (96)	h	h	>32 (0.45)
11	OH	OH	*N*-(*N*′-acetyl)piperidine	>400 (40)	8 (96)	h	h	>32 (0.45)
12	OH	OH	H	>400 (70)	4 (96)	>100	0	>32 (0.45)
13	OH	OH	NH_2	>400 (60)	5 (95)	h	h	i
14	OH	OH	OH	120 (75)	79 (92)	~20	100	1.00 (0.35)

[a] Activity measured using the ATPase assay (ref 20). [b] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. The corresponding value obtained from bicyclomycin in concurrently run experiments is provided in parentheses. [c] The percent inhibition of ATPase activity at 400 μM. The corresponding value obtained from bicyclomycin in concurrently run experiments is provided in parentheses. [d] Activity in the transcription termination assay was determined by the method of T. Platt and co-workers (ref 21). [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 (ref 22). The number in parentheses is the corresponding value obtained from bicyclomycin in concurrently run experiments. [h] Compound not tested. [i] The instability of **13** precluded the determination of the antimicrobial activity.

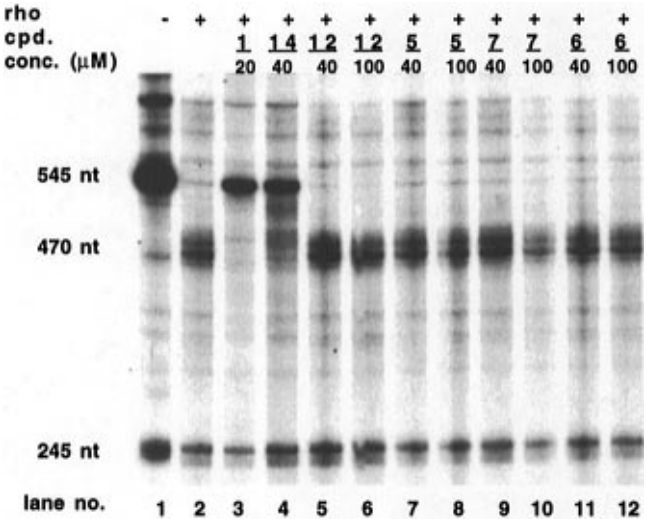
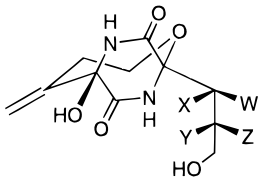


Figure 3. Autoradiograph of rho-dependent transcription termination assay for bicyclomycin and derivatives. The inhibition of *in vitro* rho-dependent transcription termination by bicyclomycin and its derivatives was determined using a modified *trp* operon template[29] and *E. coli* RNA polymerase and by measuring the incorporation of [α-^{32}P]UTP. The reactions were carried out in the absence of rho (lane 1), in the presence of rho (lane 2), and in the presence of bicyclomycin and derivatives (lanes 3−12): lane 3, **1** (20 μM); lane 4, **14** (40 μM); lane 5, **12** (40 μM); lane 6, **12** (100 μM); lane 7, **5** (40 μM); lane 8, **5** (100 μM); lane 9, **7** (40 μM); lane 10, **7** (100 μM); lane 11, **6** (40 μM); lane 12, **6** (100 μM). Relative amounts of transcripts were determined by densitometry of the autoradiograph.

termination activities. Bicyclomycin derivatives **2**−**11** did not appreciably inhibit rho-dependent ATPase activity at 400 μM, whereas **1** led to 95% inhibition of ATPase activity at this concentration.[12] Evaluation of **3**−**9** in the rho-dependent transcription termination assay gave similar results. There was no appreciable inhibition of transcription termination at 100 μM concentration, but bicyclomycin completely inhibited *trp t*′ transcript production at 20 μM.[11] Likewise, dihydrobicyclomycin derivatives **12** and **13** displayed no significant inhibition of ATPase activity (400 μM), and **12** did not inhibit transcription termination activity (100 μM). Dihydrobicyclomycin (**14**), however, inhibited both processes at 120[12] and 100 μM[11] concentrations, respectively. These results indicated that a strong molecular complementarity existed between the bicyclomycin C(1) triol group and its binding site on rho. We speculate that hydrogen bonding plays an important role in this process. A hydroxy group can serve as both a hydrogen bond donor and a hydrogen bond acceptor. The loss of biochemical activity in **7**, **8**, and **12** suggested that there are a series of hydrogen bond interactions between the C(1) triol unit in the drug and the receptor.

These results were in agreement with a recent study by Zhang and Kohn.[17] In this investigation we used the bicyclomycin derivatives **18** and **19** in which the stereochemical orientations of the C(1′) and C(2′) hydroxy groups in the C(1) triol moiety were reversed from those of **1** to determine the ATPase inhibitory properties. Inversion of the stereochemical centers at C(1′) and C(2′) led to significant loss of activity compared with bicyclomycin, indicating that the spatial orientation of these hydroxy groups was critical for drug binding.

None of the four C(3′) amine-substituted bicyclomycin derivatives (**9**−**11** and **13**) inhibited rho-dependent hy-



18 W = OH, X = H, Y = OH, Z = CH_3
19 W = H, X = OH, Y = CH_3, Z = OH

drolysis of ATP. This may be due to an interruption of the hydrogen bonding of the bicyclomycin mimic with the binding site or may simply be due to a bicyclomycin derivative ionization state change caused by protonation of the terminal amine at pH 7.9.

Consistent with our observations that **2**−**12** did not inhibit rho-dependent processes, we observed that none of these compounds exhibited antibiotic activity in the filter disc microbiological assay. The MIC values for **2**−**12** against *E. coli* W3350 cells exceeded 32 mg/mL, while the corresponding values for **1**[18] and **14** were 0.25 and 1.0 mg/mL, respectively.

The results of this study demonstrated the importance of the C(1) triol group for bicyclomycin binding to the transcription termination factor rho. These results complemented previous findings that showed that the distal C(1) triol also facilitated nucleophilic transformations at the C(5)−C(5a) exomethylene group in the drug.[9,24,25]

Conclusions

Our results demonstrate that the C(1) triol group is a critical structural element necessary for bicyclomycin binding to rho. We conclude that alteration of this moiety or placement of an enzyme irreversibly inactivating unit at this site would prohibit the bicyclomycin derivative from efficiently binding to rho.

Experimental Section

General Methods. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on 300 MHz spectrometers. Low-resolution and high-resolution (CI) mass spectral investigations were run at the University of Texas at Austin by Dr. M. Moini. 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 precoated silica gel GHLF slides (20 × 20 cm; Analtech No. 21521).

Rho protein was isolated from *E. coli* AR120 containing the overexpressing plasmid p39-AS[26] following previously published protocols.[26] Rho purity was determined by SDS-PAGE, and concentrations were determined by Lowry protein determination.[27] [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).

Preparation of C(3′)-Deoxybicyclomycin C(3′)-Thiol[15] (6). To a methanolic solution (3 mL) of **5**[15] (35 mg, 0.09 mmol) was added NaSH (5.2 mg, 0.09 mmol). The reaction mixture was stirred at room temperature (4 h), and then the solvent was removed *in vacuo*. TLC analysis (20% MeOH−$CHCl_3$) indicated the presence of two major compounds. The residue was dissolved in a minimum amount of MeOH and then purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **6** and a compound tentatively identified as **15**.

Compound 6:[15] yield, 15 mg (52%); mp 160−165 °C (lit.[15] mp 183−185 °C); R_f 0.65 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 3262 (br), 1686, 1407, 1075 cm^{-1}; ^{1}H NMR (CD_3-OD) δ 1.39 (s, 3 H), 2.53−2.68 (m, 3 H), 2.95 (d, J = 13.8 Hz, 1 H), 3.75−4.00 (m, 2 H), 4.28 (s, 1 H), 5.13 (s, 1 H), 5.56 (s, 1 H); ^{13}C NMR (CD_3OD) 25.3, 34.9, 36.7, 65.6, 71.5, 78.8, 85.9, 90.0, 116.8, 149.6, 168.7, 172.5 ppm; MS (+CI) 319 [M + 1]$^+$; M_r (+CI) 319.094 71 [M + 1]$^+$ (calcd for $C_{12}H_{19}N_2O_6S$ 319.096 38).

Compound 15: yield, 20 mg (36%); mp 163−169 °C; R_f 0.20 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 3261 (br), 1686, 1406, 1126, 1074 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.43 (s, 3 H), 2.55−2.68 (m, 2 H), 3.06 (d, J = 13.5 Hz, 1 H), 3.40 (d, J = 13.5 Hz, 1 H), 3.75−4.00 (m, 2 H), 4.16 (s, 1 H), 5.13 (s, 1 H), 5.55 (s, 1 H); ^{13}C NMR (CD_3OD) 26.2, 36.7, 53.1, 65.6, 72.2, 78.2, 83.0, 89.9, 116.8, 149.5, 168.5, 172.5 ppm; MS (+CI) 635 [M + 1]$^+$; M_r (+CI) 635.166 82 [M + 1]$^+$ (calcd for $C_{24}H_{35}N_4O_{12}S_2$ 635.169 29).

Preparation of Bicyclomycin C(3′)-Tetrahydropyranyl Ether[15] (16). To a dioxane solution (5 mL) of **1** (200 mg, 0.66 mmol) were added 3,4-dihydro-2*H*-pyran (167 mg, 2.0 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (2 mg). The solution was stirred at room temperature (4 h), and then the solvent was removed *in vacuo*. The residue was purified by column chromatography (10% MeOH−$CHCl_3$) to give a diastereomeric mixture (1:1) of **16**: yield, 172 mg (68%); mp 123−125 °C (lit.[15] mp 110−170 °C); R_f 0.31 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3402 (br), 3256 (br), 2942, 1662, 1404, 1072 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.37, 1.38 (s, 3 H), 1.40−1.95 (m, 6 H), 2.55−2.65 (m, 2 H), 3.40−3.95 (m, 6 H), 4.09, 4.13 (s, 1 H), 4.57, 4.65 (t, J = 3.0 Hz, 1 H), 5.13 (s, 1 H), 5.55 (s, 1 H); ^{13}C NMR (CD_3OD) 20.2, 20.5, 24.1, 24.8, 26.5, 26.6, 31.6, 36.7, 62.8, 63.4, 65.4, 65.6, 71.9, 72.3, 74.2, 74.5, 77.4, 78.1, 82.9, 89.6, 100.5, 100.8, 116.9, 149.5, 168.8, 172.5 ppm.

Preparation of Bicyclomycin C(1′)-*O*-Methanesulfonate (2). To an anhydrous pyridine solution (3 mL) of **16**[15] (10 mg, 0.022 mmol) was added methanesulfonyl chloride (10 mL, 0.07 mmol), and then the reaction was maintained at room temperature (5 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (SiO_2, 10% MeOH−$CHCl_3$) to give crude **17**. Compound **17** was dissolved in MeOH (3 mL), a catalytic amount of *p*-toluenesulfonic acid monohydrate (2 mg) was added, and the solution was stirred at room temperature (0.5 h). The solvent was removed *in vacuo*, and the residue was dissolved in a minimum amount of MeOH and then purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **2** as a semisolid: yield, 5 mg (61%); R_f 0.20 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3434 (br), 1662, 1174 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.43 (s, 3 H), 2.60−2.68 (m, 2 H), 3.07 (s, 3 H), 3.47 (d, J = 11.4 Hz, 1 H), 3.79 (d, J = 11.4 Hz, 1 H), 3.80−4.00 (m, 2 H), 5.15 (s, 1 H), 5.30 (s, 1 H), 5.77 (s, 1 H); ^{13}C NMR (CD_3OD) 25.3, 36.6, 39.4, 65.7, 68.4, 78.9, 83.0, 83.3, 88.6, 117.1, 149.4, 167.8, 172.4 ppm; mass spectrometry (+/−CI) did not afford a parent ion.

Preparation of C(3′)-Deoxybicyclomycin C(3′)-Azide (8). To a 50% aqueous methanolic solution (4 mL) containing **5** (30 mg, 0.079 mmol) were added NaN_3 (52 mg, 0.79 mmol), $LiClO_4$ (8.4 mg, 0.079 mmol), and NH_4Cl (4.2 mg, 0.079 mmol). The reaction solution was stirred at room temperature (36 h), and then the solvent was removed *in vacuo*. The residue was dissolved in a minimum amount of MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **8**: yield, 11 mg (44%); mp 130−143 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-

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IR (KBr) 3414 (br), 3256 (br), 2109, 1692, 1406 cm^{-1}; ^1H NMR (CD_3OD) δ 1.32 (s, 3 H), 2.55−2.70 (m, 2 H), 3.48 (s, 1 H), 3.49 (s, 1 H), 3.85−4.95 (m, 2 H), 4.02 (s, 1 H), 5.12 (s, 1 H), 5.56 (s, 1 H); ^{13}C NMR (CD_3OD) 24.6, 36.7, 60.4, 65.9, 71.9, 78.1, 81.7, 89.9, 116.8, 149.5, 168.4, 172.5 ppm; MS (+CI) 328 $[M + 1]^+$; M_r (+CI) 328.126 57 $[M + 1]^+$ (calcd for $C_{12}H_{18}N_5O_6$ 328.125 71).

Preparation of C(3′)-Deoxy-C(5),C(5a)-dihydrobicyclomycin (12). To a methanolic solution (5 mL) of **3** (10 mg, 0.035 mmol) was added a catalytic amount of 10% Pd/C (10 mg), and the solution was stirred under H_2 (30 psi) at room temperature (5 h). The solution was filtered, and the solvent was removed *in vacuo*. TLC analysis indicated the presence of one major product, **12**: yield, 9.5 mg (94%); mp 145−148 °C; R_f 0.20 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3434 (br), 1687, 1385, 1060 cm^{-1}; ^1H NMR (CD_3OD) δ 1.06 (d, J = 6.9 Hz, 3 H, C(5a)CH_3), 1.30 (s, 3 H, C(2′)CH_3), 1.35 (s, 3 H, C(2′)CH_3), 1.60−1.75 (m, 1 H, C(4)*H*H′), 1.95−2.10 (m, 1 H, C(4)H*H*′), 2.10−2.25 (m, 1 H, C(5)H), 3.70−3.90 (m, 2 H, C(1′)H, C(3)-*H*H′), 3.95−4.10 (m, 1 H, C(3)H*H*′), the proposed structural assignment was consistent with the COSY experiment; ^{13}C NMR (CD_3OD) 15.8, 28.1, 30.0, 34.9, 46.9, 62.6, 74.6, 75.4, 84.6, 89.9, 168.9, 172.2 ppm; MS (+CI) 289 $[M + 1]^+$; M_r (+CI) 289.140 95 $[M + 1]^+$ (calcd for $C_{12}H_{21}N_2O_6$ 289.139 96).

Preparation of C(3′)-Deoxy-C(5),C(5a)-dihydrobicyclomycin C(3′)-Amine (13). To a methanolic solution (1 mL) of **8** (10 mg, 0.031 mmol) was added a catalytic amount of 10% Pd/C (10 mg), and the mixture was stirred under an atmosphere of H_2 at room temperature (0.5 h). The solution was filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (SiO_2, 50% MeOH−$CHCl_3$) to afford **13**: yield, 5 mg (52%); R_f 0.15 (50% CH_3OH−$CHCl_3$); ^1H NMR (CD_3OD) δ 1.06 (d, J = 7.2 Hz, 3 H), 1.32 (s, 3 H), 1.63−1.78 (m, 1 H), 1.95−2.10 (m, 1 H), 2.10−2.28 (m, 1 H), 2.71 (d, J = 13.5 Hz, 1 H), 2.90 (d, J = 13.5 Hz, 1 H), 3.79 (dd, J = 8.4, 13.8 Hz, 1 H), 3.99 (s, 1 H), 4.04 (dd, J = 9.0, 13.8 Hz, 1 H); M_r (+CI) 304.150 68 $[M + 1]^+$ (calcd for $C_{12}H_{22}N_3O_6$ 304.150 86).

Preparation of C(5),C(5a)-Dihydrobicyclomycin[2b] (14). A methanolic solution (5 mL) containing **1** (100 mg, 0.33 mmol) and PtO_2 (5 mg) was stirred under an atmosphere of H_2 at room temperature (0.5 h). The solution was filtered, and the solvent was removed *in vacuo* to give **14** as a mixture of diastereomers (∼10:1): yield, 104 mg (∼100%); mp 188−190 °C (lit.[2b] mp 191−192 °C); FT-IR (KBr) 3413 (br), 3233 (br), 1662, 1427, 1063 cm^{-1}; ^1H NMR (CD_3OD) δ 1.06 (d, J = 7.2 Hz, 3 H), 1.32 (s, 3 H), 1.60−1.75 (m, 1 H), 1.95−2.10 (m, 1 H), 2.12−2.25 (m, 1 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.72 (d, J = 11.4 Hz, 1 H), 3.78 (dd, J = 13.5, 21.9 Hz, 1 H), 3.98−4.06 (m, 2 H); ^{13}C NMR (CD_3OD) for the major diastereomer 15.5, 23.9, 34.4, 46.5, 62.6, 68.2, 72.0, 77.8, 84.2, 89.2, 168.2, 171.8 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer 13.3, 34.9, 44.4, 62.9, 84.5, 168.0, 174.8 ppm; the remaining peaks overlapped with nearby signals and were not discerned.

Inhibitory Properties of Bicyclomycin and Bicyclomycin Derivatives in the Poly(C)-Dependent ATPase Assay.[18,20] The ability of rho to hydrolyze [γ-^{32}P]ATP was assayed in 100 μL reactions containing ATPase buffer (50 mM Tris·HCl, pH 7.9, 50 mM KCl, 12 mM $MgCl_2$, 0.1 mM DTT, 0.1 mM EDTA, 0.14 mg bovine serum albumin), 0.5 μCi [γ-^{32}P]-ATP, 250 μM ATP, 24 μM poly(C), and 100 nM rho both in the absence and presence of the bicyclomycin test compound (0−400 μM). Reactions were incubated at 32 °C for 2 min. Aliquots (2 μL) were removed and spotted onto PEI TLC sheets that had been prerun in H_2O and dried. [γ-^{32}P]ATP and $^{32}P_i$ were separated by chromatography on the PEI sheets using 0.75 M KH_2PO_4, pH 3.5 as the mobile phase and then located by autoradiography. The radioactive spots were cut out and counted by liquid scintillation according to published methods. The initial rates of reactions were determined by plotting the amount of ATP hydrolyzed versus time. Relative percent activities were calculated from the initial velocities.

***In Vitro* Rho-Dependent Transcription Termination Assay.**[11,21] *E. coli* RNA polymerase was purified according to the method of Burgess and Jendrisak[28] with minor modification. The Biogel A 5m column was replaced by a Sephacryl S400 column. The DNA substrate was a truncated form of the *trp* operon.[29] The *Hae*III−*Sal*I fragment from pWU5 plasmid (generous gift from Dr. A. J. Dombroski, University of Texas Medical School at Houston) was ligated into the *Eco*RV−*Sal*I sites of pGEM 5 resulting in pTRP5, and the isolated *Bam*HI−*Sal*I fragment from pTRP5 was used as the template for the assay. The *in vitro* transcription was carried out in a 10 μL volume containing 20 mM Tris·acetate, pH 7.9, 150 mM KCl, 4 mM $Mg(OAc)_2$, 0.1 mM DTT, 0.1 mM EDTA, 200 μM each of ATP, CTP, GTP, 20 μM of UTP, 7 μCi of [α-^{32}P]-UTP, 0.1 pmol of DNA template, 0.4 unit/μL of RNase inhibitor, 0.01 μg/μL of *E. coli* RNA polymerase, and 0.02 μg/μL of rho protein both in the absence and presence of the bicyclomycin test compound (0−100 μM). The samples were incubated at 37 °C (20 min), diluted with 0.3 M NaOAc (100 μL), 1 mM EDTA, and carrier tRNA at 0.8 μg/μL, extracted with phenol, and precipitated with EtOH. The samples were dissolved in loading buffer (80% formamide, 0.1% xylene cyanol, 0.1% bromphenol blue, and 2 mM EDTA). The RNA products were separated on a 5% polyacrylamide 8 M urea gel and visualized by autoradiography. The relative amounts of radioactive incorporation of each band was determined by densitometry.

Antimicrobial Assay of Bicyclomycin and Bicyclomycin Derivatives.[18,22] Centrifuged cells (*E. coli* W3350) from overnight LB broth cultures (50 mL) were suspended in LB broth (4 mL), and then 100 μL of cells was diluted into 2 mL of broth and mixed. The solution was poured onto 15 mL volume LB agar plates. The LB agar plate was gently rocked to distribute the cells evenly over the plate surface, and any excess cell solution was removed with a pipet. The plate was dried (15 min) in the incubator at 37 °C. An antibiotic-assay disc (Aldrich, Z134090, $^1/_4$ in.) containing 20 μL of the test compound (1, 2, 4, 8, 16, 32 mg/mL) was placed on the agar surface. The plates were incubated at 37 °C (18 h). Data plots of the zone of inhibited bacterial growth (cm^3) versus log(1000*C*), where *C* is the concentration of test compound (mg/mL), yielded linear slopes to provide the minimal inhibitory concentrations (MIC) for bicyclomycin and bicyclomycin derivatives.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of all new compounds prepared in this study (25 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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