C(5)−C(5a)-Modified Bicyclomycins: Synthesis, Structure, and Biochemical and Biological Properties

Fabien Vincent,[†] Jayasree Srinivasan,[†] Alejandro Santillán, Jr.,[†] William R. Widger,*[,‡] and Harold Kohn*[,§]

Department of Chemistry, University of Houston, Houston, Texas 77204-5641, Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204-5934, and Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360

harold_kohn@unc.edu

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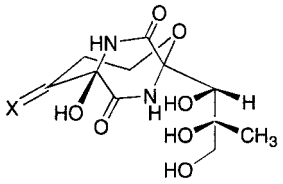
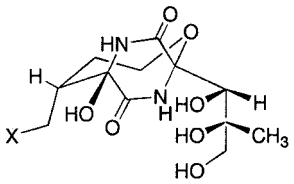
Bicyclomycin (**1**) is a novel antibiotic that targets rho transcription termination factor in *Escherichia coli*. We have demonstrated that retention of the C(5)−C(5a) exomethylene unit in **1** is not essential for inhibition. In a recent paper we proposed a working model for **1** and rho function and suggested that **1** binds in a cleft with the C(5)−C(5a) exomethylene unit directed toward the dimeric interface of two rho monomers. This report examines the bicyclomycin C(5)−C(5a) structural constraints necessary for retention of rho inhibitory activity. Three classes of C(5)−C(5a)-modified bicyclomycins have been prepared and their inhibitory activities evaluated in the poly C-dependent ATPase and filter disk antimicrobial assays. The first series consisted of 12 analogues (**8**−**19**) that contained a C(5a)-unsaturated substituent and possessed C(5*E*)-geometry. The second set were a pair of C(5a)-substituted C(5*E*)- and C(5*Z*)-geometrical isomers (**21** and **23**). The final group of compounds consisted of six C(5)−C(5a)-dihydrobicyclomycins (**24**−**28**, **34**) where the terminal substituent was systematically varied. We find that extending the C(5)−C(5a) double bond with unsaturated substituents provides bicyclomycin derivatives with excellent inhibitory activities in the biochemical assay, and that enhanced inhibitory activity is observed for the C(5*E*) geometrical isomer compared with its C(5*Z*) counterpart. Finally, C(5a)-substituted dihydrobicyclomycin inhibitory activity appears to be tightly regulated by the nature and spatial placement of the C(5a)-terminal substituent with respect to the [4.2.2]-bicyclic ring system. The observed biochemical activities for the C(5a)-extended conjugated bicyclomycin derivatives and the (5*E*) and (5*Z*) isomers were correlated with a structural model for the **1**−rho complex.

The novel, commercial antibiotic bicyclomycin (**1**) has been shown to target a broad spectrum of Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella*, and *Citrobacter*.[1−4] The primary site of action of **1** in *E. coli* is the essential cellular protein transcription termination factor rho.[5] Use of bicyclomycin irreversible inactivators[6−8] and kinetic studies of rho mutants insensitive to bicyclomycin[9] have suggested that **1** binds to a deep cleft in rho, which includes the catalytic site for ATP hydrolysis.[10]



1 X = CH_2
2 X = N~OCH_3
3 X = $CHCO_2H$
4 X = O



5 X = H
6 X = SCH_3
7 X = SC_6H_5

Learning structural parameters in **1** that govern antibiotic function remains an essential objective in our efforts to provide a detailed mechanistic description for the mode of action of this agent. Since 1972, several bicyclomycin structure−activity relationship (SAR) studies have appeared.[3,11−13] Earlier SAR investigations relied upon antimicrobial screens to assess the effect of bi-

* To whom correspondence should be addressed.
[†] Department of Chemistry, University of Houston.
[‡] Department of Biology and Biochemistry, University of Houston. E-mail: widger@uh.edu.
[§] University of North Carolina.

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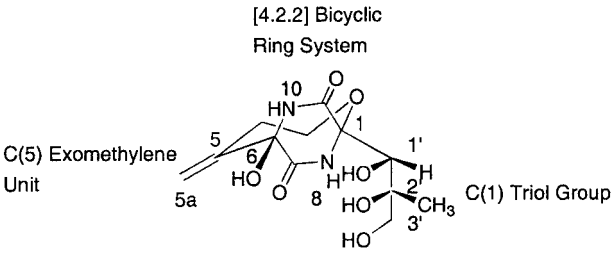

Figure 1. Sites of chemical modification in bicyclomycin (**1**).

cyclomycin structure on drug activity.[3,11,12] Our studies[13] were the first to use rho-dependent ATP hydrolysis[14] and transcriptional termination[15] assays to monitor drug binding and function.

Bicyclomycin can be structurally divided into three domains: the C(1) triol group, the [4.2.2] bicyclic ring unit, and the C(5)−C(5a) exomethylene moiety. Synthetic procedures have been developed that permit bicyclomycin modification at the numbered atoms within each domain (Figure 1).[3,11−13]

The role of the C(1) triol group for **1** function has been extensively investigated. The Müller and Williams research groups removed all or part of this group.[11,12c] Chemical deletions gave rise to compounds that lacked antibacterial activity. We showed that configurational inversion of either the C(1′) or C(2′) sites,[13a] or modification of the C(2′) and C(3′) hydroxy residues,[13b] decreased the in vitro activities by an order of magnitude or more. These results demonstrated that the C(1) triol serves as an important recognition element in bicyclomycin's binding to rho.

Modification of the piperazinedione unit in **1** led to a loss of rho-dependent ATPase and transcription termination inhibitory activities.[13c] We learned that the N(10)-methyl-substituted analogue retained approximately one-tenth the potency of **1** in rho functional assays and that additional methylation at N(8) produced a compound without activity at the tested concentrations. Selective replacement of the C(6) hydroxy group with an amino, alkoxy, hydroxylamino, thiol, or other moieties led to near total elimination of bicyclomycin inhibitory activities in rho functional assays.[13c,f] These studies documented that the [4.2.2] bicyclic ring system of bicyclomycin is also essential for drug binding.

The early SAR study of Müller and co-workers revealed that select C(5)−C(5a) exomethylene-modified bicyclomycin analogues exhibited biological activity comparable to **1**.[11] Our efforts provided additional information concerning the structural parameters necessary for rho binding and inhibition.[13d,e] First, biochemical inhibitory

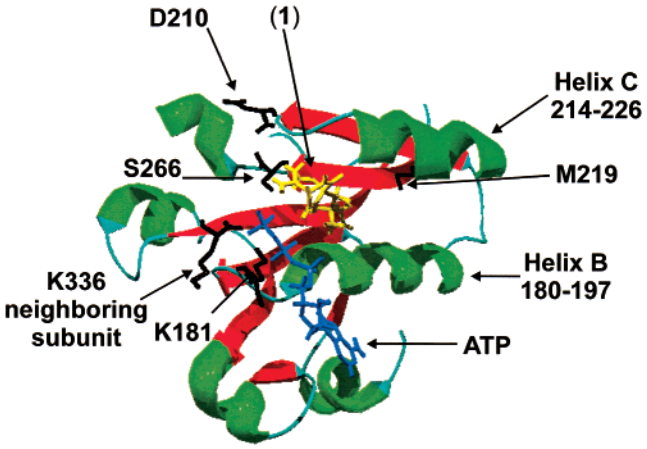

Figure 2. A model of the proposed binding site for **1** in the ATP hydrolysis domain of rho. Amino acid residues M219, D210, S266 (known to confer resistance to **1** when mutated),[5,9] and residues neighboring K336 (known to be covalently modified by **8**)[10] and K181 (known to be covalently modified by 5a-(formylanilino)dihydrobicyclomycin)[6,7] are shown. The orientation of **1** is such that the C(5a) exomethylene group is pointing toward the γ-phosphate of ATP and K181/neighboring K336.

activity of the modified bicyclomycin varied with the C(5) modification. We showed that replacement of the C(5a)-exomethylene unit in **1** with either N(OMe) to give **2** or $CHCO_2H$ to give **3** afforded compounds with nearly identical biochemical activities as **1**. Exchange of the C(5a)-exomethylene group with an oxygen atom to give **4**, however, led to a sharp drop in inhibitory activity.[13d] Second, retention of the C(5)−C(5a) double bond in modified bicyclomycins was important, but not essential, for high rho inhibition. For example, we found that while bicyclomycin was four times more potent an inhibitor than dihydrobicyclomycin (**5**), select sulfur-substituted dihydrobicyclomycins (**6**, **7**) exhibited inhibitory activities comparable to **1** in these biochemical assays.[13d] These collective findings indicated that the strict maintenance of the C(5)−C(5a) exomethylene unit in **1**-based derivatives was not essential for rho inactivation. Rather, our results indicated that structural diversity may exist at the C(5)−C(5a) site in modified bicyclomycins and that the observed inhibitory properties for C(5)−C(5a)-modified bicyclomycin likely reflect the unit's structural complementarity with the nearby amino acid residues associated with the drug-binding pocket in rho.

We proposed a working structural model for rho and bicyclomycin function.[10] We suggested that **1** binds within a cleft between the C and B helices with the C(1) triol group pointed toward the outside of rho, and the terminal C(5)−C(5a) exomethylene group pointed toward the terminal γ- phosphate of ATP and the P-loop (residues 175−183) on rho (Figure 2). Adjacent to the cleft and within a few angstroms of the cleft is the G helix of the neighboring subunit (residues 332−336). The placement of the bicyclomycin C(5)−C(5a) domain at the interface of two rho subunits highlights the role of bicyclomycin C(5) substituents in drug function and the importance of learning which substituents influence bicyclomycin binding and inhibit rho function.

We report herein synthetic methods that permit extension of the bicyclomycin C(5)−C(5a) exomethylene unit. We demonstrate that considerable structural flexibility exists at the C(5a) site in **1** for rho inhibition and predict

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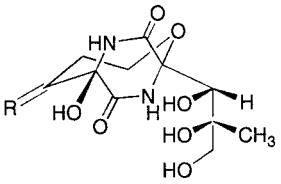
the C(5)−C(5a) structural constraints that govern rho binding and inhibition.

Results

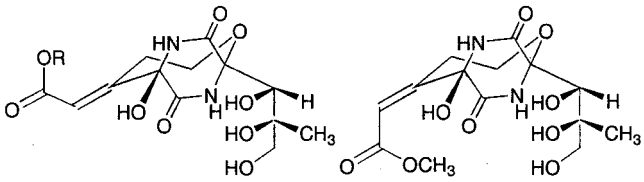
1. Experimental Plan

1.1. Choice of Compounds. Our study focused on the C(5)−C(5a) site in bicyclomycin. Three classes of compounds were prepared. The first group, **8**−**19**, consisted of C(5a)-substituted bicyclomycins with extended conjugation and defined geometry. Since our earlier studies[13d,e] showed that select C(5a)-substituted bicyclomycin analogues with 5*E* geometry exhibited pronounced inhibitory activities in rho functional assays, we chose to restrict this class of compounds to 5*E* derivatives. Furthermore, for compounds **8**−**14** and **16** we retained a C(5)- or a C(5a)-terminal substituent previously tested[13d] for rho inhibitory activity. These compounds differed only from the original bicyclomycins (**2**−**4, 20**−**22**)[11,13d] in the incorporation of an extra double bond between the terminal moiety and the [4.2.2] ring system.



8 R = C(H)-C(H)=O
9 R = C(H)-C(CH$_3$)=O
10 R = C(H)-C(H)=N~OH
11 R = C(H)-C(H)=N~OCH$_3$
12 R = C(H)-C(CH$_3$)=N~OCH$_3$
13 R = C(H)-C(H)=C(H)CO$_2$H
14 R = C(H)-C(H)=C(H)CO$_2$CH$_3$
15 R = C(H)-C(H)=C(H)CO$_2$CH$_2$CH=CH$_2$
16 R = C(H)-C(H)=C(H)CO$_2$CH$_2$C$_6$H$_5$
17 R = C(H)-C(H)=C(H)I
18 R = C(H)-C(H)=C(H)(*p*-NO$_2$C$_6$H$_4$)
19 R = C(H)-C(H)=C(H)(2-pyridyl)
20 R = N~OH

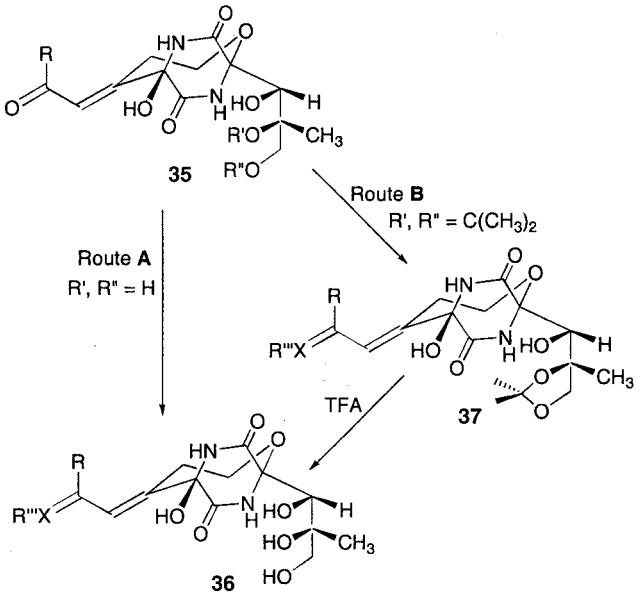
We next addressed the importance of the geometry of the bicyclomycin C(5)−C(5a) double bond for bioactivity. We wished to learn whether the drug-binding pocket could also accommodate C(5a)-substituted bicyclomycin analogues with 5*Z*-configuration. (5*Z*)-Methyl 5a-bicyclomycincarboxylate (**23**) was selected for synthesis and evaluation since the corresponding 5*E*-configurational isomer **21** displayed good inhibitory activity in the poly C-mediated ATPase assay.[13d]



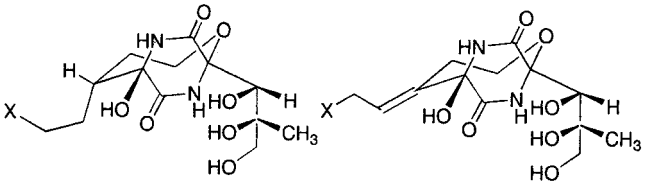
21 R = CH$_3$ (*E*-isomer)
22 R = CH$_2$C$_6$H$_5$
23 (*Z*-isomer)

The last compounds selected for study were C(5a)-substituted bicyclomycins in which the C(5)−C(5a) double bond was reduced. Previous studies have shown that while reduction of the C(5)−C(5a) double bond often led to loss of activity in rho functional assays (e.g., **1** vs **5**), select dihydrobicyclomcyin derivatives (e.g., **6**, **7**) exhibited activities comparable to **1**.[13d] In this study we prepared C(5a)-substituted bicyclomycin derivatives in which the C(5b)-terminal substituent was systematically varied from hydrogen (**24**) to nitrogen (**25**, **26**) to oxygen

Scheme 1. General Pathway for Bicyclomycins 8-19



(**27**, **28**). The activities of these compounds were compared with their corresponding C(5)−C(5a) (5*E*)-unsaturated derivatives **29**−**33**[13e] in rho functional assays. Added to the dihydrobicyclomycin list was the reduced analogue of (5*E*)-carboxylic acid **3**, compound **34**. Our objective was to gain a better appreciation of the relative importance of the bicyclomycin C(5)−C(5a) double bond for rho inhibitory activity.



24 X = H
25 X = NH$_2$
26 X = N(H)C(O)CH$_3$
27 X = OH
28 X = OC(O)CH$_3$
34 X = CH$_2$CO$_2$H
29 X = H
30 X = NH$_2$
31 X = N(H)C(O)CH$_3$
32 X = OH
33 X = OC(O)CH$_3$

1.2. Biochemical and Biological Studies. Previous studies[13] demonstrated that the poly C-dependent rho ATPase assay[14] serves as a reliable test to measure the inhibitory effects of bicyclomycin analogues and that every compound that shows poor or no activity in the poly C-dependent ATPase assay exhibits little or no antibiotic activity against W3350 *E. coli* in the filter disk assay.[16] Accordingly, our bicyclomycin derivatives were evaluated in the poly C-dependent ATPase assay. While we also determined the antibiotic activity of most newly synthesized bicyclomycins against W3350 *E. coli* using the filter disk assay, these values were not used to assess bicyclomycin binding to rho since the concentration of the different bicyclomycins within the cells could not readily be determined.

2. Synthesis and Spectral Characterization

2.1. Bicyclomycin Analogues 8−**19.** Synthesis of **8**−**19** followed the general pathway shown in Scheme 1.

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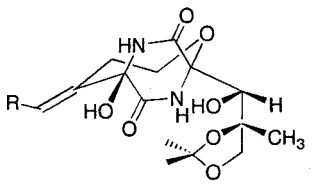
The starting C(5a)-modified bicyclomcyin **35** was either directly treated with the appropriate nucleophile to afford the targeted compound **36** (route A) or the corresponding acetonide **35** (R′,R″ = $C(CH_3)_2$) treated with the nucleophile to give **37**, and then deprotected (TFA) to yield **36** (route B).

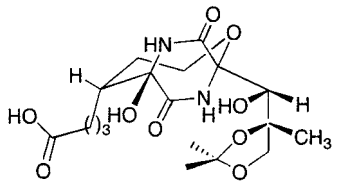
5a-Acetylbicyclomycin[11] (**9**) (Scheme 1, **35** (R = CH_3; R′, R″ = H), route A) served as the starting material for oxime **12**. The configuration of C(5)−C(5a) double bond in **9** has been assigned as the sterically less-crowded (5*E*) configuration based on chemical derivatization studies on **9** and NOE experiments conducted on the corresponding 5a-methyl bicyclomycincarboxylate (**21**).[11] Treatment of **9** with methoxylamine hydrochloride and pyridine gave **12** as a 70:30 mixture of *anti*-(5*E*,5b*E*) and *syn*-(5*E*,5b*Z*) isomers. The C(5b) configuration for the major isomer of methyl oxime **12** was determined to be *anti* by ^{13}C NMR spectroscopy. We detected a small upfield shift (∼1.1−2.1 ppm) for the C(5b) carbon in the more sterically compressed, minor *syn*-isomer. A similar shift has been reported for other *syn*-oximes, and this NMR signature is considered to be diagnostic of oxime stereochemistry.[17] Similarly, we obtained oxime **39** and methyloxime **40** from (*5E*)-5a-formylbicyclomycin C(2′),C(3′)-acetonide[7] (**38**) (Scheme 1, **35** (R = H; R′, R″ = $C(CH_3)_2$), route B), and the appropriate hydroxylamine hydrochloride. Deprotection of the acetonide group in **39** afforded **10** as a single isomer while removal of the acetonide group in **40** gave **11** as a 70:30 mixture of *anti*-(5*E*,5b*E*) and *syn*-(5*E*,5b*Z*) isomers (^1H and ^{13}C NMR analyses).



38 R = C(H)=O
39 R = C(H)=N~OH
40 R = C(H)=N~OCH_3
41 R = C(H)=C(H)CO_2CH_3
42 R = C(H)=C(H)$CO_2CH_2CH{=}CH_2$
43 R = C(H)=C(H)$CO_2CH_2C_6H_5$
44 R = C(H)=C(H)CO_2H
46 R = C(H)=C(H)(*p*-$NO_2C_6H_4$)
47 R = C(H)=C(H)(2-pyridyl)
48 R = C(H)=C(H)I
52 R = CO_2CH_3
56 R = CH_2NH_2
58 R = CH_2OH

Carboxylates **14**−**16** and carboxylic acid **13** were obtained using a two-step procedure (Scheme 1, route B). Condensation of (*5E*)-5a-formylbicyclomycin C(2′),C(3′)-acetonide (**38**) with the appropriate triphenylphosphorane afforded esters **41**−**43** as 90:10 mixtures of (5*E*,5b*E*) and (5*E*,5b*Z*) isomers, respectively. TFA removal of the acetonide group in **41**−**43** gave **14**−**16**, respectively.

We attempted to synthesize **44** by selectively removing the benzyl moiety of α,*β*,*γ*,*δ*-unsaturated ester **43** by catalytic hydrogenation. A similar approach was used for the synthesis of **3** from α,*β*-unsaturated ester **22**.[11] Use of this protocol gave saturated acid **45**, regardless of the catalyst (e.g., H_2, Pd−C; H_2, PtO_2). Acidic hydrolysis (TFA) of the acetonide-protecting group in **45** afforded **34**. Synthesis of the unsaturated acid **13** as a 90:10 mixture of (5*E*,5b*E*) and (5*E*,5b*Z*) isomers, respectively, was achieved by Pd^0-mediated deprotection[18] of allylic ester **15** in the presence of piperidine.



45

The stereochemical disposition of the newly installed C(5b)−C(5c) double bond in the C(5a)-extended bicyclomycins **13**−**16** was determined by NMR spectroscopy. First, we assigned the chemical shifts for the proton and carbon resonances for the C(5) substituent. The C(5) residue chemical shift values for the *major* (5*E*,5b*E*) isomers in **13**−**16** varied in a manner consistent with the electron density within the extended conjugated systems. In the ^1H NMR spectra, the C(5b) hydrogen resonated downfield from the C(5a) and C(5c) methine hydrogens. A similar deshielding pattern was observed in the ^{13}C NMR spectra for the corresponding carbon signals. Significantly, this pattern was not observed in the ^1H NMR spectra for the *minor* (5*E*,5b*Z*) isomers of **13**−**16**, where the C(5a) hydrogen was the most deshielded proton. Similar patterns have been observed for comparable diastereomeric α,*β*-unsaturated esters and were used to indicate structure.[19] These findings suggest that the major products were the (5*E*,5b*E*) isomers, and the minor adducts were the (5*E*,5b*Z*) isomers. Next, HMBC and HMQC NMR experiments on the major isomer for unsaturated ester **14** validated our assignments of the vinylic carbons and protons within the C(5) residue. The $J_2(^1H-^{13}C)$ and $J_3(^1H-^{13}C)$ couplings observed in the HMBC experiment established the connectivity pattern of the C(5) substituent with the [4.2.2] bicyclic ring system and the C(1) triol group. The chemical shift assignments permitted the configurational identification of the C(5b)−C(5c) double bond on the basis of the observed C(5b) and C(5c) $^1H-^1H$ vicinal coupling constants. We found that the J_{bc} coupling constant for the major isomers was 15.0 Hz and for the minor isomers it was 10.8 Hz, consistent with the proposed 5b*E*(*trans*) and 5b*Z*(*cis*) geometries, respectively.[20]

The C(5a) bicyclomycin vinyl aromatic derivatives **18** and **19** were prepared by Wittig condensation of **38** with the appropriate triphenylphosphonium salt and triethylamine to give **46** and **47** (Scheme 1, route B). Only the (5*E*,5b*E*) isomer was observed for **46** and **47** (^1H NMR analysis), suggesting that steric interactions of the appended aromatic moieties with the [4.2.2] ring system prevented formation of the corresponding (5*E*,5b*Z*)-diastereoisomers. We experienced difficulties in isolating pure **46**, prompting us to deprotect (TFA) the crude product mixture to give **18**. Use of a similar protocol gave **47** cleanly, which was then deprotected to provide the final product **19**.

Preparation of the C(5a) bicyclomycin vinyl iodide **17** was accomplished from **38** in two steps (Scheme 1, route B). Addition of chromium dichloride[21] to a THF solution containing **38**, iodoform, and *N*-methylmorpholine gave **48** as a 80:20 mixture of (5*E*,5b*E*) and (5*E*,5b*Z*) isomers,

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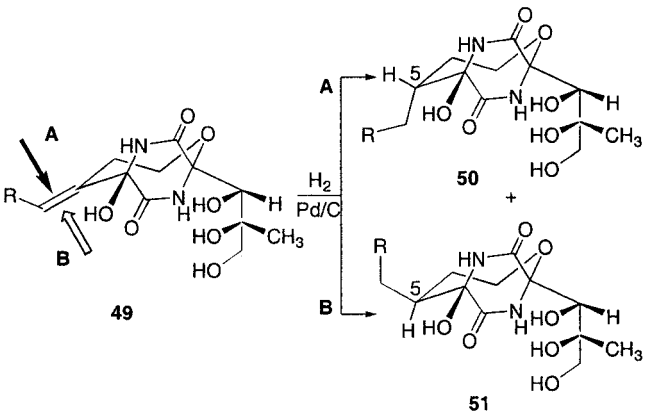
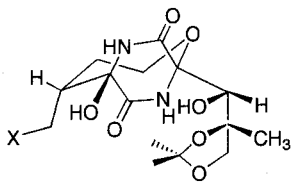
Scheme 2. General Pathway for Dihydrobicyclomycins 24-28



respectively, in 17% yield (^{1}H NMR analysis). TFA removal of the acetonide protecting group in **48** provided **17**.

2.2. (5Z)-Methyl 5a-Bicyclomycincarboxylate (23). Wittig condensation of 5-norketonebicyclomycin[11,22] (**4**) with (methoxycarbonylmethyl)tri-*n*-butylphosphonium bromide and triethylamine provided (5*E*)-**21** and (5*Z*)-**23** methyl 5a-bicyclomycincarboxylate as a 3:1 diastereomeric mixture, respectively (NMR analyses). Careful, repetitive TLC (10 X) permitted separation of the two isomers. When we increased the size of the alkoxy substituent in the phosphonium salt from methoxy to *tert*-butoxy (not shown),[23] or when we used the (methoxycarbonylmethyl)tri*phenyl*phosphonium bromide in place of the corresponding tri-*n*-*butyl*phosphonium salt, we obtained only the (5*E*) isomer.[11,13d]

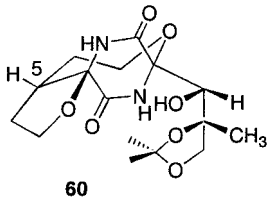
2.3. Dihydrobicyclomycins 24−28. Bicyclomycin (**1**) and bicyclomycin derivatives (**49**) undergo catalytic hydrogenation to give dihydrobicyclomycins (**50**, **51**) (Scheme 2). We employed this procedure (H_2, 10% Pd−C) to reduce the C(5)−C(5a) double bond in methyl 5a-bicyclomycincarboxylate C(2′),C(3′)-acetonide **52** to give dihydrobicyclomycin **53** as a 9:1 diastereomeric mixture.[13e] Reduction of **53** ($LiAlH_4$) provided the primary alcohol **54**.[13e] We have acylated (Ac_2O, TEA, DMAP) the primary hydroxy group in **54** to afford **55**. Removal of the acetonide group (TFA) in **54** and **55** gave **27** and **28**, respectively.



53 X = CO_2CH_3
54 X = CH_2OH
55 X = $CH_2OC(O)CH_3$
57 X = CH_2NH_2
59 X = CH_3
61 X = H
62 X = $N(H)C_6H_5$

We used comparable catalytic reductive procedures for the preparation of dihydrobicyclomycins **24**−**26**. Catalytic reduction of allylic amine **56** (H_2, 10% Pd−C) gave **57**, which was deprotected (TFA) to produce **25**. Similarly, **31** was reduced to **26**. Catalytic reduction (H_2, 10% Pd−C) of allylic alcohol **58** gave the C(5) alkyl derivative **59**. This method has been previously employed in the conversion of allylic alcohols to alkanes.[24] TFA removal of the acetonide group in **59** provided **24**. NMR analyses of **24**−**28** showed that catalytic reduction of the C(5)−C(5a) double bond gave predominately one diastereoisomer (≥90%).

Information concerning the facial orientation for the reduction of the C(5)−C(5a) double bond in **30**, **52**, and **56** and the resulting C(5) stereochemistry came from two X-ray crystallographic structural studies. Treatment of alcohol **54** with methanesulfonyl chloride and triethylamine in THF yielded tetrahydrofuran **60**. Single-crystal X-ray analysis of **60** (Supporting Information Figure 1) established the C(5) configuration as *R*. Similarly, catalytic reduction (H_2, PtO_2) of **1** gave dihydrobicyclomycin (**5**).[3] Treatment of **5** with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid furnished acetonide **61**. The X-ray crystallographic structure of **61** documented that reduction of the C(5) exomethylene group in **1** gave preferentially the (5*R*) isomer (Supporting Information Figure 2). The identical stereochemical disposition of the C(5) substituent in both **60** and **61** established that the preferred facial orientation for catalytic reduction of bicyclomycin derivatives to be pathway A in Scheme 2.



60

3. Biochemical and Biological Studies

The inhibitory activities of all newly prepared bicyclomycin derivatives were determined in the poly C-dependent ATPase assay.[14] We have provided evidence that **1** binds near the catalytic site for rho-mediated ATP hydrolysis and that this test has proven to be a reliable predictor of bicyclomycin−rho binding.[13] The antimicrobial MIC values for most of the bicyclomycin analogues were determined in the filter disk assay.[16]

3.1. C(5a)-Substituted Bicyclomycins with Extended Conjugation. The inhibitory properties of bicyclomycins **8**−**19** in the rho-dependent ATPase assay and the antimicrobial activities in the filter disk assay are listed in Table 1. Included in Table 1, wherever possible, are the activities of the corresponding parent bicyclomycins **(2**−**4**, **20**−**22)**.[13d] We define the parent as the corresponding bicyclomycin that contains the same C(5) or C(5a) terminal substituent as **8**−**19** but with one fewer carbon−carbon double bond.

Carbonyl derivatives **8** and **9** displayed excellent inhibitory activities in the poly C-dependent rho ATPase assay. The increased potency of aldehyde **8**, compared with **1**, has been partially attributed to the formation of a covalent imine bond between Lys-336 in rho and the aldehyde moiety of **8**, under the assay conditions.[8,10] We have evidence that no comparable process occurred with **9**. Incubation of **9** with rho followed by $NaBH_4$ reduction

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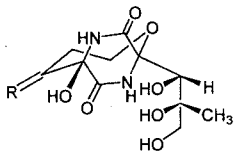
Table 1. Biochemical and Biological Activities of C(5a)-Substituted Bicyclomycins with Extended Conjugation



Cpd No.	R	ATPase[a] Activity $I_{50}(\mu M)$ (BCM)[b]	MIC[c] (mg/mL)	Cpd No.[d]	R	ATPase[a] Activity $I_{50}(\mu M)$ (BCM)[b]	MIC[c] (mg/mL)
				1	CH_2 (BCM)	60	0.25
8	C(H)-C(H)=O	35 (60)	32 (0.40)	**4**	O	160 (70)	2.70 (0.60)
9	C(H)-C(CH_3)=O	70 (50)	>32 (0.40)				
10	C(H)-C(H)=N~OH	40 (50)	2.5 (0.44)	**20**	N~OH	325 (60)	>32 (0.26)
11	C(H)-C(H)=N~OCH_3	45 (50)	2.4 (0.81)	**2**	N~OCH_3	85 (60)	0.71 (0.32)
12	C(H)-C(CH_3)=N~OCH_3	170 (45)	1.6 (0.81)				
13	C(H)-C(H)=C(H)CO_2H	500 (50)	e	**3**	C(H)CO_2H	95 (60)	>32 (0.26)
14	C(H)-C(H)=C(H)CO_2CH_3	95 (45)	>32 (0.38)	**21**	C(H)CO_2CH_3	105 (45)	0.60 (0.45)[f]
15	C(H)-C(H)=C(H)$CO_2CH_2CH=CH_2$	150 (50)	e				
16	C(H)-C(H)=C(H)$CO_2CH_2C_6H_5$	40 (40)	>32	**22**	C(H)$CO_2CH_2C_6H_5$	135 (60)	>32 (0.26)
17	C(H)-C(H)=C(H)I	50 (50)	0.83 (0.48)				
18	C(H)-C(H)=C(H)(*p*$NO_2C_6H_4$)	55 (50)	>32 (0.48)				
19	C(H)-C(H)=C(H)(2-pyridyl)	150 (50)	16 (0.48)				

[a] Inhibitory activity measured using the rho poly C-dependent ATPase assay.[14] 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. [b] BCM = bicyclomycin. [c] MIC value is the average minimum inhibitory concentration of the compound determined from duplicate tests using W3350 *E. coli*.[16] The number in parentheses is the corresponding value obtained from bicyclomycin in a concurrently run experiment. [d] Values cited are from ref 13d unless otherwise noted. [e]Compound not tested. [f]Value obtained in this study.

led to no appreciable loss of rho activity after dialysis (data not shown),[25] thus indicating that the inhibitory activity of **9** is not likely due to imine bond formation. The inhibitory activities of both **8** and **9** exceeded that of parent bicyclomycin **4**.[13d]

Oximes **10** and **11**, like aldehyde **8**, were efficient inhibitors of poly C-dependent ATP hydrolysis ($I_{50} \leq 45$ μM) and were comparable to **1** ($I_{50} = 50$ μM). The inhibitory activities of **10** and **11** exceeded their parent compounds **20** and **2**, respectively.[13d] Interestingly, when we introduced a methyl group at C(5b) to give **12**, we observed an inhibitory activity loss in the poly C-dependent rho ATPase assay.

Carboxylic acid **13** displayed poor activity in the rho poly C-dependent assay. The I_{50} value for **13** was 500 μM, making it 10 times weaker than **1** and approximately four times weaker than the parent bicyclomycin carboxylic acid **3**. Our finding that Lys-336 was the exclusive modification site for rho reductive amination probe **8**[10] suggested that this protonated lysine residue fosters bicyclomycin carboxylate **3** binding through a salt bridge. We suspect that incorporation of an additional carbon−carbon double bond between C(5a) and the carboxylic acid moiety to give **13** disrupts this interaction and leads to a loss of biochemical activity.

No clear structural pattern emerged from the rho inhibitory activities of $\alpha,\beta,\gamma,\delta$-unsaturated esters **14**−**16**. We found that in the ATPase assay, benzyl ester **16** ($I_{50} = 40$ μM) displayed rho inhibitory activity comparable with **1** ($I_{50} = 40$ μM). This activity exceeded that of methyl ester **14** ($I_{50} = 95$ μM) and of allyl ester **15** ($I_{50} = 150$ μM). By comparison, in the parent carboxylic ester series, a progressive decrease in activity was observed as the size of the alkoxy ester substituent was increased.[13d]

The I_{50} values for bicyclomycins containing a terminal iodide (**17**) and *p*-nitrophenyl (**18**) group matched the values for bicyclomycin ($I_{50} = 50-55$ μM) while the corresponding value for the terminal 2-pyridyl adduct **19** was 150 μM.

Our bicyclomycins with extended C(5a) conjugation (**8**−**19**) contained a wide range of terminal substituents. This structural diversity did not permit us to elucidate specific patterns necessary for inhibitory activity. Nonetheless, the inhibitory activities for **8**−**11**, **14**, and **16**−**18** clearly document that C(5a)-extended bicyclomycins can efficiently inhibit rho. We attempted to model bicyclomycin **18** binding to rho (Figure 3) while maintaining the same binding interactions used for **1** (Figure 2). Compound **18** was selected for study since it contained the most extensive C(5) conjugated system while retaining biochemical activity comparable to **1**. We found that if we maintained the same binding interactions for the C(1) triol and [4.4.2] bicyclic subunits in **18** as predicted for **1** (Figure 2), we incurred no adverse steric interactions for the appended (5b*E*)-*p*-nitrostyryl residue in **18** and that this residue is nestled at the interface formed by two rho

(25) Vincent, F.; Widger, W. R.; Kohn, H., unpublished results.

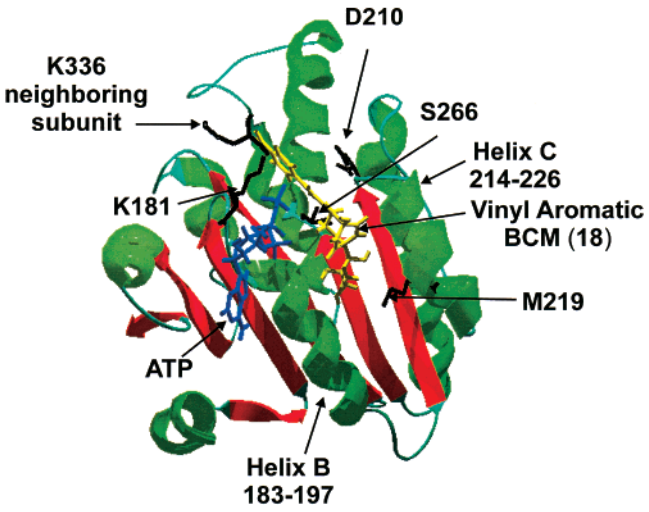


Figure 3. The proposed orientation of **18** binding to rho. Compound **18** was superimposed on the structure of **1** (Figure 2) and the C(5a) vinyl aromatic moiety was allowed to extend from the C(5a) position. The extended vinyl aromatic moiety is nestled between the adjacent subunits of rho and points in the general direction of the protein's C-terminus.

subunits[26−29] with the *p*-nitrophenyl group pointed in the general direction of the protein C-terminus.

Few of the extended C(5a)-substituted bicyclomycins that showed excellent inhibitory activity in the poly C-mediated ATPase assay exhibited significant antimicrobial activity in the filter disk assay using W3350 *E. coli*. Iodide **17** was an exception (MIC: **17** = 0.83 mg/mL, **1** = 0.48 mg/mL). Oximes **10**−**12** exhibited moderate antibiotic activity (MIC = 1.6−2.4 mg/mL) while the remaining compounds showed little or no activity. The poor correlation between bicyclomycin biochemical and biological activities has been previously observed[13d] and reinforced our decision to exclude the antimicrobial data in identifying new bicyclomycin analogues that can effectively inhibit rho function.

3.2. (5*Z*)-Methyl 5a-Bicyclomycincarboxylate (23). Preliminary information concerning the preferred geometrical structure of the C(5) double bond was gained by comparing the activities of **21** and **23**. We found that **21** was significantly more effective in inhibiting ATP hydrolysis than **23** (**21**: $I_{50} = 105\ \mu$M; **23**: $I_{50} > 400\ \mu$M; **1**: $I_{50} = 45\ \mu$M). These findings suggest that the bicyclomycin binding pocket can better accommodate 5a-bicyclomycin derivatives that have 5*E* geometry than the corresponding 5*Z* geometry. We have attempted to fit (dock) both **21** and **23** into the putative **1** binding pocket in rho (data not shown). Bicyclomycin geometry in the pocket as depicted in Figure 2 allows the *5E* isomer to be in a less hindered environment nestled between the two rho subunits, while the *5Z* geometry would be hindered by the G helix of the neighboring subunit. The orientation of **1** can be rotated on its long axis such that the C(6)OH points toward S266 instead of away from this residue. This geometry can accommodate the *5E* geometry, but the *5Z* geometry is hindered by *β*-sheet 3 containing residues 259−268. Significantly, the docking of bicyclomycin to rho is reliant on our structural model of rho. Since rho structure was derived by threading rho sequence on the structure of bovine F_1-ATP synthase and energy minimized[10] uncertainties in the model exist. One such inconsistency is the orientation of D210. A mutation D210A located in the loop between sheet 1 residues 203−209 and helix C residues 214−226 has shown loss of **1** action;[30] however, as seen in Figure 2, D210 points away from **1**. Local geometry around D210 is likely to be different, allowing some contact between D210 and **1**.

3.3. C(5a)-Substituted Dihydrobicyclomycins. The last category of compounds evaluated were the C(5a)-substituted dihydrobicyclomycins **24**−**28** and **34** (Table 2). We observed that 5a-methyldihydrobicyclomycin (**24**) inhibited poly C-dependent ATP hydrolysis ($I_{50} = 120\ \mu$M) at a level comparable with dihydrobicyclomycin (**5**) ($I_{50} = 120\ \mu$M) while **25**−**28** and **34** were less active ($I_{50} \geq 355\ \mu$M). Significantly, the C(5)−C(5a)-saturated bicyclomycins **24**−**28** exhibited equal or lower activities than their unsaturated counterparts **29**−**33**.[13e] Moreover, we found that the activities of dihydrobicyclomycins **24**−**28** paralleled those observed for **29**−**33**, respectively. The poor inhibitory activities of **24**−**28** and **34** contrasted with the excellent activities observed with **6** and **7**; several possible explanations exist for these differences. First, biochemical activities may be tightly regulated by the distance between C(5a) and the attached terminal substituent. Second, the major C(5)−C(5a) dihydrobicyclomycin epimer produced by catalytic reductive procedures (e.g., **24**−**28**) may be opposite from that obtained by Michael addition processes (e.g., **6**, **7**). Initial findings, however, suggest that both synthetic routes generate the same structural epimer as the major product. Alignment of the X-ray crystallographic structure of the Michael addition product, 5a-anilinodihydrobicyclomycin C(2′),C(3′)-acetonide (**62**),[13d] with **61** showed the same three-dimensional orientation of the C(5) substituent with respect to the [4.2.2] bicyclic ring system.

Most of the C(5a)-substituted dihydrobicyclomycins (**24**−**28**, **34**) were tested for their antibacterial activity using W3350 *E. coli* (Table 2). Only 5a-methyldihydrobicyclomycin (**24**) exhibited activity in the filter disk assay (0.62 mg/mL) comparable with bicyclomycin (0.30 mg/mL). The remaining compounds exhibited little or no activity.

Conclusions

Previous studies documented that retention of the C(5)−C(5a) exomethylene group in **1** was not essential for retention of bicyclomycin inhibitory activities in rho functional assays.[13d,e] This study determined the structural parameters that influenced rho inhibitory activity for C(5a)-substituted bicyclomycin derivatives. We learned that (1) bicyclomycins with extended C(5a)-conjugated substituents still retained excellent inhibitory activities in biochemical assays, (2) (5*E*)-substituted bicyclomycin derivatives were likely to serve as better rho inhibitors than their (5*Z*) isomers, and (3) reduction of the C(5)−C(5a) double bond generally led to a loss of rho inhibitory activity.

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(29) Yu, X.; Horiguchi, T.; Shigesada, K.; Egelman, E. H. *J. Mol. Biol.* **2000**, *299*, 1279−1287.
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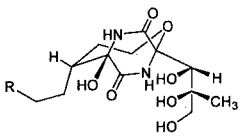
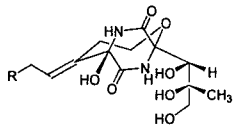
Table 2. Biochemical and Biological Activities of C(5a)-Substituted Dihydrobicyclomycins





Cpd No.	R	ATPase[a] Activity I_{50}(μM) (BCM)[b]	MIC[c] (mg/mL)	Cpd No.[d]	R	ATPase[a] Activity I_{50}(μM) (BCM)[b]	MIC[c] (mg/mL)
24	H	120 (70)	0.62 (0.30)	**29**	H	70 (70)	0.33 (0.27)
25	NH_2	>400 (45)	>32 (0.25)	**30**	NH_2	>400 (55)	>32 (0.43)
26	$N(H)C(O)CH_3$	>400 (45)	>32 (0.25)	**31**	$N(H)C(O)CH_3$	350 (55)	>32 (0.43)
27	OH	355 (70)	5.30 (0.30)	**32**	OH	120 (70)	1.07 (0.27)
28	$OC(O)CH_3$	>400 (70)	>32 (0.30)	**33**	$OC(O)CH_3$	175 (70)	2.14 (0.30)
34	CH_2CO_2H	>400 (50)	>32 (0.40)	**13**		500 (50)	e

[a] Inhibitory activity measured using the rho poly C-dependent ATPase assay.[14] 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. [b] BCM = bicyclomycin. [c] MIC value is the average minimum inhibitory concentration of the compound determined from duplicate tests using W3350 *E. coli*.[16] The number in parentheses is the corresponding value obtained from bicyclomycin in a concurrently run experiment. [d] Values cited are from ref 13e. [e] Compound not tested.

Experimental Section

General Methods. Low resolution and high-resolution (CI) mass spectral studies were run at the University of Texas at Austin by Dr. M. Moini. HPLC analyses were conducted with the following Waters Associates Units: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The products were eluted with the following gradient: C_{18} μBondapak (SS) column 3.9 × 300 mm, from an initial 100% A (water) to 40% A and 60% B (acetonitrile containing 0.1% trifluoroacetic acid) in 60 min and finally to 100% B in 65 min. The column was fitted with a μBondapak C_{18} Guardpak. A flow rate of 0.7 mL/min was used. The organic solvent utilized was HPLC grade, and was filtered (Millipore FH, 0.5 μM) and degassed prior to use. The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. Thin-layer chromatographies were run on precoated silica gel slides (20 × 20 cm; Sigma Z12272-6).

Rho protein was isolated from *E. coli* AR 120 containing the overexpressing plasmid p39-ASE, which has been corrected for the K155E substitution seen in the original p39-AS plasmid.[31] Rho purity was assessed by SDS−PAGE, and concentrations were determined by the Lowry protein determination.[32] [γ-^{32}P]ATP was purchased from Dupont New England Nuclear (Doraville, GA), and nucleotides were obtained from Sigma. Polyethyleneimine (PEI) thin-layer chromatography (TLC) plates used for ATPase assays were purchased from J. T. Baker, Inc. (Phillipsburg, NJ).

(5*E*)-5a-Acetylbicyclomycin-*O*-methyloxime (12). To an anhydrous ethanol (1 mL) solution of methoxylamine hydrochloride (3.5 mg, 0.043 mmol) and **9** (10 mg, 0.028 mmol) was added pyridine (1 drop). The resulting mixture was stirred (40 °C, 90 min) and concentrated in vacuo. The residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **12** as a colorless solid (7 mg, 66%) as a 70:30 mixture of geometric isomers: mp 107−110 °C (dec); R_f 0.47 (20% MeOH−$CHCl_3$); IR (KBr) 3434 (br), 3252 (br), 2930, 1686, 1400, 1051 cm^{-1}; ^{1}H NMR (CD_3OD) (5*E*,5b*E*)-diastereoisomer: δ 1.34 (s, 3 H, C(2′)CH_3), 1.89 (s, 3 H, C(5c)H_3), 2.57 (dd, J = 9.0, 16.2 Hz, 1 H, C(4)H*H*′), 3.25−3.33 (m, 1 H, C(4)H*H*′), 3.50 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.70−3.99 (m, 5 H, C(3)H_2, $NOCH_3$), 4.09 (s, 1 H, C(1′)H), 6.45 (s, 1 H, C(5a)H); (5*E*,5b*Z*)-diastereoisomer: δ 1.88 (s, 3 H, C(5c)H_3), 3.72 (s, 3 H, $NOCH_3$), 6.34 (s, 1 H, C(5a)H), the other signals were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) (5*E*,5b*E*)-isomer: 16.0 (C(5c)), 24.3 (C(2′)*C*H_3), 30.5 (C(4)), 62.3 ($NOCH_3$), 65.8 (C(3)), 68.6 (C(3′)), 72.3 (C(1′)), 78.4 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 126.3 (C(5a)), 145.5 (C(5)), 155.0 (C(5b)), 169.3 (C(9)), 172.6 (C(7)) ppm; (5*E*,5b*Z*)-diastereoisomer: 20.7 (C(5c)), 32.6 (C(4)), 61.7 ($NOCH_3$), 64.8 (C(3)), 83.2 (C(6)), 89.7 (C(1)), 123.7 (C(5a)), 145.0 (C(5)), 153.9 (C(5b)), 169.0 (C(9)), 172.6 (C(7)) ppm, the other signals were not detected and are believed to overlap with the major diastereoisomer; MS (+CI) 374 $[M + 1]^+$; M_r (+CI) 374.156 45 $[M + 1]^+$ (calcd for $C_{15}H_{24}N_3O_8$ 374.156 34).

(5*E*)-5a-Formylbicyclomycin-*O*-methyloxime C(2′), C(3′)-Acetonide (40). Methoxylamine hydrochloride (41 mg, 0.49 mmol) and **38** (52 mg, 0.14 mmol) were dissolved in anhydrous THF (10 mL) in the presence of molecular sieves (3 Å), and the resulting mixture was stirred (25 °C, 18 h). The reaction mixture was concentrated in vacuo. The residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **40** as a colorless solid (35 mg, 63%) as a 50:50 mixture of geometric isomers: mp 120−124 °C; R_f 0.72 (20% MeOH−$CHCl_3$); IR (KBr) 3411 (br), 3306 (br), 2986, 2938, 1695, 1384, 1044, 876 cm^{-1}; ^{1}H NMR (CD_3OD) (5*E*,5b*E*)-diastereoisomer: δ 1.38 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.65−2.74 (m, 1 H, C(4)*H*H′), 2.87−2.97 (m, 1 H, C(4)H*H*′), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.75−4.10 (m, 5 H, C(3)H_2, $NOCH_3$), 4.17 (s, 1 H, C(1′)H), 4.44 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 6.71 (d, J = 10.2 Hz, 1 H, C(5a)H), 8.06 (d, J = 10.2 Hz, C(5b)H); (5*E*,5b*Z*)-diastereoisomer: δ 7.24 and 7.38 (2 d, J = 9.9 Hz, 2 H, C(5a)H, C(5b)H), the other signals were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) (5*E*,5b*E*)-diastereoisomer: 25.1 (C(2′)*C*H_3), 26.7 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 29.6 (C(4)), 62.5 ($NOCH_3$), 65.8 (C(3)), 73.1 and 73.3 (C(3′), C(1′)), 83.3 (C(6)), 86.5 (C(2′)), 89.0 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 123.3 (C(5a)), 147.5 and 147.7 (C(5), C(5b)), 168.6 (C(9)), 171.7 (C(7)) ppm; (5*E*,5b*Z*)-diastereoisomer: 29.8 (C(4)), 62.6 ($NOCH_3$), 66.0 (C(3)), 83.5 (C(6)), 117.4 (C(5a)), 144.7 and 149.5 (C(5), C(5b)), 168.6 (C(9)) ppm, the other signals were not detected and are believed to overlap with the other diastereoisomer; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; MS (+CI) 400 $[M + 1]^+$; M_r (+CI) 400.171 06 $[M + 1]^+$ (calcd for $C_{17}H_{26}N_3O_8$ 400.171 99).

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(5*E*)-5a-Formylbicyclomycin-Oxime C(2′),C(3′)-Acetonide (39). Hydroxylamine hydrochloride (12.5 mg, 0.18 mmol) and **38** (44 mg, 0.12 mmol) were dissolved in anhydrous THF (3 mL) in the presence of molecular sieves (3 Å), and the resulting mixture was stirred (25 °C, 18 h). The reaction mixture was concentrated in vacuo, and the residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **39** as a colorless solid (39 mg, 92%) as a 95:5 mixture of geometric isomers: mp 152−155 °C; R_f 0.60 (20% MeOH−$CHCl_3$); IR (KBr) 3417 (br), 3281 (br), 2988, 2935, 2884, 1688, 1403, 1069, 876 cm^{-1}; ^1H NMR (CD_3OD) (5*E*,5b*E*)-diastereoisomer: 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$(CH_3)_2$), 2.68 (dd, J = 8.8, 16.9 Hz, 1 H, C(4)*H*H′), 2.89 (dd, J = 7.4, 16.9 Hz, 1 H, C(4)H*H*′), 3.74 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.81 (dd, J = 8.8, 13.5 Hz, 1 H, C(3)*H*H′), 3.98 (dd, J = 7.4, 13.5 Hz, 1 H, C(3)H*H*′), 4.17 (s, 1 H, C(1′)H), 4.44 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 6.75 (d, J = 10.4 Hz, 1 H, C(5a)H), 7.38 (s, 1 H, NOH), 8.05 (d, J = 10.4 Hz, 1 H, C(5b)H); (5*E*,5b*Z*)-diastereoisomer: δ 4.10 (s, 1 H, C(1′)H), 7.25 (s, 1 H, NOH), the other signals were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) 25.2 (C(2′)*C*H_3), 26.9 (C(*C*$H_3)_2$), 28.3 (C(*C*$H_3)_2$), 29.8 (C(4)), 66.1 (C(3)), 73.1 and 73.3 (C(3′), C(1′)), 83.3 (C(6)), 86.5 (C(2′)), 89.0 (C(1)), 111.7 (*C*$(CH_3)_2$), 124.1 (C(5a)), 146.0 and 148.0 (C(5), C(5b)), 168.6 (C(9)), 171.9 (C(7)) ppm, signals for the minor diastereoisomer were not detected; MS (+CI) 386 $[M + 1]^+$; M_r (+CI) 386.156 39 $[M + 1]^+$ (calcd for $C_{16}H_{24}N_3O_8$ 386.156 34).

(5*E*,5b*E*)-Bicyclomycin-5a-propenoic Acid (13). To an anhydrous solution of THF (5 mL) of **15** (18 mg, 0.044 mmol) and tetrakis(triphenylphosphine)palladium (7 mg, 0.006 mmol) was added piperidine (8 mg, 0.097 mmol), and the reaction solution was stirred (25 °C, 90 min). The reaction mixture was concentrated in vacuo, and the residue was redissolved in MeOH (0.2 mL), acidified using trifluoroacetic acid (2 drops) and purified by preparative TLC (30% MeOH−$CHCl_3$) to afford **13** (8 mg, 49%) as a white hygroscopic solid as a single isomer: R_f 0.25 (30% MeOH−$CHCl_3$); ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2′)CH_3), 2.61 (dd, J = 9.0, 16.5 Hz, 1 H, C(4)*H*H′), 3.06 (dd, J = 7.2, 16.5 Hz, 1 H, C(4)H*H*′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.64 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.76 (dd, J = 9.0, 13.0 Hz, 1 H, C(3)*H*H′), 4.02 (dd, J = 7.2, 13.0 Hz, 1 H, C(3)H*H*′), 4.09 (s, 1 H, C(1′)H), 6.06 (br d, J = 14.1 Hz, 1 H, C(5c)H), 6.80 (d, J = 11.1 Hz, 1 H, C(5a)H), 7.37 (br app t, J = 12.6 Hz, 1 H, C(5b)H); ^{13}C NMR (CD_3OD) 24.3 (C(2′)*C*H_3), 29.9 (C(4)), 65.3 (C(3)), 68.5 (C(3′)), 72.4 (C(1′)), 78.3 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 116.5 (C(5c)), 128.3 (C(5a)), 137.3 (C(5b)), 147.0 (C(5)), 165.3 (br) (C(5d)), 172.4 (C(7)) ppm, the C(9) signal was not detected; MS (ES) 395 $[M + Na]^+$; M_r (ES) $[M + Na]^+$ (calcd for $C_{14}H_{21}N_2O_9$).

Methyl C(5a)-Bicyclomycincarboxylate Mixture ((5*E*)-21 and (5*Z*)-23)). To an anhydrous dioxane solution (1 mL) of **4** (19 mg, 0.07 mmol) was added triethylamine (6.5 μL) and (methoxycarbonylmethyl)tri-*n*-butylphosphonium bromide (22 mg, 0.06 mmol), and then the solution was stirred (room temperature, 5 h). The solvent was removed in vacuo and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to give **21** and **23** as a 3:1 diastereomeric mixture, respectively (14 mg, 63%). The mixture was separated by preparative TLC using 5% MeOH−$CHCl_3$ (5X), and then 15% MeOH−$CHCl_3$ (5X) to give **21** and **23** (separation R_f 0.20).

(5*E*)-Methyl 5a-Bicyclomycincarboxylate[11] (21). mp 125−127 °C (lit.[11] mp 135−136 °C (dec)); IR (KBr) 3417, 2926, 2363, 1696, 1400, 1136 cm^{-1}; HPLC retention time (t_R): 22.4 min (214 nm); ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.71−2.80 (m, 1 H, C(4)*H*H′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.70 (s, 3 H, OCH_3), 3.65−3.86 (m, 2 H, C(4)H*H*′, C(3)*H*H′), 3.97−4.04 (m, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 6.49 (C(5a)H); ^{13}C NMR (CD_3OD) 24.2 (C(2′)*C*H_3), 29.2 (C(4)), 51.9 (OCH_3), 64.7 (C(3)), 68.4 (C(3′)), 72.2 (C(1′)), 78.1 (C(2′)), 83.4 (C(6)), 89.5 (C(1)), 120.2 (C(5a)), 158.6 (C(5)), 168.2 (OC(O)), 168.7 (C(9)), 171.6 (C(7)) ppm; MS (+CI) 361 $[M + 1]^+$; M_r (+CI) 361.123 70 $[M + 1]^+$ (calcd for $C_{14}H_{21}N_2O_9$ 361.124 71).

(5*Z*)-Methyl 5a-Bicyclomycincarboxylate (23). IR (KBr) 3427, 2935, 2358, 1689, 1395, 1136 cm^{-1}; HPLC retention time (t_R): 26.9 min (214 nm); ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.72−2.82 (m, 1 H, C(4)*H*H′), 3.51 (d, J = 11.1 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.1 Hz, 1 H, C(3′)H*H*′), 3.74 (s, 3 H, OCH_3), 3.65−3.82 (m, 2 H, C(4)H*H*′, C(3)*H*H′), 3.97−4.02 (m, 1 H, C(3)H*H*′), 4.11 (s, 1 H, C(1′)H), 6.58 (s, 1 H, C(5a)H); ^{13}C NMR (CD_3OD) 24.3 (C(2′)*C*H_3), 29.7 (C(4)), 52.1 (OCH_3), 64.7 (C(3)), 68.5 (C(3′)), 72.1 (C(1′)), 78.4 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 119.6 (C(5a)), 159.9 (C(5)), 168.3 (OC(O)), 168.8 (C(9)), 171.5 (C(7)) ppm; MS (+CI) 361 $[M + 1]^+$; M_r (+CI) 361.123 70 $[M + 1]^+$ (calcd for $C_{14}H_{21}N_2O_9$ 361.127 71).

(5*E*,5b*E*)-[5c-(4-Nitrophenyl)]-5a-ethenylbicyclomycin (18). To an anhydrous dioxane solution (3 mL) containing (4-nitrophenylmethyl)triphenylphosphonium bromide (74 mg, 0.098 mmol) and **38** (29 mg, 0.078 mmol) was added triethylamine (18 mg, 0.181 mmol), and the reaction mixture was stirred (60 °C, 18 h). The reaction mixture was concentrated in vacuo and the residue redissolved in 50% aqueous methanol (2 mL), treated with TFA (3 drops), and stirred (45 °C, 2 h). The solution was concentrated in vacuo, and the residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **18** (4 mg, 12%) as a yellow solid: mp 145−148 °C; R_f 0.52 (20% MeOH−$CHCl_3$); IR (KBr) 3429 (br), 2926 (br), 2854, 1685, 1517, 1400, 1343, 1135, 1071 cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2′)CH_3), 2.66 (dd, J = 9.3, 16.5 Hz, 1 H, C(4)*H*H′), 3.16 (dd, J = 7.2, 16.5 Hz, 1 H, C(4)H*H*′), 3.52 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.80 (dd, J = 9.3, 12.8 Hz, 1 H, C(3)*H*H′), 4.04 (dd, J = 7.2, 12.8 Hz, 1 H, C(3)H*H*′), 4.11 (s, 1 H, C(1′)H), 6.84 (d, J = 15.4 Hz, 1 H, C(5c)H), 6.91 (d, J = 11.4 Hz, 1 H, C(5a)H), 7.34 (dd, J = 11.4, 15.4 Hz, 1 H, C(5b)H), 7.72 (d, J = 9.2 Hz, 2 H, C(2″)H, C(6″)H), 8.18 (d, J = 9.2 Hz, 2 H, C(3″)H, C(5″)H); ^{13}C NMR (CD_3OD) 24.3 (C(2′)*C*H_3), 29.9 (C(4)), 65.5 (C(3)), 68.6 (C(3′)), 72.2 (C(1′)), 78.3 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 125.0 (C(3″) or C(5″)), 125.1 (C(5″) or C(3″)), 128.5 (C(6″) or C(2″)), 128.6 (C(2″) or C(6″)), 129.5 and 129.6 (C(5a), C(5b)), 134.9 (C(5c)), 144.2 and 145.4 (C(5), C(1″)), 148.4 (C(4″)), 169.1 (C(9)), 172.7 (C(7)) ppm; MS (+CI) 450 $[M + 1]^+$; M_r (+CI) 450.150 29 $[M + 1]^+$ (calcd for $C_{20}H_{24}N_3O_9$ 450.151 26).

(5*E*,5b*E*)-Methyl Bicyclomycin-5a-propenoate C(2′),C(3′)-Acetonide (41). To an anhydrous dioxane solution (2 mL) of **38** (12 mg, 0.032 mmol) was added methyl (triphenylphosphoranylidene)acetate (14 mg, 0.042 mmol), and the solution was stirred (45 °C, 2 h). The reaction mixture was concentrated in vacuo, and the residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **41** as a colorless solid (12 mg, 83%) as a 90:10 mixture of geometric isomers: mp 117−122 °C; R_f 0.72 (20% MeOH−$CHCl_3$); IR (KBr) 3439 (br), 2993 (br), 2936, 1695, 1400, 1283, 1069 cm^{-1}; ^1H NMR (CD_3OD) major isomer: δ 1.39 (s, 3 H, C(2′)CH_3), 1.42 (s, 3 H, C$(CH_3)_2$), 1.44 (s, 3 H, C$(CH_3)_2$), 2.72 (dd, J = 8.7, 16.7 Hz, 1 H, C(4)*H*H′), 2.99 (dd, J = 7.4, 16.7 Hz, 1 H, C(4)H*H*′), 3.72−3.85 (m, 5 H, C(3)*H*H′, C(3′)*H*H′, OCH_3), 4.02 (dd, J = 7.4, 13.2 Hz, 1 H, C(3)H*H*′), 4.17 (s, 1 H, C(1′)H), 4.45 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 6.09 (d, J = 15.0 Hz, 1 H, C(5c)H), 6.86 (d, J = 11.9 Hz, 1 H, C(5a)H), 7.56 (dd, J = 11.9, 15.0 Hz, 1 H, C(5b)H); minor isomer: δ 5.83 (d, J = 10.8 Hz, 1 H, C(5c)H), 6.96 (app t, J = 11.3 Hz, 1 H, C(5b)H), 7.95 (d, J = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) 25.2 (C(2′)*C*H_3), 27.0 (C(*C*$H_3)_2$), 28.2 (C(*C*$H_3)_2$), 29.9 (C(4)), 52.4 (C(O)O*C*H_3), 66.0 (C(3)), 73.2 (C(1′) or C(3′)), 73.3 (C(3′) or C(1′)), 83.4 (C(6)), 86.6 (C(2′)), 89.1 (C(1)), 111.8 (*C*$(CH_3)_2$), 125.5 (C(5c)), 127.4 (C(5a)), 140.3 (C(5b)), 149.5 (C(5)), 168.7 (C(9) or *C*(O)OCH_3), 169.0 (*C*(O)OCH_3 or C(9)), 171.9 (C(7)) ppm, signals for the minor diastereoisomer were not detected; MS (+CI) 427 $[M + 1]^+$; M_r (+CI) 427.170 96 $[M + 1]^+$ (calcd for $C_{19}H_{27}N_2O_9$ 427.171 66).

(5*E*,5b*E*)-Allyl Bicyclomycin-5a-propenoate C(2′),C(3′)-Acetonide (42). To an anhydrous dioxane solution (2 mL) of **38** (7.5 mg, 0.020 mmol) was added allyl (triphenylphosphoranylidene)acetate (20 mg, 0.056 mmol), and the reaction

mixture was stirred (25 °C, 4 h). The reaction mixture was concentrated in vacuo and the residue redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **42** as a white solid (7 mg, 71%) as a 90:10 mixture of geometric isomers: mp 120−123 °C; R_f 0.61 (10% MeOH−$CHCl_3$); IR (KBr) 3436 (br), 2936, 1695, 1653, 1400, 1281, 1173, 1062 cm^{-1}; ^1H NMR (CD_3OD) major isomer: δ 1.38 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.71 (dd, J = 8.7, 16.5 Hz, 1 H, C(4)*H*H′), 2.98 (dd, J = 7.4, 16.5 Hz, 1 H, C(4)H*H*′), 3.73 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.80 (dd, J = 8.7, 13.2 Hz, 1 H, C(3)*H*H′), 4.02 (dd, J = 7.4, 13.2 Hz, 1 H, C(3)H*H*′), 4.17 (s, 1 H, C(1′)H), 4.43 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 4.63 (dd, J = 1.4, 6.9 Hz, 2 H, C(O)OCH_2CHCH_2), 5.22 (br dd, J = 1.4, 10.2 Hz, 1 H, C(O)OCH_2CHCH$_E$$H_Z$), 5.31 (br dd, J = 1.4, 17.1 Hz, 1 H, C(O)OCH_2CHCH_EH$_Z$), 5.91−6.00 (m, 1 H, C(O)-OCH_2C*H*CH_2), 6.11 (d, J = 15.0 Hz, 1 H, C(5c)H), 6.85 (d, J = 12.0 Hz, 1 H, C(5a)H), 7.57 (dd, J = 12.0, 15.0 Hz, 1 H, C(5b)H); minor isomer: δ 5.84−5.89 (m, 1 H, C(5c)H), 7.00 (app t, J = 11.4 Hz, 1 H, C(5b)H), 7.96 (d, J = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) 25.2 (C(2′)*C*H$_3$), 27.0 (C(*C*H$_3$)$_2$), 28.2 (C(*C*H$_3$)$_2$), 29.8 (C(4)), 65.9 (C(3)), 66.4 (C(O)O*C*H$_2$CHCH$_2$), 73.0 (C(1′) or C(3′)), 73.2 (C(3′) or C(1′)), 83.4 (C(6)), 86.5 (C(2′)), 89.0 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 118.6 (C(O)OCH$_2$CH*C*H$_2$), 125.5 (C(5c)), 127.4 (C(5a)), 133.7 (C(O)OCH$_2$*C*HCH$_2$), 140.4 (C(5b)), 149.5 (C(5)), 168.0 (C(9) or *C*(O)OCH$_2$CH$_3$), 168.6 (*C*(O)OCH$_2$CHCH$_2$ or C(9)), 171.8 (C(7)) ppm, signals for the minor diastereoisomer were not detected; MS (+CI) 453 [M + 1]$^+$; M_r (+CI) 453.187 40 [M + 1]$^+$ (calcd for $C_{21}H_{28}N_2O_9$ 453.187 31).

(5*E*,5b*E*)-Benzyl Bicyclomycin-5a-propenoate C(2′),C(3′)-Acetonide (43). To an anhydrous dioxane solution (2 mL) of **38** (44 mg, 0.119 mmol) were added triethylamine (20 mg, 0.202 mmol) and (benzyloxycarbonylmethyl)triphenylphosphonium bromide (83 mg, 0.169 mmol), and the solution was stirred (25 °C, 5 h). The reaction was concentrated in vacuo and the residue redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **43** as a white solid (50 mg, 84%) as a 90:10 mixture of geometric isomers: mp 125−130 °C; R_f 0.72 (15% MeOH−$CHCl_3$); IR (KBr) 3290 (br), 2990 (br), 2935, 2880, 1695, 1400, 1278, 1137, 1067, 876 cm^{-1}; ^1H NMR (CD_3OD) major isomer: δ 1.38 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.71 (dd, J = 8.7, 16.7 Hz, 1 H, C(4)*H*H′), 2.97 (dd, J = 7.4, 16.7 Hz, 1 H, C(4)H*H*′), 3.72−3.84 (m, 2 H, C(3)*H*H′, C(3′)*H*H′), 4.00 (dd, J = 7.4, 13.2 Hz, 1 H, C(3)H*H*′), 4.17 (s, 1 H, C(1′)H), 4.44 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 5.19 (s, 2 H, OCH_2C$_6$H$_5$), 6.14 (d, J = 15.0 Hz, 1 H, C(5c)H), 6.87 (d, J = 11.7 Hz, 1 H, C(5a)H), 7.30−7.38 (m, 5 H, C(O)OCH$_2$C$_6$*H*$_5$), 7.58 (dd, J = 11.7, 15.0 Hz, 1 H, C(5b)H); minor isomer: δ 5.86 (d, J = 10.8 Hz, 1 H, C(5c)H), 7.02 (app t, J = 11.3 Hz, 1 H, C(5b)H), 8.01 (d, J = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) 25.2 (C(2′)*C*H$_3$), 27.0 (C(*C*H$_3$)$_2$), 28.2 (C(*C*H$_3$)$_2$), 29.9 (C(4)), 66.0 (C(3)), 67.5 (C(O)O*C*H$_2$C$_6$H$_5$), 73.2 and 73.3 (C(3′), C(1′)), 83.4 (C(6)), 86.5 (C(2′)), 89.1 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 125.6 (C(5c)), 127.4 (C(5a)), 129.4, 129.7 and 137.7 (C(O)OCH$_2$*C*$_6$H$_5$), 140.6 (C(5b)), 149.5 (C(5)), 168.2 and 168.6 (*C*(O)OCH$_2$C$_6$H$_5$, C(9)), 171.9 (C(7)) ppm, the remaining aromatic peak was not detected and is believed to overlap with one of the observed signals, signals for the minor diastereoisomer were not detected; MS (+CI) 503 [M + 1]$^+$; M_r (+CI) 503.202 72 [M + 1]$^+$ (calcd for $C_{25}H_{31}N_2O_9$ 503.202 96).

(5*E*,5b*E*)-[5c-(2-Pyridyl)]-5a-ethenylbicyclomycin C(2′),C(3′)-Acetonide (47). To an anhydrous dioxane solution (4 mL) of **38** (37 mg, 0.100 mmol) were added (2-pyridylmethyl)-triphenylphosphonium chloride (59 mg, 0.151 mmol) and triethylamine (15 mg, 0.151 mmol), and the solution was stirred (25 °C, 5 h). The solution was concentrated in vacuo and the residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **47** as a slightly pink solid (25 mg, 56%): mp 165−168 °C (dec); R_f 0.64 (20% MeOH−$CHCl_3$); IR (KBr) 3397 (br), 3304 (br), 2986, 2934, 2879, 1691, 1399, 1068, 768 cm^{-1}; ^1H NMR (CD_3OD) δ 1.39 (s, 3 H, C(2′)CH_3), 1.42 (s, 3 H, C(CH_3)$_2$), 1.45 (s, 3 H, C(CH_3)$_2$), 2.75 (dd, J = 9.0, 16.7 Hz, 1 H, C(4)*H*H′), 3.05 (dd, J = 7.5, 16.7 Hz, 1 H, C(4)H*H*′), 3.74 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.85 (dd, J = 9.0, 13.0 Hz, 1 H, C(3)*H*H′), 4.05 (dd, J = 7.5, 13.0 Hz, 1 H, C(3)H*H*′), 4.18 (s, 1 H, C(1′)H), 4.47 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 6.80 (d, J = 15.3 Hz, 1 H, C(5c)H), 6.93 (d, J = 11.4 Hz, 1 H, C(5a)H), 7.25 (dd, J = 4.9, 7.4 Hz, 1 H, C(4″)H), 7.47−7.56 (m, 2 H, C(5b)H, C(6″)H), 7.77 (dt, J = 1.5, 7.4 Hz, 1 H, C(5″)H), 8.47 (d, J = 4.9 Hz, 1 H, C(3″)H); ^{13}C NMR (CD_3OD) 25.2 (C(2′)*C*H$_3$), 26.9 (C(*C*H$_3$)$_2$), 28.2 (C(*C*H$_3$)$_2$), 29.8 (C(4)), 66.5 (C(3)), 73.1 and 73.3 (C(3′), C(1′)), 83.6 (C(6)), 86.6 (C(2′)), 89.1 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 124.0 (C(6″)), 124.1 (C(4″)), 129.4 (C(5b)), 129.6 (C(5a)), 135.6 (C(5c)), 139.0 (C(5″)), 144.0 (C(5)), 150.4 (C(3″)), 156.7 (C(1″)), 168.9 (C(9)), 171.9 (C(7)) ppm; MS (+CI) 446 [M + 1]$^+$; M_r (+CI) 446.192 45 [M + 1]$^+$ (calcd for $C_{22}H_{28}N_3O_7$ 446.192 73).

Dihydrobicyclomycin-5a-propionic Acid C(2′),C(3′)-Acetonide (45). To a methanolic solution (2 mL) of **43** (12 mg, 0.032 mmol) was added 10% Pd−C (14 mg, 0.042 mmol), and the resulting suspension was stirred under H_2 (1 atom) at 25 °C (3 h). The suspension was filtered (Celite), and the residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to provide **45** as a colorless solid (22 mg, 78%): mp 145−150 °C; R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3434 (br), 3412 (br), 2991 (br), 2934, 1691, 1560, 1401, 1137, 1045, 872 cm^{-1}; ^1H NMR (CD_3OD): δ 1.36 (s, 3 H, C(2′)CH_3), 1.45 (s, 6 H, C(CH_3)$_2$), 1.70−2.04 (m, 7 H, C(4)H_2, C(5)H, C(5a)H_2, C(5b)H_2), 2.22 (app quint, J = 9.0 Hz, 2 H, C(5c)H_2), 3.71 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.81 (dd, J = 7.9, 13.4 Hz, 1 H, C(3)*H*H′), 4.00 (dd, J = 7.9, 13.4 Hz, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 4.45 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′); ^{13}C NMR (CD_3OD) 25.1, 25.7, 27.0, 28.4, 29.4, and 30.9 (C(2′)*C*H$_3$, C(*C*H$_3$)$_2$, C(4), C(5a), C(5b)), 37.5 (C(5c)), 52.4 (C(5)), 63.5 (C(3)), 73.3 and 73.4 (C(3′), C(1′)), 84.5 (C(6)), 86.5 (C(2′)), 88.8 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 168.7 (C(9)), 172.1 (C(7)), 180.7 (C(O)OH) ppm; MS (+CI) 417 [M + 1]$^+$; M_r (+CI) 417.186 91 [M + 1]$^+$ (calcd for $C_{18}H_{29}N_2O_9$ 417.187 30).

(5*E*,5b*E*)-[5c-Iodo]-5a-ethenylbicyclomycin C(2′),C(3′)-Acetonide (48). In a glovebag (Aldrich Z10,608.9) under a N_2 atmosphere, an anhydrous THF solution (2 mL) of iodoform (87 mg, 0.221 mmol), *N*-methylmorpholine (22 mg, 0.217 mmol), and **38** (41 mg, 0.111 mmol) was added to an anhydrous THF mixture (3 mL) of $CrCl_2$ (95 mg, 0.772 mmol). The reaction mixture was stirred (50 °C, 1 h), filtered (glass wool), and concentrated in vacuo. The residue was redissolved in MeOH and purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **48** as a white solid (8 mg, 17%) as a 70:30 mixture of geometric isomers: mp 127−132 °C; R_f 0.72 (20% MeOH−$CHCl_3$); IR (KBr) 3454 (br), 3309 (br), 2986, 2929, 1691, 1400, 1071, 876 cm^{-1}; ^1H NMR (CD_3OD) (5*E*,5b*E*)-isomer: δ 1.38 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 2.52−2.72 (m, 1 H, C(4)*H*H′), 2.80−2.92 (m, 1 H, C(4)H*H*′), 3.72 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.77−3.85 (m, 1 H, C(3)*H*H′), 3.93−4.01 (m, 1 H, C(3)H*H*′), 4.15 (s, 1 H, C(1′)H), 4.44 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′), 6.09 (d, J = 15.0 Hz, 1 H, C(5c)H), 6.86 (d, J = 11.9 Hz, 1 H, C(5a)H), 7.56 (dd, J = 11.9, 15.0 Hz, 1 H, C(5b)H)); (5*E*,5b*Z*)-isomer: δ 4.16 (s, 1 H, C(1′)H), 5.83 (d, J = 10.8 Hz, 1 H, C(5c)H), 6.96 (app t, J = 11.3 Hz, 1 H, C(5b)H), 7.95 (d, J = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) 25.1 (C(2′)*C*H$_3$), 27.0 (C(*C*H$_3$)$_2$), 28.2 (C(*C*H$_3$)$_2$), 29.7 (C(4)), 66.2 (C(3)), 73.3 and 73.4 (C(3′), C(1′)), 82.3 (C(6)), 85.0 (C(5c)), 86.5 (C(2′)), 89.0 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 129.7 (C(5a)), 140.8 and 141.8 (C(5b), C(5)), 168.9 (C(9)), 172.2 (C(7)) ppm, the signals for the minor diastereoisomer were not detected; MS (+CI) 495 [M + 1]$^+$; M_r (+CI) 495.063 34 [M + 1]$^+$ (calcd for $C_{17}H_{24}IN_2O_7$ 495.062 83).

5a-(Acetoxy)methyldihydrobicyclomycin C(2′),C(3′)-Acetonide (55). To an anhydrous THF solution (2 mL) of **54**[13e] (15 mg, 0.04 mmol), triethylamine (12 mg, 0.12 mmol), and acetic anhydride (4 mg, 0.04 mmol) was added a catalytic amount of DMAP, and the solution was stirred (25 °C, 1 h). The solution was then cooled (0 °C), quenched with H_2O (5 mL), neutralized (aqueous dilute NaOH), and concentrated in vacuo. The residue was purified by preparative TLC (10%

MeOH−$CHCl_3$) to provide **55** as a white solid (5 mg, 23%): mp 146−150 °C; R_f 0.43 (10% MeOH−$CHCl_3$); IR (KBr) 3447 (br), 3315 (br), 2990, 2937, 2884, 1688 (br), 1459, 1400 (br), 1243 (br), 1196, 1139, 1074, 1044, 977, 877, 770 cm^{-1}; ^1H NMR (CD_3OD) δ 1.36 (s, 3 H, C(2′)CH_3), 1.38−1.45 (m, 1 H, C(5a)*H*H′), 1.44 (s, 6 H, C(CH_3)$_2$), 1.72−1.82 (m, 1 H, C(4)*H*H′), 1.98−2.05 (m, 1 H, C(4)H*H′*), 2.01 (s, 3 H, OC(O)CH_3), 2.08−2.17 (m, 1 H, C(5)H), 2.21−2.32 (m, 1 H, C(5a)H*H′*), 3.71 (d, J = 8.6 Hz, 1 H, C(3′)*H*H′), 3.82 (dd, J = 8.3, 13.7 Hz, 1 H, C(3)*H*H′), 4.01 (dd, J = 8.1, 13.7 Hz, 1 H, C(3)H*H′*), 4.09 (s, 1 H, C(1′)H), 4.10−4.15 (m, 2 H, C(5b)H_2), 4.45 (d, J = 8.6 Hz, 1 H, C(3′)H*H′*), the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) 20.8 (C(O)*C*H_3), 24.9 (C(2′)*C*H_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 29.5 (C(4)), 31.9 (C(5a)), 63.8 (C(5b)), 64.5 (C(3)), 73.2 (C(3′) or C(1′)), 73.3 (C(1′) or C(3′)), 84.2 (C(6)), 86.4 (C(2′)), 88.8 (C(1)), 111.7 (*C*(CH_3)$_2$), 171.7, 172.0 and 172.9 (C(7), C(9), *C*(O)CH_3) ppm, the C(5) signal was not detected and is believed to be beneath the solvent peak; MS (+CI) 417 [M + 1]$^+$; M_r (+CI) 417.187 35 [M + 1]$^+$ (calcd for $C_{18}H_{29}N_2O_9$ 417.187 31).

5a-(Amino)methyldihydrobicyclomycin C(2′),C(3′)-Acetonide (57). A methanolic suspension (2 mL) of **56** (10 mg, 0.03 mmol) and 10% Pd−C (catalytic amount) was stirred (25 °C, 30 min) under H_2 (1 atm). The suspension was filtered (Celite) and concentrated in vacuo. The residue was purified by preparative TLC (3 × 30% MeOH−$CHCl_3$) to provide **57** as a white solid (6 mg, 60%): mp 171−175 °C; R_f 0.00 (20% MeOH−$CHCl_3$); IR (KBr) 3439 (br), 2994 (br), 2946, 1687, 1638, 1401, 1257, 1196, 1138, 1046 (br), 877, 669 cm^{-1}; ^1H NMR (CD_3OD) δ for the major diastereomer: 1.37 (s, 3 H, C(2′)CH_3), 1.45 (s, 6 H, C(CH_3)$_2$), 1.56−1.64 (m, 1 H, C(5a)*H*H′), 1.74−1.82 (m, 1 H, C(4)*H*H′), 2.01−2.18 (m, 3 H, C(4)H*H′*, C(5)H, C(5a)H*H′*), 3.04−3.12 (m, 2 H, C(5b)H_2), 3.71 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.85 (dd, J = 8.3, 13.8 Hz, 1 H, C(3)*H*H′), 3.99 (dd, J = 8.4, 13.8 Hz, 1 H, C(3)H*H′*), 4.11 (s, 1 H, C(1′)H), 4.43 (d, J = 8.4 Hz, 1 H, C(3′)H*H′*), the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^1H NMR (DMF-d_7) δ for the major diastereomer: 1.35 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.42 (s, 3 H, C(CH_3)$_2$), 1.62−1.81 (m, 2 H, C(4)*H*H′, C(5a)*H*H′), 1.98−2.09 (m, 1 H, C(4)H*H′*), 2.22−2.32 (m, 2 H, C(5)H, C(5a)H*H′*), 3.14 (br t, J = 6.9 Hz, 2 H, C(5b)H_2), 3.73 (d, J = 8.3 Hz, 1 H, C(3′)*H*H′), 3.82 (dd, J = 8.4, 13.4 Hz, 1 H, C(3)*H*H′), 4.01 (dd, J = 8.6, 13.4 Hz, 1 H, C(3)H*H′*), 4.11 (br s, 1 H, C(1′)H), 4.41 (d, J = 8.3 Hz, 1 H, C(3′)H*H′*), 6.00 (br s, 1 H, C(1′)OH), 8.07−8.19 (br s, 2 H, N(8)H, N(10)H), the C(5b)NH_2 and C(6)OH signals were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer: 25.0 (C(2′)*C*H_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 29.7 (C(5a) or C(4)), 33.3 (C(4) or C(5a)), 40.0 (C(5b)), 63.7 (C(3)), 73.0 ((C3′) or C(1′)), 73.2 (C(1′) or C(3′)), 84.3 (C(6)), 86.4 (C(2′)), 88.8 (C(1)), 111.6 (*C*(CH_3)$_2$), 168.1 and 171.7 (C(7), C(9)) ppm, the C(5) signal was not detected and is believed to be beneath the solvent peak; ^{13}C NMR (DMF-d_7) for the major diastereomer: 24.2 (C(2′)*C*H_3), 26.3 (C(*C*H_3)$_2$), 27.9 (C(*C*H_3)$_2$), 28.2 (C(5a) or C(4)), 31.6 (C(4) or C(5a)), 39.1 (C(5b)), 48.7 (C(5)), 62.5 (C(3)), 72.2 (C(3′) or C(1′)), 72.6 (C(1′) or C(3′)), 83.4 (C(6)), 85.6 (C(2′)), 87.5 (C(1)), 110.1 (*C*(CH_3)$_2$), 166.5 and 169.4 (C(7), C(9)) ppm, no signals were detected in CD_3OD or DMF-d_7 NMR spectra (^1H, ^{13}C) for any minor diastereomer; MS (+CI) 374 [M + 1]$^+$; M_r (+CI) 374.192 18 [M + 1]$^+$ (calcd for $C_{16}H_{28}N_3O_7$ 374.192 73).

5a-Methyldihydrobicyclomycin C(2′),C(3′)-Acetonide (59). A methanolic suspension (2 mL) of **58** (15 mg, 0.04 mmol) and 10% Pd−C (catalytic amount) was stirred (25 °C, 30 min) under H_2 (1 atm). The suspension was filtered (Celite) and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−$CHCl_3$) to provide **59** (9 mg, 62%) as a diastereomeric mixture (∼9:1): mp 181−184 °C; R_f 0.75 (20% MeOH−$CHCl_3$); IR (KBr) 3427 (br), 3302, 3193 (br), 2983, 2938, 2884, 1685, 1669, 1460, 1401, 1381, 1247, 1185, 1141, 1074, 1047, 878, 757 cm^{-1}; ^1H NMR (CD_3OD) δ for the major diastereomer: 0.90 (t, J = 7.4 Hz, 3 H, C(5a)CH_3), 1.12−1.17 (m, 1 H, C(5a)*H*H′), 1.36 (s, 3 H, C(2′)CH_3), 1.44 (s, 3 H, C(CH_3)$_2$), 1.45 (s, 3 H, C(CH_3)$_2$), 1.72−1.80 (m, 1 H, C(4)*H*H′), 1.82−2.08 (m, 3 H, C(4)H*H′*, C(5)H, C(5a)H*H′*), 3.71 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.80 (dd, J = 8.0, 13.4 Hz, 1 H, C(3)*H*H′), 3.99 (dd, J = 8.7, 13.4 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: 4.08 (s, 1 H, C(1′)H), the other signals were not detected and are believed to overlap with the major diastereomer; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer: 13.0 (C(5a)*C*H_3)), 22.2 (C(5a)), 24.9 (C(2′)*C*H_3), 26.8 (C(*C*H_3)$_2$), 28.2 (C(*C*H_3)$_2$), 30.0 (C(4)), 54.2 (C(5)), 63.2 (C(3)), 73.2 (C(3′)), 73.3 (C(1′)), 84.4 (C(6)), 86.4 (C(2′)), 88.6 (C(1)), 111.6 (*C*(CH_3)$_2$), 168.7 and 170.0 (C(7), C(9)) ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer: 53.1 (C(5)), 73.1 (C(3′) or C(1′)), the other signals were not detected and are believed to overlap with the major diastereomer; the structural assignments were in agreement with the APT experiment; MS (+CI) 359 [M + 1]$^+$; M_r (+CI) 359.181 97 [M + 1]$^+$ (calcd for $C_{16}H_{27}N_2O_7$ 359.181 83).

General Procedure for the Preparation of Bicyclomycins 10, 11, 14−17, 19, 24, 25, 27, 28, and 34. To a 50% aqueous methanolic solution (2 mL) of the bicyclomycin C(2′),C(3′)-acetonide (1 equiv) was added TFA (3 drops). The solution was stirred (22−45 °C, 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.

(5*E*)-5a-Formylbicyclomycin Oxime (10). Using **39** (26 mg, 0.067 mmol) gave **10** (18 mg, 75%) as a colorless solid: mp 104−106 °C; R_f 0.19 (20% MeOH−$CHCl_3$); IR (KBr) 3438 (br), 3277 (br), 2957, 2891, 1685, 1405, 1206, 1141, 1063 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 2.60 (dd, J = 9.0, 16.5 Hz, 1 H, C(4)*H*H′), 2.96 (dd, J = 7.5, 16.5 Hz, 1 H, C(4)H*H′*), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.77 (dd, J = 9.0, 12.9 Hz, 1 H, C(3)*H*H′), 3.95−4.02 (m, 1 H, C(3)H*H′*), 4.09 (s, 1 H, C(1′)H), 6.73 (d, J = 10.2 Hz, 1 H, C(5a)H), 7.36 (s, 1 H, NOH), 8.04 (d, J = 10.2 Hz, 1 H, C(5b)H); ^{13}C NMR (CD_3OD) 24.3 (C(2′)*C*H_3), 30.0 (C(4)), 65.1 (C(3)), 68.6 (C(3′)), 72.2 (C(1′)), 78.3 (C(2′)), 83.4 (C(6)), 89.6 (C(1)), 124.1 (C(5a)), 146.4 and 148.1 (C(5), C(5b)), 168.9 (C(9)), 172.4 (C(7)) ppm; MS (+CI) 346 [M + 1]$^+$; M_r (+CI) 346.126 44 [M + 1]$^+$ (calcd for $C_{13}H_{22}N_3O_8$ 346.125 04).

(5*E*)-5a-Formylbicyclomycin *O*-Methyloxime (11). Using **40** (4 mg, 0.009 mmol) gave **11** (4 mg, 100%) as a colorless solid as a 70:30 mixture of geometric isomers: mp 102−106 °C; R_f 0.46 (20% MeOH−$CHCl_3$); IR (KBr) 3417 (br), 3286 (br), 2979, 2941, 1691, 1401, 1138, 1041, 776 cm^{-1}; ^1H NMR (CD_3OD) (5*E*,5b*E*)-isomer: δ 1.34 (s, 3 H, C(2′)CH_3), 2.59 (dd, J = 9.3, 16.4 Hz, 1 H, C(4)*H*H′), 2.95 (dd, J = 7.2, 16.4 Hz, 1 H, C(4)H*H′*), 3.50 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.74−3.79 (m, 1 H, C(3)*H*H′), 3.84 (s, 3 H, $NOCH_3$), 3.94−4.02 (m, 1 H, C(3)H*H′*), 4.09 (s, 1 H, C(1′)H), 6.69 (d, J = 10.5 Hz, 1 H, C(5a)H), 8.05 (d, J = 10.5 Hz, C(5b)H); (5*E*,5b*Z*)-isomer: δ 3.88 (s, 3 H, $NOCH_3$), 7.22 (d, J = 9.9 Hz, 1 H, C(5a)H or C(5b)H), 7.37 (d, J = 9.9 Hz, 1 H, C(5b)H or C(5a)H); the other signals were not detected and are believed to overlap with the major diastereoisomer; ^{13}C NMR (CD_3OD) (5*E*,5b*E*)-isomer: 24.3 (C(2′)*C*H_3), 30.0 (C(4)), 62.5 ($NOCH_3$), 65.0 (C(3)), 68.6 (C(3′)), 72.2 (C(1′)), 78.4 (C(2′)), 83.4 (C(6)), 89.6 (C(1)), 123.4 (C(5a)), 147.8 (C(5), C(5b)), 168.6 (C(9)), 172.2 (C(7)) ppm; (5*E*,5b*Z*)-isomer: 29.8 (C(4)), 62.5 ($NOCH_3$), 64.9 (C(3)), 83.4 (C(6)), 117.6 (C(5a)), 144.5 and 149.9 (C(5), C(5b)) ppm, the other signals were not detected and are believed to overlap with the major diastereoisomer; MS (+CI) 360 [M + 1]$^+$; M_r (+CI) 360.140 19 [M + 1]$^+$ (calcd for $C_{14}H_{22}N_3O_8$ 360.140 07).

(5*E*,5b*E*)-Methyl Bicyclomycin-5a-propenoate (14). Using **41** (14 mg, 0.034 mmol) gave **14** (12 mg, 83%) as a colorless solid as a 90:10 mixture of geometric isomers: mp 150 °C (dec); R_f 0.29 (15% MeOH−$CHCl_3$); IR (KBr) 3447 (br), 2956 (br), 2929, 1696, 1400, 1288, 1066 cm^{-1}; ^1H NMR (CD_3OD) major isomer: δ 1.35 (s, 3 H, C(2′)CH_3), 2.64 (dd, J = 9.3, 16.8 Hz, 1 H, C(4)*H*H′), 3.06 (dd, J = 7.4, 16.8 Hz, 1 H, C(4)H*H′*), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.66 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.74−3.80 (m, 4 H, C(3)*H*H′, OCH_3), 4.02 (dd, J =

7.4, 13.2 Hz, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 6.08 (d, *J* = 15.1 Hz, 1 H, C(5c)H), 6.85 (d, *J* = 11.7 Hz, 1 H, C(5a)H), 7.56 (dd, *J* = 11.7, 15.1 Hz, 1 H, C(5b)H); minor isomer: *δ* 5.84 (d, *J* = 10.8 Hz, 1 H, C(5c)H), 6.97 (app t, *J* = 11.4 Hz, 1 H, C(5b)H), 7.95 (d, *J* = 12.0 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) 24.3 (C(2′)CH_3), 30.1 (C(4)), 52.3 (C(O)OCH_3), 65.1 (C(3)), 68.3 (C(3′)), 72.2 (C(1′)), 78.4 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 125.3 (C(5c)), 127.5 (C(5a)), 140.5 (C(5b)), 149.8 (C(5)), 168.9 and 169.0 (*C*(O)OCH$_3$, C(9)), 172.3 (C(7)) ppm, signals for the minor diastereoisomer were not detected; the structural assignments were in agreement with the ^{1}H−^{1}H COSY, HMBC and HMQC experiments; MS (+CI) 387 [M + 1]$^+$; M_r (+CI) 387.142 01 [M + 1]$^+$ (calcd for $C_{16}H_{23}N_2O_9$ 387.140 35).

(5*E*, 5b*E*)-Allyl Bicyclomycin-5a-propenoate (15). Using **42** (35 mg, 0.077 mmol) gave **15** (25 mg, 78%) as a colorless solid: mp 121−124 °C (dec); R_f 0.29 (10% MeOH−$CHCl_3$); IR (KBr) 3419 (br), 2958, 1696, 1647, 1400, 1281, 1173, 1062 cm^{-1}; ^{1}H NMR (CD_3OD) major isomer: *δ* 1.34 (s, 3 H, C(2′)CH_3), 2.64 (dd, *J* = 9.0, 16.5 Hz, 1 H, C(4)*H*H′), 3.06 (dd, *J* = 7.4, 16.5 Hz, 1 H, C(4)H*H*′), 3.51 (d, *J* = 11.2 Hz, 1 H, C(3′)*H*H′), 3.65 (d, *J* = 11.2 Hz, 1 H, C(3′)H*H*′), 3.76 (dd, *J* = 9.0, 13.0 Hz, 1 H, C(3)*H*H′), 4.01 (dd, *J* = 7.4, 13.0 Hz, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 4.63 (dd, *J* = 1.5, 6.9 Hz, 2 H, C(O)OCH_2-CHCH$_2$), 5.22 (br dd, *J* = 1.5, 10.5 Hz, 1 H, C(O)OCH$_2$-CHCH$_E$*H*$_Z$), 5.31 (br dd, *J* = 1.5, 17.4 Hz, 1 H, C(O)OCH$_2$-CHC*H*$_E$H$_Z$), 5.91−6.00 (m, 1 H, C(O)OCH$_2$C*H*CH$_2$), 6.11 (d, *J* = 15.0 Hz, 1 H, C(5c)H), 6.85 (d, *J* = 12.0 Hz, 1 H, C(5a)H), 7.57 (dd, *J* = 12.0, 15.0 Hz, 1 H, C(5b)H); minor isomer: *δ* 5.84 (d, *J* = 10.8 Hz, 1 H, C(5c)H), 6.99 (app t, *J* = 11.3 Hz, 1 H, C(5b)H), 7.96 (d, *J* = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) 24.3 (C(2′)CH_3), 30.0 (C(4)), 65.0 (C(3)), 66.4 (C(O)OCH_2CHCH$_2$), 68.5 (C(3′)), 72.2 (C(1′)), 78.3 (C(2′)), 83.5 (C(6)), 89.6 (C(1)), 118.6 (C(O)OCH$_2$-C*H*CH$_2$), 125.4 (C(5c)), 127.5 (C(5a)), 133.7 (C(O)OCH$_2$*C*HCH$_2$), 140.6 (C(5b)), 149.9 (C(5)), 168.1 and 168.9 (*C*(O)OCH$_2$CHCH$_2$, C(9)), 172.2 (C(7)) ppm, signals for the minor diastereoisomer were not detected; MS (+CI) 413 [M + 1]$^+$; M_r (+CI) 413.155 50 [M + 1]$^+$ (calcd for $C_{18}H_{24}N_2O_9$ 413.156 01).

(5*E*,5b*E*)-Benzyl Bicyclomycin-5a-propenoate (16). Using **43** (12 mg, 0.024 mmol) gave **16** (9 mg, 82%) as a colorless solid as a 90:10 mixture of geometric isomers: mp 134−139 °C (dec); R_f 0.23 (10% MeOH−$CHCl_3$); IR (KBr) 3421 (br), 3267 (br), 2936, 2890, 1695, 1401, 1279, 1146, 1061 cm^{-1}; ^{1}H NMR (CD_3OD) major isomer: *δ* 1.35 (s, 3 H, C(2′)CH_3), 2.63 (dd, *J* = 9.1, 16.7 Hz, 1 H, C(4)*H*H′), 3.05 (dd, *J* = 7.4, 16.7 Hz, 1 H, C(4)H*H*′), 3.50 (d, *J* = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, *J* = 11.4 Hz, 1 H, C(3′)H*H*′), 3.75 (dd, *J* = 9.1, 13.2 Hz, 1 H, C(3)*H*H′), 4.00 (dd, *J* = 7.4, 13.2 Hz, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 5.19 (s, 2 H, C(O)OCH_2C$_6$H$_5$), 6.13 (d, *J* = 15.0 Hz, 1 H, C(5c)H), 6.86 (d, *J* = 11.7 Hz, 1 H, C(5a)H), 7.30−7.40 (m, 5 H, C(O)OCH$_2$C$_6$*H*$_5$), 7.58 (dd, *J* = 11.7, 15.0 Hz, 1 H, C(5b)H); minor isomer: *δ* 5.85 (d, *J* = 10.8 Hz, 1 H, C(5c)H), 7.02 (app t, *J* = 11.3 Hz, 1 H, C(5b)H), 8.02 (d, *J* = 11.8 Hz, 1 H, C(5a)H), the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) 24.3 (C(2′)CH_3), 30.0 (C(4)), 65.0 (C(3)), 67.5 (OCH_2-C$_6$H$_5$), 68.6 (C(3′)), 72.1 (C(1′)), 78.3 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 125.5 (C(5c)), 127.5 (C(5a)), 129.4, 129.7 and 137.7 (C(O)OCH$_2$*C*$_6$H$_5$), 140.5 (C(5b)), 149.9 (C(5)), 168.3 and 168.9 (*C*(O)OCH$_2$C$_6$H$_5$, C(9)), 172.2 (C(7)) ppm, the remaining aromatic peak was not detected and is believed to overlap with one of the observed signals, signals for the minor diastereoisomer were not detected; MS (+CI) 387 [M + 1]$^+$; M_r (+CI) 387.172 92 [M + 1]$^+$ (calcd for $C_{22}H_{27}N_2O_9$ 387.171 66).

(5*E*,5b*E*)-[5c-Iodo]-5a-ethenylbicyclomycin (17). Using **48** (6 mg, 0.012 mmol) gave **17** (4 mg, 63%) as a white solid as a 70:30 mixture of geometric isomers: mp 145 °C (dec); R_f 0.40 (20% MeOH−$CHCl_3$); IR (KBr) 3428 (br), 2964, 2922, 2852, 1685, 1406, 1138, 620 cm^{-1}; ^{1}H NMR (CD_3OD) (5*E*,5b*E*)-isomer: *δ* 1.34 (s, 3 H, C(2′)CH_3), 2.48 (dd, *J* = 8.7, 16.8 Hz, 1 H, C(4)*H*H′), 2.91 (dd, *J* = 7.2, 16.8 Hz, 1 H, C(4)H*H*′), 3.50 (d, *J* = 11.0 Hz, 1 H, C(3′)*H*H′), 3.65 (d, *J* = 11.0 Hz, 1 H, C(3′)H*H*′), 3.70−3.83 (m, 1 H, C(3)*H*H′), 3.92−4.00 (m, 1 H, C(3)H*H*′), 4.08 (s, 1 H, C(1′)H), 6.62 (d, *J* = 11.4 Hz, 1 H, C(5a)H), 6.73 (d, *J* = 14.2 Hz, 1 H, C(5c)H), 7.33 (dd, *J* = 11.4, 14.2 Hz, 1 H, C(5b)H); (5*E*,5b*Z*)-isomer: *δ* 2.59 (dd, *J* = 9.6, 16.2 Hz, 1 H, C(4)*H*H′), 3.50 (d, *J* = 11.1 Hz, 1 H, C(3′)*H*H′), 4.09 (s, 1 H, C(1′)H), 6.63 (d, *J* = 10.5 Hz, 1 H, C(5a)H), 6.89 (d, *J* = 7.4 Hz, 1 H, C(5c)H), 7.04 (dd, *J* = 7.4, 10.5 Hz, 1 H, C(5b)H), the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; ^{13}C NMR (CD_3OD) (5*E*,5b*E*)-isomer: 24.3 (C(2′)CH_3), 29.8 (C(4)), 65.2 (C(3)), 68.6 (C(3′)), 72.3 (C(1′)), 78.3 (C(2′)), 84.9 (C(5c)), 89.6 (C(1)), 129.8 (C(5a)), 141.1 and 141.9 (C(5), C(5b)), 169.4 (C(9)), 172.4 (C(7)) ppm, the C(6) signal was not observed; (5*E*,5b*Z*)-isomer: 88.2 (C(5c)), 130.0 (C(5a)), 134.9 (C(5b)), 145.5 (C(5)), 169.0 (C(9)), 172.4 (C(7)) ppm, the remaining peaks were not detected and are believed to overlap with the other diastereoisomer; MS (+CI) 455 [M + 1]$^+$; M_r (+CI) 455.031 31 [M + 1]$^+$ (calcd for $C_{14}H_{20}IN_2O_7$ 455.031 53).

(5*E*,5b*E*)-[5c-(2-Pyridyl)]-5a-ethenylbicyclomycin (19). Using **47** (20 mg, 0.045 mmol) gave **19** (13 mg, 71%) as a slightly pink solid: mp 150 °C (dec); R_f 0.38 (20% MeOH−$CHCl_3$); IR (KBr) 3447 (br), 2956 (br), 2929, 1696, 1400, 1288, 1066 cm^{-1}; ^{1}H NMR (CD_3OD) *δ* 1.35 (s, 3 H, C(2′)CH_3), 2.66 (dd, *J* = 9.0, 16.4 Hz, 1 H, C(4)*H*H′), 3.14 (dd, *J* = 7.4, 16.4 Hz, 1 H, C(4)H*H*′), 3.51 (d, *J* = 11.1 Hz, 1 H, C(3′)*H*H′), 3.67 (d, *J* = 11.1 Hz, 1 H, C(3′)H*H*′), 3.80 (dd, *J* = 9.0, 12.8 Hz, 1 H, C(3)*H*H′), 4.04 (dd, *J* = 7.4, 12.8 Hz, 1 H, C(3)H*H*′), 4.11 (s, 1 H, C(1′)H), 6.80 (d, *J* = 15.0 Hz, 1 H, C(5c)H), 6.92 (d, *J* = 11.4 Hz, 1 H, C(5a)H), 7.25 (dd, *J* = 5.7, 7.8 Hz, 1 H, C(4″)H), 7.48−7.58 (m, 2 H, C(5b)H, C(6″)H), 7.77 (dt, *J* = 0.9, 7.8 Hz, 1 H, C(5″)H), 8.47 (d, *J* = 5.7 Hz, 1 H, C(3″)H); ^{13}C NMR (CD_3OD) 24.3 (C(2′)CH_3), 29.9 (C(4)), 65.5 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.4 (C(2′)), 83.6 (C(6)), 89.6 (C(1)), 124.0 (C(6″)), 124.1 (C(4″)), 129.6 (C(5b)), 129.7 (C(5a)), 135.6 (C(5c)), 138.9 (C(5″)), 144.2 (C(5)), 150.2 (C(3″)), 156.7 (C(1″)), 169.1 (C(9)), 172.7 (C(7)) ppm, the structural assignments were in agreement with the ^{1}H−^{1}H COSY and HMQC experiments; MS (+CI) 406 [M + 1]$^+$; M_r (+CI) 406.161 36 [M + 1]$^+$ (calcd for $C_{19}H_{24}N_3O_7$ 446.161 43).

5a-Methyldihydrobicyclomycin (24). Using **59** (8 mg, 0.02 mmol) gave **24** as a diastereomeric mixture (∼9:1): yield, 5 mg (70%); mp 151−154 °C; R_f 0.38 (20% MeOH−$CHCl_3$); IR (KBr) 3410 (br), 3249 (br), 2965, 2936, 2880, 1686 (br), 1459, 1403 (br), 1148, 1123, 1081, 1052, 1015, 878, 766 cm^{-1}; ^{1}H NMR (CD_3OD) *δ* (major diastereomer) 0.90 (t, *J* = 7.4 Hz, 3 H, C(5a)CH_3), 1.10−1.18 (m, 1 H, C(5a)*H*H′), 1.32 (s, 3 H, C(2′)CH_3), 1.80−2.05 (m, 4 H, C(4)H_2, C(5)H, C(5a)H*H*′), 3.50 (d, *J* = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, *J* = 11.4 Hz, 1 H, C(3′)H*H*′), 3.75 (dd, *J* = 7.4, 13.7 Hz, 1 H, C(3)*H*H′), 3.98 (dd, *J* = 8.9, 13.7 Hz, 1 H, C(3)H*H*′), 4.01 (s, 1 H, C(1′)H); ^{1}H NMR (CD_3OD) *δ* (minor diastereomer) 4.03 (s, 1 H, C(1′)H), the other signals were not detected and are believed to overlap with the major diastereomer; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) (major diastereomer) 13.0 (C(5a)*C*H$_3$), 21.8 (C(5a)), 24.2 (C(2′)CH_3), 29.6 (C(4)), 54.1 (C(5)), 61.6 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.1 (C(2′)), 84.4 (C(6)), 89.2 (C(1)), 169.3 and 172.6 (C(7), C(9)) ppm; ^{13}C NMR (CD_3OD) (minor diastereomer) 20.1 (C(5a)), the other signals were not detected and are believed to overlap with the major diastereomer; MS (+CI) 319 [M + 1]$^+$; M_r (+CI) 319.150 06 [M + 1]$^+$ (calcd for $C_{13}H_{23}N_2O_7$ 319.150 53).

5a-(Amino)methyldihydrobicyclomycin (25). Using **57** (11 mg, 0.03 mmol) gave **25** as a white solid (7 mg, 71%): mp 158−161 °C; R_f 0.00 (30% MeOH−$CHCl_3$); IR (KBr) 3448 (br), 3231 (br), 3024, 2939 (br), 1682 (br), 1637, 1531, 1402 (br), 1346, 1289, 1183, 1132, 1024 cm^{-1}; ^{1}H NMR (CD_3OD) *δ* (major diastereomer) 1.33 (s, 3 H, C(2′)CH_3), 1.55−1.62 (m, 1 H, C(5a)*H*H′), 1.75−1.86 (m, 1 H, C(4)*H*H′), 2.02−2.18 (m, 3 H, C(4)H*H*′, C(5)H, C(5a)H*H*′), 3.06 (t, *J* = 6.8 Hz, 2 H, C(5b)H$_2$), 3.51 (d, *J* = 11.3 Hz, 1 H, C(3′)*H*H′), 3.67 (d, *J* = 11.3 Hz, 1 H, C(3′)H*H*′), 3.80 (dd, *J* = 7.7, 13.7 Hz, 1 H, C(3)*H*H′), 3.98 (dd, *J* = 9.2, 13.7 Hz, 1 H, C(3)H*H*′), 4.04 (s, 1 H, C(1′)H), the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) (major diastereomer)

24.2 (C(2′)*C*H$_3$), 29.5 (C(4)), 33.2 (C(5a)), 40.1 (C(5b)), 62.2 (C(3)), 68.5 (C(3′)), 72.0 (C(1′)), 78.2 (C(2′)), 84.3 (C(6)), 89.3 (C(1)), 168.8 and 172.3 (C(7), C(9)) ppm, the C(5) signal was not detected and is believed to be hidden in the solvent peak; no signals were detected in CD$_3$OD spectra (^{1}H, ^{13}C) for the minor diastereomer; MS (+CI) 334 [M + 1]$^+$; M_r (+CI) 333.153 86 [M]$^+$ (calcd for $C_{13}H_{23}N_3O_7$ 333.153 60).

5a-(Hydroxy)methyldihydrobicyclomycin (27). Using **54**[13e] (10 mg, 0.03 mmol) gave **27** as a white solid (9 mg, 100%): mp 157−160 °C; R_f 0.05 (20% MeOH−CHCl$_3$); IR (KBr) 3427 (br), 3234 (br), 3006, 2953, 2896, 1685, 1401 (br), 1208 (br), 1140, 1043, 842, 803, 724 cm^{-1}, ^{1}H NMR (CD$_3$OD) δ (major diastereomer) 1.31−1.41 (m, 1 H, C(5a)*H*H′), 1.33 (s, 3 H, C(2′)CH$_3$), 1.76−1.88 (m, 1 H, C(4)*H*H′), 1.97−2.06 (m, 1 H, C(4)H*H′*), 2.08−2.18 (m, 2 H, C(5)H, C(5a)H*H′*), 3.52 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.54−3.64 (m, 2 H, C(5b)H$_2$), 3.66 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.78 (dd, J = 7.8, 13.8 Hz, 1 H, C(3)*H*H′), 3.96−4.03 (m, 1 H, C(3)H*H′*), 4.02 (s, 1 H, C(1′)H), the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD$_3$OD) (major diastereomer) 24.2 (C(2′)*C*H$_3$), 31.5 and 32.4 (C(4), C(5a)), 61.5 (C(5b)), 62.3 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.1 (C(2′)), 84.3 (C(6)), 89.3 (C(1)), 169.0 and 172.4 (C(7), C(9)) ppm, the C(5) signal was not detected and is believed to be hidden in the solvent peak; no signals were detected in the CD$_3$OD spectra (^{1}H, ^{13}C) for any minor diastereomer; MS (+CI) 335 [M + 1]$^+$; M_r (+CI) 335.144 50 [M + 1]$^+$ (calcd for $C_{13}H_{23}N_2O_8$ 335.145 44).

5a-(Acetoxy)methyldihydrobicyclomycin (28). Using **55** (6 mg, 0.01 mmol) gave **28** as a white solid (2 mg, 37%): mp 139−142 °C; R_f 0.39 (20% MeOH−CHCl$_3$); IR (KBr) 3410 (br), 3258 (br), 3299, 1686 (br), 1459, 1400 (br), 1265 (br), 1204, 1141, 1042, 800, 767, 657 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ (major diastereomer) 1.32 (s, 3 H, C(2′)CH$_3$), 1.41−1.50 (m, 1 H, C(5a)*H*H′), 1.77−1.86 (m, 1 H, C(4)*H*H′), 1.96−2.04 (m, 1 H, C(4)H*H′*), 2.01 (s, 3 H, C(O)CH$_3$), 2.06−2.12 (m, 1 H, C(5)H), 2.18−2.27 (m, 1 H, C(5a)H*H′*), 3.49 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.78 (dd, J = 7.8, 13.9 Hz, 1 H, C(3)*H*H′), 4.00 (dd, J = 8.9, 13.9 Hz, 1 H, C(3)H*H′*), 4.02 (s, 1 H, C(1′)H), 4.07−4.15 (m, 2 H, C(5b)H$_2$), the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD$_3$OD) (major diastereomer) 20.8 (C(O)*C*H$_3$), 24.2 (C(2′)*C*H$_3$), 29.1 (C(4)), 31.4 (C(5a)), 62.3 (C(5b)), 64.4 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.1 (C(2′)), 84.2 (C(6)), 89.4 (C(1)), 168.8, 172.2 and 172.9 (C(7), C(9), *C*(O)CH$_3$) ppm, the C(5) signal was not detected and is believed to be hidden in the solvent peak; no signals were detected in CD$_3$OD spectra (^{1}H, ^{13}C) for the minor diastereomer; MS (+CI) 377 [M + 1]$^+$; M_r (+CI) 377.155 40 [M + 1]$^+$ (calcd for $C_{15}H_{25}N_2O_9$ 377.156 01).

5a-Dihydrobicyclomycin Propionic Acid (34). Using **45** (18 mg, 0.043 mmol) gave **34** (13 mg, 80%) as a hygroscopic solid: R_f 0.12 (20% MeOH−CHCl$_3$); ^{1}H NMR (CD$_3$OD) δ 1.33 (s, 3 H, C(2′)CH$_3$), 1.49−2.03 (m, 7 H, C(4)H$_2$, C(5)H, C(5a)H$_2$, C(5b)H$_2$), 2.16−2.25 (m, 2 H, C(5c)H$_2$), 3.52 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 11.4 Hz, 1 H, C(3′)H*H′*), 3.76 (dd, J = 7.5, 13.5 Hz, 1 H, C(3)*H*H′), 3.96−4.03 (m, 2 H, C(3)H*H′*, C(1′)H); ^{13}C NMR (CD$_3$OD) 24.3, 25.8, 29.0, and 30.4 (C(2′)-*C*H$_3$, C(4), C(5a), C(5b)), 37.7 (C(5c)), 52.2 (C(5)), 61.9 (C(3)), 68.6 (C(3′)), 72.4 C(1′)), 78.3 (C(2′)), 84.5 (C(6)), 89.4 (C(1)), 172.6 (C(7)) ppm, the C(9) and C(O)OH carbons were not detected; MS (+CI) 399 [M + Na]$^+$; M_r (+CI) 399.139 14 [M + Na]$^+$ (calcd for $C_{15}H_{24}N_2O_9Na$ 399.137 95).

5a-(Acetamido)methyldihydrobicyclomycin (26). A methanolic suspension (2 mL) of **31** (10 mg, 0.03 mmol) and 10% Pd−C (catalytic amount) was stirred (25 °C, 30 min) under an atmosphere of H$_2$. The suspension was filtered (Celite) and concentrated in vacuo. The residue was purified by preparative TLC (2 × 25% MeOH−CHCl$_3$) to provide **26** as a white solid (8 mg, 80%): mp 140−143 °C; R_f 0.28 (30% MeOH−CHCl$_3$); IR (KBr) 3432 (br), 2945 (br), 1685 (br), 1641, 1570, 1433, 1405, 1304 (br), 1206, 1142, 1050 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ (major diastereomer) 1.26−1.38 (m, 1 H, C(5a)*H*H′), 1.33 (s, 3 H, C(2′)CH$_3$), 1.77−1.86 (m, 1 H, C(4)*H*H′), 1.92−2.10 (m, 3 H, C(4)H*H′*, C(5)H, C(5a)H*H′*), 2.01 (s, 3 H, C(O)CH$_3$), 3.13−3.26 (m, 2 H, C(5b)H$_2$), 3.51 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.66 (d, J = 11.3 Hz, 1 H, C(3′)H*H′*), 3.77 (dd, J = 8.0, 13.7 Hz, 1 H, C(3)*H*H′), 3.95−4.02 (m, 1 H, C(3)H*H′*), 4.02 (s, 1 H, C(1′)H), the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD$_3$OD) (major diastereomer) 22.6 (C(O)*C*H$_3$)), 24.2 (C(2′)-*C*H$_3$), 29.5 (C(4)), 31.1 (C(5a)), 39.0 (C(5b)), 62.1 (C(3)), 68.5 (C(3′)), 72.2 (C(1′)), 78.1 (C(2′)), 84.2 (C(6)), 89.3 (C(1)), 169.0, 172.3 and 173.2 (C(7), C(9), *C*(O)CH$_3$)) ppm, the C(5) signal was not detected and is believed to be hidden in the solvent peak; no signals were detected in the spectra (^{1}H, ^{13}C NMR) for the minor diastereomer; MS (+CI) 376 [M + 1]$^+$; M_r (+CI) 376.171 02 [M + 1]$^+$ (calcd for $C_{15}H_{26}N_3O_8$ 376.171 99).

Compound 60. To an anhydrous THF solution (2 mL) of **54** (19 mg, 0.05 mmol) and triethylamine (26 mg, 0.25 mmol) was added methanesulfonyl chloride (29 mg, 0.25 mmol). The suspension was stirred (25 °C, 30 min), filtered (glass wool), and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−CHCl$_3$) to provide a mixture of unidentified products (R_f 0.70, 20% MeOH−CHCl$_3$) and **60** as a white solid (8 mg, 44%): R$_f$ 0.68 (20% MeOH−CHCl$_3$); ^{1}H NMR (DMF-d_7) δ 1.38 (s, 3 H, C(2′)CH$_3$), 1.44 (s, 6 H, C(CH$_3$)$_2$), 1.61−1.73 (m, 1 H, C(5a)*H*H′), 1.85−1.93 (m, 1 H, C(4)*H*H′), 2.08−2.21 (m, 2 H, C(4)H*H′*, C(5a)H*H′*), 2.33−2.41 (m, 1 H, C(5)H), 3.72 (d, J = 8.2 Hz, 1 H, C(3′)*H*H′), 3.82 (dd, J = 9.6, 13.5 Hz, 1 H, C(3)*H*H′), 3.99−4.14 (m, 2 H, C(5b)H$_2$), 4.10 (d, J = 8.0 Hz, 1 H, C(1′)H), 4.20 (dd, J = 7.4, 13.5 Hz, 1 H, C(3)H*H′*), 4.48 (d, J = 8.2 Hz, 1 H, C(3′)H*H′*), 5.85 (d, J = 8.0 Hz, 1 H, C(1′)OH), 8.17 (s, 1 H, N(8)H), 9.07 (br s, 1 H, N(10)H); ^{13}C NMR (DMF-d_7) 24.8 (C(2′)*C*H$_3$), 26.6 (C(*C*H$_3$)$_2$), 28.5 (C(*C*H$_3$)$_2$), 31.3 (C(4)), 39.9 (C(5a)), 53.7 (C(5)), 67.4 (C(5b)), 68.7 (C(3)), 72.3 (C(3′)), 73.1 (C(1′)), 86.0 (C(2′)), 89.3 (C(1)), 91.5 (C(6)), 110.6 (*C*(CH$_3$)$_2$), 166.3 (C(7)), 169.7 (C(9)) ppm, the structural assignments were in agreement with the ^{1}H−^{1}H COSY, the HMBC and the HMQC experiments; MS (+CI) 357 [M + 1]$^+$; M_r (+CI) 357.165 24 [M + 1]$^+$ (calcd for $C_{16}H_{25}N_2O_7$ 357.166 18).

X-ray Crystallographic Study of 60. Compound **60** was recrystallized from methanol. Crystals of **60** belong to the space group $P2_12_12_1$ (orthorhombic) with a = 6.610 (1) Å, b = 10.120 (1) Å, c = 24.963 (3) Å; V = 1670 Å^3, D_{calcd} = 1.42 g cm^{-3}, and Z = 4. Data were collected at −50 °C, and the structure was refined to R_f = 0.036, R_w = 0.030 for 1628 reflections with $I > 3\,\sigma(I)$.

C(5),C(5a)-Dihydrobicyclomycin C(2′),C(3′)-Acetonide (61). To an anhydrous 2,2-dimethoxypropane:dimethylformamide (3:1) solution (4 mL) of **5** (13 mg, 0.04 mmol) was added *p*-toluenesulfonic acid (catalytic amount). The solution was stirred (45 °C, 2 h) and concentrated in vacuo. The residue was purified by preparative TLC (10% MeOH−CHCl$_3$) to provide **61** as a diastereomeric mixture (∼9:1): yield, 7 mg (48%); mp 148−151 °C; R$_f$ 0.46 (10% MeOH−CHCl$_3$); IR (KBr) 3523, 3397 (br), 3503, 3204, 2987, 2924, 2882, 1700, 1673, 1400 (br), 1245, 1193, 1141, 1075, 1051, 959, 878, 810, 772 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ (major diastereomer) 1.05 (d, J = 7.2 Hz, 3 H, C(5a)H$_3$), 1.35 (s, 3 H, C(2′)CH$_3$), 1.45 (s, 6 H, C(CH$_3$)$_2$), 1.62−1.73 (m, 1 H, C(4)*H*H′), 1.96−2.06 (m, 1 H, C(4)H*H′*), 2.12−2.25 (m, 1 H, C(5)H), 3.70 (d, J = 8.4 Hz, 1 H, C(3′)-*H*H′), 3.82 (dd, J = 8.6, 13.7 Hz, 1 H, C(3)*H*H′), 4.03 (dd, J = 8.7, 13.7 Hz, 1 H, C(3)H*H′*), 4.08 (s, 1 H, C(1′)H), 4.46 (d, J = 8.4 Hz, 1 H, C(3′)H*H′*); no signals were detected for the minor diastereomer and are believed to be overlap with the major diastereomer; ^{13}C NMR (CD$_3$OD) (major diastereomer) 16.0 (C(5a)), 24.9 (C(2′)*C*H$_3$), 26.8 (C(*C*H$_3$)$_2$), 28.3 (C(*C*H$_3$)$_2$), 34.9 (C(4)), 47.1 (C(5)), 64.4 (C(3)), 73.2 and 73.3 (C(1′), C(3′)), 84.5 (C(6)), 86.4 (C(2′)), 89.0 (C(1)), 111.7 (*C*(CH$_3$)$_2$), 167.8 and 171.6 (C(7), C(9)) ppm; ^{13}C NMR (CD$_3$OD) (minor diastereomer) 14.5 (C(5a)), 34.7 (C(4)), 44.6 (C(5)), 63.7 (C(3)), 73.2 (C(1′) or C(3′)), 86.5 (C(2′)) ppm, the other signals were not detected and are believed to overlap with the major diastereomer; MS (+CI) 345 [M + 1]$^+$; M_r (+CI) 345.166 62 [M + 1]$^+$ (calcd for $C_{15}H_{25}N_2O_7$ 345.166 18).

X-ray Crystallographic Study of 61. Compound **61** was recrystallized from methanol. Crystals of **61** belong to the space group $P2_1$ (monoclinic) with a = 10.935 (1) Å, b = 7.296 (1) Å, c = 11.372 (1) Å; V = 859 Å^3, D_{calcd} = 1.40 g cm^{-3}, and

$Z = 2$. Data were collected at −50 °C, and the structure was refined to $R_f = 0.052$, $R_w = 0.047$ for 1741 reflections with $I > 3\sigma(I)$.

Inhibitory Properties of Bicyclomycin and Bicyclomycin Derivatives in the Poly C-Dependent Rho ATPase Assay.[14] The ability of rho to hydrolyze [γ^{32}P]ATP was assayed in 100 μL reactions containing ATPase buffer (40 mM Tris·HCl, pH 7.9, 50 mM KCl, 12 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 0.07 mg bovine serum albumin), 0.25 mM ATP, 0.5 μCi of [γ-^{32}P]ATP, 40 nM poly C, and 100 nM rho. Reactions were preincubated at 32 °C for 90 s prior to the addition of ATP. Aliquots (2 μL) were removed at various times (15, 30, 45, 60, 75 s) during the reaction and spotted onto PEI TLC sheets. [γ-^{32}P]ATP and $^{32}P_i$ were separated by chromatography on PEI sheets using 0.75 M KH_2PO_4, pH 3.5, as the mobile phase. The developed TLC plates were used to expose PhosphorImager plates (10−20 min) and scanned using a Fuji BAS Bio Imaging Analyzer and the radioactive spots analyzed using the Macintosh BAS analysis program. The initial rates of reactions were determined by plotting the amount of ATP hydrolyzed versus time. Relative percent activities were calculated from the initial velocities.

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

Docking Experiments of 1 and 18 with Rho. The docking of **1** to rho was done manually using Swiss pdb Viewer to the putative structure of rho derived by threading rho sequence on the crystal structure of F_1-ATP synthase followed by energy minimization.[9] Care was taken to keep the C(5a)-exomethylene moiety pointing toward residues K181 and neighboring K336 and positioning the bicyclomycin analogue relatively close to the amino acid residues when mutated are know to confer resistance to **1**. Also, care was taken to place **1** in a position that produced little steric hindrance from surrounding residues. The position of **1** was not energy minimized.

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Supporting Information Available: ORTEP views of **60** (Figure S1) and **61** (Figure S2) showing the atom numbering scheme along with tables of crystal data, bond lengths and angles, atomic coordinates, and anisotropic thermal parameters, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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