Role of the [4.2.2] Bicyclic Unit in Bicyclomycin: Synthesis, Structure, Chemical, Biochemical, and Biological Properties

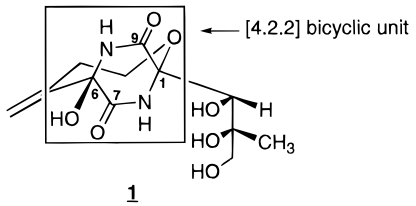
Alejandro Santillán, Jr.,[†] Hyeung-geun Park,[†] Xiangdong Zhang,[†] Oh-Seuk Lee,[†] William R. Widger,[‡] and Harold Kohn*,[†]

Department of Chemistry, University of Houston, Houston, Texas 77204-5641, and Department of Biochemical and Biophysical Sciences, University of Houston, Houston, Texas 77204-5934

Received May 29, 1996[⊗]

Twelve bicyclomycin derivatives were synthesized to determine the effect of modification of the [4.2.2] bicyclic unit in bicyclomycin (**1**) on drug function. Few bicyclomycin derivatives have been described in which the [4.2.2] ring system has been modified. The compounds evaluated were divided into two categories: the two *N*-methyl-modified bicyclomycins (**2**, **3**) and the ten C(6)-substituted bicyclomycins (**4**−**13**). Substituents introduced at the C(6) site included alkoxy, thioalkoxy, thiophenoxy, anilino, and hydrogen. A procedure was developed to synthesize select C(6)-substituted bicyclomycins. Bicyclomycin was first converted to bicyclomycin C(2′),C(3′)-acetonide (**16**) and then treated with methanesulfonyl chloride to give *in situ* the corresponding C(6) mesylate **17**. Treatment of **17** with the appropriate nucleophile followed by removal of the C(2′),C(3′)-acetonide group gave the desired C(6)-substituted bicyclomycin. The chemical properties of C(6) *O*-methylbicyclomycin (**4**) were examined. Treatment of THF−H_2O mixtures of **4** with excess EtSH maintained at "pH" 8.0−9.0 led to no detectable reaction, while at more basic "pH" values **4** underwent stereospecific conversion to the bis-spiro derivative **33** and no appreciable EtSH addition to the C(5)−C(5a) exomethylene unit. These results were compared to the reactivity of **1** with EtSH. The stability (pH 7.4, 37 °C) of C(6)-substituted bicyclomycins **4**, **6**, and **10**−**13** in aqueous solutions were examined. We observed that most of these compounds (**4**, **6**, **10**−**12**) underwent near complete change (>75%) within 200 h. The [4.2.2] bicyclic-modified bicyclomycins were evaluated in the rho-dependent ATPase assay and their antimicrobial activities determined using a filter disc assay. Most of the compounds were also tested in the transcription termination assay. We observed that all structural modifications conducted within the [4.2.2] bicyclic unit led to a loss of rho-dependent ATPase (I_{50} > 400 μM) and to transcription termination (I_{50} > 100 μM) inhibitory activities, as well as a loss of antimicrobial activity (MIC > 32 mg/mL). Only N(10)-methylbicyclomycin (**2**) displayed moderate inhibitory activities in these assays. These findings indicated that the [4.2.2] bicyclic unit played an important role in the antibiotic-rho recognition process. Potential factors that govern this interaction are briefly discussed. We concluded that placement of an irreversible inactivating unit at the *N*- and *O*-sites within the [4.2.2] bicyclic unit in **1** would likely prohibit the bicyclomycin derivative from efficiently binding to rho.

X-ray crystallographic analysis of bicyclomycin (**1**) and its analogues showed that the [4.2.2] bicyclic unit within **1** is a rigid system and that the piperazinedione ring adopts a twist-boat conformation in the solid state.[1] Functional groups contained in this unit include the C(1) aminal and C(6) hemiaminal centers and the C(7) and C(9) amide groups. Few bicyclomycin derivatives have been reported in which the [4.2.2] ring system has been modified.[2] In this study, we prepared select bicyclomycin analogues that probed the hydrogen bonding and steric requirements of this unit for binding to the receptor site in rho. We found that all structural modifications led to significant losses in biochemical activity, indicating a strong complementarity between the bicyclic system in **1** and the bicyclomycin binding pocket in rho.



1

Results and Discussion

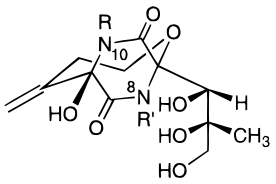
A. Choice of Substrates. The prepared compounds were divided into two categories: the two amide-modified bicyclomycins **2** and **3** and the ten C(6)-substituted bicyclomycins **4**−**13**.

[†] Department of Chemistry.
[‡] Department of Biochemical and Biophysical Sciences.
[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.

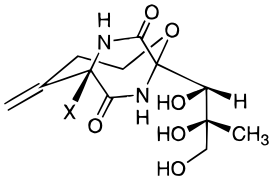
(1) (a) Tokuma, Y.; Koda, S.; Miyoshi, T.; Morimoto, Y. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 18−23. (b) Kohn, H.; Abuzar, S.; Korp, J. D.; Zektzer, A. S.; Martin, G. E. *J. Heterocycl. Chem.* **1988**, *25*, 1511−1517. (c) Zhang, Z.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 9815−9826.

(2) Müller, B. W.; Zak, O.; Kump, W.; Tosch, W.; Wacker, O. *J. Antibiot.* **1979**, *32*, 689−705.

S0022-3263(96)01003-1 CCC: $12.00



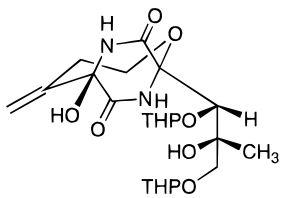
2 R = CH_3, R' = H
3 R, R' = CH_3



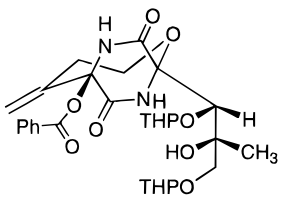
4 X = OCH_3
5 X = OCH_2CH_3
6 X = OCH_2CF_3
7 X = $OCH(CH_3)_2$
8 X = $OC(O)CH_3$
9 X = OC(O)Ph
10 X = SCH_2CH_3
11 X = SPh
12 X = NHPh
13 X = H

B. Synthesis. Compounds **2**, **3**, and **8** were previously reported by Müller and co-workers.[2] The C(6) benzoate ester **9** was prepared using a method similar to the one described for **8**. Conversion of **1** to the C(1′),C(3′) bis(tetrahydropyranyl ether) **14**[2] followed by treatment with benzoyl chloride and (dimethylamino)-pyridine gave crude **15**. Deprotection of the tetrahydropyranyl groups with aqueous acetic acid gave **9**.

The novel C(6)-substituted compounds **4**−**7** and **10**−**13** were prepared by a common procedure (Scheme 1).



14

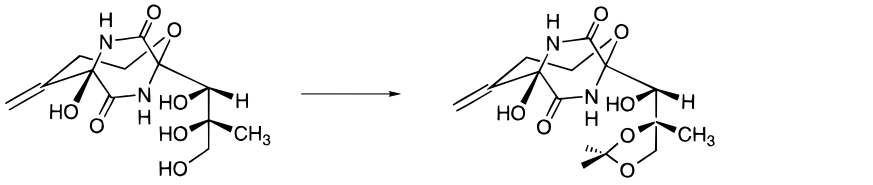


15

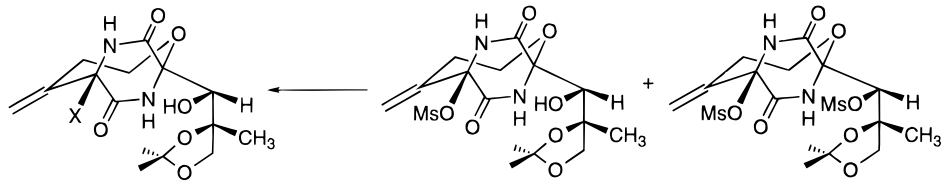
Bicyclomycin was first converted to the known bicyclomycin C(2′),C(3′)-acetonide (**16**).[3,4] Addition of methanesulfonyl chloride (3 equiv) to a THF solution of **16** and triethylamine gave a binary mixture tentatively identified as the C(6) mesylate **17** and the C(6),C(1′) dimesylate **18**. The approximate ratio of **17** to **18** was 9:1 (TLC analysis). Treatment of **17** and **18** *in situ* with excess amounts of the appropriate nucleophile followed by purification of the reaction mixtures by preparative TLC afforded **19**−**26**. Removing the C(2′),C(3′)-acetonide group in **19**−**26** with trifluoroacetic acid in aqueous methanol yielded the desired C(6)-substituted bicyclomycins **4**−**7** and **10**−**13**, respectively.

C. Spectral Studies. Satisfactory spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, low- and high-resolution mass) data were obtained for all compounds in this study.[5] The site of the *N*-methyl group in **2** was confirmed by one-dimensional NOE NMR spectroscopy. Irradiation of the C(4)*H*H′ peak (δ 2.20) led to a 5% intensity increase of the *N*(10)-CH_3 signal. Correspondingly, irradiation of *N*(10)-CH_3 resonance (δ 2.87) led to a 3% intensity increase of the C(4)*H*H′ signal. The NMR study indicates that the C(4) methylene hydrogens were in close proxim-

Scheme 1. Synthesis of Compounds 4−7 and 10−13

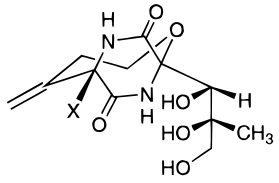


1 **16**



17 **18**

19 X = OCH_3
20 X = OCH_2CH_3
21 X = OCH_2CF_3
22 X = $OCH(CH_3)_2$
23 X = SCH_2CH_3
24 X = SPh
25 X = NHPh
26 X = H



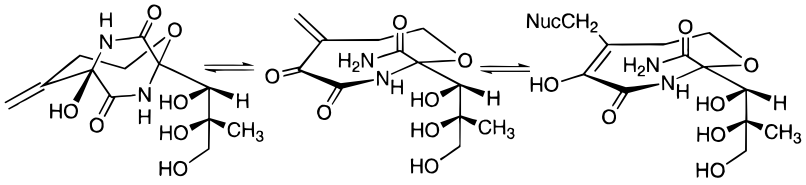
4 X = OCH_3
5 X = OCH_2CH_3
6 X = OCH_2CF_3
7 X = $OCH(CH_3)_2$
10 X = SCH_2CH_3
11 X = SPh
12 X = NHPh
13 X = H

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments for C(6)-Substituted Bicyclomycins

	^{1}H NMR[a]				^{13}C NMR[b]				
compd no.	C(4)*H*H′	C(4)H*H*′	C(5a)*H*H′	C(5a)H*H*′	C(3)	C(4)	C(5)	C(5a)	C(6)
1	2.57−2.65 (m)		5.14 (s)	5.55 (s)	65.5	36.7	149.5	117.0	83.0
4	2.58−2.62 (m)		5.13 (s)	5.50 (s)	65.5	36.9	148.5	117.0	87.9
5	2.58−2.62 (m)		5.13 (s)	5.54 (s)	65.5	36.9	148.6	117.0	87.6
6	2.60−2.65 (m)		5.20 (s)	5.28 (s)	65.4	36.8	147.6	117.5	87.7
7	2.55−2.60 (m)		5.11 (s)	5.52 (s)	65.5	36.8	148.1	117.1	88.5
8	2.55−2.75 (m)		5.23 (s)	5.34 (s)	65.1	36.6	146.4	117.1	84.9
9	2.60−2.90 (m)		5.35 (s)	5.75 (s)	65.2	36.8	146.8	117.2	85.4
10	2.56 (dd, 6.7, 16.0)	2.73 (dd, 9.3, 16.0)	5.18 (s)	5.46 (s)	66.0	37.6	150.4	118.2	71.3
11	2.61 (dd, 7.1, 16.1)	2.78 (dd, 9.2, 16.1)	5.29 (s)	5.61 (s)	66.0	38.0	150.4	118.2	*c*
12	2.62 (dd, 6.9, 15.9)	2.77 (dd, 9.3, 15.9)	5.29 (s)	5.62 (s)	66.6	37.2	151.2	117.3	74.5
13	2.56−2.59 (m)		5.06 (s)	5.14 (s)	64.9	36.1	147.4	118.2	62.7

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant(s) in hertz. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD. [b] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. All spectra were recorded at 75 MHz, and the solvent used was CD_3OD. [c] The C(6) signal was not detected and is believed to accidentally overlap the peak at 68.4 ppm.

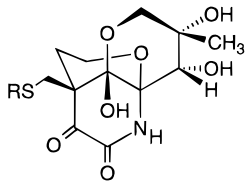
Scheme 2. Partial Mechanism for Bicyclomycin Covalent Modification Processes



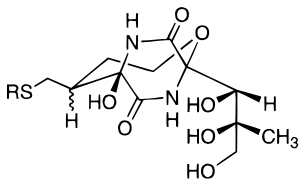
1 **27** **28**

ity to the *N*(10)-methyl group in **2**. These results confirm Müller's original structural assignment for this compound.[2] The observed ^{1}H and ^{13}C NMR chemical shift values for **4**−**13** were similar to those of **1**.[6] Table 1 lists key NMR signals for these compounds. Only the C(6) ^{13}C NMR chemical shift value in **4**−**13** varied with the structure of the C(6) substituent. The differences in C(6) chemical shift resonance values with C(6) substituent was consistent with the literature,[7] but the magnitude of these differences was attenuated. For example, replacement of the hydrogen substituent in C(6)-deoxybicyclomycin (**13**) by an ethanethiolate group or an ethoxy unit produced only modest increases in the C(6) chemical shift value (e.g., **10**, +8.6 ppm; **5**, +24.9 ppm).

D. Chemical Studies. Bicyclomycin function has been proposed to proceed by modification of nucleophilic amino acid residues in protein(s) necessary for bacterial survival.[6b,8−14] Both Iseki[8] and Kohn[6b,10−14] have suggested that covalent modification processes in **1** occur by Michael addition of the nucleophile to the ring-opened enone **27** to give **28** (Scheme 2). We have shown that the product profile depended upon the solution "pH".[13,14] At near neutral "pH" values, bicyclomycin reacted with thiols to give the novel rearranged adduct **29**,[11,14] while at elevated "pH" values ("pH" 10−11) the reaction yielded C(5)−C(5a) substituted adduct **30** as the predominant product.

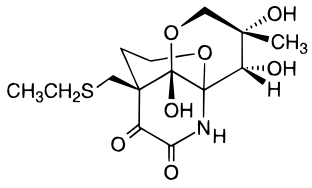


29



30

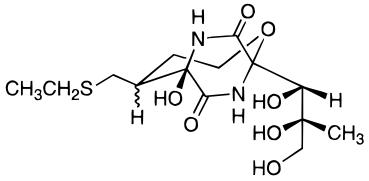
In light of these findings, we examined the reactivity of the C(6) methoxy derivative **4** in THF−H_2O (3:1) mixtures. When a solution containing **4** was treated with excess EtSH at "pH" 8.0−9.0 (22 °C, 7 d), there were no noticeable products but the starting material was recovered. The corresponding reaction with **1** gave **31** after 12 h.[11,14] This result provided support to the notion that



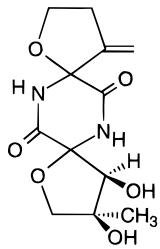
31

bicyclomycin C(5)−C(5a) functionalization proceeded through the ring-opened enone **27**. Additional support for the interconversion of **1** to **27** in thiolate-mediated bicyclomycin transformations was obtained by comparing the reactivity of **1** and **4** at higher "pH" values ("pH" 9.9−10.5). Treatment of **1** with excess EtSH gave **32**[11,14] and **31**[14] while **4** produced **33**, along with several unidentified adducts. NMR analyses of **33** indicated the presence of only a single diastereomer. Previous acid-promoted methods for the conversion of **1** to **33** produced a mixture of bis-spiro diastereomers.[15,16]

(3) Kamiya, T.; Maeno, S.; Kitaura, Y. Belgium Patent 847 475.
(4) Zhang, Z.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 9815−9826.
(5) Park, H. Ph.D. Thesis, University of Houston, 1995.
(6) (a) Park, H.-g.; Vela, M. A.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 471−478. (b) Kohn, H.; Abuzar, S. *J. Am. Chem. Soc.* **1988**, *110*, 3661−3663.
(7) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York; 1972.
(8) Someya, S.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402−407.
(9) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733−740.
(10) Pisabarro, A. G.; Canada, F. J.; Vasquez, D.; Arriaga, P.; Rodriguez-Tebar, A. *J. Antibiot.* **1986**, *34*, 914−921.
(11) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089−4090.
(12) Abuzar, S.; Kohn, H. *J. Org. Chem.* **1989**, *54*, 4000−4003.
(13) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1989**, *111*, 4895−4903.
(14) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1990**, *112*, 3114−3121.
(15) Maag, H.; Blount, J. F.; Coffen, D. L.; Steppe, T. V.; Wong, F. *J. Am. Chem. Soc.* **1978**, *100*, 6786−6788.

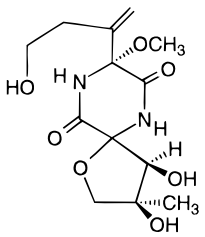


32



33

The stability of C(6)-substituted bicyclomycins **4**, **6**, and **10**−**12** in aqueous solutions (pH 7.4, 37 °C) was compared to that of bicyclomycin (Table 2). For **11**, DMSO (10%) was added to the aqueous solution to solubilize the bicyclomycin derivative. We observed that these bicyclomycin derivatives underwent change (>75%) within 200 h (HPLC and TLC analyses). The stability of the C(6)-substituted bicyclomycin was a function of the C(6)-X substituent. We found that the approximate order of stability was **4** > **12** > **6** > **10** > **11**. This order paralleled the basicity of the C(6)-X substituent.[17] Correspondingly, bicyclomycin underwent near complete change (>75%) within 57 h. The HPLC and TLC product profiles for **1** indicated the formation of several polar, unidentified adducts. The product profiles for **4**, **6**, **10**, **11**, and **12** showed the presence of diastereomeric **33**. Furthermore, we observed for **4**, **10**, **11**, and **12** one or two additional products whose HPLC retention times varied with the C(6)-X substituent. Neither bicyclomycin nor its reaction products were detected in the HPLC and TLC product profiles for **4**, **6**, and **10**−**12**. This finding indicated that these compounds did not hydrolyze to **1**. Repetition of the reaction using **4** on a semipreparative scale led to the isolation of diastereomeric **33** and the new adduct. NMR and MS studies indicated that the new compound was diastereomeric **34**. Key ^{13}C NMR resonances for **34** were the C(6) methoxy carbon signal at 51.1 ppm and the C(3) peak at 61.3 ppm. Significantly, the C(3) signal in **34** (DMF-d_7) was upfield (Δ 2.7 ppm) from the corresponding resonance in **1**. These results demonstrated that cleavage of both C(1)−O(2) and C(6)−X bonds within **4**, **6**, **10**−**12** could occur and that scission of the C(1)−O(2) bond could proceed prior to cleavage of the C(6)−X bond. Consistent with this general pathway, maintenance of an aqueous solution of **13** at 37 °C produced initially only one major compound.



34

E. Biochemical and Biological Studies. An early investigation showed no appreciable antibiotic activity in bicyclomycin derivatives **2**, **3**, and **8**,[2] a finding supported by this study. Table 3 shows that successive *N*-methylation of the piperazinedione ring led to lower inhibitory activities in the rho ATPase assay.[18] For example the I_{50} values of **1**[19] and **2** were 60 and 350 μM, respectively. Similarly, N(8),N(10)-dimethylbicyclomycin (**3**) showed less than 5% inhibition of ATPase activity at a 400 μM concentration while bicyclomycin (**1**) inhibited ATP hydrolysis by 95% at this concentration. A similar relationship of activity to *N*-methyl substitution was observed in the transcription termination assay.[20] The estimated I_{50} values for **1**[21] and **2** in this assay were 5 and >100 μM, respectively. These findings suggested either that hydrogen bond interactions between the N(8) and N(10) positions in the drug and the receptor site in rho helped to promote drug binding or that increases in the steric size of the *N*-substituents led to significant decreases in the binding of the bicyclomycin derivative to rho.

Table 2. Stability of C(6)-Substituted Bicyclomycins[a]

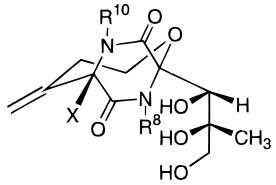
compd no.	C(6)−X	$t_{1/2}$ (h)
1	OH	28.6
4	OCH_3	99.6
6	OCH_2CF_3	54.7
10	SCH_2CH_3	26.5
11[b]	SPh	8.6
12	NHPh	81.5
13	H	202.1

[a] The stability of the C(6)-substituted bicyclomycins was determined in aqueous pH 7.4 buffered solutions (40 mM Tris) at 37 °C by HPLC. [b] DMSO (10%) was added to the reaction.

When we modified the C(6) site in bicyclomycin, inhibitory activity was lost in both assays. In the case of **6** and **11**, DMSO (10%) was added to the aqueous buffered solutions to solubilize the compounds. Control studies with bicyclomycin showed that this amount of DMSO did not affect its ability to inhibit rho-dependent ATPase, transcription termination processes, or its antimicrobial activity (Table 3). No appreciable inhibition of ATPase activity was observed for **4**−**13** at 400 μM. Similarly, in the rho-dependent transcription termination assay, **4**−**7** and **9**−**13** at 100 μM did not prevent the synthesis of rho-dependent transcripts (Figure 1). These results indicated that the C(6) hydroxy group played a key role in the drug's interaction with rho. Significantly, both increases (**4**−**12**) and a decrease (**13**) in the size of the C(6) substituent compared with that of **1** led to pronounced losses of inhibitory activity of the bicyclomycin analogue in the rho-dependent ATPase and transcription termination assays (Table 3). This finding demonstrated that steric interactions were not solely responsible for the lack of biochemical activities for the C(6)-substituted bicyclomycins. Two other factors may account for the inability of these compounds to inhibit rho-dependent processes. First, a key hydrogen-bonding donor interaction may exist between the C(6) hydroxy group in **1** and the amino acid residue(s) in the bicyclomycin binding domain in rho. Of the ten C(6)-substituted derivatives prepared (**4**−**13**) only the bulky C(6) anilino group in **12**, like **1**, can donate a hydrogen bond to an amino acid receptor. Comparable disruptions of hydrogen-bonding interactions have been proposed to explain the loss of biochemical activities for bicyclomycin C(1) triol-modified derivatives.[22] Second, of the ten C(6)-substi-

(16) Kohn, H.; Abuzar, S. *J. Org. Chem.* **1988**, *53*, 2769−2773.
(17) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row: New York, 1987; pp 297−298.
(18) Sharp, J. A.; Galloway, J. L.; Platt, T. *J. Biol. Chem.* **1983**, *258*, 3482−3486.
(19) Park, H.-g.; Zhang, X.; Moon, H.-s.; Zwiefka, A.; Cox, K.; Gaskell, S. J.; Widger, W. R.; Kohn, H. *Arch. Biochem. Biophys.* **1995**, *323*, 447−454.
(20) Wu, A. M.; Christie, G. E.; Platt, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2913−2917.
(21) Magyar, A.; Zhang, X.; Kohn, H.; Widger, W. R. *J. Biol. Chem.* **1996**, *271*, 25369−25374.

Table 3. Biochemical and Biological Activities of [4.2.2] Bicyclic-Modified Bicyclomycins



				inhibition of ATPase activity[a]		TT activity[d]		
compd no.	R^8	R^{10}	X	I_{50} (μM) (BCM)[b]	400 μM (%) (BCM)[c]	I_{50} (μM)[e]	100 μM (%)[f]	MIC[g] (mg/mL)
1	H	H	OH	60	95	~5	100	0.25
1[h]	H	H	OH	75	85	~5	100	0.27
2	H	CH_3	OH	350 (75)	55 (86)	>100	29	3.0 (0.15)
3	CH_3	CH_3	OH	>400 (65)	3 (90)	i	i	>32 (0.32)
4	H	H	OCH_3	>400 (60)	2 (94)	>100	1	>32 (0.32)
5	H	H	OCH_2CH_3	>400 (60)	1 (94)	>100	5	>32 (0.32)
6[h]	H	H	OCH_2CF_3	>400 (75)	9 (85)	>100	2	>32 (0.29)
7	H	H	$OCH(CH_3)_2$	>400 (60)	5 (88)	>100	2	>32 (0.32)
8	H	H	$OC(O)CH_3$	>400 (70)	9 (86)	i	i	16 (0.65)
9	H	H	OC(O)Ph	>400 (70)	5 (86)	>100	0	>32 (0.32)
10	H	H	SCH_2CH_3	>400 (60)	3 (88)	>100	5	>32 (0.29)
11[h]	H	H	SPh	>400 (75)	7 (85)	>100	5	>32 (0.29)
12	H	H	NHPh	>400 (60)	1 (88)	>100	3	>32 (0.32)
13	H	H	H	>400 (60)	1 (88)	>100	10	>32 (0.32)

[a] Activity measured using the ATPase assay (ref 18). [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 20). [e] The I_{50} value is the concentration that gave 50% rho-dependent RNA transcripts. [f] The percentage of inhibition 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 disc assay (ref 23). The number in parentheses is the corresponding value obtained from bicyclomycin in a concurrently run experiment. [h] DMSO (10%) was used as a cosolvent. [i] Compound not tested.

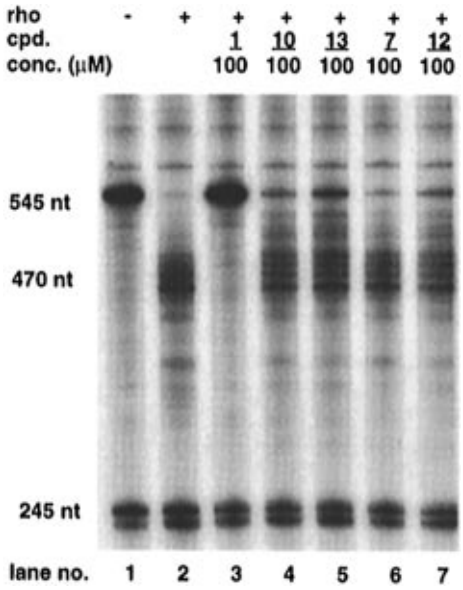


Figure 1. Autoradiograph of rho-dependent transcription termination assay for bicyclomycin and derivatives. The inhibition of *in vitro* rho-dependent transcription termination by bicyclomycin and its derivatives was determined using a modified *trp* operon template[20] and *E. coli* RNA polymerase and by measuring the [α-^{32}P]UTP incorporation in the RNA transcripts. The reactions were carried out without rho (lane 1), in the presence of rho (lane 2), and in the presence of bicyclomycin and derivatives (lanes 3−7): lane 3, **1** (100 μM); lane 4, **10** (100 μM); lane 5, **13** (100 μM); lane 6, **7** (100 μM); lane 7, **12** (100 μM). Relative amounts of transcripts were determined by densitometry of the autoradiograph.

tuted bicyclomycin derivatives, only **12** can undergo C(6) ring opening to give a neutral adduct. With bicyclomycin, the corresponding process yields enone **27** (Scheme 2). The identity of the bicyclomycin species (e.g., **1**, **27**) that binds to rho is not known. If the C(6) hemiaminal ring opening in **1** is important for drug binding to rho, then bicyclomycin derivatives **4**−**13** should exhibit decreased inhibitory activity in the rho-dependent ATPase and transcription termination assays.

The biochemical results were consistent with the observed biological properties[23] for these compounds (Table 3). We found that for the two amide-modified bicyclomycins **2** and **3** only **2** retained partial antibiotic activity (MIC = 3 mg/mL), compared with **1** (MIC = 0.25 mg/mL). The loss of activity of **2** and **3**, compared with **1**, in the filter disc antimicrobial assay paralleled the decreases observed in the rho-dependent ATPase assays. Correspondingly, none of the C(6)-substituted bicyclomycins **4**−**13** exhibited any detectable antibiotic activity (MIC > 32 mg/mL). This result was consistent with the inactivity of these compounds in the rho-dependent ATPase and transcription termination assays.

Conclusions

Our studies demonstrated that structural modification of the piperazinedione unit in bicyclomycin led to pronounced changes in the biochemical and biological activities of the substrate. The finding that compounds **3**−**13** were devoid of both biological and biochemical activities at the concentrations used and that only **2** even partially retained these activities suggested that strong binding interaction(s) existed between the piperazinedione unit and the binding site in rho. We concluded that incorporation of an irreversible inactivating unit[24] within the piperazinedione ring system in bicyclomycin would not permit sufficient binding to rho to allow the location of bicyclomycin rho binding.

(22) Park, H.-g.; Zhang, X.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7750.
(23) Ericsson, H. M.; Sherris, J. C. *Acta Pathol. Microbiol. Scand.* **1971**, Suppl. 217, 1−90.

Experimental Section

General Methods. Procedures identical to those previously described were used in the synthesis and evaluation bicyclomycin-modified derivatives in the poly(C)-dependent ATPase, rho-dependent transcription termination, and antimicrobial assays.[22]

Preparation of Bicyclomycin C(6)-*O*-Benzoate (9). To a CH_2Cl_2 solution (4 mL) containing **14**[2] (24 mg, 0.05 mmol) were added benzoyl chloride (8.5 mg, 0.06 mmol) and (dimethylamino)pyridine (12.5 mg, 0.1 mmol). The solution was stirred at room temperature (12 h), and then the solvent was evaporated *in vacuo*. The residue was diluted with ethyl acetate (50 mL) and washed sequentially with a saturated aqueous $CuSO_4$ solution (5 mL), H_2O (5 mL), and a saturated aqueous NaCl solution (5 mL). The solution was dried over anhydrous Na_2SO_4 (3 g) and then evaporated *in vacuo* to obtain crude **15**. The crude **15** was dissolved in 50% aqueous acetic acid (10 mL), and the solution was stirred at room temperature (4 h). The solvent was evaporated *in vacuo* and purified by preparative TLC (10% MeOH−$CHCl_3$) to obtain **9**: yield, 8 mg (39%); mp 182 °C (dec); R_f 0.40 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3428 (br), 1698, 1560, 1409 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H), 2.60−2.90 (m, 2 H), 3.53 (d, J = 11.1 Hz, 1 H), 3.68 (d, J = 11.1 Hz, 1 H), 3.80−4.05 (m, 2 H), 4.13 (s, 1 H), 5.35 (s, 1 H), 5.75 (s, 1 H), 7.51−8.08 (m, 5 H); ^{13}C NMR (CD_3OD) 24.1, 36.8, 65.2, 68.5, 72.1, 78.2, 85.4, 89.3, 117.2, 129.8, 130.7, 130.8, 135.0, 146.8, 165.0, 167.4 ppm, the C(7) signal was not detected; MS (+CI) 407 $[M + 1]^+$; M_r (+CI) 407.143 64 $[M + 1]^+$ (calcd for $C_{19}H_{23}N_2O_8$ 407.145 44).

General Procedure for the Preparation of C(6)-*O*-Alkylbicyclomycin C(2′),C(3′)-Acetonides 19−22. To an anhydrous THF solution (4 mL) of **16**[3,4] (1 equiv) and triethylamine (3 equiv) was added methanesulfonyl chloride (3 equiv). The reaction mixture was stirred at room temperature (10 min), filtered (glass wool), and concentrated *in vacuo*. The residue was dissolved in the desired alcohol (4 mL), and the "pH" of the reaction was adjusted to 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 purified by PTLC (5−10% MeOH−$CHCl_3$) to produce the desired product.

Using this procedure, we prepared the following compounds.

C(6)-*O*-Methylbicyclomycin C(2′),C(3′)-Acetonide (19). Using **16** (42 mg, 0.12 mmol), triethylamine (37 mg, 0.37 mmol), and methanesulfonyl chloride (44 mg, 0.37 mmol) gave **16** (5 mg, 12% recovery) and **19** as a white solid (20 mg, 46%): mp 105−108 °C; R_f 0.60 (10% MeOH−$CHCl_3$); IR (KBr) 3439, 3295, 2990, 2940, 1698, 1397, 1208, 1117, 1073, 1046, 877 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.43 (s, 3 H), 1.45 (s, 3 H), 2.60−2.65 (m, 2 H), 3.40 (s, 3 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.81−3.87 (m, 1 H), 3.91−3.97 (m, 1 H), 4.16 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 5.14 (s, 1 H), 5.52 (s, 1 H); ^{13}C NMR (CD_3OD) 25.2, 26.2, 26.8, 36.7, 53.0, 66.6, 72.6, 73.2, 86.5, 87.8, 89.1, 111.7, 117.0, 148.3, 169.4, 170.0 ppm; MS (+FAB) 357 $[M + 1]^+$; M_r (+CI) 357.165 44 $[M + 1]^+$ (calcd for $C_{16}H_{25}N_2O_7$ 357.166 18).

C(6)-*O*-Ethylbicyclomycin C(2′),C(3′)-Acetonide (20). Using **16** (40 mg, 0.12 mmol), triethylamine (36 mg, 0.35 mmol), and methanesulfonyl chloride (40 mg, 0.35 mmol) gave **16** (2 mg, 5% recovery) and **20** as a white solid (18 mg, 44%): mp 99−102 °C; R_f 0.44 (5% MeOH−$CHCl_3$); IR (KBr) 3434, 3007, 2986, 2937, 2898, 1698, 1385, 1244, 1197, 1135, 1116, 1073, 1045, 875, 789 cm^{-1}; ^1H NMR (CD_3OD) δ 1.26 (t, J = 7.1 Hz, 3 H), 1.38 (s, 3 H), 1.42 (s, 3 H), 1.45 (s, 3 H), 2.58−2.63 (m, 2 H), 3.59−3.66 (m, 2 H), 3.72 (d, J = 8.4 Hz, 1 H), 3.84−3.87 (m, 1 H), 3.90−3.93 (m, 1 H), 4.16 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 5.14 (s, 1 H), 5.56 (s, 1 H); ^{13}C NMR (CD_3OD) 15.4, 25.1, 26.2, 26.8, 36.6, 61.9, 66.6, 72.6, 73.2, 86.5, 87.5, 89.0, 111.6, 117.0, 148.4, 169.3, 170.3 ppm; MS (−CI) 369 $[M - 1]^-$; M_r (−CI) 369.164 62 $[M - 1]^-$ (calcd for $C_{17}H_{25}N_2O_7$ 369.166 18).

C(6)-*O*-(Trifluoroethyl)bicyclomycin C(2′),C(3′)-Acetonide (21). Using **16** (22 mg, 0.06 mmol), triethylamine (20 mg, 0.19 mmol), and methanesulfonyl chloride (22 mg, 0.19 mmol) gave **16** (6 mg, 27% recovery) and **21** as a white solid (6 mg, 22%): mp 98−101 °C; R_f 0.65 (10% MeOH−$CHCl_3$); IR (KBr) 3327, 3301, 2991, 2939, 2885, 1700, 1459, 1385, 1285, 1176, 1133, 1074, 1046, 965, 876, 811, 790 cm^{-1}; ^1H NMR (CD_3OD) δ 1.39 (s, 3 H), 1.42 (s, 3 H), 1.45 (s, 3 H), 2.62−2.65 (m, 2 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.77−3.84 (m, 1 H), 3.94−4.05 (m, 1 H), 4.04−4.17 (m, 2 H), 4.19 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 5.21 (s, 1 H), 5.54 (s, 1 H); ^{13}C NMR (CD_3OD) 25.4, 26.8, 28.1, 36.6, 63.7 (q, J = 35.4 Hz), 66.5, 72.0, 73.0, 86.6, 87.6, 89.4, 111.6, 117.5, 125.1 (q, J = 274.1 Hz), 147.5, 168.4, 169.7 ppm; MS (+FAB) 425 $[M + 1]^+$; M_r (+FAB) 425.154 26 $[M + 1]^+$ (calcd for $C_{17}H_{24}N_2O_7F_3$ 424.153 56).

C(6)-*O*-Isopropylbicyclomycin C(2′),C(3′)-Acetonide (22). Using **16** (29 mg, 0.09 mmol), triethylamine (26 mg, 0.25 mmol), and methanesulfonyl chloride (29 mg, 0.25 mmol) gave **16** (6 mg, 21% recovery) and **22** as a white solid (10 mg, 31%): mp 78−82 °C; R_f 0.61 (5% MeOH−$CHCl_3$); IR (KBr) 3445, 3322, 2984, 2936, 2882, 1694, 1457, 1385, 1244, 1190, 1137, 1072, 1044, 876, 786 cm^{-1}; ^1H NMR (CD_3OD) δ 1.24 (d, J = 6.2 Hz, 3 H), 1.30 (d, J = 6.2 Hz, 3 H), 1.38 (s, 3 H), 1.42 (s, 3 H), 1.45 (s, 3 H), 2.56−2.61 (m, 2 H), 3.72 (d, J = 8.4 Hz, 1 H), 3.77−3.85 (m, 1 H), 3.88−3.99 (m, 2 H), 4.15 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 5.12 (s, 1 H), 5.53 (s, 1 H); ^{13}C NMR (CD_3OD) 23.9, 24.6, 25.1, 26.8, 28.2, 36.6, 66.5, 71.5, 72.6, 73.2, 86.5, 88.3, 88.9, 111.6, 117.0, 148.8, 169.2, 171.0 ppm; MS (+CI) 385 $[M + 1]^+$; M_r (+CI) 385.196 35 $[M + 1]^+$ (calcd for $C_{18}H_{29}N_2O_7$ 385.197 48).

General Procedure for the Preparation of C(6) S- and N-Substituted Bicyclomycin C(2′),C(3′)-Acetonides 23−25. To an anhydrous THF solution (4 mL) of **16** (1 equiv) and triethylamine (3 equiv) was added methanesulfonyl chloride (3 equiv). The reaction mixture was stirred at room temperature (10 min), filtered (glass wool), and concentrated *in vacuo*. The residue was dissolved in isopropyl alcohol (2 mL) and treated with the desired nucleophile (3−10 equiv). The "pH" of the reaction mixture was adjusted to approximately 5.5 with dilute aqueous NaOH, and the solution was stirred at room temperature (20 min). The "pH" of the reaction mixture was adjusted to 7.0 with dilute aqueous NaOH, and the solution was 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 ((**23**, **25**): 5% MeOH−$CHCl_3$; (**24**): 3 × 50% ethyl acetate−hexanes) to afford the desired product.

Using this procedure, we prepared the following compounds.

C(6)-*S*-Ethylbicyclomycin C(2′),C(3′)-Acetonide (23). Using **16** (50 mg, 0.15 mmol), triethylamine (44 mg, 0.44 mmol), methanesulfonyl chloride (50 mg, 0.44 mmol), and ethanethiol (90 mg, 1.46 mmol) gave **23** as a white solid (15 mg, 26%): mp 89−93 °C; R_f 0.41 (80% ethyl acetate−hexanes); IR (KBr) 3419, 3321, 2986, 2934, 2878, 1696, 1385, 1244, 1195, 1073, 1044, 875, 787 cm^{-1}; ^1H NMR (CD_3CN) δ 1.18 (t, J = 7.7 Hz, 3 H), 1.27 (s, 3 H), 1.35 (s, 3 H), 1.38 (s, 3 H), 2.46−2.65 (m, 4 H), 3.64−3.71 (m, 1 H), 3.70 (d, J = 8.4 Hz, 1 H), 3.90−3.98 (m, 1 H), 3.97 (d, J = 8.7 Hz, 1 H), 4.30 (d, J = 8.4 Hz, 1 H), 4.36 (d, J = 8.7 Hz, 1 H), 5.14 (s, 1 H), 5.39 (s, 1 H), 7.06, 7.95 (br, 2 H); ^{13}C NMR (CD_3CN) 13.5, 24.0, 24.9, 26.6, 27.8, 37.1, 66.2, 68.9, 73.0, 74.6, 85.3, 86.9, 111.1, 117.1, 149.7, 167.6, 169.2 ppm, the APT spectrum was consistent with the proposed structural assignment; MS (+CI) 387 $[M + 1]^+$; M_r (+CI) 387.159 02 $[M + 1]^+$ (calcd for $C_{17}H_{27}N_2O_6S$ 387.158 98).

C(6)-*S*-Phenylbicyclomycin C(2′),C(3′)-Acetonide (24). Using **16** (38 mg, 0.11 mmol), triethylamine (34 mg, 0.33 mmol), methanesulfonyl chloride (38 mg, 0.33 mmol), and thiophenol (110 mg, 1.0 mmol) gave **24** as a white solid (10 mg, 21%): mp 83−86 °C; R_f 0.48 (80% ethyl acetate−hexanes); IR (KBr) 3426, 3312, 2985, 2934, 2878, 1687, 1384, 1247, 1198, 1075, 1040, 874, 853, 753, 693, 473 cm^{-1}; ^1H NMR (CD_3CN) δ 0.81 (s, 3 H), 1.29 (s, 3 H), 1.31 (s, 3 H), 2.55 (dd, J = 6.3, 16.1 Hz, 1 H), 2.67 (dd, J = 7.5, 16.1 Hz, 1 H), 3.60 (d, J = 8.7 Hz,

(24) For a discussion of enzyme irreversible inactivators, see: (a) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 1992; pp 178−219. (b) Bayley, H.; Staros, J. V. Photoaffinity Labeling and Related Techniques. In *Azides and Nitrenes: Reactivity and Utility*; Scriven, E. F. V., Ed.; Academic Press: Orlando, 1984; pp 433−490. (c) Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479−12520.

1 H), 3.72 (dd, J = 6.3, 14.7 Hz, 1 H), 3.75 (d, J = 9.5 Hz, 1 H), 3.91−3.98 (m, 1 H), 4.10 (d, J = 8.7 Hz, 1 H), 4.74 (d, J = 9.5 Hz, 1 H), 5.24 (s, 1 H), 5.50 (s, 1 H), 7.30−7.38 (m, 4 H), 7.54−7.58 (m, 3 H); ^{13}C NMR (CD_3CN) 22.7, 27.1, 28.4, 38.0, 66.4, 74.1, 75.3, 77.4, 85.1, 85.4, 111.6, 118.4, 130.5, 130.9, 131.1, 137.3, 149.7, 167.4, 169.5 ppm, the APT spectrum was consistent with the proposed structural assignment; MS (+CI) 435 $[M + 1]^+$; M_r (+CI) 435.157 67 $[M + 1]^+$ (calcd for $C_{21}H_{27}N_2O_6S$ 435.158 98).

C(6)-*N*-Phenylbicyclomycin C(2′),C(3′)-Acetonide (25). Using **16** (38 mg, 0.11 mmol), triethylamine (34 mg, 0.33 mmol), methanesulfonyl chloride (38 mg, 0.33 mmol), and aniline (31 mg, 0.33 mmol) gave **25** as a white solid (13 mg, 28%): mp 125−128 °C; R_f 0.60 (10% MeOH−$CHCl_3$); IR (KBr) 3376, 3310, 2988, 2934, 2878, 1688, 1604, 1506, 1399, 1315, 1254, 1194, 1072, 1044, 877, 751, 695 cm^{-1}; ^{1}H NMR (CD_3-OD) δ 1.43 (s, 6 H), 1.46 (s, 3 H), 2.62 (dd, J = 7.0, 15.9 Hz, 1 H), 2.77 (dd, J = 9.0, 15.9 Hz, 1 H), 3.75 (d, J = 8.3 Hz, 1 H), 3.78−3.86 (m, 1 H), 4.07 (dd, J = 7.0, 12.8 Hz, 1 H), 4.25 (s, 1 H), 4.49 (d, J = 8.3 Hz, 1 H), 5.30 (s, 1 H), 5.65 (s, 1 H), 6.70−6.79 (m, 3 H), 7.04−7.17 (m, 2 H); ^{13}C NMR (CD_3OD) 25.3, 26.9, 28.1, 37.0, 67.6, 72.4, 73.2, 74.4, 86.7, 89.5, 111.6, 116.9, 117.4, 120.0, 129.7, 145.1, 151.1, 170.0, 171.1 ppm; MS (+CI) 418 $[M + 1]^+$; M_r (+CI) 418.198 49 $[M + 1]^+$ (calcd for $C_{21}H_{28}N_3O_6$ 418.197 81).

Preparation of C(6)-Deoxybicyclomycin C(2′),C(3′)-Acetonide (26). To an anhydrous THF solution (4 mL) of **16** (42 mg, 0.12 mmol) and triethylamine (37 mg, 0.37 mmol) was added methanesulfonyl chloride (42 mg, 0.37 mmol). The reaction mixture was stirred at room temperature (10 min), filtered (glass wool), and concentrated *in vacuo*. The residue was suspended in H_2O (4 mL) and treated with $NaBH_4$ (23 mg, 0.61 mmol). The "pH" of the reaction mixture was adjusted to approximately 5.5 with dilute aqueous HCl, and the solution was stirred at room temperature (20 min). The "pH" of the reaction mixture was adjusted to 7.0 with dilute aqueous HCl, and the solution was concentrated *in vacuo*. The residue was purified by PTLC (10% MeOH−$CHCl_3$) to provide **16** (8 mg, 19% recovery) and **26** as a white solid (10 mg, 31%): mp 88−92 °C; R_f 0.52 (10% MeOH−$CHCl_3$); IR (KBr) 3414, 3308, 2986, 2936, 1693, 1405, 1382, 1198, 1074, 1044, 874 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 2.57−2.60 (m, 2 H), 3.73 (d, J = 8.7 Hz, 1 H), 3.79−3.86 (m, 1 H), 3.91−3.97 (m, 1 H), 4.15 (s, 1 H), 4.43 (s, 1 H), 4.46 (d, J = 8.7 Hz, 1 H), 5.08 (s, 1 H), 5.16 (s, 1 H); ^{13}C NMR (CD_3-OD) 24.8, 26.1, 27.8, 34.6, 60.6, 63.7, 70.5, 71.2, 85.2, 86.9, 109.2, 117.1, 146.0, 166.9, 168.0 ppm, the APT spectrum was consistent with the proposed structural assignment; MS (+CI) 327 $[M + 1]^+$; M_r (+CI) 327.155 14 $[M + 1]^+$ (calcd for $C_{15}H_{23}N_2O_6$ 327.155 61).

General Procedure for the Preparation of C(6)-Substituted Bicyclomycins 4−7 and 10−13. To a 50% aqueous methanolic solution (3 mL) containing the C(6)-substituted C(2′),C(3′)-acetonide was added trifluoroacetic acid (3 drops). The reaction solution was stirred at room temperature (3 h) and concentrated *in vacuo*. The residue was purified by PTLC (20% MeOH−$CHCl_3$) to provide the desired product.

Using this procedure, we prepared the following compounds.

C(6)-*O*-Methylbicyclomycin (4). Using **19** (11 mg, 0.03 mmol) gave **4** as a white solid (6 mg, 61%): mp 105−108 °C; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3428, 3257, 2943, 1692, 1403, 1154, 1118, 1047, 788, 586 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.58−2.62 (m, 2 H), 3.39 (s, 3 H), 3.50 (d, J = 11.1 Hz, 1 H), 3.66 (d, J = 11.1 Hz, 1 H), 3.77−3.82 (m, 1 H), 3.89−3.94 (m, 1 H), 4.08 (s, 1 H), 5.13 (s, 1 H), 5.50 (s, 1 H); ^{13}C NMR (CD_3OD) 24.1, 36.9, 53.0, 65.5, 68.5, 72.0, 78.2, 87.9, 89.4, 117.0, 148.5, 169.8, 170.3 ppm; MS (+CI) 317 $[M + 1]^+$; M_r (+CI) 317.134 87 $[M + 1]^+$ (calcd for $C_{13}H_{21}N_2O_7$ 317.134 76).

C(6)-*O*-Ethylbicyclomycin (5). Using **20** (18 mg, 0.05 mmol) gave **5** as a white solid (8 mg, 50%): mp 98−101 °C; R_f 0.55 (20% MeOH−$CHCl_3$); IR (KBr) 3433, 3271, 2980, 2940, 2902, 1687, 1403, 1154, 1115, 1046, 874, 788, 680, 585, 443 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.26 (t, J = 7.1 Hz, 3 H), 1.33 (s, 3 H), 2.58−2.62 (m, 2 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.60−3.70 (m, 3 H), 3.74−3.83 (m, 1 H), 3.88−3.98 (m, 1 H), 4.08 (s, 1 H), 5.13 (s, 1 H), 5.54 (s, 1 H); ^{13}C NMR (CD_3OD) 15.4, 24.1, 36.9, 61.8, 65.5, 68.5, 71.9, 78.2, 87.6, 89.3, 117.0, 148.6, 169.6, 170.6 ppm; MS (+CI) 331 $[M + 1]^+$; M_r (+CI) 331.151 35 $[M + 1]^+$ (calcd for $C_{14}H_{23}N_2O_7$ 331.150 53).

C(6)-*O*-(Trifluoroethyl)bicyclomycin (6). Using **21** (12 mg, 0.03 mmol) gave **6** as a white solid (7 mg, 64%): mp 109−113 °C; R_f 0.51 (20% MeOH−$CHCl_3$); IR (KBr) 3452, 3268, 2948, 2889, 1699, 1399, 1285, 1167, 1129, 1073, 1044, 964, 938, 874, 809, 787, 714, 687, 594, 485, 434 cm^{-1}; ^{1}H NMR (CD_3-OD) δ 1.33 (s, 3 H), 2.60−2.65 (m, 2 H), 3.49 (d, J = 11.3 Hz, 1 H), 3.65 (d, J = 11.3 Hz, 1 H), 3.74−3.82 (m, 1 H), 3.92−3.99 (m, 1 H), 4.06−4.17 (m, 2 H), 4.09 (s, 1 H), 5.20 (s, 1 H), 5.28 (s, 1 H); ^{13}C NMR (CD_3OD) 24.1, 36.8, 63.6 (q, J = 34.8 Hz), 65.4, 68.3, 71.9, 78.2, 87.7, 89.3, 117.5, 125.2 (q, J = 274.4 Hz), 147.6, 168.6, 170.0 ppm; MS (+CI) 385 $[M + 1]^+$; M_r (+CI) 385.121 69 $[M + 1]^+$ (calcd for $C_{14}H_{20}N_2O_7F_3$ 385.122 26).

C(6)-*O*-Isopropylbicyclomycin (7). Using **22** (12 mg, 0.03 mmol) gave **7** as a white solid (7 mg, 84%): mp 99−102 °C; R_f 0.64 (20% MeOH−$CHCl_3$); IR (KBr) 3427, 3282, 2977, 2939, 2884, 1691, 1458, 1403, 1295, 1263, 1157, 1107, 1067, 1044, 941, 873, 786, 682, 589 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.23 (d, J = 6.0 Hz, 3 H), 1.29 (d, J = 6.0 Hz, 3 H), 1.34 (s, 3 H), 2.55−2.60 (m, 2 H), 3.50 (d, J = 11.3 Hz, 1 H), 3.66 (d, J = 11.3 Hz, 1 H), 3.73−3.80 (m, 1 H), 3.88−3.97 (m, 2 H), 4.08 (s, 1 H), 5.11 (s, 1 H), 5.52 (s, 1 H); ^{13}C NMR (CD_3OD) 23.4, 23.9, 24.2, 36.8, 65.5, 68.5, 71.4, 71.9, 78.2, 88.5, 89.2, 117.1, 148.1, 169.6, 171.3 ppm, all peak assignments (Table 1) were in agreement with the APT experiment; MS (+CI) 345 $[M + 1]^+$; M_r (+CI) 345.166 08 $[M + 1]^+$ (calcd for $C_{15}H_{25}N_2O_7$ 345.166 18).

C(6)-*S*-Ethylbicyclomycin (10). Using **23** (17 mg, 0.04 mmol) gave **10** as a white solid (4 mg, 26%): mp 99−104 °C; R_f 0.29 (10% MeOH−$CHCl_3$); IR (KBr) 3446, 3281, 2976, 2935, 2882, 1686, 1457, 1401, 1176, 1139, 1068, 1044, 954, 867, 807, 783, 680, 584, 529, 501, 451 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.24 (t, J = 7.2 Hz, 3 H), 1.33 (s, 3 H), 2.56 (dd, J = 6.7, 16.0 Hz, 1 H), 2.59−2.69 (m, 2 H), 2.73 (dd, J = 9.3, 16.0 Hz, 1 H), 3.49 (d, J = 11.1 Hz, 1 H), 3.64 (d, J = 11.1 Hz, 1 H), 3.74 (dd, J = 9.3, 13.2 Hz, 1 H), 3.99 (dd, J = 6.7, 13.2 Hz, 1 H), 4.07 (s, 1 H), 5.18 (s, 1 H), 5.46 (s, 1 H); ^{13}C NMR (CD_3OD) 13.7, 24.2, 25.3, 37.6, 66.0, 68.5, 71.3, 71.8, 78.3, 89.3, 118.2, 150.4, 170.0, 170.3 ppm; MS (+FAB) 347 $[M + 1]^+$; M_r (+FAB) 347.127 99 $[M + 1]^+$ (calcd for $C_{14}H_{23}N_2O_6S$ 347.127 68).

C(6)-*S*-Phenylbicyclomycin (11). Using **24** (15 mg, 0.03 mmol) gave **11** as a white solid (3 mg, 22%): mp 88−92 °C; R_f 0.39 (10% MeOH−$CHCl_3$); IR (KBr) 3428, 3276, 2955, 2934, 2878, 1686, 1457, 1397, 1291, 1249, 1213, 1176, 1140, 1067, 1042, 1025, 980, 863, 782, 753, 691, 597, 521, 473 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.25 (s, 3 H), 2.61 (dd, J = 7.1, 16.1 Hz, 1 H), 2.78 (dd, J = 9.2, 16.1 Hz, 1 H), 3.45 (d, J = 11.3 Hz, 1 H), 3.59 (d, J = 11.3 Hz, 1 H), 3.75 (dd, J = 9.2, 13.4 Hz, 1 H), 3.98−4.03 (m, 2 H), 5.29 (s, 1 H), 5.61 (s, 1 H), 7.30−7.33 (m, 3 H), 7.58−7.61 (m, 2 H); ^{13}C NMR (CD_3OD) 24.0, 38.0, 66.0, 68.4, 71.7, 78.2, 89.0, 118.2, 130.0, 130.2, 136.4, 150.4, 169.3, 169.7 ppm, the remaining two peaks were not detected and are believed to accidentally overlap with the signals at 68.4 and 130.0 ppm; MS (+CI) 395 $[M + 1]^+$; M_r (+CI) 395.126 38 $[M + 1]^+$ (calcd for $C_{18}H_{23}N_2O_6S$ 395.127 68).

C(6)-*N*-Phenylbicyclomycin (12). Using **25** (6 mg, 0.01 mmol) gave **12** as a white solid (3.0 mg, 55%): mp 108−111 °C; R_f 0.28 (10% MeOH−$CHCl_3$); IR (KBr) 3389, 3245, 2934, 2883, 1686, 1603, 1506, 1405, 1314, 1256, 1164, 1068, 1023, 875, 753, 694, 438 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.37 (s, 3 H), 2.62 (dd, J = 6.9, 15.9 Hz, 1 H), 2.77 (dd, J = 9.3, 15.9 Hz, 1 H), 3.53 (d, J = 11.4 Hz, 1 H), 3.69 (d, J = 11.4 Hz, 1 H), 3.80 (dd, J = 9.3, 12.9 Hz, 1 H), 4.05 (dd, J = 6.9, 12.9 Hz, 1 H), 4.16 (s, 1 H), 5.29 (s, 1 H), 5.62 (s, 1 H), 6.71−6.81 (m, 3 H), 7.07−7.15 (t, J = 8.0 Hz, 2 H); ^{13}C NMR (CD_3OD) 24.2, 37.2, 66.6, 68.6, 72.0, 74.5, 78.4, 89.6, 116.8, 117.3, 119.9, 129.7, 145.1, 151.2, 170.2, 171.2 ppm; MS (+CI) 378 $[M + 1]^+$; M_r (+CI) 378.167 13 $[M + 1]^+$ (calcd for $C_{18}H_{24}N_3O_6$ 378.166 51).

C(6)-Deoxybicyclomycin (13). Using **26** (14 mg, 0.04 mmol) gave **13** as a white solid (9 mg, 73%): mp 117−121 °C; R_f 0.41 (20% MeOH−$CHCl_3$); IR (KBr) 3408, 3277, 2934, 1686, 1414, 1293, 1071, 1041 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.56−2.59 (m, 2 H), 3.49 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.76−3.82 (m, 1 H), 3.89−3.95 (m, 1 H), 4.07 (s, 1 H), 4.40 (s, 1 H), 5.06 (s, 1 H), 5.14 (s, 1 H); ^{13}C NMR (CD_3OD) 24.2, 36.1, 62.7, 64.9, 68.5, 72.1, 78.2, 88.9, 118.2,

147.4, 169.6, 171.7 ppm; MS (+CI) 287 $[M + 1]^+$; M_r (+CI) 287.123 60 $[M + 1]^+$ (calcd for $C_{12}H_{19}N_2O_6$ 287.124 31).

General Procedure for the Reaction of Bicyclomycin (1) and C(6)-*O*-Methylbicyclomycin (4) with EtSH. To a degassed (Ar, 5 min) solution of **1** or **4** (1 equiv) and THF−H_2O (3:1, 4 mL) was added EtSH (16 equiv). The "pH" of the reaction mixture was then adjusted with aqueous dilute NaOH, and the solution was stirred at room temperature under an Ar atmosphere. The "pH" of the solution was then measured at the end of the reaction, the solvent was removed *in vacuo*, and the residue was purified by PTLC (20% MeOH−$CHCl_3$) to afford the product(s).

Using this procedure, we ran the following reactions.

Reaction of Bicyclomycin (1) with EtSH at "pH" 9. Using **1** (18 mg, 0.06 mmol) and EtSH (59 mg, 0.95 mmol) led to a drop in the solution "pH" to 8.2 during the reaction (12 h). TLC analysis showed only the presence of **31**. Purification of the reaction provided **31**[10,11] as a white solid (9 mg, 44%): mp 210−215 °C (lit.[10,11] mp 216−218 °C); R_f 0.68 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.15 (s, 3 H), 1.24 (t, J = 7.5 Hz, 3 H), 1.81−1.93 (m, 1 H), 2.30 (dt, J = 6.5, 14.1 Hz, 1 H), 2.58 (q, J = 7.5 Hz, 2 H), 2.90 (d, J = 14.1 Hz, 1 H), 3.00 (d, J = 14.1 Hz, 1 H), 3.64 (d, J = 12.0 Hz, 1 H), 3.68−3.78 (m, 1 H), 3.90 (s, 1 H), 4.03 (d, J = 12.0 Hz, 1 H), 3.92−4.06 (m, 1 H).

Reaction of C(6)-*O*-Methylbicyclomycin (4) with EtSH at pH 9. Using **4** (16 mg, 0.05 mmol) and EtSH (50 mg, 0.81 mmol) led to a drop in the solution "pH" to 8.0 during the reaction (7 d). TLC analysis showed no reaction. Purification of the reaction led to the recovery of **4** (8 mg, 50%): R_f 0.43 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.58−2.62 (m, 2 H), 3.39 (s, 3 H), 3.50 (d, J = 11.3 Hz, 1 H), 3.66 (d, J = 11.3 Hz, 1 H), 3.78−3.83 (m, 1 H), 3.89−3.95 (m, 1 H), 4.09 (s, 1 H), 5.13 (s, 1 H), 5.50 (s, 1 H).

Reaction of Bicyclomycin (1) with EtSH at "pH" 10.5. Using **1** (25 mg, 0.08 mmol) and EtSH (82 mg, 1.32 mmol) led to a drop in the solution "pH" to 9.9 during the reaction (12 h). TLC analysis showed only the presence of **31** and **32**. Purification of the reaction led to **31** and **32**.

Compound 31:[10,11] yield, 5 mg (17%); mp 212−217 °C (lit.[10,11] mp 216−218 °C); R_f 0.68 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.15 (s, 3 H), 1.24 (t, J = 7.3 Hz, 3 H), 1.80−1.94 (m, 1 H), 2.32 (dt, J = 6.6, 14.1 Hz, 1 H), 2.58 (q, J = 7.3 Hz, 2 H), 2.89 (d, J = 14.0 Hz, 1 H), 3.00 (d, J = 14.0 Hz, 1 H), 3.64 (d, J = 12.2 Hz, 1 H), 3.68−3.78 (m, 1 H), 3.90 (s, 1 H), 4.03 (d, J = 12.2 Hz, 1 H), 3.97−4.03 (m, 1 H).

Compound 32:[13] yield, 8 mg (27%); mp 155−165 °C (lit.[13] semisolid); R_f 0.45 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.23 (t, J = 7.2 Hz, 3 H), 1.32 (s, 3 H), 2.05−2.26 (m, 4 H), 2.42−2.55 (m, 2 H), 3.15 (d, J = 11.7 Hz, 1 H), 3.51 (d, J = 11.4 Hz, 1 H), 3.67 (d, J = 11.4 Hz, 1 H), 3.73−3.84 (m, 1 H), 3.90−4.00 (m, 1 H), 4.03 (s, 1 H).

Reaction of C(6)-*O*-Methylbicyclomycin (4) with EtSH at pH 10.5. Using **4** (17 mg, 0.05 mmol) and EtSH (53 mg, 0.86 mmol) led to a drop in the solution "pH" to 10.4 during the reaction (12 h). TLC analysis showed the presence of **33** as the major product and several unidentified compounds along with a small amount of **4**. Purification of the reaction led to **33** (5 mg, 33%): mp 185−195 °C; R_f 0.68 (20% MeOH−$CHCl_3$); IR (KBr) 3232, 2965, 2933, 1686, 1561, 1422, 1261, 1158, 1092, 1050, 1023, 1002, 913, 884, 825, 644, 553, 490 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.77−2.91 (m, 2 H), 3.82 (d, J = 9.3 Hz, 1 H), 3.96 (d, J = 9.3 Hz, 1 H), 4.07−4.22 (m, 2 H), 4.42 (s, 1 H), 5.34 (t, J = 2.1 Hz, 1 H), 5.40 (t, J = 2.1 Hz, 1 H); ^{13}C NMR (CD_3OD) 21.1, 32.6, 68.9, 77.7, 78.6, 78.8, 90.1, 111.4, 151.0, 167.8, 169.9 ppm, the remaining signal was not detected and may accidentally overlap with the peak at 90.1 ppm; MS (+CI) 285 $[M + 1]^+$; M_r (+CI) 285.107 85 $[M + 1]^+$ (calcd for $C_{12}H_{17}N_2O_6$ 285.108 66).

General Procedure for the Determination of the Stability of C(6)-Substituted Bicyclomycin Derivatives. The stability of bicyclomycins (**1**, **4**, **6**, **10**−**13**) in aqueous pH 7.4 buffered solution (40 mM Tris) was determined by HPLC. A 2 mg/mL solution of the bicyclomycin derivative was maintained at 37 ± 1 °C. DMSO (10%) was added to the reaction of **11**. The pH of the reaction mixture was determined both before and after the reaction. In the cases of **1**, **4**, **10**, **11**, and **13**, the pH at the end of the reaction was 7.4 ± 0.1. In the reactions involving **6** and **12** the pH at the end of the reaction was 7.0 and 6.8, respectively. Linear plots of ln A_0/A of the starting material and products at λ = 214 nm versus time were obtained for an average of seven points per bicyclomycin derivative over the course of the reaction. Using a least-squared program the half-life for consumption of the bicyclomycin derivative was calculated (Table 2). Most reactions were monitored for at least 2 half-lives, and the products were identified by coinjection of authentic samples with the reaction mixtures (HPLC) and further verified by TLC analysis.

Stability of C(6)-*O*-Methylbicyclomycin (4). An aqueous pH 7.4 buffered (2 mL, 40 mM Tris) solution of **4** (12 mg, 0.38 mmol) was maintained at 37 ± 1 °C (100 h). During the course of the reaction the pH changed from 7.4 to 7.3. The reaction was concentrated *in vacuo*, and the residue was purified by PTLC (2 × 15% MeOH−$CHCl_3$) to afford the following compounds.

Compound 4: yield, 5 mg (42% recovery); R_f 0.50 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.57−2.65 (m, 2 H), 3.39 (s, 3 H), 3.50 (d, J = 11.3 Hz, 1 H), 3.66 (d, J = 11.3 Hz, 1 H), 3.72−3.84 (m, 1 H), 3.90−3.96 (m, 1 H), 4.09 (s, 1 H), 5.13 (s, 1 H), 5.50 (s, 1 H).

Compound 33 (diastereomeric mixture): yield, 2 mg (19%); R_f 0.59 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.70−2.85 (m, 2 H), 3.80−3.84 (m, 1 H), 3.94−3.97 (m, 1 H), 4.05-4.22 (m, 2 H), 4.41−4.42 (m, 1 H), 5.25−5.41 (m, 2 H).

Compound 34 (diastereomeric mixture): yield, 3 mg (25%) as a semisolid; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3433, 1681, 1418, 1295, 1148, 1099, 1021, 976, 879, 810 cm^{-1}; ^{1}H NMR (DMF-d_7) major diastereomer δ 1.35 (s, 3 H), 2.45 (t, J = 7.4 Hz, 2 H), 3.25 (s, 3 H), 3.60−3.70 (m, 2 H), 3.77 (d, J = 9.2 Hz, 1 H), 3.93 (d, J = 9.2 Hz, 1 H), 4.48 (s, 1 H), 4.54 (br s, 1 H), 5.16 (s, 1 H), 5.41 (s, 1 H), 5.72 (br s, 1 H), 5.96 (br s, 1 H), 7.75 (s, 1 H), 8.77 (s, 1 H); minor diastereomer δ 1.19 (s, 3 H), 3.89 (d, J = 9.2 Hz, 1 H), 4.13 (d, J = 9.2 Hz, 1 H), 5.51 (s, 1 H), the other signals were not detected; ^{13}C NMR (DMF-d_7) major diastereomer 21.0, 51.1, 61.3, 76.8, 78.0, 78.1, 84.2, 89.4, 114.3, 146.0, 164.9, 166.9 ppm, the remaining signal is believed to accidentally overlap with the solvent peak at 35.2 ppm; minor diastereomer 20.7, 76.1, 88.1, 114.8, 145.6, 166.9, 168.3 ppm, the other signals were not detected; MS (+CI) 317 $[M + 1]^+$; M_r (+CI) 317.133 90 $[M + 1]^+$ (calcd for $C_{13}H_{21}N_2O_7$ 317.134 88).

Acknowledgment. We thank the National Institutes of Health (Grant GM37934) and the Robert A. Welch Foundation (Grant E-607) for 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, and Dr. A. Magyar (University of Houston) for his help in the transcription termination assays.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds for all new compounds (47 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.

JO961003Q