5a-Methyl-Substituted Bicyclomycins: Synthesis and Chemical, Biochemical, and Biological Properties

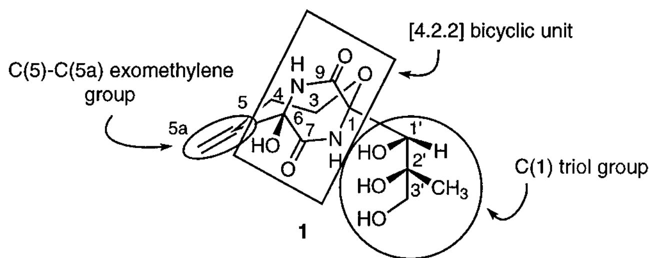
Alejandro Santillán, Jr.,† Xiangdong Zhang,† William R. Widger,‡ and Harold Kohn*,†

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 November 7, 1997

A select series of 5a-methyl-substituted bicyclomycins (**2**−**10**, **34**) have been prepared to identify interactions that influence antibiotic binding to the *Escherichia coli* rho transcription termination factor and to aid in identifying the bicyclomycin binding domain. The regioselective reduction of methyl 5a-bicyclomycincarboxylate C(2′),C(3′)-acetonide (**13**) using lithium triethylborohydride provided 5a-(hydroxy)methylbicyclomycin C(2′),C(3′)-acetonide (**14**). Alcohol **14** served as the key synthetic intermediate in preparing the targeted compounds. Replacing or modifying the terminal hydroxy group in **14** gave the corresponding hydrogen, acyl, halogen, azide, amine, and amide derivatives, which were then treated with trifluoroacetic acid to remove the C(2′),C(3′)-acetonide protecting group to give **2**−**10**. The chemical reactivity of 5a-(chloro)methylbicyclomycin (**6**) with the nucleophile EtSH was compared with bicyclomycin (**1**). We found that allylic chloride **6** underwent S_N2 displacement with EtSH, while **1** furnished C(5)−C(5a) exomethylene group modified adducts, suggesting that **6** may serve as a site selective irreversible alkylation probe. Evaluation of **2**−**6**, **8**−**10**, and **34** in rho functional assays showed that 5a-methylbicyclomycin (**2**), 5a-(hydroxy)-methylbicyclomycin (**3**), 5a-[2,6-bis(trifluoromethyl)benzoxy]methylbicyclomycin (**5**), 5a-(azido)-methylbicyclomycin (**8**), 5a-(ethylmercapto)methylbicyclomycin (**34**), and **6** all exhibited inhibitory properties comparable with **1**. The activities of these compounds and the remaining bicyclomycins within this series provided information of the structural interactions that occur with drug binding. Finally, we found that **2** displayed comparable antimicrobial activity with **1** in the filter disc assay. Compound **2** is the most biologically active bicyclomycin derivative reported to date.

Bacterial resistance to conventional antibiotics is a major health concern. The increasing number of antibiotic-resistant microbes has led to the search of structurally unique antibacterial agents that have novel modes of action;[1] bicyclomycin (**1**) is one such agent. This drug possesses a broad spectrum of antimicrobial activity against Gram-negative bacteria including *Escherichia coli, Klebsiella, Shigella, Salmonella, Citrobacter, Enterobacter cloacae,* and *Neisseria gonorrheae*[2−4] and the Gram-positive bacterium *Micrococcus luteus*.[5]



Recently, we demonstrated that in *E. coli* the essential cellular protein, rho transcription termination factor,[6] is the primary site of action of **1**.[7] Mechanistic information obtained on the pathway for bicyclomycin inhibition of rho transcriptional processes showed that bicyclomycin inhibits rho by a reversible pathway and prevents the production of rho-dependent RNA transcripts.[7−9] Our studies indicated that the antibiotic affected rho's translocation to the DNA-RNA elongation complex by interfering with the RNA binding to the secondary RNA binding (tracking) site and its ability to hydrolyze ATP.

We determined the bicyclomycin structural units required for rho binding and inactivation by dividing the antibiotic into three sectors: C(1) triol group, [4.2.2] bicyclic unit, and C(5)−C(5a) exomethylene moiety.[10−15]

* Phone no.: (713)743-3240. Fax no.: (713)743-2709. E-mail address: Hkohn@uh.edu.

† Department of Chemistry.

‡ Department of Biochemical and Biophysical Sciences.

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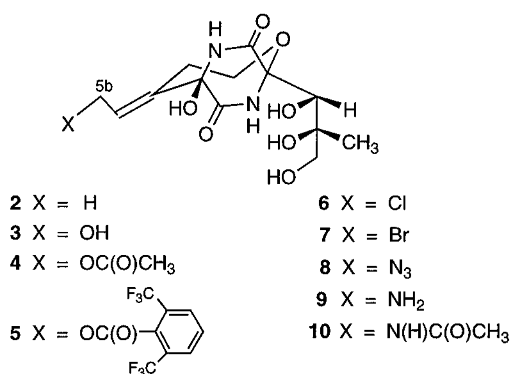
S0022-3263(97)02055-0 CCC: $15.00 © 1998 American Chemical Society
Published on Web 02/03/1998

Each unit was structurally modified, and the effect on bicyclomycin function was assessed using in vitro biochemical assays. These tests measured the bicyclomycin analogue's ability to inhibit rho-dependent transcription termination[16] and rho-dependent ATPase activity.[17] We found that the C(1) triol and [4.2.2] piperazinedione units in bicyclomycin were critical structural elements necessary for rho recognition and drug function and structural changes at these sites led to inactive bicyclomycin analogues.[10−13,15] Correspondingly, we found that select bicyclomycins modified at the C(5)−C(5a) site retained excellent inhibitory activities in the in vitro biochemical assays.[14] These results led us to conclude that structural alteration of C(5)−C(5a) exomethylene unit in **1** may provide potent bicyclomycin analogues.

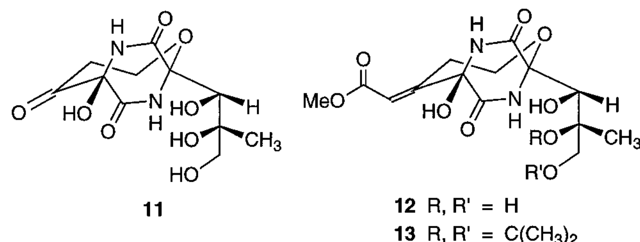
In this paper, we report on the synthesis and chemical, biochemical, and biological properties of a series of bicyclomycin analogues in which the C(5)−C(5a) exomethylene unit has been extended by one carbon. We show that many of these compounds bind efficiently to rho, retain excellent inhibitory activities in in vitro and in vivo assays, and hold promise as biomechanistic probes to determine the site of the drug-rho interaction.

Results and Discussion

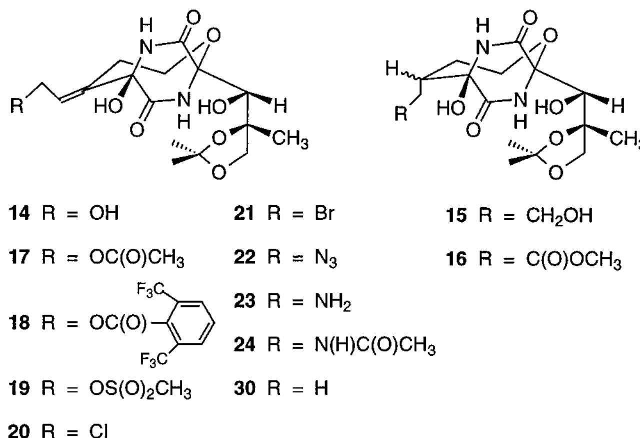
A. Choice of Substrates. We restricted our initial choice of compounds to the 5a-methyl-substituted bicyclomycins **2**−**10**. We expected that **2**−**10** would permit us to identify interactions (e.g., hydrogen bonding, electrostatic, steric) that influence drug-rho binding. Of the nine compounds, **4**−**7** may serve as alkylating agents leading to the selective monoalkylation of rho. These four bicyclomycin derivatives differ in their reactivity toward nucleophiles. We expected the order of reactivity to be **7** > **6** > **5** > **4**. Krantz and co-workers[18] have shown that acetoxy- and bis(trifluoromethyl)benzoxy-substituted compounds can function as highly selective substrates for protein labeling provided suitable protein residues can catalyze the nucleophilic substitution process.



B. Synthesis. Müller and co-workers were the first to introduce a carbon moiety at the C(5a) site in bicyclomycin.[19] These researchers showed that treatment of C(5) norketonebicyclomycin[19,20] (**11**) with methyl(triphenylphosphoranylidene)acetate provided methyl 5a-bicyclomycincarboxylate (**12**). We utilized **12** to prepare



2−**10**. Methyl ester **12** was converted to acetonide **13** by treatment with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid in DMF. Reduction of **13** with lithium aluminum hydride gave a 2:1 mixture of 5a-(hydroxy)methylbicyclomycin C(2′),C(3′)-acetonide (**14**) and 5a-(hydroxy)methyldihydrobicyclomycin C(2′),C(3′)-acetonide (**15**), respectively (data not shown). Compounds **14** and **15** were found to coelute on TLC plates (20% MeOH−$CHCl_3$). Use of lithium triethylborohydride (8 equiv) in place of lithium aluminum hydride for the reduction of **13** led to improved yields of **14**. The reaction was observed to proceed with near complete regioselectivity. Inspection of the ^{1}H NMR spectrum for the reaction product showed only trace levels (<5%) of the fully reduced 5a-(hydroxy)methyldihydrobicyclomycin C(2′),C(3′)-acetonide (**15**). The overall yield of allylic alcohol **14** from bicyclomycin was 35%. Catalytic reduction (10% Pd−C, H_2) of **13** to give **16** followed by treatment with lithium aluminum hydride provided an authentic sample of **15**.



Removal of the acetonide group in **14** using trifluoroacetic acid (TFA) gave 5a-(hydroxy)methylbicyclomycin (**3**). Synthesis of 5a-(acetoxy)methylbicyclomycin (**4**) was accomplished by first acylating allylic alcohol **14** using acetic anhydride, triethylamine, and a catalytic amount of DMAP to provide **17** and then by deprotecting the triol

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group with TFA. Compound **5** was prepared by coupling **14** with 2,6-bis-(trifluoromethyl)benzoic acid using carbonyl diimidazole and triethylamine in the presence of DMAP to give **18** followed by treatment with TFA.

We prepared the 5a-(halo)methylbicyclomycins **6** and **7** by converting allylic alcohol **14** in situ to the allylic mesylate **19** using methanesulfonyl chloride and triethylamine in THF. Mesylate displacement with lithium chloride in THF provided **20**. Deprotection of the triol group in **20** gave **6**. Our efforts to prepare **7** by substituting LiBr for LiCl in this procedure were only partially successful. In situ mesylation using **14** with methanesulfonyl chloride followed by treatment with LiBr gave a 3:1 mixture of **21**:**20**, respectively (data not shown). Accordingly, we substituted methanesulfonic anhydride for methanesulfonyl chloride to eliminate the chloride ion content in the reaction. This protocol gave a 9:1 mixture of **21**:**20**, respectively. The source of the chloride ion, which led to the minor production of **20**, was not identified. Even more surprising was our finding that treatment of the 9:1 mixture of **21** and **20** with TFA gave a 1:1 mixture tentatively identified as **7** and **6**. We suspect that the allylic bromide **7** was converted, in part, to **6** during the reaction and workup, which produced the near equal amounts of these two compounds. The source of the adventitious chloride ion in this transformation is unknown. Attempts to separate **6** and **7** were not successful, which prevented us from fully characterizing **7**.

The improved synthesis of 5a-(bromo)methylbicyclomycin C(2′),C(3′)-acetonide (**21**) provided us with an activated bicyclomycin with both excellent chemical reactivity and stability. Bicyclomycin **21** served as a key intermediate for the synthesis of the remaining 5a-methyl-substituted bicyclomycins. Dissolution of **21** and sodium azide in DMF provided **22** with no formation of other side products (TLC analysis). Triol group deprotection of acetonide **22** provided 5a-(azido)methylbicyclomycin (**8**). Synthesis of 5a-(amino)methylbicyclomycin (**9**) was achieved by first condensing anhydrous ammonia in a THF solution of allylic bromide **21** maintained at −78 °C to give **23** followed by acid-catalyzed removal of the acetonide group. Treatment of **23** with acetic anhydride and triethylamine gave **24**, which was converted to **10** with TFA.

We explored an alternative approach to 5a-(amino)methylbicyclomycin C(2′),C(3′)-acetonide (**23**). Attempted chemoselective reduction of the azide group in **22** using Staudinger conditions[21] did not yield **23** but rather the novel pyrrole **25**. The NMR data for **25** supported both the presence of a pyrrole ring and the cleavage of the N(10)−C(6) bond in the rearranged bicyclomycin. The ^{1}H NMR spectrum exhibited a pair of downfield doublets (J = 2.4 Hz) at δ 5.88 and δ 6.84 indicative of a 2,3-disubstituted pyrrole. In the APT spectrum we detected two unsubstituted aromatic carbon signals at 111.3 and 123.9 ppm and two substituted aromatic carbon resonances at 123.0 and 126.2 ppm. Comparison of the ^{13}C NMR chemical shifts for the C(1) and C(1′) carbons in **25** with those typically observed for N(10)−C(6) ring closed bicyclomycin acetonides revealed that these signals resonated ~3 and 7 ppm, respectively, downfield, while the ^{1}H NMR C(1′) methine peak in **25**

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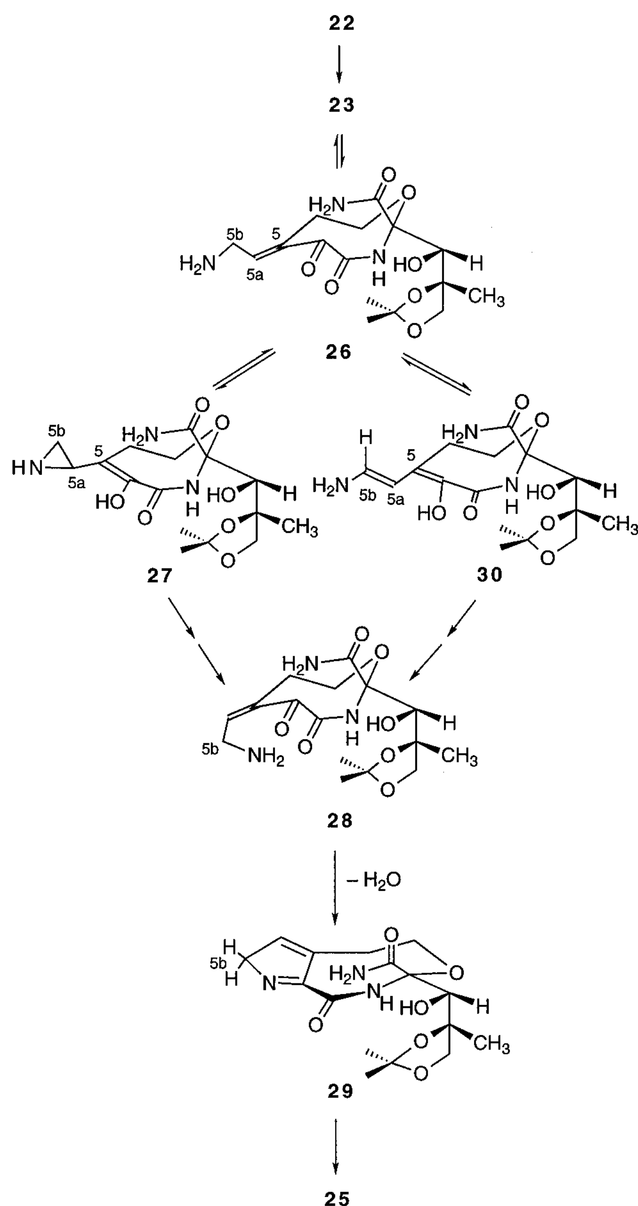
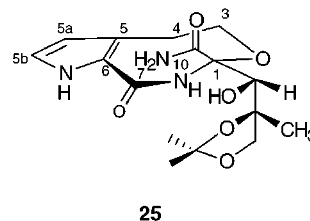
Scheme 1. Pathways for Formation of Compound 25



appeared upfield (~0.42 ppm) from the chemical shift value usually observed for this signal. The magnitude and direction of the ^{13}C and ^{1}H NMR shifts for **25** were reminiscent of sterically induced polarization effects (γ-effect)[22] that accompany the relief of the steric strain.



Two pathways for the formation of **25** are depicted in Scheme 1. Both pathways proceeded by initial Staudinger

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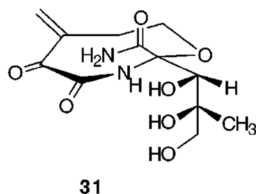
reduction of **22** to generate the desired allylic amine **23**. In one route, reversible ring opening of **23** to the (5*E*)-alkene **26** was followed by intramolecular Michael addition of the primary amine to the C(5a) site to create aziridine **27**. Rotation about the C(5)−C(5a) bond followed by enol-assisted cleavage of the aziridine ring provided the (5*Z*)-alkene **28**. Intramolecular condensation of **28** gave **29**, which then aromatized to **25**. In the second pathway, tautomerization of the ring-opened allylic amine **26** provided **30**, which then underwent C(5)−C(5a) bond rotation and isomerization to generate **28**. Cyclization of **28** followed by aromatization gave **25**. These two pathways can be distinguished. Dissolution of **23** in warm (55 °C) alkaline D_2O−THF mixtures gave **25** with no detectable incorporation of deuterium at the C(5b) site (^{1}H NMR: δ 6.84). These findings are consistent with the intermediate formation of aziridine **27** since if the reaction proceeded through **30**, deuterium incorporation would have likely occurred at the C(5b) site in **28** leading to the C(5b) deuterium analogue of **25**.

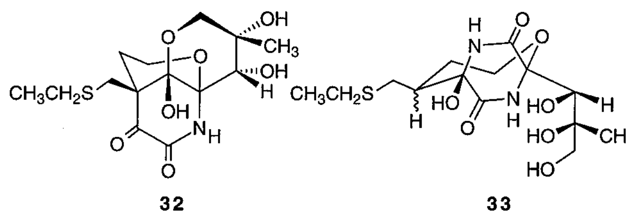
The parent compound for this series of compounds, 5a-methylbicyclomycin (**2**), was prepared by reduction of **21** with lithium triethylborohydride to give **30** followed by treatment with acid.

C. Spectral Studies. Satisfactory spectroscopic (IR, ^{1}H, ^{13}C NMR, low- and high-resolution mass) data were obtained for all new compounds. The ^{1}H and ^{13}C NMR signals for the C(5b) protons and carbon in the 5a-methyl-substituted bicyclomycins varied with substituent and were consistent with values reported in the literature.[23,24] Introduction of a substituted alkyl unit at C(5a) in **1** led to upfield shifts (4−9 ppm) for the C(5) signal and downfield shifts (7−14 ppm) for the C(5a) resonance in compounds **2**−**10**.[24] We assigned the C(5) configuration for bicyclomycins **2**−**10** as the sterically less-hindered (*E*) configuration on the basis of Müller's earlier assignment for methyl 5a-carboxylatebicyclomycin (**12**).[19]

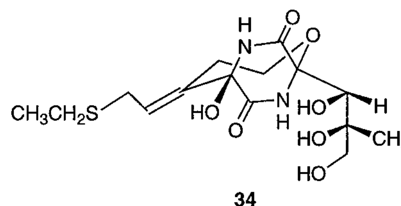
D. Chemical Studies. Bicyclomycin has been shown to undergo addition reactions at the C(5)−C(5a) exo-methylene site with thiols and secondary amines in THF:H_2O (3:1) mixtures at near neutral and basic pH values.[25−27] This transformation proceeds by nucleophilic addition to the ring-opened Michael acceptor **31**. In light



of these findings, the reactivity of EtSH with bicyclomycin (**1**) was compared with 5a-(chloro)methylbicyclomycin (**6**). We wished to determine if a reaction occurs at either the C(5b) (chloro)methyl allylic or the C(5a) vinylic sites in **6**. Treating **1** with excess EtSH at pH 7.4−8.5 at room temperature gave the rearranged adduct **32**[11,15] whereas at "pH" 10−11 the C(5)−C(5a) substituted adduct **33** predominated.[11,15] Correspondingly, treatment of a THF:



H_2O (3:1) solution of **6** with EtSH (10 equiv) at pH 8.0 gave no reaction (TLC analysis) after 18 h (22 °C) and the recovery of **6**. Elevation of the solution "pH" to 10 initiated ethanethiolate displacement of the allylic chloride to provide **34** (22 °C, 2 h). Analysis of the reaction by TLC prior to workup showed a single product and the loss of starting material. These experiments demonstrated that only the S_N2 displacement of the allylic chloride substituent in **6** occurred and suggested that **6** may serve as a selective irreversible rho inactivator.



E. Biochemical and Biological Studies. The inhibitory properties of 5a-methylbicyclomycins **2**−**6**, **8**−**10**, and **34** in the rho-dependent ATPase assay are listed in Table 1. All the compounds effectively inhibited (40−95%) rho-dependent ATPase activity at 400 μM. At this concentration, bicyclomycin inhibited the hydrolysis of ATP by 86−95%. Inspection of the I_{50} values indicated that 5a-(chloro)methylbicyclomycin (**6**) (I_{50} = 45 μM) was more effective than bicyclomycin (I_{50} = 60 μM) in inhibiting ATP hydrolysis. Additionally, **2**, **3**, **5**, **8**, and **34** (I_{50} = 70−120 μM) showed inhibitory properties that approached that of bicyclomycin whereas **4** retained only moderate activity (I_{50} = 175 μM) relative to bicyclomycin. The 5a-(amino)methyl-substituted bicyclomycins **9** and **10** showed only residual activity in the ATPase assay (I_{50} $\geq$ 350 μM). Similar biochemical findings were observed in the rho-dependent transcription termination assay. The estimated I_{50} values for **2**, **6**, **8**, and **34** (I_{50} = 5−10 μM) were comparable to that found for bicyclomycin (I_{50} $\approx$ 5 μM), whereas **3** and **5** showed only moderate activity (I_{50} = 10−40 μM) and **9** and **10** exhibited very little activity (I_{50} $\geq$ 40 μM). The activity of **4** was not determined in this assay.

In a previous study, we observed that increases in the size of the carboxylate ester or the N-substituted oxime moieties in substituted 5a-carboxylate **35** and C(5) oxime **36** bicyclomycins led to progressive decreases in the inhibitory activities of the bicyclomycin analogues.[14] We did not see a comparable pattern for the 5a-methyl-substituted bicyclomycins (Table 1). Increases in the steric size of the 5a-methyl unit led to both decreases (**3** vs **4**, **8** vs **10**) and increases (**4** vs **5**) in the inhibitory ATPase activity. Significantly, both **5** and **6** showed excellent inhibitory activities in the rho functional assays. These two 5a-methyl-substituted bicyclomycins contain moieties at the C(5b) site susceptible to S_N2 displacement. This finding coupled with the appreciable stability of **5**

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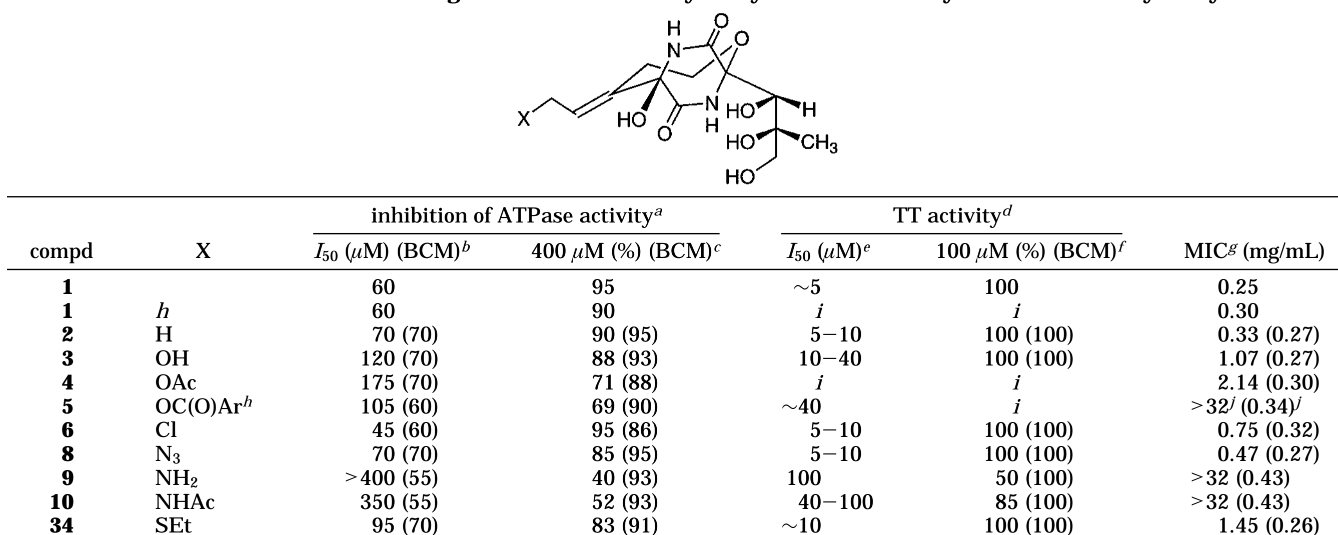
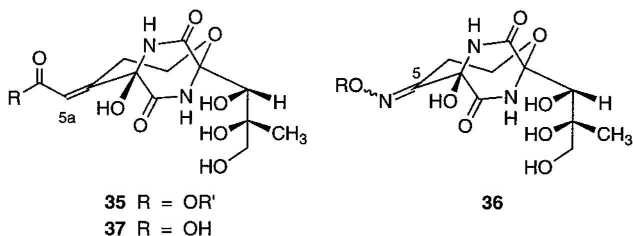
Table 1. Biochemical and Biological Activities of Bicyclomycin and 5a-Methyl-Substituted Bicyclomycins



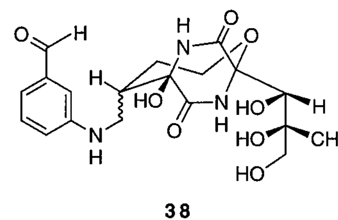
compd	X	inhibition of ATPase activity[a] I_{50} (μM) (BCM)[b]	inhibition of ATPase activity[a] 400 μM (%) (BCM)[c]	TT activity[d] I_{50} (μM)[e]	TT activity[d] 100 μM (%) (BCM)[f]	MIC[g] (mg/mL)
1		60	95	~5	100	0.25
1	*h*	60	90	*i*	*i*	0.30
2	H	70 (70)	90 (95)	5–10	100 (100)	0.33 (0.27)
3	OH	120 (70)	88 (93)	10–40	100 (100)	1.07 (0.27)
4	OAc	175 (70)	71 (88)	*i*	*i*	2.14 (0.30)
5	OC(O)Ar[h]	105 (60)	69 (90)	~40	*i*	>32[j] (0.34)[j]
6	Cl	45 (60)	95 (86)	5–10	100 (100)	0.75 (0.32)
8	N_3	70 (70)	85 (95)	5–10	100 (100)	0.47 (0.27)
9	NH_2	>400 (55)	40 (93)	100	50 (100)	>32 (0.43)
10	NHAc	350 (55)	52 (93)	40–100	85 (100)	>32 (0.43)
34	SEt	95 (70)	83 (91)	~10	100 (100)	1.45 (0.26)

[a] Activity measured using the ATPase assay (ref 17). [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 16). [e] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. [f] The percentage of transcription termination at 100 μM. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [g] MIC value is the average minimum inhibitory concentration of the tested compound determined from duplicate tests using a filter disk assay (ref 30). 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. [j] The sample was applied to the filter disk using 100% DMSO.

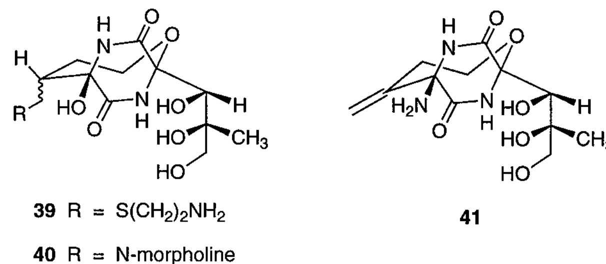
and **6** in ATPase buffered solutions[28] made these analogues ideal candidates for further study as potential site selective irreversible alkylating probes.



The inactivity of 5a-(amino)methylbicyclomycin (**9**) in the ATPase and transcription termination assay provided insights into the structure of the bicyclomycin binding domain in rho. Previously, we reported that **37** is a potent rho inhibitor (ATPase assay $I_{50} = 95$ μM; transcription termination assay $I_{50} \approx 10$ μM).[14] We expect that **37** exists as the carboxylate anion under the employed assay conditions (pH = 7.9). Similarly, **9** should exist as the protonated amine. The finding that **37** inhibited rho-dependent ATPase and transcription termination processes demonstrates that adverse electrostatic interactions alone were not responsible for the loss of activity of **9**. Rather, we suspect that binding may benefit from an electrostatic interaction from a nearby positively charged amino acid residue. This notion is in agreement with the recent report that the reductive amination probe 5a-(3-formylanilino)dihydrobicyclomycin (**38**) is selectively modified by a lysine residue in the rho bicyclomycin binding pocket.[29] If this is the case, then



the positively charged 5a-(amino)methyl residue in **9** may prevent the bicyclomycin from binding to rho. Consistent with this theory we have shown that the I_{50} value for the three amines, dihydrobicyclomycin 5a-2-aminoethyl sulfide (**39**),[14] 5a-morpholinodihydrobicyclomycin (**40**),[14] and 6-aminobicyclomycin (**41**),[15] all exceed 400 μM in the rho-dependent ATPase assay.



The biochemical results did not correlate with the biological studies (Table 1). For example, the MIC value[30] for 5a-(chloro)methylbicyclomycin (**6**) (0.75 mg/mL) was nearly three times higher than that found for bicyclomycin (0.25–0.32 mg/mL), even though **6** was a more effective inhibitor than **1** in the ATPase assay. Similarly, we observed that **5** exhibited no detectable antibiotic activity and yet it possessed an I_{50} value of 105 μM in the ATPase assay. These findings reinforced our decision to exclude the antimicrobial data in our identification of bicyclomycin analogues that effectively inhibit

(28) Santillán, A., Jr. Ph.D. Dissertation, University of Houston, December, 1997.

(29) Cho, H.; Park, H.-g.; Zhang, X.; Riba, I.; Gaskell, S. J.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1997**, *62*, 5432–5440.

(30) Ericsson, H. M.; Sherris, J. C. *Acta Pathol. Microbiol. Scand.* **1971**, suppl., *217*, 1–90.

rho function.[10] This series of compounds also provided **2**, which is the most biologically active bicyclomycin *derivative* reported to date (MIC = 0.33 mg/mL). The antibacterial activities of **2**, **6**, and **8** provide encouraging results on the potential of 5a-methyl substituted bicyclomycins as second generation drug candidates.

Conclusions

Synthetic methods have been developed to extend the C(5)−C(5a) exomethylene unit in bicyclomycin. Select substituents can be introduced at this site of the antibiotic without loss of in vitro biochemical and in vivo biological activities. The use of these and related analogues as biomechanistic probes to determine the site of drug binding to rho is being investigated.

Experimental Methods[31]

Preparation of Methyl 5a-Bicyclomycincarboxylate C(2′),C(3′)-Acetonide (13). To an anhydrous 2,2-dimethoxypropane:dimethylformamide (3:1) solution (4 mL) of **12**[19] (530 mg, 1.47 mmol) was added a catalytic amount of *p*-toluenesulfonic acid. The solution was stirred (45 °C, 2 h) and concentrated in vacuo. The residue was purified by column chromatography using 5% MeOH−$CHCl_3$ as the eluant to provide **13** as a white solid (472 mg, 89%): mp 120−124 °C; R_f 0.49 (10% MeOH−$CHCl_3$); IR (KBr) 3422 (br), 3288 (br), 2991, 2939, 2885, 1699 (br), 1434 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.41 (s, 3 H), 1.44 (s, 3 H), 2.92 (dd, J = 8.9, 16.4 Hz, 1 H), 3.59−3.83 (m, 2 H), 3.69 (s, 3 H), 3.73 (d, J = 8.6 Hz, 1 H), 4.00 (dd, J = 8.0, 13.4 Hz, 1 H), 4.17 (s, 1 H), 4.45 (d, J = 8.6 Hz, 1 H), 6.50 (s, 1 H); ^{13}C NMR (CD_3OD) 25.1, 26.8, 28.1, 29.2, 52.0, 65.6, 72.8, 73.1, 83.3, 86.5, 89.0, 111.6, 120.1, 158.1, 168.0, 168.5, 171.2 ppm; MS (+CI) 401 $[M + 1]^+$; M_r (+CI) 401.155 83 $[M + 1]^+$ (calcd for $C_{17}H_{25}N_2O_9$ 401.156 01).

Preparation of 5a-(Hydroxy)methylbicyclomycin C(2′),C(3′)-Acetonide (14). To an anhydrous THF solution (4 mL) of **13** (104 mg, 0.26 mmol) maintained at −78 °C was added lithium triethylborohydride (331 mg, 3.12 mmol). The solution was stirred (18 h) during which time the solution temperature was allowed to warm to room temperature. The solution was cooled (0 °C), quenched with H_2O (6 mL), neutralized (TFA), and concentrated in vacuo. The residue was purified by column chromatography using 15% MeOH−$CHCl_3$ as the eluant. The residue was further purified by preparative TLC (20% MeOH−$CHCl_3$) to provide **14** as a white solid (75 mg, 75%): mp 179−184 °C; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3421 (br), 3301 (br), 2990, 2938, 2884, 1688 (br), 1457 cm^{-1}; ^1H 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)$_2$), 2.53 (dd, J = 8.6, 16.8 Hz, 1 H, C(4)*H*H′), 2.70 (dd, J = 7.4, 16.8 Hz, 1 H, C(4)-H*H′*), 3.73 (d, J = 8.6 Hz, 1 H, C(3′)*H*H′), 3.80 (dd, J = 8.6, 13.1 Hz, 1 H, C(3)*H*H′), 3.95 (dd, J = 7.4, 13.1 Hz, 1 H, C(3)-H*H′*), 4.14 (d, J = 6.6 Hz, 2 H, C(5b)H_2), 4.15 (s, 1 H, C(1′)H), 4.45 (d, J = 8.6 Hz, 1 H, C(3′)H*H′*), 6.29 (t, J = 6.6 Hz, 1 H, C(5a)H), the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) 24.9 (C(2′)-*C*H_3), 26.8 (C(*C*H_3)$_2$), 28.1 (C(*C*H_3)$_2$), 29.3 (C(4)), 59.2 (C(5b)), 66.2 (C(3)), 73.1 (C(1′)), 73.2 (C(3′)), 83.1 (C(6)), 86.4 (C(2′)), 88.8 (C(1)), 111.6 (*C*(CH_3)$_2$), 130.7 (C(5a)), 140.9 (C(5)), 168.6 and 172.5 (C(7), C(9)) ppm, the structural assignments were in agreement with the APT experiment; inspection of the ^1H NMR spectrum showed trace levels of **15**; MS (+CI) 373 $[M + 1]^+$; M_r (+CI) 373.159 86 $[M + 1]^+$ (calcd for $C_{16}H_{25}N_2O_8$ 373.161 09).

Preparation of Methyl 5a-Dihydrobicyclomycincarboxylate C(2′),C(3′)-Acetonide (16). A methanolic suspension (4 mL) of **13** (22 mg, 0.06 mmol) and 10% Pd−C (catalytic amount) was stirred (room temperature, 45 min) under an atmosphere of H_2. The suspension was filtered (Celite) and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−$CHCl_3$) to provide **16** as a diastereomeric mixture (∼9:1): yield, 17 mg (77%); mp 177−181 °C; R_f 0.51 (10% MeOH−$CHCl_3$); IR (KBr) 3439 (br), 3306 (br), 2990, 2940, 2886, 1733, 1687, 1437 cm^{-1}; ^1H NMR (CD_3OD) δ for the major diastereomer, 1.35 (s, 3 H, C(2′)CH_3), 1.45 (s, 6 H, C(CH_3)$_2$), 1.65−1.76 (m, 1 H, C(4)*H*H′), 1.98−2.07 (m, 1 H, C(4)H*H′*), 2.13 (dd, J = 9.8, 16.4 Hz, 1 H, C(5a)*H*H′), 2.53−2.62 (m, 1 H, C(5)H), 2.94 (dd, J = 4.1, 16.4 Hz, 1 H, C(5a)-H*H′*), 3.65 (s, 3 H, OCH_3), 3.70 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.83 (dd, J = 8.7, 13.8 Hz, 1 H, C(3)*H*H′), 4.02 (dd, J = 8.4, 13.8 Hz, 1 H, C(3)H*H′*), 4.09 (s, 1 H, C(1′)H), 4.45 (d, J = 8.4 Hz, 1 H, C(3′)H*H′*); ^1H NMR (CD_3OD) δ for the minor diastereomer, no signals were detected and are believed to overlap with the major diastereomer, the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^1H NMR (DMF-d_7) δ for the major diastereomer, 1.34 (s, 3 H), 1.42 (s, 6 H), 1.69−1.78 (m, 1 H), 1.98−2.06 (m, 1 H), 2.17 (dd, J = 9.8, 16.4 Hz, 1 H), 2.56−2.62 (m, 1 H), 2.92−3.00 (m, 1 H), 3.64 (s, 3 H), 3.72 (d, J = 8.1 Hz, 1 H), 3.83 (dd, J = 8.6, 13.6 Hz, 1 H), 4.04 (dd, J = 8.4, 13.6 Hz, 1 H), 4.11 (d, J = 8.0 Hz, 1 H), 4.41 (d, J = 8.1 Hz, 1 H), 5.98 (d, J = 8.0 Hz, 1 H), 7.13 (br s, 1 H), 8.08 and 8.95 (br s, 2 H); ^1H NMR (DMF-d_7) δ for the minor diastereomer, no signals were detected and are believed to overlap with the major diastereomer; ^{13}C NMR (DMF-d_7) for the major diastereomer, 24.2, 26.3, 27.9, 31.3, 47.6, 51.4, 62.9, 72.2, 72.6, 82.6, 85.6, 87.7, 110.2, 166.0, 169.1, 173.0 ppm, the remaining signal was not detected and is believed to be beneath the solvent peak; ^{13}C NMR (DMF-d_7) for the minor diastereomer, 32.4, 46.3 ppm, the other signals were not detected and are believed to overlap with the major diastereomer; MS (+CI) 403 $[M + 1]^+$; M_r (+CI) 403.171 03 $[M + 1]^+$ (calcd for $C_{17}H_{27}N_2O_9$ 403.171 66).

Preparation of 5a-(Hydroxy)methyldihydrobicyclomycin C(2′),C(3′)-Acetonide (15). To an anhydrous THF solution (2 mL) of **16** (54 mg, 0.13 mmol) was added lithium aluminum hydride (38 mg, 0.94 mmol). The suspension was stirred (room temperature, 24 h), cooled (0 °C), quenched with H_2O (10 mL), and neutralized (aqueous dilute HCl). The solution was concentrated in vacuo and the residue purified by preparative TLC (20% MeOH−$CHCl_3$) to provide **15** as a white solid (16 mg, 32%): mp 174−177 °C; R_f 0.38 (20% MeOH−$CHCl_3$); IR (KBr) 3459 (br), 3382, 3307, 3276, 2986, 2936, 2862, 1705, 1682, 1455 cm^{-1}; ^1H NMR (CD_3OD) δ 1.28−1.36 (m, 1 H), 1.36 (s, 3 H), 1.44 (s, 6 H), 1.74−1.84 (m, 1 H), 1.97−2.08 (m, 1 H), 2.12−2.22 (m, 2 H), 3.56−3.64 (m, 2 H), 3.71 (d, J = 8.4 Hz, 1 H), 3.82 (dd, J = 8.3, 13.7 Hz, 1 H), 4.01 (dd, J = 8.6, 13.7 Hz, 1 H), 4.09 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H); ^1H NMR (DMF-d_7) δ 1.25−1.34 (m, 1 H, C(5a)*H*H′), 1.34 (s, 3 H), 1.41 (s, 3 H, C(CH_3)$_2$), 1.42 (s, 3 H, C(CH_3)$_2$), 1.76−1.86 (m, 1 H, C(4)*H*H′), 1.94−2.04 (m, 1 H, C(4)H*H′*), 2.12−2.21 (m, 2 H, C(5)H, C(5a)H*H′*), 3.72 (d, J = 8.3 Hz, 1 H, C(3′)*H*H′), 3.80 (dd, J = 8.1, 13.4 Hz, 1 H, C(3)*H*H′), 3.99 (dd, J = 8.6, 13.4 Hz, 1 H, C(3)H*H′*), 4.10 (d, J = 8.0 Hz, 1 H, C(1′)H), 4.41 (d, J = 8.3 Hz, 1 H, C(3′)H*H′*), 4.56 (br s, 1 H, C(5b)OH), 5.98 (d, J = 8.0 Hz, 1 H, C(1′)OH), 6.83 (br s, 1 H, C(6)OH), 7.98 and 8.77 (br s, 2 H, N(8)H, C(10)H), the $^1H-^1H$ COSY experiment verified that the C(5b)H_2 signal was beneath the H_2O peak and supported the structural assignments; ^{13}C NMR (CD_3OD) 24.9, 26.8, 28.3, 31.9, 32.9, 61.5, 63.8, 73.2, 73.3, 84.3, 86.4, 88.7, 111.6, 168.3, 171.9 ppm, the remaining signal was not detected and is believed to be beneath the solvent peak; ^{13}C NMR (DMF-d_7) 24.1, 26.3, 27.9, 30.7, 32.1, 48.3, 60.4, 62.2, 72.3, 72.9, 83.5, 85.5, 87.3, 110.1, 167.1, 169.7 ppm; no signals were detected in CD_3OD or DMF-d_7 spectra (^1H, ^{13}C) for any minor diastereomer; MS (+CI) 375 $[M + 1]^+$; M_r (+CI) 375.175 58 $[M + 1]^+$ (calcd for $C_{16}H_{27}N_2O_8$ 375.176 74).

Preparation of 5a-(Acetoxy)methylbicyclomycin C(2′),C(3′)-Acetonide (17). To an anhydrous THF solution (1 mL) of **14** (16 mg, 0.04 mmol), triethylamine (22 mg, 0.22 mmol), and acetic anhydride (13 mg, 0.13 mmol) was added a catalytic amount of DMAP. The solution was stirred (room temperature, 1 h), diluted with H_2O (2 mL), neutralized (aqueous dilute NaOH), and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−$CHCl_3$)

(31) For the general experimental procedures employed, see: ref 12.

to provide **17** as a white solid (8 mg, 46%): mp 133−137 °C; R_f 0.43 (10% MeOH−CHCl$_3$); IR (KBr) 3441 (br), 2990, 2935, 2886, 1688 (br), 1458 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 2.01 (s, 3 H), 2.57 (dd, J = 8.5, 16.8 Hz, 1 H), 2.74 (dd, J = 7.6, 16.8 Hz, 1 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.81 (dd, J = 8.5, 13.4 Hz, 1 H), 3.96 (dd, J = 7.6, 13.4 Hz, 1 H), 4.15 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 4.65 (d, J = 6.7 Hz, 2 H), 6.23 (t, J = 6.7 Hz, 1 H); ^{13}C NMR (CD$_3$OD) 20.7, 25.0, 26.8, 28.1, 29.5, 61.6, 66.1, 73.0, 73.2, 83.0, 86.5, 88.9, 111.6, 125.5, 143.8, 168.5, 172.2, 172.5 ppm; MS (+CI) 415 [M + 1]$^+$; M_r (+CI) 415.171 14 [M + 1]$^+$ (calcd for $C_{18}H_{27}N_2O_9$ 415.171 66).

Preparation of 5a-[2,6-Bis(trifluoromethyl)benzoxy]methylbicyclomycin C(2′),C(3′)-Acetonide (18). To an anhydrous THF solution (1 mL) of **14** (15 mg, 0.04 mmol), 1,1′-carbonyldiimidazole (7 mg, 0.04 mmol), and 2,6-bis(trifluoromethyl)benzoic acid (11 mg, 0.04 mmol) was added a catalytic amount of DMAP. The solution was stirred (room temperature, 24 h), diluted with H$_2$O (2 mL), neutralized (aqueous dilute NaOH), and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−CHCl$_3$) to provide **14** (5 mg, 33% recovery) and **18** as a white solid (8 mg, 49%): mp 166−170 °C; R_f 0.50 (10% MeOH−CHCl$_3$); IR (KBr) 3440 (br), 3313 (br), 2991, 2940, 2887, 1738, 1698 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.38 (s, 3 H), 1.41 (s, 3 H), 1.44 (s, 3 H), 2.63 (dd, J = 8.8, 16.7 Hz, 1 H), 2.87 (dd, J = 7.4, 16.7 Hz, 1 H), 3.73 (d, J = 8.3 Hz, 1 H), 3.84 (dd, J = 8.8, 13.2 Hz, 1 H), 4.00 (dd, J = 7.4, 13.2 Hz, 1 H), 4.17 (s, 1 H), 4.45 (d, J = 8.3 Hz, 1 H), 4.96 (d, J = 7.1 Hz, 1 H), 4.97 (d, J = 7.1 Hz, 1 H), 6.37 (t, J = 7.1 Hz, 1 H), 8.05 (s, 2 H), 8.12 (s, 1 H); ^{13}C NMR (CD$_3$OD) 25.0, 26.8, 28.1, 29.5, 63.5, 66.0, 72.9, 73.2, 83.0, 86.5, 89.0, 111.6, 124.3, 124.0 (q, J = 270.5 Hz), 124.4 (q, J = 270.5 Hz), 128.3, 129.2, 129.3, 129.7, 133.0 (q, J = 33.2 Hz), 135.2 (q, J = 33.2 Hz), 145.3, 166.2, 168.6, 172.1 ppm; MS (+CI) 613 [M + 1]$^+$; M_r (+CI) 613.163 09 [M + 1]$^+$ (calcd for $C_{25}H_{27}F_6N_2O_9$ 613.162 08).

Preparation of 5a-(Chloro)methylbicyclomycin C(2′),C(3′)-Acetonide (20). To an anhydrous THF solution (1 mL) of **14** (20 mg, 0.05 mmol) and triethylamine (8 mg, 0.08 mmol) maintained at 0 °C was added methanesulfonyl chloride (9 mg, 0.08 mmol). The reaction mixture was stirred (0 °C, 30 min), filtered (glass wool), and treated with LiCl (23 mg, 0.54 mmol). The suspension was stirred (room temperature, 40 min), filtered (cotton plug), and concentrated in vacuo. The residue was suspended in H$_2$O (2 mL), neutralized (aqueous dilute NaOH), and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−CHCl$_3$) to provide **20** as a white solid (9 mg, 43%): mp 91−96 °C; R_f 0.51 (10% MeOH−CHCl$_3$); IR (KBr) 3433 (br), 2986, 2937, 2884, 1687 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.38 (s, 3 H, C(2′)CH$_3$), 1.42 (s, 3 H, C(CH$_3$)$_2$), 1.44 (s, 3 H, C(CH$_3$)$_2$), 2.58 (dd, J = 8.6, 16.7 Hz, 1 H, C(4)*H*H′), 2.77 (dd, J = 7.6, 16.7 Hz, 1 H, C(4)H*H′*), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.81 (dd, J = 8.6, 13.3 Hz, 1 H, C(3)*H*H′), 3.97 (dd, J = 7.6, 13.3 Hz, 1 H, C(3)H*H′*), 4.15 (s, 1 H, C(1′)H), 4.16 (d, J = 8.0 Hz, 1 H, C(5b)*H*H′), 4.17 (d, J = 8.0 Hz, 1 H, C(5b)H*H′*), 4.45 (d, J = 8.4 Hz, 1 H, C(3′)H*H′*), 6.38 (t, J = 8.0 Hz, 1 H, C(5a)H), the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD$_3$OD) 25.0 (C(2′)*C*H$_3$), 26.8 (C(*C*H$_3$)$_2$), 28.1 (C(*C*H$_3$)$_2$), 29.1 (C(4)), 39.9 (C(5b)), 66.0 (C(3)), 73.1 and 73.2 (C(1′), C(3′)), 83.0 (C(6)), 86.4 (C(2′)), 88.9 (C(1)), 111.6 (*C*(CH$_3$)$_2$), 126.8 (C(5a)), 144.6 (C(5)), 168.6 and 172.1 (C(7), C(9)) ppm, the structural assignments were in agreement with the APT experiment; MS (+FAB) 393 ([M + 1]$^+$, 31%), 391 ([M + 1]$^+$, 100%); M_r (+CI) 391.126 38 [M + 1]$^+$ (calcd for $C_{16}H_{24}{}^{35}ClN_2O_7$ 391.127 20).

Preparation of 5a-(Bromo)methylbicyclomycin C(2′),C(3′)-Acetonide (21). To an anhydrous THF solution (1 mL) of **14** (26 mg, 0.07 mmol) and triethylamine (10.6 mg, 0.11 mmol) was added methanesulfonic anhydride (18.6 mg, 0.11 mmol). The reaction mixture was stirred (room temperature, 30 min), filtered (glass wool), and treated with LiBr (61 mg, 0.07 mmol). The suspension was stirred (room temperature, 30 min) and filtered (cotton plug). The solution was cooled (0 °C) and quenched with H$_2$O (6 mL) and neutralized (aqueous dilute NaOH). The solution was concentrated in vacuo and the residue purified by preparative TLC (15% MeOH−CHCl$_3$) to provide **14** (7 mg, 27% recovery) and a mixture of **21**:**20** (~9:1): yield, 14 mg (46%); mp 111−114 °C; R_f 0.36 (10% MeOH−CHCl$_3$); IR (KBr) 3439 (br), 3304 (br), 2991, 2917, 2881, 1701 (br) cm^{-1}; ^{1}H NMR (CD$_3$OD) δ for **21**: 1.38 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 2.57 (dd, J = 8.8, 16.7 Hz, 1 H), 2.79 (dd, J = 7.6, 16.7 Hz, 1 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.81 (dd, J = 8.8, 13.3 Hz, 1 H), 3.97 (dd, J = 7.6, 13.3 Hz, 1 H), 4.06 (d, J = 8.6 Hz, 2 H), 4.16 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 6.46 (t, J = 8.6 Hz, 1 H); ^{1}H NMR (CD$_3$OD) δ for **20**, 4.15−4.20 (m, 2 H), 6.38 (t, J = 8.4 Hz, 1 H), the other signals were not detected and are believed to overlap with **21**; ^{13}C NMR (CD$_3$OD) for **21**: 25.0, 26.8, 27.0, 28.1, 28.8, 65.7, 73.0, 73.2, 83.0, 86.4, 88.9, 111.6, 126.8, 145.0, 168.6, 172.0 ppm; ^{13}C NMR (CD$_3$OD) for **20**: 40.0 ppm, the other signals were not detected and are believed to overlap with **21**; MS (+CI) 437 ([M + 1]$^+$, 100%), 435 ([M + 1]$^+$, 96%); M_r (+CI) 435.075 88 [M + 1]$^+$ (calcd for $C_{16}H_{24}{}^{79}BrN_2O_7$ 435.076 69).

Preparation of 5a-(Azido)methylbicyclomycin C(2′),C(3′)-Acetonide (22). To an anhydrous DMF solution (1 mL) of **21** (12 mg, 0.03 mmol) was added NaN$_3$ (9.0 mg, 0.14 mmol). The solution was stirred (room temperature, 3 h) and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−CHCl$_3$) to provide **22** as a white solid (9 mg, 82%): mp 149−152 °C; R_f 0.37 (10% MeOH−CHCl$_3$); IR (KBr) 3427 (br), 3307 (br), 2987, 2936, 2884, 2103, 1692 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.38 (s, 3 H), 1.41 (s, 3 H), 1.44 (s, 3 H), 2.57 (dd, J = 8.9, 16.7 Hz, 1 H), 2.75 (dd, J = 7.5, 16.7 Hz, 1 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.80 (dd, J = 8.9, 13.3 Hz, 1 H), 3.90 (d, J = 7.3 Hz, 2 H), 3.97 (dd, J = 7.5, 13.3 Hz, 1 H), 4.16 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 6.28 (t, J = 7.3 Hz, 1 H); ^{13}C NMR (CD$_3$OD) 25.0, 26.8, 28.1, 29.3, 66.2, 73.1, 73.2, 83.1, 86.4, 88.9, 111.6, 124.5, 145.1, 168.6, 172.3 ppm, the C(5b) signal was not detected and is believed to be beneath the solvent peak; ^{13}C NMR (DMF-d_7) 24.6, 26.6, 28.1, 28.9, 48.0, 65.2, 72.6, 72.9, 82.7, 85.9, 87.9, 110.5, 122.9, 145.7, 167.4, 170.2 ppm; MS (+FAB) 398 [M + 1]$^+$; M_r (+CI) 398.167 20 [M + 1]$^+$ (calcd for $C_{16}H_{24}N_5O_7$ 398.167 57).

Preparation of 5a-(Amino)methylbicyclomycin C(2′),C(3′)-Acetonide (23). To an anhydrous THF solution (1 mL) of **21** (17 mg, 0.04 mmol, **21**:**20** ~9:1) maintained at −78 °C was condensed anhydrous NH$_3$ (excess) which was passed through a NaOH drying tube. The solution was stirred (10 min) and warmed to room temperature under a stream of N$_2$ (30 min). The suspension was concentrated in vacuo, and the residue was purified by preparative TLC (3 × 30% MeOH−CHCl$_3$) to provide **20** (2 mg, 12%) and **23** as a white solid (10 mg, 69%): mp 168−171 °C; R_f 0.05 (30% MeOH−CHCl$_3$); IR (KBr) 3434 (br), 2992, 2938, 1689, 1638, 1475 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.39 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 2.56 (dd, J = 8.8, 16.9 Hz, 1 H), 2.75 (dd, J = 7.4, 16.9 Hz, 1 H), 3.65 (d, J = 7.1 Hz, 2 H), 3.74 (d, J = 8.4 Hz, 1 H), 3.80 (dd, J = 8.8, 13.3 Hz, 1 H), 3.99 (dd, J = 7.4, 13.3 Hz, 1 H), 4.17 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 6.21 (t, J = 7.1 Hz, 1 H); ^{13}C NMR (CD$_3$OD) 25.1, 26.8, 28.0, 29.4, 38.1, 65.6, 72.9, 73.2, 82.9, 86.5, 88.9, 111.6, 122.4, 145.8, 168.5, 172.0 ppm; MS (+CI) 372 [M + 1]$^+$; M_r (+CI) 372.176 12 [M + 1]$^+$ (calcd for $C_{16}H_{26}N_3O_7$ 372.177 08).

Preparation of 5a-(Acetamido)methylbicyclomycin C(2′),C(3′)-Acetonide (24). To an anhydrous THF solution (1 mL) of **23** (12 mg, 0.03 mmol) and triethylamine (13 mg, 0.13 mmol) was added acetic anhydride (13 mg, 0.13 mmol). The solution was stirred (room temperature, 10 min) and concentrated in vacuo. The residue was purified by preparative TLC (2 × 30% MeOH−CHCl$_3$) to provide **24** as a white solid (8 mg, 60%): mp 125−128 °C; R_f 0.42 (20% MeOH−CHCl$_3$); IR (KBr) 3439 (br), 2991, 2938, 2886, 1696, 1654, 1560 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 1.91 (s, 3 H), 2.56 (dd, J = 8.9, 16.9 Hz, 1 H), 2.75 (dd, J = 7.4, 16.9 Hz, 1 H), 3.72 (d, J = 8.9 Hz, 1 H), 3.77−3.88 (m, 1 H), 3.81 (d, J = 6.9 Hz, 1 H), 3.82 (d, J = 6.9 Hz, 1 H), 3.95 (dd, J = 7.4, 13.1 Hz, 1 H), 4.15 (s, 1 H), 4.45 (d, J = 8.9 Hz, 1 H), 6.11 (t, J = 6.9 Hz, 1 H); ^{13}C NMR (CD$_3$OD) 22.4, 25.0, 26.8, 28.1, 29.1, 38.0, 66.2, 73.0, 73.2, 83.0, 86.4, 88.9,

111.6, 127.4, 141.8, 168.6, 172.4, 173.0 ppm; MS (+CI) 414 $[M + 1]^+$; M_r (+CI) 414.186 35 $[M + 1]^+$ (calcd for $C_{18}H_{28}N_3O_8$ 414.187 64).

Preparation of Compound 25. A warm (55 °C) THF solution (3 mL) of **22** (15 mg, 0.04 mmol), triphenylphosphine (15 mg, 0.06 mmol), and H_2O (3 mg, 0.19 mmol) was stirred (55 °C, 24 h) and then concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH–$CHCl_3$) to provide **25** as a white solid (4 mg, 30%): mp 154–157 °C; R_f 0.40 (10% MeOH–$CHCl_3$); IR (KBr) 3377 (br), 2986, 2937, 2900, 1687, 1627, 1561, 1430 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 1.35 (s, 6 H, C(CH_3)$_2$), 2.78–2.87 (m, 1 H, C(4)*H*H′), 3.14–3.25 (m, 1 H, C(4)H*H′*), 3.69 (d, J = 9.0 Hz, 1 H, C(3′)*H*H′), 3.75 (s, 1 H, C(1′)H), 4.05–4.12 (m, 1 H, C(3)-*H*H′), 4.09 (d, J = 9.0 Hz, 1 H, C(3′)H*H′*), 4.36–4.45 (m, 1 H, C(3)H*H′*), 5.88 (d, J = 2.4 Hz, 1 H, C(5a)H), 6.84 (d, J = 2.4 Hz, 1 H, C(5b)H), the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) 21.6 (C(2′)*C*H$_3$), 26.7 (C(*C*H$_3$)$_2$), 27.6 (C(*C*H$_3$)$_2$), 30.3 (C(4)), 62.4 (C(3)), 75.1 (C(3′)), 79.7 (C(1′)), 84.2 (C(2′)), 91.6 (C(1)), 111.2 (*C*(CH$_3$)$_2$), 111.3 (C(5a)), 123.0 (C(5)), 123.9 (C(5b)), 126.2 (C(6)), 168.4 and 174.5 (C(7), C(9)) ppm, the structural assignments were in agreement with the APT experiment; MS (+CI) 354 $[M + 1]^+$; M_r (+CI) 354.165 61 $[M + 1]^+$ (calcd for $C_{16}H_{24}N_3O_6$ 354.166 51).

Preparation of Compound 25 Using NaOD–D_2O. To a warm (55 °C) THF solution (2 mL) of **23** (4 mg, 0.01 mmol) and D_2O (3 drops) was added NaOD (3 drops, ~0.1 M). The solution was stirred (55 °C, 10 h) and then concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH–$CHCl_3$) to provide **25** as a white solid (2 mg, 53%): R_f 0.36 (10% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.35 (s, 6 H), 2.80–2.87 (m, 1 H), 3.14–3.25 (m, 1 H), 3.68 (d, J = 8.7 Hz, 1 H), 3.75 (s, 1 H), 4.06–4.12 (m, 1 H), 4.09 (d, J = 8.7 Hz, 1 H), 4.36–4.45 (m, 1 H), 5.88 (d, J = 2.1 Hz, 1 H), 6.84 (d, J = 2.1 Hz, 1 H).

Preparation of 5a-Methylbicyclomycin C(2′),C(3′)-Acetonide (30). To an anhydrous THF solution (2 mL) of **21** (11 mg, 0.03 mmol) maintained at −78 °C was added lithium triethylborohydride (21 mg, 0.20 mmol). The solution was stirred (15 h) during which time the solution was allowed to warm to room temperature. The solution was cooled (0 °C), diluted with H_2O (4 mL), neutralized (aqueous dilute HCl), and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH–$CHCl_3$) to provide **30** as a white solid (5 mg, 56%): mp 156–159 °C; R_f 0.36 (10% MeOH–$CHCl_3$); IR (KBr) 3427 (br), 3333 (br), 2986, 2935, 2880, 1687, 1458 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.41 (s, 3 H), 1.44 (s, 3 H), 1.67 (d, J = 7.1 Hz, 3 H), 2.50 (dd, J = 8.6, 16.7 Hz, 1 H), 2.72 (dd, J = 7.7, 16.7 Hz, 1 H), 3.73 (d, J = 8.4 Hz, 1 H), 3.78 (dd, J = 8.6, 12.8 Hz, 1 H), 3.95 (dd, J = 7.7, 12.8 Hz, 1 H), 4.15 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 6.22 (q, J = 7.1 Hz, 1 H); ^{13}C NMR (CD_3OD) 13.2, 25.0, 26.8, 28.2, 28.5, 66.5, 73.1, 73.2, 83.4, 86.4, 88.8, 111.6, 125.8, 139.9, 168.7, 172.1 ppm; MS (+CI) 357 $[M + 1]^+$; M_r (+CI) 357.165 31 $[M + 1]^+$ (calcd for $C_{16}H_{25}N_2O_7$ 357.166 18).

General Procedure for the Preparation of 5a-Methyl-Substituted Bicyclomycins 2–10. To a 50% aqueous methanol solution (2 mL) of the 5a-methyl-substituted bicyclomycin C(2′),C(3′)-acetonide (1 equiv) was added trifluoroacetic acid (3 drops). The solution was stirred (room temperature, 2 h) and concentrated in vacuo. The residue was purified by preparative TLC (20% MeOH–$CHCl_3$) to provide the desired product.

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

Preparation of 5a-Methylbicyclomycin (2). Using **30** (8 mg, 0.02 mmol) gave **2** as a white solid (4 mg, 56%): mp 144–146 °C; R_f 0.30 (20% MeOH–$CHCl_3$); IR (KBr) 3396 (br), 3270 (br), 2970, 2937, 2883, 1687 (br), 1656, 1458 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.67 (d, J = 6.8 Hz, 3 H), 2.43 (dd, J = 9.3, 16.4 Hz, 1 H), 2.78 (dd, J = 7.4, 16.4 Hz, 1 H), 3.50 (d, J = 11.3 Hz, 1 H), 3.65 (d, J = 11.3 Hz, 1 H), 3.73 (dd, J = 9.3, 12.9 Hz, 1 H), 3.94 (dd, J = 7.4, 12.9 Hz, 1 H), 4.08 (s, 1 H), 6.20 (q, J = 6.8 Hz, 1 H); ^{13}C NMR (CD_3OD) 13.6, 24.2, 28.7, 65.5, 68.5, 72.1, 78.2, 83.3, 89.4, 125.9, 140.1, 169.0, 173.3 ppm; MS (+CI) 317 $[M + 1]^+$; M_r (+CI) 317.134 48 $[M + 1]^+$ (calcd for $C_{13}H_{21}N_2O_7$ 317.134 88).

Preparation of 5a-(Hydroxy)methylbicyclomycin (3). Using **14** (10 mg, 0.03 mmol) gave **3** as a white solid (9 mg, 100%): mp 116–118 °C; R_f 0.10 (20% MeOH–$CHCl_3$); IR (KBr) 3454 (br), 1685 (br), 1508 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.46 (dd, J = 9.1, 16.7 Hz, 1 H), 2.75 (dd, J = 7.3, 16.7 Hz, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.75 (dd, J = 9.1, 13.1 Hz, 1 H), 3.94 (dd, J = 7.3, 13.1 Hz, 1 H), 4.08 (s, 1 H), 4.14 (d, J = 6.3 Hz, 2 H), 6.26 (t, J = 6.3 Hz, 1 H); ^{13}C NMR (CD_3OD) 24.2, 29.4, 59.2, 65.3, 68.5, 72.1, 78.2, 83.1, 89.4, 130.9, 141.0, 168.9, 172.8 ppm; MS (+CI) 333 $[M + 1]^+$; M_r (+CI) 333.130 28 $[M + 1]^+$ (calcd for $C_{13}H_{21}N_2O_8$ 333.129 79).

Preparation of 5a-(Acetoxy)methylbicyclomycin (4). Using **17** (6 mg, 0.01 mmol) gave **4** as a white solid (3 mg, 55%): mp 132–135 °C; R_f 0.39 (20% MeOH–$CHCl_3$); IR (KBr) 3435 (br), 3277 (br), 2949, 1686 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.01 (s, 3 H), 2.50 (dd, J = 9.0, 16.5 Hz, 1 H), 2.81 (dd, J = 7.6, 16.5 Hz, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.76 (dd, J = 9.0, 13.3 Hz, 1 H), 3.95 (dd, J = 7.6, 13.3 Hz, 1 H), 4.08 (s, 1 H), 4.65 (d, J = 6.7 Hz, 2 H), 6.21 (t, J = 6.7 Hz, 1 H); ^{13}C NMR (CD_3OD) 20.7, 24.1, 29.6, 61.7, 65.1, 68.4, 72.0, 78.2, 83.1, 89.4, 125.5, 144.1, 168.8, 172.5, 172.6 ppm; MS (+CI) 375 $[M + 1]^+$; M_r (+CI) 375.140 34 $[M + 1]^+$ (calcd for $C_{15}H_{23}N_2O_9$ 375.140 36).

Preparation of 5a-[2,6-Bis(trifluoromethyl)benzoxy]-methylbicyclomycin (5). Using **18** (20 mg, 0.03 mmol) gave **5** as a white solid (12 mg, 64%): mp 149–151 °C; R_f 0.48 (20% MeOH–$CHCl_3$); IR (KBr) 3434 (br), 3263 (br), 2946, 2896, 1736, 1686 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.57 (dd, J = 9.2, 16.7 Hz, 1 H), 2.91 (dd, J = 7.4, 16.7 Hz, 1 H), 3.51 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.80 (dd, J = 9.2, 13.3 Hz, 1 H), 3.99 (dd, J = 7.4, 13.3 Hz, 1 H), 4.09 (s, 1 H), 4.97 (d, J = 7.1 Hz, 2 H), 6.36 (t, J = 7.1 Hz, 1 H), 8.05 (s, 2 H), 8.12 (s, 1 H); ^{13}C NMR (CD_3OD) 24.1, 29.7, 63.6, 65.0, 68.5, 72.0, 78.2, 83.1, 89.4, 124.2 (q, J = 271.3 Hz), 124.4, 124.5 (q, J = 271.3 Hz), 128.3, 129.1, 129.2, 129.6, 132.9 (q, J = 33.2 Hz), 135.2 (q, J = 33.2 Hz), 145.3, 166.3, 168.8, 172.5 ppm; MS (+CI) 573 $[M + 1]^+$; M_r (+CI) 573.132 08 $[M + 1]^+$ (calcd for $C_{22}H_{23}F_6N_2O_9$ 573.130 78).

Preparation of 5a-(Chloro)methylbicyclomycin (6). Using **20** (15 mg, 0.04 mmol) gave **6** as a white solid (8 mg, 59%): mp 119–126 °C; R_f 0.38 (20% MeOH–$CHCl_3$); IR (KBr) 3428 (br), 3282 (br), 2979, 2946, 2892, 1687 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.50 (dd, J = 9.0, 16.7 Hz, 1 H, C(4)*H*H′), 2.83 (dd, J = 7.3, 16.7 Hz, 1 H, C(4)H*H′*), 3.50 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 11.3 Hz, 1 H, C(3′)H*H′*), 3.75 (dd, J = 9.0, 13.2 Hz, 1 H, C(3)*H*H′), 3.97 (dd, J = 7.3, 13.2 Hz, 1 H, C(3)H*H′*), 4.08 (s, 1 H, C(1′)H), 4.16 (d, J = 8.0 Hz, 2 H, C(5b)H_2), 6.36 (t, J = 8.0 Hz, 1 H, C(5a)H), the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) 24.2, 29.1, 40.0, 65.0, 68.5, 72.1, 78.2, 83.1, 89.5, 126.9, 144.9, 168.8, 172.4 ppm; MS (+CI) 353 ($[M + 1]^+$, 30%), 351 ($[M + 1]^+$, 100%); M_r (+CI) 351.095 34 $[M + 1]^+$ (calcd for $C_{13}H_{20}{}^{35}ClN_2O_7$ 351.095 90).

Attempted Synthesis of 5a-(Bromo)methylbicyclomycin (7). Using **21** (20 mg, 0.04 mmol, **21**:**20** ~9:1) gave a mixture tentatively assigned as **7**:**6** (~1:1): yield, a mixture composed of ~7 mg of **7** (40%) and ~6 mg of **6**; R_f 0.36 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ for **7**, 1.34 (s, 3 H), 2.50–2.55 (m, 1 H), 2.80–2.88 (m, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.71–3.79 (m, 1 H), 3.93–4.00 (m, 1 H), 4.07 (d, J = 8.1 Hz, 2 H), 4.08 (s, 1 H), 6.44 (t, J = 8.1 Hz, 1 H); ^1H NMR (CD_3OD) δ for **6**: 4.16 (d, J = 8.1 Hz, 2 H), 6.36 (t, J = 8.1 Hz, 1 H), the other signals were not detected and are believed to overlap with **7**; ^{13}C NMR (CD_3OD) for **7**, 24.1, 27.1, 28.9, 64.7, 68.4, 72.0, 78.2, 83.1, 89.4, 126.9, 145.3, 168.8, 172.4 ppm; ^{13}C NMR (CD_3OD) for **6**: 29.1, 40.0, 65.0, 144.9 ppm, the other signals were not detected and are believed to overlap with **7**.

Preparation of 5a-(Azido)methylbicyclomycin (8). Using **22** (9.0 mg, 0.02 mmol) gave **8** as a white solid (6.0 mg, 74%): mp 128–132 °C; R_f 0.37 (20% MeOH–$CHCl_3$); IR (KBr)

3414 (br), 3256 (br), 2978, 2944, 2884, 2105, 1687 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.51 (dd, J = 9.1, 16.7 Hz, 1 H), 2.80 (dd, J = 7.6, 16.7 Hz, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.75 (dd, J = 9.1, 13.3 Hz, 1 H), 3.90 (d, J = 7.2 Hz, 2 H), 3.96 (dd, J = 7.6, 13.3 Hz, 1 H), 4.09 (s, 1 H), 6.26 (t, J = 7.2 Hz, 1 H); ^{13}C NMR (CD_3OD) 24.2, 29.4, 48.5, 65.2, 68.5, 72.0, 78.2, 83.1, 89.4, 124.6, 145.2, 168.8, 172.6 ppm; MS (+CI) 358 $[M + 1]^+$; M_r (+CI) 358.137 93 $[M + 1]^+$ (calcd for $C_{13}H_{20}N_5O_7$ 358.136 27).

Preparation of 5a-(Amino)methylbicyclomycin (9). Using **23** (10 mg, 0.03 mmol) gave **9** as a white solid (6.0 mg, 67%): mp 154−158 °C; R_f 0.00 (30% MeOH−$CHCl_3$); IR (KBr) 3427 (br), 3250 (br), 2949 (br), 1687 (br), 1638, 1459 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.50 (dd, J = 9.6, 17.1 Hz, 1 H), 2.78 (dd, J = 7.2, 17.1 Hz, 1 H), 3.49 (d, J = 11.4 Hz, 1 H), 3.63 (d, J = 7.0 Hz, 2 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.76 (dd, J = 9.6, 13.4 Hz, 1 H), 3.97 (dd, J = 7.2, 13.4 Hz, 1 H), 4.10 (s, 1 H), 6.18 (t, J = 7.0 Hz, 1 H); ^{13}C NMR (CD_3OD) 24.1, 29.6, 38.1, 64.6, 68.4, 72.0, 78.2, 83.1, 89.4, 122.4, 146.1, 168.8, 172.3 ppm; MS (+CI) 332 $[M + 1]^+$; M_r (+CI) 332.147 16 $[M + 1]^+$ (calcd for $C_{13}H_{22}N_3O_7$ 332.145 78).

Preparation of 5a-(Acetamido)methylbicyclomycin (10). Using **24** (8.0 mg, 0.02 mmol) gave **10** as a white solid (6.0 mg, 83%): mp 142−146 °C; R_f 0.05 (30% MeOH−$CHCl_3$); IR (KBr) 3427 (br), 1686 (br), 1560 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.91 (s, 3 H), 2.48 (dd, J = 9.0, 16.5 Hz, 1 H), 2.82 (dd, J = 7.6, 16.5 Hz, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.72−3.80 (m, 1 H), 3.82 (d, J = 7.1 Hz, 2 H), 3.95 (dd, J = 7.6, 13.3 Hz, 1 H), 4.08 (s, 1 H), 6.09 (t, J = 7.1 Hz, 1 H); ^{13}C NMR (CD_3OD) 22.5, 24.2, 29.3, 38.0, 65.3, 68.5, 72.1, 78.2, 83.1, 89.4, 127.5, 142.5, 168.9, 172.1, 173.0 ppm; MS (+CI) 374 $[M + 1]^+$; M_r (+CI) 374.154 80 $[M + 1]^+$ (calcd for $C_{15}H_{24}N_3O_8$ 374.156 34).

General Procedure for the Reaction of 6 with Ethanethiol. To a degassed (Ar, 5 min) THF−H_2O (3:1, 2 mL) solution containing **6** (1 equiv) was added EtSH (8−10 equiv). The "pH" of the solution was adjusted with aqueous dilute NaOH and stirred (room temperature) under an Ar atmosphere. The solvent was removed in vacuo, and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to afford the product(s).

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

Reaction of 6 with Ethanethiol at "pH" 8. The "pH" of a THF−H_2O solution containing **6** (6 mg, 0.02 mmol) and EtSH (8 mg, 0.12 mmol) was adjusted to 8. During the course of the reaction (18 h) the solution "pH" dropped to 7. TLC analysis indicated only the presence of the starting bicyclomycin **6**. The reaction solution was worked up to provide **6** as a white solid (2 mg, 33% recovery): R_f 0.33 (20% MeOH−$CHCl_3$); ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.51 (dd, J = 9.0, 16.5 Hz, 1 H), 2.84 (dd, J = 7.4, 16.5 Hz, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.75 (dd, J = 9.0, 13.1 Hz, 1 H), 3.97 (dd, J = 7.4, 13.1 Hz, 1 H), 4.08 (s, 1 H), 4.16 (d, J = 7.9 Hz, 2 H), 6.36 (t, J = 7.9 Hz, 1 H).

Reaction of 6 with Ethanethiol at "pH" 10.5. The "pH" of a THF−H_2O solution containing **6** (8 mg, 0.02 mmol) and EtSH (14 mg, 0.23 mmol) was adjusted to 10.5. During the course of the reaction (2 h) the solution "pH" dropped to 8.1. TLC analysis indicated full consumption of starting bicyclomycin **6**. The reaction solution was worked up to provide **34** as a white solid (5 mg, 58%): mp 148−150 °C; R_f 0.46 (20% MeOH−$CHCl_3$); IR (KBr) 3402 (br), 3276 (br), 2971, 2929, 2878, 1687 (br), 1499 cm^{-1}; ^1H NMR (CD_3OD) δ 1.20 (t, J = 7.4 Hz, 3 H), 1.34 (s, 3 H), 2.46 (q, J = 7.4 Hz, 2 H), 2.44−2.53 (m, 1 H), 2.81 (dd, J = 7.4, 16.8 Hz, 1 H), 3.20 (d, J = 8.1 Hz, 2 H), 3.50 (d, J = 11.3 Hz, 1 H), 3.65 (d, J = 11.3 Hz, 1 H), 3.73 (dd, J = 9.3, 12.9 Hz, 1 H), 3.95 (dd, J = 7.4, 12.9 Hz, 1 H), 4.08 (s, 1 H), 6.23 (t, J = 8.1 Hz, 1 H); ^{13}C NMR (CD_3OD) 15.1, 24.2, 25.8, 29.0, 29.1, 65.4, 68.5, 72.0, 78.2, 83.2, 89.4, 127.9, 141.9, 168.9, 173.0 ppm; MS (+CI) 377 $[M + 1]^+$; M_r (+CI) 377.138 16 $[M + 1]^+$ (calcd for $C_{15}H_{25}N_2O_7S$ 377.138 25).

Acknowledgment. We thank the National Institutes of Health (Grant GM37934) and the Robert A. Welch Foundation (Grant No. E0607) 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. Hyeung-geun Park (Seoul National University) for his preliminary studies on the reduction of **13** to **14** and **15**.

Supporting Information Available: ^1H and ^{13}C NMR spectra of all new compounds prepared in this study (51 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.

JO972055Y