Role of the C(5)−C(5a) Exomethylene Group in Bicyclomycin: Synthesis, Structure, and Biochemical and Biological Properties

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Thirty-two C(5)−C(5a) exomethylene-modified bicyclomycin derivatives were prepared to determine the effect of structural modification of this unit on bicyclomycin (**1**) function. The compounds were grouped into three categories: the C(5)-unsaturated bicyclomycins, the C(5a)-substituted C(5)−C(5a)-dihydrobicyclomycin derivatives, and the C(5)-modified norbicyclomycins. An efficient three-step procedure was developed to synthesize C(5a)-substituted C(5),C(5a)-dihydrobicyclomycins. Bicyclomycin was converted to bicyclomycin C(2′),C(3′)-acetonide (**36**) and then treated with a nucleophile in 50% aqueous methanol ("pH" 10.5) to give the C(5a)-substituted C(5),C(5a)-dihydrobicyclomycin C(2′),C(3′)-acetonide. Removal of the acetonide group (trifluoroacetic acid in 50% aqueous methanol) in the final step provided the desired bicyclomycin derivative. All the compounds were evaluated using the rho-dependent ATPase assay and their antimicrobial activities determined using a filter disc assay. Most of the compounds were also tested in the transcription termination assay. We observed that many of the C(5)-unsaturated bicyclomycins effectively inhibited ATP hydrolysis at 400 μM and inhibited the production of rho-dependent transcripts at 100 μM. The biochemical activities of C(5a)-bicyclomycincarboxylic acid (**5**), methyl C(5a)-bicyclomycincarboxylate (**6**), ethyl C(5a)-bicyclomycincarboxylate (**7**), and bicyclomycin C(5)-norketone *O*-methyloxime (**11**) were all similar to **1**. Compounds **6**, **7**, and **11** exhibited diminished antibiotic activity compared to **1**, and **5** displayed no detectable activity. Several C(5a)-substituted C(5),C(5a)-dihydrobicyclomycins showed significant inhibition of rho-dependent ATPase and transcription termination activities. The inhibitory properties of C(5),C(5a)-dihydrobicyclomycin C(5a)-methyl sulfide (**18**), C(5),C(5a)-dihydrobicyclomycin C(5a)-phenyl sulfide (**23**), and C(5)−C(5a)-dihydrobicyclomycin-5,5a-diol (**31**) approached those of **1**. Compounds **18**, **23**, and **31** did not exhibit antibiotic activity. Two of the four C(5)-modified norbicyclomycin adducts showed moderate inhibitory activities in the ATPase assay, and none showed significant antibiotic activity. Our findings showed that the C(5)−C(5a) exomethylene unit retention in **1** was not essential for inhibition of *in vitro* rho activity. The structure−activity relationship data indicated that bicyclomycins that contained a small unsaturated C(5) unit or C(5),C(5a)-dihydrobicyclomycins that possessed a small, nonpolar C(5a) substituent effectively inhibited rho function in *in vitro* biochemical assays. We concluded that the C(5)−C(5a) unit in **1** was not a critical structural element necessary for drug binding to rho and that irreversible, inactivating units placed at this site would permit the bicyclomycin derivative to bind efficiently to rho.

Studies of the mechanism of action of bicyclomycin (**1**) have focused on the role of the C(5)−C(5a) exomethylene group.[1−11] The pioneering investigations of Iseki and co-workers showed that methyl mercaptan reacted at the C(5a) site in **1** at basic pH values.[1] Subsequently, Kohn and Abuzar demonstrated that treatment of bicyclomycin with both sulfur and nitrogen nucleophiles produced a spectrum of C(5a)-modified derivatives at near neutral to basic pH values.[3−7] These findings led to proposals that bicyclomycin expresses its activity by reacting with nucleophilic species necessary for bacterial function upon hemiaminal ring-opening to enone **2** (Scheme 1).[1,5] The importance of these transformations for bicyclomycin antibiotic activity remains unresolved. We have shown that **1** targets the rho transcription termination factor in *Escherichia coli*.[12] There is evidence that prolonged incubation (37 °C, 8−40 h) of rho with excess bicyclomycin in the absence of ATP and poly C gave multiple bicyclomycin−rho adducts.[13] Correspondingly, kinetic studies demonstrated that **1** was a *reversible*, noncompetitive inhibitor of ATP in rho-dependent ATPase processes.[13] Furthermore, similar inhibitory properties were observed for bicyclomycin and dihydrobicyclomycin[14] (**4**) in both the rho-dependent ATPase[13] and transcription termination assays.[15] Dihydrobicyclomycin is a bicyclo-

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(14) Kamiya, T.; Maeno, S.; Hashimoto, M.; Mine, Y. *J. Antibiot.* **1972**, *25*, 576−581.
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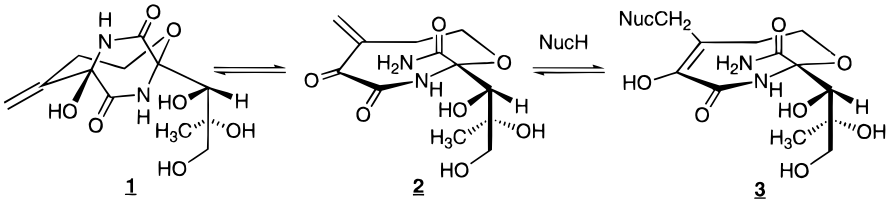
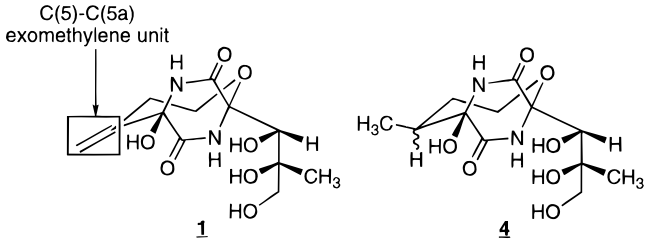
Scheme 1. Partial Pathway of Nucleophilic-Mediated Bicyclomycin Transformations



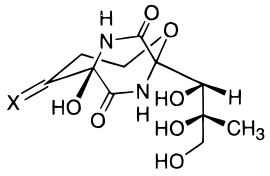
mycin analogue that cannot undergo covalent modification at the C(5)−C(5a) exomethylene site.

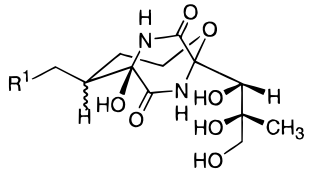


In this study, we asked whether structural modifications of the C(5)−C(5a) exomethylene group in bicyclomycin affects binding of the bicyclomycin analogue to rho. We found that retention of this unit is not essential for rho binding or for *in vitro* rho inhibition.

Results

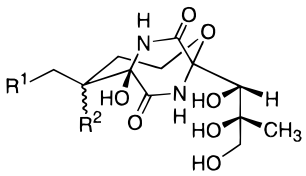
A. Selection of Compounds. Thirty-two bicyclomycin derivatives were prepared in our structure−activity relationship (SAR) study of the C(5)−C(5a) exomethylene group in bicyclomycin. These can be grouped as C(5)-unsaturated bicyclomycins **5**−**14**, C(5),C(5a)-dihydrobicyclomycin derivatives **4** and **15**−**31**, and C(5)-modified norbicyclomycins **32**−**35**.

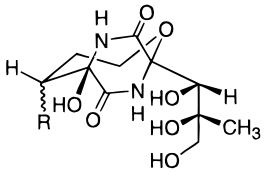




1	X = CH_2
5	X = $C(H)CO_2H$
6	X = $C(H)CO_2CH_3$
7	X = $C(H)CO_2CH_2CH_3$
8	X = $C(H)CO_2CH_2C_6H_5$
9	X = $C(H)CO_2C(CH_3)_3$
10	X = NOH
11	X = $NOCH_3$
12	X = $NOCH_2CH_3$
13	X = $NOCH_2Ph$
14	X = O

4	R^1 = H
15	R^1 = CO_2H
16	R^1 = CO_2CH_3
17	R^1 = $CO_2CH_2CH_3$
18	R^1 = SCH_3
19	R^1 = $S(CH_2)_2CH_3$
20	R^1 = $S(CH_2)_5CH_3$
21	R^1 = $S(CH_2)_2OH$
22	R^1 = $S(CH_2)_2NH_2$
23	R^1 = SC_6H_5
24	R^1 = $S(O)CH_3$
25	R^1 = $S(O)_2CH_3$
26	R^1 = *N*-morpholine
27	R^1 = NHC_6H_5
28	R^1 = 6-aminoquinoline





29	R^1, R^2 = O
30	R^1 = H, R^2 = OH
31	R^1 = OH, R^2 = OH

32	R = OH
33	R = $OS(O)_2CH_3$
34	R = $OS(O)_2CH_2Ph$
35	R = NHPh

B. Synthesis. Compounds **5**−**7**, **10**, **11**, **14**, **29**, and **31** were originally prepared by Müller and co-workers,[16] **4** was first synthesized by Kamiya and associates,[14] and we prepared **18**,[3−7] **26**,[6] **32**,[17] and **35**.[17] We employed these procedures to synthesize **4**, **6**, **7**, **10**, **11**, **14**, **31**, **32**, and **35**. Compound **14** was prepared by ozonolysis of **1**.[16,17] Treatment of **14** with (carbomethoxymethylene)-triphenylphosphorane, (carbethoxymethylene)triphenylphosphorane, and ((*tert*-butoxycarbonyl)methylene)-triphenylphosphorane gave Wittig products **6**, **7**, and **9**, respectively, in modest yields (42−70%). Compound **8** was produced in 42% yield by adding triethylamine to a mixture of ((benzyloxycarbonyl)methyl)triphenylphosphonium bromide and **14**. Hydrogenation (10% Pd/C, H_2 (1 atm)) of **8** gave free acid **5** (50%). The C(5)-oxime derivative **10** was prepared by treating **14** with $NH_2OH\cdot$HCl and pyridine in ethanol. The corresponding methyloxime (**11**) and ethyloxime (**12**) derivatives were prepared by an analogous procedure using *O*-methylhydroxylamine hydrochloride and *O*-ethylhydroxylamine hydrochloride, respectively.

Catalytic hydrogenation (10% Pd/C, H_2 (30 psi)) of **6** and **7** gave the C(5),C(5a)-reduced analogues **16** and **17**, respectively. Reduction of **8** under these conditions led to both the hydrogenation of the exomethylene group and the removal of the benzyl moiety to give free acid **15**.

Compounds **18**−**23** and **26**−**28** were prepared in three steps using an improved procedure for the synthesis of C(5a)-substituted C(5),C(5a)-dihydrobicyclomycin derivatives (Scheme 2). Beginning with bicyclomycin, protection of the C(2′) and C(3′) hydroxy groups with 2,3-dimethoxypropane gave the known acetonide **36**[18] (95%). Dissolution of **36** and the appropriate nucleophile in 50% aqueous methanol adjusted to "pH" 10.5 gave the C(5a)-substituted dihydrobicyclomycin C(2′),C(3′)-acetonides **37**−**45** in good yields (40−100%). The Michael addition reactions proceeded within 1 h (**37**−**42**) and 24 h (**43**−**45**) at room temperature and in most cases gave a mixture of diastereomers. In two cases (**43**, **45**), we observed only one diastereomer. Deprotection of the acetonide group at room temperature furnished the C(5a)-substituted C(5),C(5a)-dihydrobicyclomycins **18**−**23** and **26**−**28**. This procedure provided higher yields and cleaner product mixtures than the direct addition of the nucleophile to bicyclomycin in basic tetrahydrofuran−water mixtures.[6,7]

Oxidation of **18** using 30% H_2O_2 in 50% aqueous methanol rapidly (0.5 h) gave sulfoxide **24** in near quantitative yield. Use of longer reaction times (5 h) and higher concentrations of H_2O_2 with **18** gave the sulfone **25** in 58% yield.

Epoxide **29** was originally prepared by Müller and co-workers using an aqueous 30% H_2O_2 solution that

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(17) Zhang, Z.; Park, H.-g.; Kohn, H. *J. Org. Chem.* **1995**, *60*, 5346−5351.
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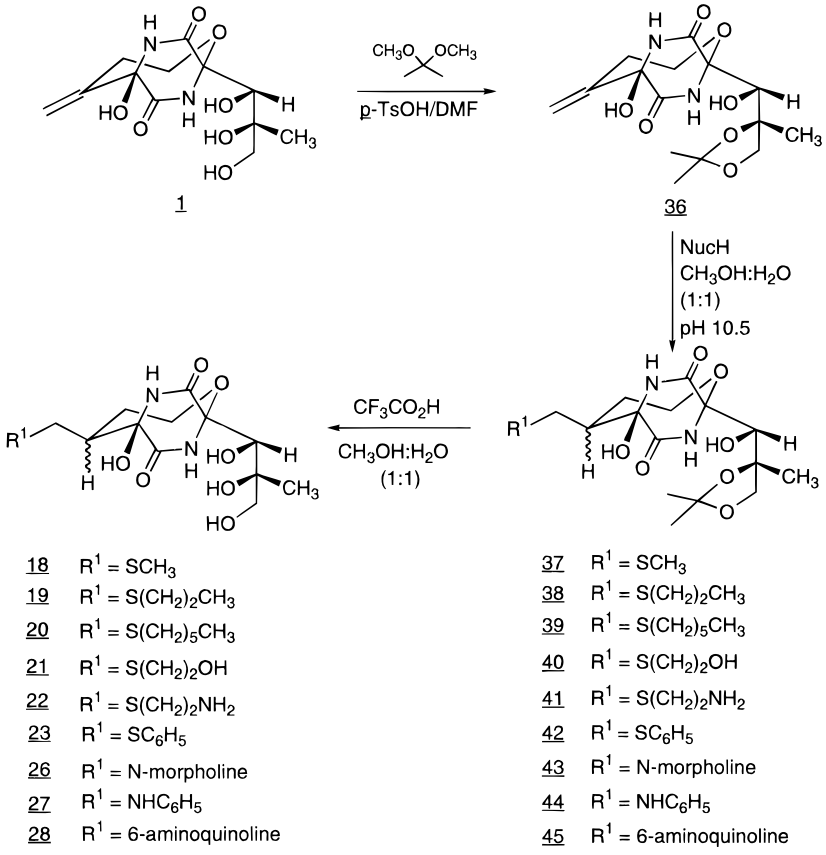
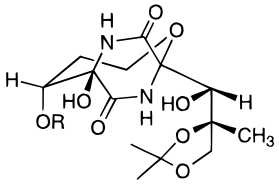
Scheme 2. Preparation of Compounds 18−23 and 26−28



18	$R^1 = SCH_3$	37	$R^1 = SCH_3$
19	$R^1 = S(CH_2)_2CH_3$	38	$R^1 = S(CH_2)_2CH_3$
20	$R^1 = S(CH_2)_5CH_3$	39	$R^1 = S(CH_2)_5CH_3$
21	$R^1 = S(CH_2)_2OH$	40	$R^1 = S(CH_2)_2OH$
22	$R^1 = S(CH_2)_2NH_2$	41	$R^1 = S(CH_2)_2NH_2$
23	$R^1 = SC_6H_5$	42	$R^1 = SC_6H_5$
26	R^1 = N-morpholine	43	R^1 = N-morpholine
27	$R^1 = NHC_6H_5$	44	$R^1 = NHC_6H_5$
28	R^1 = 6-aminoquinoline	45	R^1 = 6-aminoquinoline

contained bicyclomycin, a catalytic amount of $Na_2WO_4 \cdot 2H_2O$, and acetic acid.[16] Our analyses of the ^{1}H NMR spectrum of the reaction mixture indicated the presence of the desired product, **29**, along with a compound tentatively designated as the epoxide ring-opened acetate (data not shown). Accordingly, we substituted the non-nucleophilic acid, trifluoroacetic acid for acetic acid and observed only **29** (67%). Catalytic reduction (10% Pd/C) of **29** produced **30** as a single diastereomer.

Ozonolysis of **1** at −78 °C in methanol followed by direct catalytic hydrogenation (10% Pd/C, 3 h) led to the efficient stereospecific production of **32**.[17] Similarly, use of **36** in this procedure gave **46** in 96% yield.[17] Subsequent treatment of **46** with methanesulfonyl chloride and α-toluenesulfonyl chloride in pyridine resulted in **47** and **48**, respectively. Removal of the acetonide groups in **47** and **48** in acidic methanol−water gave **33** and **34**, respectively.



46	R = H
47	$R = S(O_2)CH_3$
48	$R = S(O_2)CH_2Ph$

C. Structural Characterization. Satisfactory spectroscopic data were obtained for all new compounds. Table 1 contains key ^{1}H NMR and ^{13}C NMR chemical shift values for the C(5)−C(5a) exomethylene-modified bicyclomycin derivatives. As expected, the NMR chemical shift values for the C(4) methylene protons and the C(4) carbon resonances were sensitive to the modification of C(5)−C(5a) exomethylene group. We noted a distinctive downfield shift from **1** (∼Δ 0.5−1.3 ppm) in the ^{1}H NMR for one of the two C(4) methylene protons in the C(5)-unsaturated bicyclomycins **5**−**14**. We also noted the diagnostic appearance of the C(5a) proton in the vinylic carboxylic acid derivatives **5**−**9** between δ 6.39−6.53. In the ^{13}C NMR, the C(4) resonance for the C(5) vinylic carboxylic acid derivatives **5**−**9** and the C(5) imine analogues **10**−**13** appeared upfield (Δ 6.2−10.9 ppm) from that observed for **1**.

The C(5a)-substituted dihydrobicyclomycin derivatives **15**−**28** showed an average upfield shift from **1** (∼Δ 0.32−0.77 ppm) for the C(4) methylene protons upon saturation of the C(5)−C(5a) exomethylene group; the appearance of two distinct signals for the diastereotopic C(5a) methylene protons in the ^{1}H NMR, the observation of the C(5) proton resonance in the ^{1}H NMR at δ 1.85−2.80, and the detection of the C(5) carbon signal in the ^{13}C NMR between 45 and 56 ppm. Analysis of the NMR spectra also showed that the chemical shift values for the C(5a) methylene protons and C(5a) carbon resonances varied with C(5a) substituent in a predictable fashion.[19,20] We observed that placement of a sulfur-containing moiety at C(5a) in **18**−**22** led to signals for the C(5a) methylene group at δ 1.85−3.16 and 29.9−31.9 ppm in the ^{1}H and ^{13}C NMR spectra, respectively. Correspondingly, incorporation of an amino substituent at C(5a) in **26**−**28** led to the appearance of a signal for the C(5a) hydrogens at

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(20) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972.

Table 1. Key ^{1}H NMR Spectral Properties for Bicyclomycin and C(5)−C(5a) Exomethylene-Modified Bicyclomycins

compd no.	^{1}H NMR[a]							^{13}C NMR[b]				
	C(3)*H*H′	C(3)H*H′*	C(4)*H*H′	C(4)H*H′*	C(5)H	C(5a)*H*H′	C(5a)H*H′*	C(3)	C(4)	C(5)	C(5a)	C(6)
1	3.75−3.86 (m)	3.87−4.00 (m)	2.55−2.75 (m)			5.14 (s)	5.55 (s)	65.4	36.6	149.4	117.0	82.9
5	3.80−4.00 (m)		2.60−2.72 (m)	3.80−4.00 (m)		6.51 (s)		65.6	30.4	144.4	129.1	83.2
6	3.50−3.88 (m)	3.95−4.05 (m)	2.65−2.80 (m)	3.50−3.88 (m)		6.48 (s)		64.6	29.4	158.4	120.2	83.5
7	3.70−3.85 (m)	3.95−4.05 (m)	2.65−2.80 (m)	3.70−3.85 (m)		6.49 (s)		64.6	29.4	158.2	120.6	83.5
8	3.68−3.85 (m)	3.92−4.03 (m)	2.71−2.79 (m)	3.68−3.85 (m)		6.53 (s)		64.7	29.5	158.7	120.3	83.5
9	3.69−3.82 (m)	3.92−4.05 (m)	2.62−2.78 (m)	3.69−3.82 (m)		6.39 (s)		64.8	29.2	156.7	122.3	83.5
10	3.80 (dd, 9.4, 13.3)	3.98 (dd, 6.9, 13.3)	2.55 (dd, 9.4, 16.8)	3.39 (dd, 6.9, 16.8)				62.3	25.7	156.3		82.4
11	3.70−3.82 (m)	3.84−4.02 (m)	2.48−2.62 (m)	3.25−3.34 (m)				62.2	26.4	157.3		82.4
12	3.72−3.84 (m)	3.90−4.00 (m)	2.48−2.62 (m)	3.27−3.38 (m)				62.3	26.6	156.8		82.4
13	3.68−3.80 (m)	3.86−3.98 (m)	2.50−2.62 (m)	3.28−3.40 (m)				62.1	26.8	157.9		82.5
14	3.90−4.10 (m)		2.75−2.87 (m)	3.02−3.14 (m)				59.5	42.5	203.9		85.6
4	3.78 (dd, 13.5, 21.9)	3.90−4.06 (m)	1.60−1.75 (m)	1.95−2.10 (m)	2.12−2.25 (m)			62.6	34.4	46.5	15.5	84.2
15	3.78 (dd, 8.1, 13.8)	3.95−4.10 (m)	1.72−1.90 (m)	1.95−2.15 (m)	2.50−2.62 (m)	1.95−2.15 (m)	2.81 (dd, 6.0, 15.6)	63.4	33.5	*c*	39.8	84.3
16	3.79 (dd, 8.4, 13.8)	3.95−4.05 (m)	1.65−1.83 (m)	1.95−2.10 (m)	2.50−2.63 (m)	2.17 (dd, 9.9, 16.2)	2.92 (dd, 3.6, 16.2)	64.3	32.0	52.2	35.2	83.5
						2.24 (dd, 9.6, 15.6)			*32.6*		*33.6*	*83.2*
17	3.79 (dd, 8.1, 13.8)	3.95−4.07 (m)	1.70−1.83 (m)	1.95−2.10 (m)	2.50−2.65 (m)	2.15 (dd, 10.2, 16.5)	2.91 (dd, 3.6, 16.5)	63.1	32.0	*c*	35.4	83.5
						2.23 (dd, 9.3, 15.9)		*64.3*	*32.6*		*33.9*	
18	3.70−4.10 (m)		2.05−2.30 (m)		2.05−2.30 (m)	2.05−2.30 (m)	3.06 (d, 11.7)	62.0	34.1	51.9	29.9	83.6
						2.40 (dd, 11.9, 13.1)	*3.08 (d, 13.1)*	*63.3*	*32.9*		*30.4*	
19	3.70−4.05 (m)		2.00−2.30 (m)		2.00−2.30 (m)	2.00−2.30 (m)	3.11 (d, 11.4)	62.0	35.2	52.5	31.7	83.7
							3.13 (d, 13.8)	*63.3*				*83.5*
20	3.70−4.05 (m)		2.05−2.35 (m)		2.05−2.35 (m)	2.05−2.35 (m)	3.12 (d, 11.7)	62.0	33.1	52.5	31.7	83.7
							3.14 (d, 12.3)	*63.3*		*50.3*		*83.5*
21	3.60−4.05 (m)		1.85−2.30 (m)		1.85−2.30 (m)	1.85−2.30 (m)	3.13 (d, 12.6)	62.2	35.5	52.8	31.9	83.6
						2.38 (dd, 11.3, 13.2)	*3.16 (d, 11.3)*	*62.0*	*35.4*	*50.5*	*30.9*	*83.5*
22	3.75−4.05 (m)		1.90−2.30 (m)		1.90−2.30 (m)	1.90−2.30 (m)	3.13 (d, 12.0)	63.3	36.0	52.7	31.4	83.7
						2.36 (dd, 11.1, 13.2)	*3.15 (d, 13.2)*	*62.0*		*50.4*	*30.6*	*83.5*
23	3.62−4.05 (m)		2.05−2.35 (m)		2.05−2.35 (m)	2.56 (dd, 12.0, 14.1)	3.45−4.07 (m)	63.1	33.1	51.8	29.9	83.7
						2.73 (dd, 11.4, 13.5)		*62.0*	*32.2*		*30.5*	*83.6*
24	3.75−3.90 (m)	4.00−4.13 (m)	1.90−2.25 (m)		2.50−2.80 (m)	2.50−2.80 (m)	3.30−3.40 (m)	62.6	31.3	54.6	46.1	82.8
								63.2	*33.2*	*56.1*	*47.6*	*83.3*
25	3.80−4.10 (m)		1.95−2.35 (m)		2.60−2.75 (m)	2.90−3.05 (m)	3.76 (d, 16.2)	63.0	32.3	54.5	46.7	83.0
									32.7	*53.5*	*45.7*	*82.9*
26	3.85 (dd, 8.7, 13.8)	4.00−4.10 (m)	1.55−1.72 (m)	1.80−1.95 (m)	2.25−2.40 (m)	2.25−2.40 (m)	2.63−2.83 (m)	65.4	31.9	45.4	61.4	85.5
27	3.70−4.05 (m)		1.85−2.10 (m)		2.30−2.45 (m)	2.96 (dd, 9.3, 13.8)	3.50−3.70 (m)	62.7	30.0	50.6	44.2	84.0
						3.08 (dd, 9.0, 13.8)					*44.4*	
28	3.65−4.20 (m)		1.90−2.15 (m)		2.40−2.55 (m)	3.07 (dd, 9.6, 13.8)	3.65−3.95 (m)	62.5	29.9	50.4	43.5	83.8
29	3.87 (dd, 8.1, 13.8)	4.07−4.15 (m)	1.91 (dd, 8.1, 16.5)	2.14 (dd, 9.0, 16.5)		2.51 (d, 5.4)	3.20 (d, 5.4)	62.6	36.2	64.2	50.3	80.6
30	3.65−3.75 (m)	4.05−4.16 (m)	1.88−1.97 (m)					61.1	42.1	80.3	23.2	84.7
31	3.88 (dd, 8.4, 13.8)	3.95−4.05 (m)	1.85 (dd, 8.4, 16.5)	2.09 (dd, 8.1, 16.5)		3.53 (d, 12.0)	3.77 (d, 12.0)	66.0	36.3	80.6	61.7	85.2
32	3.70−3.85 (m)	4.15−4.30 (m)	1.87−2.05 (m)	2.15−2.60 (m)	3.90−4.10 (m)			60.4	34.7	79.1		83.7
33	3.78−3.88 (m)	4.05−4.20 (m)	2.10−2.35 (m)		4.80−4.85 (m)			58.9	34.4	87.4		82.3
34	3.70−3.80 (m)	4.00−4.10 (m)	2.00−2.30 (m)		4.80−5.02 (m)			58.9	34.2	87.1		82.3
35	3.72−3.82 (m)	4.05−4.18 (m)	1.92−2.20 (m)		3.72−3.82 (m)			61.8	32.7	64.7		83.2

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant(s) in hertz. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD. The entries listed in italics correspond to the signals detected for the minor diastereomer observed in the product mixture. [b] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. All spectra were recorded at 75 MHz, and the solvent used was CD_3OD. The entries listed in italics correspond to the signals detected for the minor diastereomer observed in the product mixture. [c] The signal was not observed.

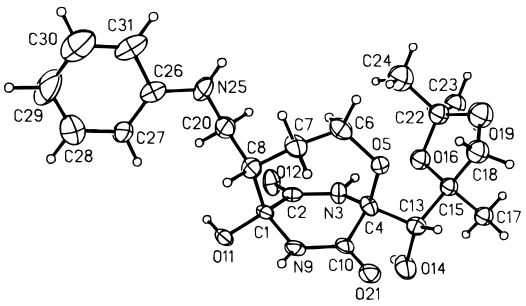


Figure 1. ORTEP drawing of compound **44** showing the atom numbering scheme. Thermal ellipsoids are 40% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. Only the major orientation of the disordered C15 group is shown. Selected bond distances (Å) are as follows: C(1)−C(8), 1.580(11); C(7)−C(8), 1.571(12); C(8)−C(20), 1.542(12); C(20)−N(25), 1.459(11); N(25)−C(26), 1.385(12). Selected angles (deg) are as follows: C(1)−C(8)−C(7), 115.7(6); C(8)−C(20)−N(25), 110.9(7); C(20)−N(25)−C(26), 119.7(8); N(25)−C(26)−C(27), 125.6(9); N(25)−C(26)−C(31), 115.9(9).

δ 2.25−3.95 in the ^{1}H NMR and 43.5−61.4 ppm in the ^{13}C NMR. Consistent with literature trends, the C(5a) methylene protons for epoxide **29** (δ 2.51, 3.20) were located upfield from **31** (δ 3.53, 3.77).[19]

Modification of the C(5)−C(5a) exomethylene group by either catalytic hydrogenation or Michael addition led to the introduction of a new chiral center at C(5). Hydrogenation of **1**, **6**, and **7** gave **4**, **16**, and **17**, respectively, as diastereomeric mixtures that were enriched in one stereoisomer (^{1}H and ^{13}C NMR analyses). The stereoselectivity of the Michael addition reaction to acetonide **36** to give **37**−**45** depended upon the nucleophile. The amine addition reactions proceeded with higher diastereoselectivity (≥9:1) than the thiol reactions (1.2−5:1). X-ray crystallographic analysis of purified **44** identified the major product as the C(5)-*S* adduct (Figure 1). The precise factors responsible for this trend have not been identified because an insufficient number of reactions were conducted to allow us to sort out the different variables (e.g., size, polarity, nucleophilicity) that existed within these experiments.

Oxidation of the 3:1 binary mixture of C(5a) methyl sulfide **18** gave sulfoxide **24**. NMR analyses showed only two distinct isomers present in an approximate 1:1 ratio. We have attributed this set of signals to the two diastereomers formed by oxidation of the major sulfide **18** to the chiral sulfoxides. We suspect that the signals from oxidation of the minor sulfide adduct present in the starting material were not detected. To confirm our suspicions, we inspected the ^{1}H and ^{13}C NMR spectra for the corresponding achiral methyl sulfone adduct **25**. In this case, NMR analyses, once again, showed a 3:1 diastereomeric product mixture stemming from the C(5) chirality introduced in the initial Michael addition of methanethiol to **36**.

Several other C(5)−C(5a) transformations proceeded stereospecifically to give a single isomer (NMR analyses). These were the epoxidation of **1** to give **29**, the catalytic (Pd/C) reduction of **29** to give pentol **30,** and the OsO_4-H_2O_2 oxidation of **1** to give hexol **31**.

D. Biochemical and Biological Properties. For convenience sake, we have divided our discussion of the biochemical and biological properties of bicyclomycin C(5)−C(5a) exomethylene-modified derivatives into three parts. The first describes the C(5)-unsaturated adducts **5**−**14**. The inhibitory properties of these compounds in the poly(C)-dependent rho ATPase assay[21] are listed in Table 2A. Most of the compounds effectively inhibited (56−90%) ATP hydrolysis at 400 μM. Bicyclomycin at this concentration inhibited ATP hydrolysis by 86−95%. Inspection of the I_{50} values for these compounds indicated that **5**−**7** and **11** exhibited inhibitory activity (I_{50} = 85−100 μM) that approached that of bicyclomycin (I_{50} = 60 μM),[13] while **8** and **12**−**14** displayed moderate activities (I_{50} = 135−175 μM). All of the compounds were evaluated in the rho-dependent transcription termination assay[22] and led to comparable findings. The estimated I_{50} values for **6** and **11** were the same as those for **1** (I_{50} ~5 μM),[15] while those for **5**, **7**, **8**, and **12** were only slightly higher (I_{50} ~5−10 μM) (Figure 2).

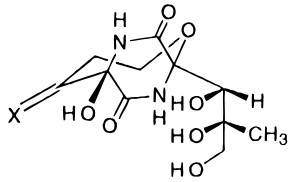
Inspection of these biochemical data revealed that the smaller C(5)−C(5a)-unsaturated substituents were the most effective inhibitors of rho-dependent ATPase and transcription termination activities. For the four esters **6**−**9**, compounds **6** and **7** were the most effective inhibitors, and for the substituted oximes **11**−**13**, compound **11** was the most active. We found that compounds that exhibited high I_{50} values in the ATPase assay (e.g., **9**, **10**, **14**) always displayed high I_{50} values in the transcription termination assay.

The biochemical data for this subset of compounds were poor prognosticators of the antimicrobiological data. Although compounds **5**−**8**, **11**, and **12** all efficiently inhibited rho-dependent processes in the biochemical assays, only **6** and **11** displayed significant (MIC ≤ 0.71 mg/mL) antimicrobial activities.[23] The fact that the biochemical test data do not correspond with the biological results reinforced our decision not to use the antibiotic activity data as a measure of the bicyclomycin derivative's binding to rho.[24] We suspect that many of these compounds were either not present in sufficiently high concentrations within the bacteria or they underwent chemical or metabolic change to give inactive bicyclomycin derivatives.

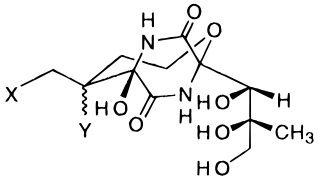
The second group of compounds were the 18 C(5)−C(5a) saturated bicyclomycin derivatives **4** and **15**−**31**. All of these underwent ATPase assay. We observed that **18**, **23**, and **31** exhibited I_{50} values (I_{50} = 75−90 μM) similar to those of bicyclomycin (I_{50} = 60 μM), while **4**, **15**−**17**, **19**, **20**, **27**, and **28** possessed I_{50} values of 180 μM or less and **21**, **22**, **24**−**26**, **29**, and **30** exhibited little activity in the ATPase assay. Most of the compounds tested were also evaluated using the transcription termination assay. Compounds that showed pronounced inhibitory properties in the ATPase assay also prevented the synthesis of rho-dependent transcripts, for example, **16**−**20**, **23**, **27**, and **28** all showed ATPase I_{50} values between 80 and 180 μM and prevented rho transcript synthesis at 100 μM. The activities of **18** (I_{50} ~5 μM) and **23** (I_{50} = 10−20 μM) in the transcription termination assay were comparable to bicyclomycin (I_{50} ~5 μM) and dihydrobicyclomycin (I_{50} ~20 μM).[15] Correspondingly, compounds **22**, **24**, and **25** showed low activity in both the ATPase and transcription termination assays, with only one exception. We observed that **25** exhibited

(21) Sharp, J. A.; Galloway, J. L.; Platt, T. *J. Biol. Chem.* **1983**, *258*, 3482−3486.
(22) Wu, A. M.; Christie, G. E.; Platt, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2913−2917.
(23) Ericsson, H. M.; Sherris, J. C. *Acta Pathol. Microbiol. Scand.* **1971**, Suppl. 217, 1−90.
(24) Park, H.-g.; Zhang, X.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7750. Santillán, A., Jr.; Park, H.-g.; Zhang, X.; Lee, O.-S.; Widger, W. R.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 7756.

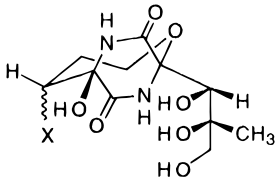
Table 2. Biochemical and Biological Activities of C(5)−C(5a) Exomethylene-Modified Bicyclomycins



1, 5–14



4, 15–31



32–35

			inhibition of ATPase activity[a]		TT activity[d]		
compd	X	Y	I_{50} (μM) (BCM)[b]	400 μM (%) (BCM)[c]	I_{50} (μM)[e]	100 μM (%) [f]	MIC [g] (mg/mL)
			A. C(5)-Unsaturated Bicyclomycin Analogues				
1	CH_2		60	95	∼5	100	0.25
5	$C(H)CO_2H$		95 (60)	86 (93)	∼10	100	>32 (0.26)
6	$C(H)CO_2CH_3$		100 (70)	71 (90)	∼5	100	0.60 (0.45)
7	$C(H)CO_2CH_2CH_3$		100 (70)	72 (90)	5−10	100	2.70 (0.45)
8	$C(H)CO_2CH_2C_6H_5$		135 (60)	82 (93)	5−10	100	>32 (0.26)
9	$C(H)CO_2C(CH_3)_3$		>400 (60)	41 (93)	>100	33	>32 (0.32)
10	NOH		325 (60)	56 (93)	>100	44	>32 (0.26)
11	$NOCH_3$		85 (60)	90 (95)	∼5	100	0.71 (0.32)
12	$NOCH_2CH_3$		160 (60)	78 (93)	5−10	100	2.80 (0.26)
13	$NOCH_2Ph$		175 (60)	65 (95)	10−40	100	>32 (0.32)
14	O		160 (70)	71 (86)	40−100	81	2.70 (0.60)
			B. C(5)−C(5a) Dihydrobicyclomycin Analogues				
4	H	H	120 (75)	79 (92)	∼20	100	1 (0.35)
15	CO_2H	H	120 (60)	78 (93)	40−100	100	>32 (0.26)
16	CO_2CH_3	H	180 (70)	71 (90)	10−40	100	3.6 (0.45)
17	$CO_2CH_2CH_3$	H	180 (70)	72 (90)	10−40	100	10.5 (0.45)
18	SCH_3	H	75 (60)	84 (95)	∼5	100	>32 (0.32)
19	$S(CH_2)_2CH_3$	H	160 (75)	68 (86)	10−40	100	>32 (0.60)
20	$S(CH_2)_5CH_3$	H	150 (75)	75 (86)	10−40	100	>32 (0.60)
21	$S(CH_2)_2OH$	H	270 (60)	62 (93)	10−40	100	>32 (0.26)
22	$S(CH_2)_2NH_2$	H	>400 (60)	30 (93)	40−100	58	>32 (0.26)
23	SC_6H_5	H	75 (60)	86 (95)	10−20	100	>32 (0.32)
24	$S(O)CH_3$	H	>400 (70)	30 (86)	>100	6	>32 (0.60)
25	$S(O)_2CH_3$	H	>400 (70)	49 (86)	40−100	80	>32 (0.60)
26	*N*-morpholine	H	>400 (60)	3 (93)	*h*	*h*	>32 (0.26)
27	*N*-aniline	H	120 (50)	81 (96)	∼40	80	>32 (0.45)
28	6-aminoquinoline	H	170 (75)	70 (92)	10−40	100	>32 (0.35)
29	X,Y = O		300 (75)	62 (86)	>100	5	2.7 (0.60)
30	H	OH	>400 (75)	9 (86)	*h*	*h*	>32 (0.60)
31	OH	OH	90 (60)	78 (93)	*h*	*h*	>32 (0.26)
			C. C(5)-Modified Norbicyclomycin Analogues				
32	OH		110 (60)	74 (86)	>100	39	8−16 (0.60)
33	$OS(O)_2CH_3$		180 (60)	68 (90)	∼40	90	16−32 (0.32)
34	$OS(O)_2CH_2Ph$		>400 (60)	44 (90)	*h*	*h*	16−32 (0.32)
35	NHPh		400 (60)	51 (90)	>100	0	>32 (0.32)

[a] Activity measured using the ATPase assay (ref 21). [b] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [c] The percent inhibition of ATPase activity at 400 μM. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [d] Activity in the transcription termination assay was determined by the method of T. Platt and co-workers (ref 22). [e] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. [f] The percentage of transcription termination at 100 μM. [g] MIC value is the average minimum inhibitory concentration of the tested compound determined from duplicate tests using a filter disc assay (ref 23). The number in parentheses is the corresponding value obtained from bicyclomycin in a concurrently run experiment. [h] Compound not tested.

surprising activity in the transcription termination assay (I_{50} ∼40−100 μM) but displayed only weak inhibitory activity in the ATPase assay (I_{50} > 400 μM). The biochemical significance of these findings in terms of rho function is under investigation.

There were several patterns within the ATPase data. (1) For the three simple C(5a) thioalkyl derivatives **18**−**20**, the most effective inhibitor was the methanethiol adduct **18**. The I_{50} value for **18** was 75 μM, and the values for the corresponding propanethiol (**19**) and hexanethiol (**20**) adducts were 160 and 150 μM, respectively. This trend was similar to the steric effect previously observed for esters **6**−**9** and the C(5)−C(5a)-unsaturated oximes **11**−**13**. (2) Incorporation of a polar substituent at C(5a) led to a drop in the inhibitory activity of the bicyclomycin derivative in the ATPase assay. Three examples within the data set agreed with this trend. First, the C(5a)-substituted compounds, **19**, **21**, and **22**, were all of comparable size. Of these three, the most effective inhibitor in the ATPase assay was the propanethiol adduct **19**. The I_{50} values for **19**, **21**, and **22** were 160, 270, and >400 μM, respectively. Second, conversion of the methyl sulfide **18** (I_{50} = 75 μM) to the more polar sulfoxide adduct **24** decreased inhibitory ATPase activity (I_{50} > 400 μM). Third, C(5a) aromatic amines **27** and **28** inhibited ATP hydrolysis (I_{50} = 120−170 μM) more efficiently than the more basic C(5a) morpholine adduct **26** (I_{50} > 400 μM). (3) Reduction of the olefinic unit at C(5) usually led to a decrease in the inhibitory properties of the bicyclomycin derivatives. For example, the I_{50} values for the vinylic adducts **1** and **5**−**7** ranged from 60 to 100 μM, while the I_{50} values for the

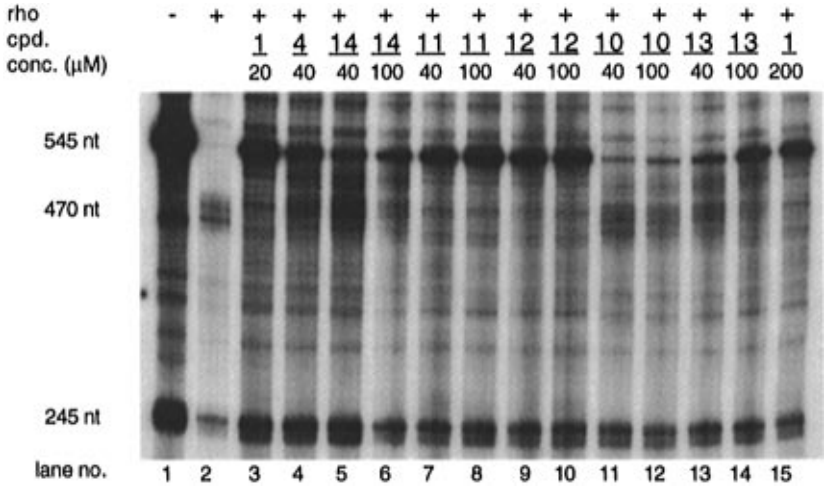


Figure 2. Autoradiograph of rho-dependent transcription termination assay for bicyclomycin and derivatives. The inhibition of *in vitro* rho-dependent transcription termination by bicyclomycin and its derivatives was determined using a modified *trp* operon template[22] and *E. coli* RNA polymerase and measuring the incorporation of [α-^{32}P]UTP. The reactions were carried out in the absence of rho (lane 1), in the presence of rho (lane 2), and in the presence of bicyclomycin and derivatives (lanes 3−15): lane 3, **1** (20 μM); lane 4, **4** (40 μM); lane 5, **14** (40 μM); lane 6, **14** (100 μM); lane 7, **11** (40 μM); lane 8, **11** (100 μM); lane 9, **12** (40 μM); lane 10, **12** (100 μM); lane 11, **10** (40 μM); lane 12, **10** (100 μM); lane 13, **13** (40 μM); lane 14, **13** (100 μM); lane 15, **1** (200 μM). Relative amounts of transcripts were determined by densitometry of the autoradiograph.

corresponding saturated analogues **4** and **15**−**17** were from 120 to 180 μM.

The C(5)−C(5a)-saturated bicyclomycin derivatives **29**−**31** have a heteroatom attached to C(5). Similar activity trends were observed for this limited array of compounds. Introduction of a polar substituent at C(5) reduced the activity in the ATPase assay. The I_{50} values for epoxide **29** (I_{50} = 300 μM) and pentol **30** (I_{50} >400 μM) were larger than that for dihydrobicyclomycin (**4**) (I_{50} = 120 μM). An exception to this trend was the surprising activity of hexol **31** (I_{50} = 90 μM).

There was little correlation between the antimicrobial and the biochemical activities (ATPase, transcription termination) for the C(5)−C(5a)-saturated bicyclomycin derivatives **4** and **15**−**31**. Despite the significant activities of many of these compounds in the rho-dependent ATPase and transcription termination assays (e.g., **18**, **23**, **31**), few exhibited activity against W3350 *E. coli* (MIC > 32 mg/mL). Of the compounds evaluated, only **4**, **16**, **17**, and **29** exhibited moderate activity.

The final group evaluated was the C(5)-modified norbicyclomycins **32**−**35**. Of these four compounds, **32** and **33** displayed moderate inhibitory activities in the ATPase assay (I_{50} = 110−180 μM) and **34** and **35** showed little activity (I_{50} > 400 μM) (Table 2C). None of the four showed significant antibiotic activity.

Discussion

The early SAR study of Müller and co-workers[16] demonstrated that structural modifications of the C(5)−C(5a) exomethylene site in bicyclomycin, like those made at the C(1) triol[10,24,25] and [4.2.2] bicyclic units,[24] often led to reduced antibiotic activity. Nonetheless, these investigators found that the C(5)-unsaturated derivatives **6**, **7**, and **11** all possessed notable antimicrobial activities against Gram-negative bacteria.[16] This finding suggested to us that other structural modifications made at this site would not disrupt the binding interactions of the bicyclomycin derivative with rho. Our study of the C(5)−C(5a) exomethylene site in **1** was divided into the C(5)−C(5a)-unsaturated adducts **5**−**14**, the C(5),C(5a)-dihydrobicyclomycin derivatives **4** and **15**−**31**, and the C(5)-modified norbicyclomycins **32**−**35**.

Many of the C(5)−C(5a)-unsaturated bicyclomycin derivatives retained pronounced inhibitory properties in the poly(C)-dependent rho ATPase assay. Significantly, the activities of compounds **5**−**7** and **11** (I_{50} = 85−100 μM) were comparable to that of bicyclomycin (I_{50} = 60 μM).[13] Evaluation of **5**−**8**, **10**−**12**, and **14** in the rho-dependent transcription termination assay revealed that **5**−**8** and **11**−**13** all prevented the synthesis of rho-dependent transcripts at 100 μM concentration. Moreover, the I_{50} values for **6** and **11** (~5 μM) equaled that of bicyclomycin (5 μM).[15] These findings demonstrated that in these two biochemical assays select C(5)-unsaturated units can be introduced into bicyclomycin in place of the vinyl group apparently without reducing the inhibitory properties of these analogues. Consistent with their excellent activities in the ATPase and transcription termination assays, compounds **6**, **7**, and **11** displayed antimicrobial activity against *E. coli* W3350.

Inspection of the biochemical and biological properties for the C(5a)-substituted C(5)−C(5a)-saturated bicyclomycin derivatives **4** and **15**−**31** provided additional insights into the SAR requirements for bicyclomycin function. We observed that many of the compounds (**4**, **15**−**21**, **23**, **27**, **28**) inhibited rho-dependent ATPase and transcription termination activities. This finding demonstrated that incorporation of a C(5)−C(5a)-unsaturated unit within the bicyclomycin ring skeleton was not essential for activity in these assays. Two patterns emerged from this study. First, enhanced inhibitory activities were observed for smaller C(5a) substituents compared with larger C(5a) groups (e.g., **18** vs **19**, **20**; **27** vs **28**). Second, replacement of nonpolar C(5a) substituents by polar groups led to a drop in the inhibitory properties of the bicyclomycin derivative (e.g., **18** vs **24**; **19** vs **21**, **22**). Nonetheless, of the 18 compounds evaluated in this group only **4**, **16**, and **17** displayed antibiotic

(25) Park, H.-g.; Vela, M. A.; Kohn, H. *J. Am. Chem. Soc.* **1994**, *116*, 471−478.

activity against *E. coli* W3350. This result demonstrated the value of using biochemical assays to assemble the bicyclomycin SAR profile, which is required for the design of bicyclomycin-based rho irreversible inactivators. We have attributed the lack of biological results—biochemical test data correspondence to the poor transport of the bicyclomycin analogues across the bacterial cell wall, the rapid efflux of these adducts out of the cell, or to the metabolism of the bicyclomycin derivative within the bacteria—to species with a low affinity to bind to rho.

These cumulative findings permit several speculations. First, the SAR study suggested that the structural domain surrounding the C(5)−C(5a) unit in the bicyclomycin binding region in rho is relatively nonpolar. Furthermore, the enhanced inhibitory properties observed with smaller C(5a) substituents vs larger C(5a) groups indicated that larger substituents may destabilize the bicyclomycin derivative−rho binding complex. This spatial constraint for drug binding may account, in part, for the excellent inhibitory properties observed for the near planar C(5)−C(5a)-unsaturated bicyclomycin derivatives **5**−**8**, **11**, and **12**.[26] Second, the SAR results indicated that the C(5)−C(5a) region in **1** offered an excellent opportunity for the introduction of an irreversible, inactivating unit. We observed that a wide range of structural modifications can be conducted at this site without significantly impairing the ability of the bicyclomycin analogue to inhibit *in vitro* rho function. Of particular interest was the finding that the C(5) aryl derivatives **23** and **27** retained significant inhibitory activities in both the ATPase and transcription termination assays. The size of these C(5a) substituents approximates many conventional photoaffinity and reductive amination probes.[27] Third, we noted superlative inhibitory activities in the rho-dependent ATPase and transcription termination assays for C(5a) bicyclomycin carboxylic esters **6** and **7**, oximes **11** and **12**, and C(5a) sulfides **18** and **23**. These activities indicated that irreversible transformations that may proceed at the C(5)−C(5a) exomethylene unit in **1** did not play a significant role in inhibiting rho-dependent ATPase and transcription termination processes under *in vitro* assay conditions. This result was consistent with the previous finding that both bicyclomycin and dihydrobicyclomycin effectively inhibited these rho-dependent functions.[13,15]

Conclusions

We observed that select C(5)−C(5a) exomethylene-modified bicyclomycin analogues retained activity in rho-dependent assays. The finding that both substituted C(5)-unsaturated and C(5),C(5a)-dihydrobicyclomycin derivatives inhibited rho-dependent ATPase and transcription termination processes showed that the exomethylene unit in **1** was not essential for drug binding to rho. Our data indicated that the inclusion of relatively small, nonpolar substituents at the C(5) and the C(5a) sites provided maximal rho inhibition in biochemical tests. We concluded that the C(5)−C(5a) site in bicyclomycin is an excellent choice for the introduction of an irreversible, inactivating unit.[27]

Experimental Section

General Methods. Procedures identical to those previously described[24] were used in the synthesis and evaluation of bicyclomycin C(5)−C(5a) exomethylene-modified derivatives in the poly(C)-dependent ATPase,[21] rho-dependent transcription termination,[22] and antimicrobial assays.[23]

Preparation of Bicyclomycin C(5)-Norketone[16,17] (14). An anhydrous ethanolic solution (40 mL) of **1** (200 mg, 0.67 mmol) was treated with O_3 at −78 °C (3 min). The solution was degassed with Ar (10 min), and then methyl sulfide (0.5 mL) was added at −78 °C. The solution was slowly allowed to warm to 0 °C during which time the solution became cloudy. The solvent was removed *in vacuo*, and the residue was dried under vacuum to give **14** as a white solid: yield, 191 mg (95%); mp 163−169 °C (lit.[16] mp 171−175 °C); R_f 0.10 (40% MeOH−$CHCl_3$); FT-IR (KBr) 3344 (br), 1731, 1693, 1410 cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 3 H), 2.75−2.87 (m, 1 H), 3.02−3.15 (m, 1 H), 3.51 (d, J = 11.4 Hz, 1 H), 3.69 (d, J = 11.4 Hz, 1 H), 3.90−4.10 (m, 2 H), 4.16 (s, 1 H); MS (+CI) 305 $[M + 1]^+$; M_r (+CI) 304.090 58 $[M]^+$ (calcd for $C_{11}H_{16}N_2O_8$ 304.090 67).

Preparation of Methyl C(5a)-Bicyclomycincarboxylate[16] (6). An anhydrous dioxane solution (4 mL) of **14** (45 mg, 0.15 mmol) and (carbomethoxymethylene)triphenylphosphorane (59 mg, 0.18 mmol) was stirred at room temperature (12 h). The solvent was removed *in vacuo*, and the residue was purified by column chromatography (SiO_2, 20% MeOH−$CHCl_3$) to give **6**: yield, 23 mg (49%); mp 130−132 °C (lit.[16] mp 135−136 °C); R_f 0.55 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3414 (br), 3250 (br), 1693, 1400, 1062 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.65−2.80 (m, 1 H), 3.50−3.88 (m, 7 H), 3.95−4.05 (m, 1 H), 4.10 (s, 1 H), 6.48 (s, 1 H); ^{13}C NMR (CD_3OD) 24.2, 29.4, 52.0, 64.6, 68.4, 72.0, 78.2, 83.5, 89.5, 120.2, 158.4, 168.1, 168.8, 171.5 ppm; MS (+CI) 361 $[M + 1]^+$; M_r (+CI) 361.124 53 $[M + 1]^+$ (calcd for $C_{14}H_{21}N_2O_9$ 361.124 71).

Preparation of Ethyl C(5a)-Bicyclomycincarboxylate[16] (7). The preceding reaction was repeated using **14** (50 mg, 0.16 mmol) and (carbethoxymethylene)triphenylphosphorane (55 mg, 0.17 mmol) to give **7**: yield, 36 mg (60%); mp 120−125 °C (lit.[16] mp 116−120 °C); R_f 0.58 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 3270 (br), 1699, 1394, 1190 cm^{-1}; ^1H NMR (CD_3OD) δ 1.26 (t, J = 7.2 Hz, 3 H), 1.34 (s, 3 H), 2.65−2.80 (m, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.70−3.85 (m, 2 H), 3.95−4.05 (m, 1 H), 4.10 (s, 1 H), 4.15 (q, J = 7.2 Hz, 2 H), 6.49 (s, 1 H); ^{13}C NMR (CD_3OD) 14.5, 24.1, 29.4, 61.5, 64.6, 68.4, 72.0, 78.2, 83.5, 89.4, 120.6, 158.2, 167.6, 168.8, 171.5 ppm; MS (+CI) 375 $[M + 1]^+$; M_r (+CI) 375.141 09 $[M + 1]^+$ (calcd for $C_{15}H_{23}N_2O_9$ 375.140 36).

Preparation of Benzyl C(5a)-Bicyclomycincarboxylate (8). To an anhydrous dioxane solution (5 mL) of **14** (100 mg, 0.33 mmol) and ((benzyloxycarbonyl)methyl)triphenylphosphonium bromide (180 mg, 0.37 mmol) was added triethylamine (42 μL, 0.66 mmol), and then the solution was stirred at room temperature (12 h). The solvent was removed *in vacuo*, and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **8**: yield, 60 mg (42%); mp 127−129 °C; R_f 0.60 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 3256 (br), 1693, 1385, 1174 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.71−2.79 (m, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.66 (d, J = 11.4 Hz, 1 H), 3.68−3.85 (m, 2 H), 3.92−4.03 (m, 1 H), 4.10 (s, 1 H), 5.15 (s, 2 H), 6.53 (s, 1 H), 7.30−7.40 (m, 5 H); ^{13}C NMR (CD_3OD) 24.2, 29.5, 64.7, 67.2, 68.4, 72.1, 78.2, 83.5, 89.5, 120.3, 129.2, 129.5, 137.5, 158.7, 167.4, 168.7, 171.5 ppm, the remaining aromatic carbon signal was not detected and is believed to overlap with the other aromatic peaks; M_r (+CI) 437.154 75 $[M + 1]^+$ (calcd for $C_{20}H_{25}N_2O_9$ 437.156 01).

Preparation of *tert*-Butyl C(5a)-Bicyclomycincarboxylate (9). Using the conditions for the preparation of **6** and utilizing **14** (50 mg, 0.16 mmol) and ((*tert*-butoxycarbonyl)-methylene)triphenylphosphorane (67.8 mg, 0.18 mmol) gave **9** as a white solid: yield, 45 mg (70%); mp 133−135 °C; R_f

(26) Our findings do not permit us to differentiate this effect from stabilizing interactions that may be derived from the π-electrons localized at C(5) within the bicyclomycin derivative and the receptor.

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

0.58 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3430, 3264, 2980, 1707 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 1.46 (s, 9 H, C(CH_3)$_3$), 2.62−2.78 (m, 1 H, C(4)*H*H′), 3.50 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.66 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.69−3.82 (m, 2 H, C(3)*H*H′, C(4)H*H*′), 3.92−4.05 (m, 1 H, C(3)H*H*′), 4.10 (s, 1 H, C(1′)H), 6.39 (s, 1 H, C(5a)H); ^{13}C NMR (CD_3OD) 24.2 (C(2′)CH_3), 28.4 (C(CH_3)$_3$), 29.2 (C(4)), 64.8 (C(3)), 72.0 (C(1′)), 78.2 (C(2′)), 82.1, 83.5 (C(6), $C(CH_3)_3$), 89.5 (C(1)), 122.3 (C(5a)), 156.7 (C(5)), 167.1 (OC(O)), 168.9 (C(9)), 171.7 (C(7)) ppm; the proposed structural assignments were consistent with the COSY, HMQC, and HMBC NMR experimental data; MS (+CI) 403 $[M + 1]^+$; M_r (+CI) 403.171 70 $[M + 1]^+$ (calcd for $C_{17}H_{27}N_2O_9$ 403.171 66).

Preparation of C(5a)-Bicyclomycincarboxylic Acid[16] (5). To a methanolic solution (2 mL) of **8** (5 mg, 0.012 mmol) was added a catalytic amount of 10% Pd/C (2 mg). The solution was stirred at room temperature under an atmosphere of H_2 during which time the reaction was monitored by TLC (50% MeOH−$CHCl_3$). After 2 h the starting material was completely consumed. The solution was filtered, and the solvent was removed *in vacuo*. The residue was purified by preparative TLC (50% MeOH−$CHCl_3$) to afford **5**: yield, 2 mg (50%); mp 168−175 °C (lit.[16] mp 173−174 °C); R_f 0.10 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3444 (br), 3256 (br), 1696, 1559, 1394 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.60−2.72 (m, 1 H), 3.51 (d, J = 11.4 Hz, 1 H), 3.67 (d, J = 11.4 Hz, 1 H), 3.80−4.00 (m, 3 H), 4.08 (s, 1 H), 6.51 (s, 1 H), the carboxylic acid proton was not detected; ^{13}C NMR (CD_3OD) 24.2, 30.4, 65.6, 68.5, 72.4, 78.1, 83.2, 89.5, 129.1, 144.4, 168.9, 172.3, 175.6 ppm; M_r (+CI) 346.101 11 (calcd for $C_{13}H_{18}N_2O_9$ 346.101 23).

Preparation of Bicyclomycin C(5)-Norketone Oxime[16] (10). To an ethanolic solution of **14** (100 mg, 0.33 mmol) were added NH_4OH·HCl (23 mg, 0.33 mmol) and pyridine (27 μL). The mixture was stirred at 40 °C (2 h) and then concentrated *in vacuo*. The residue was purified by preparative TLC (30% MeOH−$CHCl_3$) to give **10**: yield, 93 mg (86%); mp 180−183 °C (lit.[16] mp 185−188 °C); R_f 0.15 (30% MeOH−$CHCl_3$); ^1H NMR (CD_3OD) δ 1.35 (s, 3 H), 2.55 (dd, J = 9.4, 16.8 Hz, 1 H), 3.39 (dd, J = 6.9, 16.8 Hz, 1 H), 3.52 (d, J = 11.4 Hz, 1 H), 3.67 (d, J = 11.4 Hz, 1 H), 3.80 (dd, J = 9.4, 13.3 Hz, 1 H), 3.98 (dd, J = 6.9, 13.3 Hz, 1 H), 4.11 (s, 1 H); ^{13}C NMR (CD_3-OD) 24.1, 25.7, 62.3, 68.5, 72.0, 78.2, 82.4, 89.6, 156.3, 168.5, 170.8 ppm; MS (+CI) 320 $[M + 1]^+$.

Preparation of Bicyclomycin C(5)-Norketone *O*-Methyloxime[16] (11). Using the preceding protocol and utilizing **14** (35 mg, 0.12 mmol), *O*-methylhydroxylamine hydrochloride (1 mg, 0.13 mmol), and pyridine (11 μL, 0.13 mmol) gave **11**: yield, 30 mg (75%); mp 137−139 °C (lit.[16] mp 145−148 °C); R_f 0.52 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3414, 3263, 2980, 1694 cm^{-1} (lit.[16] IR (KBr) 3480, 3260, 2980, 1705 cm^{-1}); ^1H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.48−2.62 (m, 1 H), 3.25−3.34 (m, 1 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.70−3.82 (m, 1 H), 3.84−4.02 (m, 1 H), 3.92 (s, 3 H), 4.10 (s, 1 H); ^{13}C NMR (CD_3OD) 24.1, 26.4, 62.2, 62.9, 68.3, 72.1, 78.2, 82.4, 89.5, 157.3, 168.4, 170.3 ppm; MS (+CI) 334 $[M + 1]^+$; M_r (+CI) 334.124 27 $[M + 1]^+$ (calcd for $C_{12}H_{20}N_3O_8$ 334.125 04).

Preparation of Bicyclomycin C(5)-Norketone *O*-Ethyloxime (12). Using the procedure for **10** and utilizing **14** (36 mg, 0.12 mmol), *O*-ethylhydroxylamine hydrochloride (13 mg, 0.13 mmol), and pyridine (11 μL, 0.13 mmol) gave **12**: yield, 32 mg (77%); mp 141−143 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3408, 3266, 2982, 1694 cm^{-1}; ^1H NMR (CD_3OD) δ 1.25 (t, J = 6.9 Hz, 3 H), 1.34 (s, 3 H), 2.48−2.62 (m, 1 H), 3.27−3.38 (m, 1 H), 3.49 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.72−3.84 (m, 1 H), 3.90−4.00 (m, 1 H), 4.10 (s, 1 H), 4.18 (q, J = 6.9 Hz, 2 H); ^{13}C NMR (CD_3OD) 14.7, 24.1, 26.6, 62.3, 68.4, 71.5, 72.0, 78.2, 82.4, 89.6, 156.8, 168.4, 170.5 ppm; MS (+CI) 348 $[M + 1]^+$; M_r (+CI) 348.140 85 $[M + 1]^+$ (calcd for $C_{13}H_{22}N_3O_8$ 348.140 69).

Preparation of Bicyclomycin C(5)-Norketone *O*-Benzyloxime (13). Using the procedure for **10** and utilizing **14** (36 mg, 0.12 mmol), *O*-benzylhydroxylamine hydrochloride (21 mg, 0.13 mmol), and pyridine (11 μL, 0.13 mmol) gave **13**: yield, 33 mg (67%); mp 147−150 °C; R_f 0.58 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3391, 3325, 2982, 1728, 1690 cm^{-1}; ^1H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.50−2.62 (m, 1 H), 3.28−3.40 (m, 1 H), 3.49 (d, J = 11.4 Hz, 1 H), 3.64 (d, J = 11.4 Hz, 1 H), 3.68−3.80 (m, 1 H), 3.86−3.98 (m, 1 H), 4.09 (s, 1 H), 5.17 (s, 2 H), 7.22−7.40 (m, 5 H); ^{13}C NMR (CD_3OD) 24.1, 26.8, 62.1, 68.4, 72.0, 77.9, 78.2, 82.5, 89.6, 129.0, 129.1, 129.5, 138.3, 157.9, 168.5, 170.4 ppm; MS (+CI) 410 $[M + 1]^+$; M_r (+CI) 410.157 13 $[M + 1]^+$ (calcd for $C_{18}H_{24}N_3O_8$ 410.156 34).

Preparation of C(5),C(5a)-Dihydrobicyclomycincarboxylic Acid (15). A methanolic solution (2 mL) containing **8** (5 mg, 0.012 mmol) and Pd/C (2 mg) was stirred at room temperature under 30 psi of H_2 (2 h). The solution was filtered, and the solvent was removed *in vacuo* to give **15** as a single diastereomer: yield, 4 mg (∼100%); mp 190−200 °C; R_f 0.10 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3434 (br), 1685, 1207 cm^{-1}; ^1H NMR (CD_3OD) δ 1.32 (s, 3 H, C(2′)CH_3), 1.72−1.90 (m, 1 H, C(4)*H*H′), 1.95−2.15 (m, 2 H, C(4)H*H*′, C(5a)-*H*H′), 2.50−2.62 (m, 1 H, C(5)H), 2.81 (dd, J = 6.0, 15.6 Hz, 1 H, C(5a)H*H*′), 3.53 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.68 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.78 (dd, J = 8.1, 13.8 Hz, 1 H, C(3)*H*H′), 3.95−4.10 (m, 2 H, C(3)H*H*′, C(1′)H), the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) 24.2, 33.5, 39.8, 63.4, 68.5, 72.3, 78.1, 84.3, 89.5, 168.2, 172.4, 181.1 ppm, the C(5) signal was not detected and is believed to be beneath the solvent signals; MS (+CI) 349 $[M + 1]^+$; M_r (+CI) 349.124 74 $[M + 1]^+$ (calcd for $C_{13}H_{21}N_2O_9$ 349.124 71).

Preparation of Methyl C(5),C(5a)-Dihydrobicyclomycincarboxylate (16). Using the preceding procedure and **6** (10 mg, 0.028 mmol) and 10% Pd/C (5 mg) gave **16** as a mixture of diastereomers (∼5:1): yield, 9 mg (90%); mp 160−162 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3433 (br), 3294 (br), 1686, 1407, 1140, 1045 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 1.65−1.83 (m, 1 H, C(4)*H*H′), 1.95−2.10 (m, 1 H, C(4)H*H*′), 2.17 (dd, J = 9.9, 16.2 Hz, 1 H, C(5a)*H*H′), 2.50−2.63 (m, 1 H, C(5)H), 2.92 (dd, J = 3.6, 16.2 Hz, 1 H, C(5a)H*H*′), 3.53 (d, J = 11.4 Hz, 1 H, C(3′)-*H*H′), 3.62−3.72 (m, 4 H, C(3′)H*H*′, C(O)OCH_3), 3.79 (dd, J = 8.4, 13.8 Hz, 1 H, C(3)*H*H′), 3.95−4.05 (m, 2 H, C(3)H*H*′, C(1′)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.24 (dd, J = 9.6, 15.6 Hz, 1 H, C(5a)*H*H′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 32.0, 35.2, 52.2, 63.1, 64.3, 68.5, 72.3, 78.1, 83.5, 89.6, 168.0, 171.8, 174.7 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 32.6, 33.6, 72.2, 83.2, 89.3 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 363 $[M + 1]^+$; M_r (+CI) 363.139 78 $[M + 1]^+$ (calcd $C_{14}H_{23}N_2O_9$ 363.140 36).

Preparation of Ethyl C(5),C(5a)-Dihydrobicyclomycincarboxylate (17). Using the procedure described for **15** and utilizing **7** (14 mg, 0.037 mmol) and 10% Pd/C (7 mg) gave **17** as a mixture of diastereomers (∼5:1) after purification by column chromatography (SiO_2, 20% MeOH−$CHCl_3$): yield, 12 mg (86%); mp 149−151 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3420 (br), 3269 (br) 1693, 1399, 1044 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.23 (t, J = 7.2 Hz, 3 H, C(O)OCH_2CH_3), 1.32 (s, 3 H, C(2′)CH_3), 1.70−1.83 (m, 1 H, C(4)*H*H′), 1.95−2.10 (m, 1 H, C(4)H*H*′), 2.15 (dd, J = 10.2, 16.5 Hz, 1 H, C(5a)*H*H′), 2.50−2.65 (m, 1 H, C(5)H), 2.91 (dd, J = 3.6, 16.5 Hz, 1 H, C(5a)H*H*′), 3.53 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.68 (d, J = 11.4 Hz, 1 H, C(3)H*H*′), 3.79 (dd, J = 8.1, 13.8 Hz, 1 H, C(3)*H*H′), 3.95−4.20 (m, 4 H, C(3)H*H*′, C(1′)H, C(O)OCH_2CH_3); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.17 (t, J = 7.2 Hz, 3 H, C(O)OCH_2CH_3), 2.23 (dd, J = 9.3, 15.9 Hz, 1 H, C(5a)*H*H′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR for the major diastereomer, 14.5, 24.2, 32.0, 35.4, 61.7, 63.1, 68.5, 72.3, 78.1, 83.5, 89.6, 168.0, 171.8, 174.3 ppm, the C(5) signal was not detected and is believed to be beneath the solvent signals; ^{13}C NMR for the minor diastereomer, 32.6, 33.9, 64.3 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 377 $[M + 1]^+$; M_r (+CI) 377.155 64 $[M + 1]^+$ (calcd for $C_{15}H_{25}N_2O_9$ 377.156 01).

General Procedure for the Preparation of C(5a)-Substituted Dihydrobicyclomycin C(2′),C(3′)-Acetonides. To a 50% aqueous methanolic solution (2−8 mL) of **36** (1 equiv)

was added the desired nucleophile (2−10 equiv), and then the "pH" was adjusted to 10.5 with a dilute aqueous NaOH solution. The solution was stirred at room temperature (10−1440 min), and then the "pH" was adjusted to 7.0 with an aqueous 5% HCl solution. The solvent was removed *in vacuo*, and the residue was purified either by column chromatography (SiO_2, 5% MeOH−$CHCl_3$) or by preparative TLC (10−40% MeOH−$CHCl_3$) to afford the desired product.

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

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-Methyl Sulfide[7] (37). Using **36** (20 mg, 0.06 mmol) and sodium methanethiolate (20 mg, 0.29 mmol) gave **37** as a mixture of diastereomers (∼3:1): yield, 23 mg (∼100%); mp 164−165 °C (lit.[7] mp 122−125 °C); R_f 0.50 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3289 (br), 1686, 1419, 1045 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3 H), 1.44 (s, 6 H), 1.85−2.00 (m, 1 H), 2.00−2.30 (m, 6 H), 3.10−3.30 (m, 1 H), 3.71 (d, J = 8.7 Hz, 1 H), 3.75−4.08 (m, 2 H), 4.10 (s, 1 H), 4.45 (d, J = 8.7 Hz, 1 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.40 (dd, J = 11.7, 13.2 Hz, 1 H), 4.47 (d, J = 8.1 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) 15.6, 24.9, 26.8, 28.2, 30.4, 34.4, 52.0, 63.6, 73.2, 73.3, 83.6, 86.3, 88.7, 111.6, 168.2, 171.6 ppm; MS (+CI) 391 $[M + 1]^+$; M_r (+CI) 391.152 67 $[M + 1]^+$ (calcd for $C_{16}H_{27}N_2O_7S$ 391.153 90).

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-Propyl Sulfide (38). Using **36** (50 mg, 0.15 mmol) and propanethiol (34 mg, 0.45 mmol) afforded **38** as a mixture of diastereomers (∼4:1): yield, 44 mg (72%); mp 100−115 °C; R_f 0.60 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3415 (br), 3307 (br), 1687, 1381, 1046 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 0.97 (t, J = 7.5 Hz, 3 H), 1.36 (s, 3 H), 1.44 (s, 6 H), 1.52−1.68 (m, 2 H), 1.85−2.00 (m, 1 H), 2.05−2.24 (m, 3 H), 2.35−2.58 (m, 2 H), 3.10−3.20 (m, 1 H), 3.71 (d, J = 8.4 Hz, 1 H), 3.81 (dd, J = 8.7, 13.5 Hz, 1 H), 4.01 (dd, J = 8.4, 13.5 Hz, 1 H), 4.10 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 0.98 (t, J = 7.5 Hz, 3 H), 4.47 (d, J = 8.1 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer, 13.6, 23.8, 24.9, 26.8, 28.2, 30.4, 32.0, 35.1, 52.6, 63.5, 73.2, 83.6, 86.3, 88.7, 111.6, 168.1, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 167.1, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 419 $[M + 1]^+$; M_r (+CI) 419.184 14 $[M + 1]^+$ (calcd for $C_{18}H_{31}N_2O_7S$ 419.185 20).

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-Hexyl Sulfide (39). Using **36** (50 mg, 0.15 mmol) and hexanethiol (100 mg, 0.45 mmol) gave **39** as a mixture of diastereomers (∼5:1): yield, 43 mg (64%); mp 100−108 °C; R_f 0.60 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3414 (br), 3302 (br), 1686, 1409, 1046 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 0.90 (t, J = 6.9 Hz, 3 H), 1.25−1.48 (m, 15 H), 1.50−1.65 (m, 2 H), 1.85−2.00 (m, 1 H), 2.05−2.25 (m, 3 H), 2.40−2.55 (m, 2 H), 3.15 (d, J = 9.9 Hz, 1 H), 3.71 (d, J = 8.4 Hz, 1 H), 3.81 (dd, J = 7.8, 13.5 Hz, 1 H), 4.00 (dd, J = 8.7, 13.5 Hz, 1 H), 4.10 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.47 (d, J = 8.4 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer, 14.4, 23.6, 24.9, 26.8, 28.2, 29.5, 30.4, 30.6, 32.0, 32.6, 33.1, 52.6, 63.5, 73.2, 83.7, 86.3, 88.7, 111.6, 168.2, 171.6 ppm; MS (+CI) 461 $[M + 1]^+$; M_r (+CI) 462.231 52 $[M + 1]^+$ (calcd for $C_{21}H_{37}N_2O_7S$ 461.232 15).

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-2-Hydroxyethyl Sulfide (40). Using **36** (50 mg, 0.15 mmol) and 2-hydroxyethanethiol (35 mg, 0.45 mmol) afforded **40** as a mixture of diastereomers (∼2:1): yield, 28 mg (46%); mp 110−115 °C; R_f 0.36 (15% MeOH−$CHCl_3$); FT-IR (KBr) 3414 (br), 3314 (br), 1687, 1406, 1044 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3 H), 1.44 (s, 6 H), 1.85−2.28 (m, 4 H), 2.52−2.72 (m, 2 H), 3.17 (d, J = 10.8 Hz, 1 H), 3.62−3.72 (m, 3 H), 4.10 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.41 (dd, J = 11.7, 13.2, 1 H), 4.47 (d, J = 8.4 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.8, 28.2, 30.4, 32.3, 35.6, 52.9, 62.2, 63.6, 73.2, 83.6, 86.4, 88.8, 111.6, 168.1, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 50.4, 83.5, 89.0, 167.2, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 421 $[M + 1]^+$; M_r (+CI) 421.163 65 $[M + 1]^+$ (calcd for $C_{17}H_{29}N_2O_8S$ 421.164 46).

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-2-Aminoethyl Sulfide (41). Using **36** (5 mg, 0.015 mmol) and 2-aminoethanethiol (5.7 mg, 0.075 mmol) gave **41** as a mixture of diastereomers (∼1.2:1): yield, 6 mg (98%); mp 150−153 °C; R_f 0.60 (40% MeOH−$CHCl_3$); FT-IR (KBr) 3414 (br), 3305 (br), 1687, 1383, 1045 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.37 (s, 3 H), 1.45 (s, 3 H), 1.46 (s, 3 H), 1.85−2.30 (m, 4 H), 2.55−2.80 (m, 2 H), 2.96 (t, J = 6.3 Hz, 2 H), 3.12−3.32 (m, 1 H), 3.71 (d, J = 8.4 Hz, 1 H), 3.75−4.10 (m, 3 H), 4.47 (d, J = 8.4 Hz, 1 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.40 (dd, J = 11.4, 13.2 Hz, 1 H), 4.46 (d, J = 8.4 Hz, 1 H), the remaining peaks overlapped with nearby signals; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.8, 28.3, 29.9, 31.7, 33.2, 40.7, 50.2, 63.6, 73.2, 83.5, 86.4, 89.1, 111.7, 168.0, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.6, 40.6, 52.6, 83.6, 86.4, 88.8, 167.2, 173.6 ppm, the remaining peaks overlapped with nearby signals. The assignments for the ^1H and ^{13}C NMR signals for the two diastereomers may be interchanged due to the near equal amounts of both isomers present in the NMR sample; MS (+CI) 420 $[M + 1]^+$; M_r (+CI) 420.179 08 $[M + 1]^+$ (calcd for $C_{17}H_{30}N_3O_7S$ 420.180 45).

Dihydrobicyclomycin C(2′),C(3′)-Acetonide C(5a)-Phenyl Sulfide (42). Using **36** (100 mg, 0.29 mmol) and thiophenol (65 mg, 0.58 mmol) gave **42** as a mixture of diastereomers (∼3:1): yield, 78 mg (59%); mp 180−181 °C; R_f 0.68 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3427 (br), 3290 (br), 1699, 1380, 1046 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3 H), 1.45 (s, 6 H), 1.90−2.35 (m, 3 H), 2.52 (dd, J = 11.4, 13.8 Hz, 1 H), 3.70−3.85 (m, 3 H), 4.01 (dd, J = 8.4, 13.8 Hz, 1 H), 4.09 (s, 1 H), 4.45 (d, J = 8.4 Hz, 1 H), 7.15- 7.40 (m, 5 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.76 (dd, J = 11.7, 13.8 Hz, 1 H), 4.10 (s, 1 H), 4.46 (d, J = 8.4 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.8, 26.8, 28.2, 30.3, 33.5, 51.9, 63.6, 73.2, 73.3, 83.7, 86.3, 88.7, 111.6, 127.0, 129.9, 130.2, 137.3, 168.0, 171.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 89.0 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 453 $[M + 1]^+$; M_r (+CI) 452.161 45 $[M]^+$ (calcd for $C_{21}H_{28}N_2O_7S$ 452.161 72).

C(5a)-Morpholinodihydrobicyclomycin C(2′),C(3′)-Acetonide[6] (43). Using **36** (50 mg, 0.15 mmol) and morpholine (100 mg, 1.15 mmol) afforded **43** as a single diastereomer: yield, 25 mg (40%); mp 140−143 °C (lit.[6] mp135−140 °C); R_f 0.55 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3451 (br), 3321 (br), 1692, 1407, 1052 cm^{-1}; ^1H NMR (CD_3OD) δ 1.32 (s, 3 H) 1.35 (s, 3H), 1.46 (s, 3 H), 1.50−1.70 (m, 1 H), 1.80−2.00 (m, 1 H), 2.25−2.40 (m, 2 H), 2.42−2.55 (m, 2 H), 2.65−2.85 (m, 3 H), 3.65−3.80 (m, 5 H), 3.85−4.20 (m, 3 H), 4.48 (d, J = 8.4 Hz, 1 H); ^{13}C NMR (CD_3OD) 24.8, 26.8, 28.4, 31.9, 45.2, 54.2, 61.5, 66.0, 67.8, 73.3, 73.5, 85.5, 86.3, 89.3, 117.7, 165.8, 171.8 ppm; MS (+CI) 430 $[M + 1]^+$; M_r (+CI) 430.218 01 $[M + 1]^+$ (calcd for $C_{19}H_{32}N_3O_8$ 430.218 94).

C(5a)-Anilinodihydrobicyclomycin C(2′),C(3′)-Acetonide (44). Using **36** (100 mg, 0.29 mmol) and aniline (270 mg, 2.9 mmol) gave **44** as a mixture of diastereomers (∼9:1): yield, 52 mg (41%); mp 125−128 °C; R_f 0.60 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3395 (br), 3331 (br), 1687, 1383, 1045 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3 H, C(2′)-CH_3), 1.46 (s, 6 H, C(CH_3)$_2$), 1.80−2.10 (m, 2 H, C(4)H_2), 2.30−2.45 (m, 1 H, C(5)H), 2.94 (dd, J = 8.4, 13.5 Hz, 1 H, C(5a)*H*H′), 3.63 (dd, J = 4.5, 13.5 Hz, 1 H, C(5a)H*H*′), 3.71 (d, J = 8.4 Hz, 1 H, C(3′)*H*H′), 3.75−3.95 (m, 1 H, C(3)*H*H′), 4.00−4.10 (m, 2 H, C(3)H*H*′, C(1′)H), 4.46 (d, J = 8.4 Hz, 1 H, C(3′)H*H*′); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.11 (dd, J = 9.3, 13.5 Hz, 1 H, C(5a)*H*H′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR

(CD_3OD) for the major diastereomer, 24.8, 26.8, 28.2, 30.4, 44.6, 50.6, 64.2, 73.2, 73.4, 84.1, 86.3, 88.8, 111.7, 114.4, 118.3, 130.1, 149.6, 167.8, 171.9 ppm, no signal was detected for the minor diastereomer; MS (+CI) 435 $[M]^+$; M_r (+CI) 435.200 27 $[M]^+$ (calcd for $C_{21}H_{29}N_3O_7$ 435.200 55).

C(5a)-(6-Aminoquinolino)dihydrobicyclomycin C(2′),C-(3′)-Acetonide (45). Using **36** (20 mg, 0.06 mmol) and 6-aminoquinoline (17.3 mg, 0.12 mmol) afforded **45** as a single diastereomer: yield, 7.3 mg (26%); mp 140−145 °C; R_f 0.60 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3327 (br), 1697, 1382, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.46 (s, 6 H), 1.82−2.20 (m, 2 H), 2.40−2.50 (m, 1 H), 3.06 (dd, J = 9.6, 13.8 Hz, 1 H), 3.70−4.15 (m, 5 H), 4.46 (d, J = 8.7 Hz, 1 H), 6.85 (d, J = 2.1 Hz, 1 H), 7.22 (dd, J = 2.1, 9.1 Hz, 1 H), 7.36 (dd, J = 4.2, 8.4 Hz, 1 H), 7.72 (d, J = 9.1 Hz, 1 H), 8.09 (d, J = 8.4 Hz, 1 H), 8.44 (d, J = 4.2 Hz, 1 H); ^{13}C NMR (CD_3OD) 24.9, 26.8, 28.2, 30.3, 43.9, 50.5, 63.9, 73.2 (2 C), 83.8, 86.4, 88.8, 103.2, 111.7, 122.5, 124.0, 128.5, 132.2, 136.8, 142.0, 145.0, 148.5, 168.0, 171.9 ppm; MS (+CI) 487 $[M + 1]^+$; M_r (+CI) 487.219 35 $[M + 1]^+$ (calcd for $C_{24}H_{31}N_4O_7$ 487.219 28).

General Procedure for the Preparation of C(5a)-Substituted Dihydrobicyclomycins. To a 50% aqueous methanolic solution (1−2 mL) containing the C(5a)-substituted dihydrobicyclomycin C(2′),C(3′)-acetonide was added trifluoroacetic acid (1 drop), and the solution was stirred at either room temperature or 60 °C (1−2 h) and then the solvent was removed *in vacuo*. The residue was purified by either column chromatography (SiO_2, 15−20% MeOH−$CHCl_3$) or thin-layer chromatography (10−50% MeOH−$CHCl_3$) to give the desired product.

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

Dihydrobicyclomycin C(5a)-Methyl Sulfide[3,7] (18). Using **37** (20 mg, 0.052 mmol) gave **18** as a mixture of diastereomers (∼3:1): yield, 16 mg (89%); mp 125−128 °C (lit.[7] semisolid); R_f 0.25 (15% MeOH−$CHCl_3$); FT-IR (KBr) 3413 (br), 3270 (br), 1686, 1405, 1139, 1039 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H), 2.05−2.30 (m, 7 H), 3.06 (d, J = 11.7 Hz, 1 H), 3.51 (d, J = 11.4 Hz, 1 H), 3.67 (d, J = 11.4 Hz, 1 H), 3.70−4.10 (m, 3 H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.40 (dd, J = 11.9, 13.1 Hz, 1 H), 3.08 (d, J = 13.1 Hz, 1 H), the remaining peaks overlapped with nearby signals and were not detected; ^{13}C NMR (CD_3OD) for the major diastereomer, 15.6, 24.2, 29.9, 34.1, 51.9, 62.0, 68.5, 72.2, 78.1, 83.6, 89.4, 168.9, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.4, 32.9, 63.3, 72.3 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 351 $[M + 1]^+$; M_r (+CI) 351.121 38 $[M + 1]^+$ (calcd for $C_{13}H_{23}N_2O_7S$ 351.122 60).

Dihydrobicyclomycin C(5a)-Propyl Sulfide (19). Using **38** (35 mg, 0.084 mmol) afforded **19** as a mixture of diastereomers (∼4:1): yield, 26 mg (82%); mp 115−118 °C; R_f 0.30 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3415 (br), 3269 (br), 1687, 1405, 1049 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 0.97 (t, J = 7.2 Hz, 3 H, $SCH_2CH_2CH_3$), 1.32 (s, 3 H, C(2′)-CH_3), 1.50−1.70 (m, 2 H, $SCH_2CH_2CH_3$), 2.00−2.30 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.35−2.55 (m, 2 H, $SCH_2CH_2CH_3$), 3.11 (d, J = 11.4 Hz, 1 H, C(5a)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*′), 3.70−4.05 (m, 3 H, C(3)H_2, C(1′)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 0.98 (t, J = 7.2 Hz, 3 H, $SCH_2CH_2CH_3$), 3.13 (d, J = 13.8 Hz, 1 H, C(5a)H*H*′), the remaining peaks overlapped with nearby signals and were not detected and the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 13.7, 23.9, 24.2, 29.9, 31.7, 35.2, 52.5, 62.0, 68.5, 72.2, 78.1, 83.7, 89.3, 168.8, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 50.3, 63.3, 72.3, 83.7, 89.6, 167.6, 174.1 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 379 $[M + 1]^+$; M_r (+CI) 379.153 21 $[M + 1]^+$ (calcd for $C_{15}H_{27}N_2O_7S$ 379.153 90).

Dihydrobicyclomycin C(5a)-Hexyl Sulfide (20). Using **39** (35 mg, 0.076 mmol) gave **20** as a mixture of diastereomers (∼5:1): yield, 26 mg (81%); mp 100−105 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3427 (br), 3263 (br), 1698, 1403, 1037 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 0.90 (t, J = 6.9 Hz, 3 H, $S(CH_2)_5CH_3$), 1.20−1.45 (m, 9 H, SCH_2-$CH_2(CH_2)_3CH_3$, C(2′)CH_3), 1.50−1.65 (m, 2 H, $SCH_2CH_2(CH_2)_3$-CH_3), 2.05−2.35 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.40−2.60 (m, 2 H, $SCH_2(CH_2)_4CH_3$), 3.12 (d, J = 11.7 Hz, 1 H, C(5a)-H*H*′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.66 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.70−4.05 (m, 3 H, C(3)H_2, C(1′)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.14 (d, J = 12.3 Hz, 1 H, C(5a)H*H*′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 14.4, 23.6, 24.2, 29.5, 29.9, 30.6, 31.7, 32.6, 33.1, 52.5, 62.0, 68.5, 72.2, 78.1, 83.7, 89.3, 168.9, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 50.3, 63.3, 78.7, 83.5, 89.6, 174.1 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 421 $[M + 1]^+$; M_r (+CI) 421.200 13 $[M + 1]^+$ (calcd for $C_{18}H_{33}N_2O_7S$ 421.200 85).

Dihydrobicyclomycin C(5a)-2-Hydroxyethyl Sulfide (21). Using **40** (3.5 mg, 0.008 mmol) gave **21** as a mixture of diastereomers (∼2:1): yield, 3.0 mg (94%); mp 110−115 °C; R_f 0.30 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3426, 1686, 1406 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.31 (s, 3 H, C(2′)CH_3), 1.85−2.30 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.50−2.70 (m, 2 H, SCH_2CH_2OH), 3.13 (d, J = 12.6 Hz, 1 H, C(5a)H*H*′), 3.50 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.60−4.05 (m, 6 H, C(3′)H*H*′, SCH_2CH_2OH, C(3)H_2, C(1′)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.38 (dd, J = 11.3, 13.2 Hz, 1 H, C(5a)*H*H′), 3.16 (d, J = 11.3 Hz, 1 H, C(5a)H*H*′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 29.9, 31.9, 35.5, 52.8, 62.2, 63.3, 68.5, 72.1, 78.1, 83.6, 89.3, 168.8, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.5, 30.9, 35.4, 50.5, 62.0, 72.2, 83.5, 89.6, 167.6, 174.1 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 381 $[M + 1]^+$; M_r 381.134 13 $[M + 1]^+$ (calcd for $C_{14}H_{25}N_2O_8S$ 381.133 16).

Dihydrobicyclomycin C(5a)-2-Aminoethyl Sulfide (22). Using **41** (35 mg, 0.08 mmol) afforded **22** as a mixture of diastereomers (∼1.2:1): yield, 1 mg (19%); mp 140−145 °C; R_f 0.10 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3414 (br), 3274 (br), 1686, 1407 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 1.90−2.30 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.50−2.70 (m, 2 H, $SCH_2CH_2NH_2$), 2.79 (t, J = 6.3 Hz, 2 H, $SCH_2CH_2NH_2$), 3.13 (d, J = 12.0 Hz, 1 H, C(5a)H*H*′), 3.51 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.67 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.75−4.05 (m, 3 H, C(3)H_2, C(1′)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.36 (dd, J = 11.1, 13.2 Hz, 1 H, C(5a)*H*H′), 3.15 (d, J = 13.2 Hz, 1 H, C(5a)H*H*′), 3.55 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.71 (d, J = 11.4 Hz, 1 H, C(3′)-H*H*′), the remaining peaks overlapped with nearby signals, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.0, 31.4, 36.0, 41.6, 52.7, 63.3, 68.5, 72.2, 78.1, 83.7, 89.4, 168.7, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.3, 30.6, 50.4, 62.0, 72.3, 83.5, 89.6, 167.7, 174.1 ppm, the remaining peaks overlapped with nearby signals; the assignments for the ^1H and ^{13}C NMR signals for the two diastereomers may be interchanged due to the near equal amounts of both isomers present in the NMR sample; MS (+CI) 380 $[M + 1]^+$; M_r (+CI) 380.149 24 $[M + 1]^+$ (calcd for $C_{14}H_{26}N_3O_7S$ 380.149 15).

Dihydrobicyclomycin C(5a)-Phenyl Sulfide (23). Using **42** (30 mg, 0.066 mmol) gave **23** as a mixture of diastereomers (∼3:1): yield, 20 mg (74%); mp 125−128 °C; R_f 0.26 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3407 (br), 3264 (br), 1687, 1405, 1027 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 2.05−2.35 (m, 3 H, C(4)H_2, C(5)H), 2.56 (dd, J = 12.0, 14.1 Hz, 1 H, C(5a)*H*H′), 3.45−4.07 (m, 6 H, C(5a)H*H*′, C(3′)H_2, C(3)H_2, C(1′)H), 7.10−7.40 (m, 5 H, C_6H_5); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.73 (dd, J = 11.4, 13.5 Hz, 1 H, C(5a)*H*H′), the remaining peaks overlapped with nearby signals and were not detected, the ^1H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 29.9, 33.1, 51.8, 63.1, 68.5, 72.3, 78.1, 83.7, 89.4, 126.9, 129.9, 130.0, 137.3,

168.8, 172.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.5, 32.2, 62.0, 72.4, 83.6, 89.6, 167.8, 174.1 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 413 $[M + 1]^+$; M_r (+CI) 413.137 95 $[M + 1]^+$ (calcd for $C_{18}H_{25}N_2O_7S$ 413.138 25).

C(5a)-Morpholinodihydrobicyclomycin[6] (26). Using **43** (10 mg, 0.023 mmol) afforded **26** as a single diastereomer: yield, 6 mg (65%); mp 135−139 °C (lit.[6] semisolid); R_f 0.10 (10% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.32 (s, 3 H), 1.55−1.72 (m, 1 H), 1.80−1.95 (m, 1 H), 2.25−2.40 (m, 2 H), 2.42−2.58 (m, 2 H), 2.63−2.83 (m, 3 H), 3.56 (d, J = 11.4 Hz, 1 H), 3.60−3.80 (m, 5 H), 3.85 (dd, J = 8.7, 13.8 Hz, 1 H), 4.00−4.10 (m, 2 H); ^{13}C NMR (CD_3OD) 24.2, 31.9, 45.4, 54.3, 61.4, 65.4, 67.8, 68.5, 72.4, 78.1, 85.5, 90.2, 166.3, 171.2 ppm.

C(5a)-Anilinodihydrobicyclomycin (27). Using **44** (30 mg, 0.07 mmol) afforded **27** as a mixture of diastereomers (∼9:1): yield, 20 mg (74%); mp 145−148 °C; R_f 0.20 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3402 (br), 1686, 1406, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 1.85−2.10 (m, 2 H, C(4)H_2), 2.30−2.45 (m, 1 H, C(5)H), 2.96 (dd, J = 9.3, 13.8 Hz, 1 H, C(5a)*H*H′), 3.50−4.05 (m, 6 H, C(3)H_2, C(3′)H_2, C(5a)H*H*′, C(1′)H), 6.55−6.70 (m, 2 H, PhH_2), 7.02−7.15 (m, 3 H, PhH_3); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.08 (dd, J = 9.0, 13.8 Hz, 1 H, C(5a)*H*H′), the remaining peaks overlapped with nearby signals and were not detected, the ^{1}H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.0, 44.2, 50.6, 62.7, 68.6, 72.4, 78.1, 84.0, 89.5, 114.3, 118.2, 130.0, 149.7, 168.6, 172.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 44.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 396 $[M + 1]^+$; M_r (+CI) 396.176 61 $[M + 1]^+$ (calcd for $C_{18}H_{26}N_3O_7$ 396.177 08).

C(5a)-(6-Aminoquinolino)dihydrobicyclomycin (28). Using **45** (7.3 mg, 0.015 mmol) gave **28** as a single diastereomer: yield, 6.6 mg (97%); mp 168−170 °C; R_f 0.12 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3383 (br), 3256 (br), 1686, 1384, 1140, 1044 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H, C(2′)CH_3), 1.90−2.15 (m, 2 H, C(4)H_2), 2.40−2.55 (m, 1 H, C(5)H), 3.07 (dd, J = 9.6, 13.8 Hz, 1 H, C(5a)*H*H′), 3.50−3.90 (m, 4 H, C(3′)H_2, C(3)*H*H′, C(5a)H*H*′), 4.00−4.15 (m, 2 H, C(3)H*H*′, C(1′)H), 6.84 (d, J = 2.6 Hz, 1 H, C(5″)H), 7.19 (dd, J = 2.6, 9.1 Hz, 1 H, C(7″)H), 7.32 (dd, J = 4.2, 8.4 Hz, 1 H, C(3″)H), 7.70 (d, J = 9.1 Hz, 1 H, C(8″)H), 8.02 (d, J = 8.4 Hz, 1 H, C(4″)H), 8.41 (d, J = 4.2 Hz, 1 H, C(2″)H), the ^{1}H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) 24.2, 29.9, 43.5, 50.4, 62.5, 68.5, 72.3, 78.1, 83.8, 89.4, 103.4, 122.9, 123.4, 129.4, 132.1, 135.9, 143.2, 145.8, 148.3, 168.6, 172.4 ppm; MS (+CI) 447 $[M + 1]^+$; M_r (+CI) 447.187 54 $[M + 1]^+$ (calcd for $C_{21}H_{27}N_4O_7$ 447.187 98).

Preparation of Dihydrobicyclomycin C(5a)-Methyl Sulfoxide (24). To a 50% aqueous methanolic solution (1 mL) of **18** (10 mg, 0.027 mmol) was added 30% H_2O_2 (31 μL, 0.27 mmol). The solution was stirred at room temperature (30 min), and then the solvent was removed *in vacuo* to give **24** as a mixture of diastereomers (∼1:1): yield, 11 mg (∼100%); mp 158−163 °C; R_f 0.10 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3409 (br), 3276 (br), 1687, 1406, 1138, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.90−2.25 (m, 2 H), 2.50−2.80 (m, 5 H), 3.30−3.40 (m, 1 H), 3.53, 3.55 (2d, J = 11.4 Hz, 1 H), 3.71 (d, J = 11.4 Hz, 1 H), 3.75−3.90 (m, 1 H), 4.00−4.13 (m, 2 H); ^{13}C NMR (CD_3OD) 23.9, 31.3, 33.2, 38.1, 39.1, 46.1, 47.6, 54.6, 56.1, 62.6, 63.2, 68.2, 71.9, 72.0, 77.9, 82.8, 83.3, 89.3, 89.4, 167.4, 167.7, 171.3, 171.4 ppm; MS (+CI) 367 $[M + 1]^+$; M_r (+CI) 367.116 99 $[M + 1]^+$ (calcd for $C_{13}H_{23}N_2O_8S$ 367.117 51).

Preparation of Dihydrobicyclomycin C(5a)-Methyl Sulfone (25). A 30% aqueous H_2O_2 solution (0.5 mL) of **18** (20 mg, 0.055 mmol) was stirred at room temperature (5 h). The solvent was removed *in vacuo*, and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to give **25** as a mixture of diastereomers (∼3:1): yield, 12 mg (58%); mp 148−152 °C; R_f 0.40 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 3263 (br), 1688, 1406, 1132 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 1.95−2.35 (m, 2 H, C(4)H_2), 2.60−2.75 (m, 1 H, C(5)H), 2.90−3.05 (m, 4 H, C(5a)*H*H′, S(O)$_2CH_3$), 3.52 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.69 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), 3.76 (d, J = 16.2 Hz, 1 H, C(5a)H*H*′), 3.80−4.10 (m, 3 H, C(3)H_2, C(1′)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.54 (d, J = 11.4 Hz, 1 H, C(3′)*H*H′), 3.70 (d, J = 11.4 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected, the ^{1}H NMR assignments were confirmed by COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 32.3, 41.6, 46.7, 54.5, 63.0, 68.5, 72.2, 78.2, 83.0, 89.5, 171.6 ppm, the signal for the C(9) peak was not detected; ^{13}C NMR (CD_3OD) for the minor diastereomer, 32.7, 45.7, 53.5, 62.6, 82.9 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 381 $[M - 1]^-$; M_r (−CI) 381.097 89 $[M - 1]^-$ (calcd for $C_{13}H_{23}N_2O_8S$ 381.096 78).

Preparation of Bicyclomycin C(5),C(5a)-Epoxide[16] (29). To an aqueous solution (1 mL) of **1** (10 mg, 0.033 mmol) was added a catalytic amount of $Na_2WO_4{\cdot}2H_2O$ (1 mg) and 30% H_2O_2 (5 μL). The pH was adjusted to 6.0 with dilute aqueous trifluoroacetic acid. The solution was stirred at room temperature (18 h), and the solvent was removed *in vacuo*. The residue was purified by column chromatography (SiO_2, 20% MeOH−$CHCl_3$) to afford **29** as a single diastereomer: yield, 7 mg (67%); mp 175−183 °C (lit.[16] mp 194−197 °C); R_f 0.30 (20% CH_3OH−$CHCl_3$); FT-IR (KBr) 3405 (br), 3279 (br), 1687, 1405, 1082 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 1.91 (dd, J = 8.1, 16.5 Hz, 1 H), 2.14 (dd, J = 9.0, 16.5 Hz, 1 H), 2.51 (d, J = 5.4 Hz, 1 H), 3.20 (d, J = 5.4 Hz, 1 H), 3.55 (d, J = 11.4 Hz, 1 H), 3.71 (d, J = 11.4 Hz, 1 H), 3.87 (dd, J = 8.1, 13.8 Hz, 1 H), 4.07−4.15 (m, 2 H); ^{13}C NMR (CD_3OD) 24.2, 36.2, 50.3, 62.6, 64.2, 68.5, 72.3, 78.2, 80.6, 90.1, 167.9, 170.9 ppm; MS (+CI) 319 $[M + 1]^+$; M_r (+CI) 319.114 17 $[M + 1]^+$ (calcd for $C_{12}H_{19}N_2O_8$ 319.114 14).

Preparation of Dihydrobicyclomycin-5-ol (30). To a methanolic solution (1 mL) of **29** (6 mg, 0.012 mmol) was added a catalytic amount of PtO_2 (1 mg), and the reaction was stirred at room temperature under an atmosphere of H_2 (2 h). The reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to afford **30** as a single diastereomer: yield, 6 mg (99%); mp 115−120 °C; R_f 0.20 (10% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.29 (s, 3 H), 1.33 (s, 3 H), 1.88−1.97 (m, 2 H), 3.52 (d, J = 11.4 Hz, 1 H), 3.65−3.75 (m, 2 H), 4.02 (s, 1 H), 4.05−4.16 (m, 1 H); ^{13}C NMR (CD_3OD) 23.2, 24.2, 42.1, 61.1, 68.5, 72.3, 78.1, 80.3, 84.7, 89.7, 172.3 ppm, the remaining signal was not detected; MS (+CI) 321 $[M + 1]^+$; M_r (+CI) 321.129 39 $[M + 1]^+$ (calcd for $C_{12}H_{21}N_2O_8$ 321.129 79).

Preparation of Norbicyclomycin 5-Mesylate C(2′),C-(3′)-Acetonide (47). To an anhydrous pyridine solution (3 mL) of **46** (50 mg, 0.15 mmol) was added methanesulfonyl chloride (30 μL, 0.39 mmol), and then the solution was stirred at room temperature (1.5 h). The solvent was removed *in vacuo*, and the residue was purified by flash chromatography on SiO_2 (5% MeOH−$CHCl_3$) to provide a pale yellow solid: yield, 45 mg (73%); mp 161−163 °C; R_f 0.46 (10% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.37 (s, 3 H), 1.45 (s, 6 H), 2.10−2.45 (m, 2 H), 3.22 (s, 3 H), 3.72 (d, J = 8.4 Hz, 1 H), 3.80−3.91 (m, 1 H), 4.07−4.15 (m, 1 H), 4.14 (s, 1 H), 4.44 (d, J = 8.4 Hz, 1 H), 4.80−4.86 (m, 1 H); ^{13}C NMR (CD_3OD) 25.2, 26.8, 28.1, 34.3, 38.8, 60.0, 72.6, 73.0, 82.3, 86.5, 87.1, 89.0, 111.6, 169.3 ppm; MS (+CI) 425 $[M + 1]^+$; M_r (+CI) 425.123 86 $[M + 1]^+$ (calcd for $C_{15}H_{25}N_2O_{10}$ 425.122 99).

Preparation of Norbicyclomycin 5-Benzylsulfonate C(2′),C(3′)-Acetonide (48). Using the preceding procedure and utilizing **46** (40 mg, 0.12 mmol) and α-toluenesulfonyl chloride (59 mg, 0.31 mmol) gave **48** as a pale yellow solid: yield, 35 mg (58%); mp 139−141 °C; R_f 0.50 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3430, 3297, 2988, 1696, 1047 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.38 (s, 3 H), 1.46 (s, 6 H), 2.00−2.30 (m, 2 H), 3.72 (d, J = 8.4 Hz, 1 H), 3.73−3.85 (m, 1 H), 3.92−4.12 (m, 1 H), 4.15 (s, 1 H), 4.44 (d, J = 8.4 Hz, 1 H), 4.64 (d, J = 14.1 Hz, 1 H), 4.74 (d, J = 14.1 Hz, 1 H), 4.80−5.02 (m, 1 H), 7.32−7.56 (m, 5 H); ^{13}C NMR (CD_3OD) 25.2, 26.9, 28.1, 34.1, 58.2, 60.0, 72.7, 73.0 (2 C), 82.3, 86.5, 86.7, 89.1, 111.6, 129.7, 129.8, 132.1, 169.3 (2 C) ppm; MS (+CI) 501 $[M + 1]^+$; M_r (+CI) 501.153 64 $[M + 1]^+$ (calcd for $C_{21}H_{29}N_2O_{10}$ 501.154 29).

Preparation of Norbicyclomycin 5-Mesylate (33). An aqueous methanolic solution (1:1, 5 mL) of **47** (30 mg, 0.071

mmol) was acidified ("pH" 1.9) with dilute aqueous sulfuric acid (0.2 N) and then heated at 60 °C (0.5 h). The solution was neutralized with an aqueous saturated $NaHCO_3$ solution. The solvent was removed *in vacuo*, and the residue was purified by preparative TLC (20% MeOH−$CHCl_3$) to give **33** as a white solid: yield, 15 mg (55%); mp 161−165 °C; R_f 0.52 (20% MeOH−$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.33 (s, 3 H), 2.10−2.35 (m, 2 H), 3.22 (s, 3 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.65 (d, J = 11.4 Hz, 1 H), 3.78−3.88 (m, 1 H), 4.04 (s, 1 H), 4.05−4.20 (m, 1 H), 4.80−4.85 (m, 1 H); ^{13}C NMR (CD_3OD) 24.2, 34.4, 38.8, 58.9, 68.4, 72.1, 78.2, 82.3, 87.4, 89.3, 169.7 (2 C) ppm; MS (+CI) 384 $[M]^+$; M_r (+CI) 385.091 69 $[M + 1]^+$ (calcd for $C_{12}H_{21}N_2O_{10}S$ 385.091 69).

Preparation of Norbicyclomycin 5-Benzylsulfonate (34). Using the preceding procedure and **48** (34 mg, 0.068 mmol) gave **34** as a pale yellow solid: yield, 17 mg (54%); mp 153−155 °C; R_f 0.54 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3474, 2988, 1696, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.34 (s, 3 H), 2.00−2.30 (m, 2 H), 3.50 (d, J = 11.4 Hz, 1 H), 3.64 (d, J = 11.4 Hz, 1 H), 3.70−3.80 (m, 1 H), 4.04 (s, 1 H), 4.00−4.10 (m, 1 H), 4.62 (d, J = 14.1 Hz, 1 H), 4.75 (d, J = 14.1 Hz, 1 H), 4.80−5.02 (m, 1 H), 7.30−7.60 (m, 5 H); ^{13}C NMR (CD_3OD) 24.2, 34.2, 58.2, 58.9, 68.4, 72.2, 78.2, 82.3, 87.1, 89.4, 129.7, 129.8, 132.1, 169.7 (2 C) ppm; MS (+CI) 461 $[M + 1]^+$; M_r (+CI) 461.122 46 $[M + 1]^+$ (calcd for $C_{18}H_{25}N_2O_{10}S$ 461.122 99).

X-ray Crystallographic Study of C(5a)-Anilinodihydrobicyclomycin C(2′),C(3′)-Acetonide (44).[28] Crystals of **44** belong to the space group $P2_12_12_1$ (orthorhombic) with a = 6.921 (1) Å, b = 10.979 (2) Å, c = 29.915 (7) Å, V = 2273 Å^3, D_{calcd} = 1.37 g cm^{-3}, and Z = 4. Data were collected at −50 °C, and the structure was refined to R_f = 0.071, R_w = 0.059 for 1532 reflections with $I > 3\sigma(I)$.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds prepared in this study (78 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(28) The authors have deposited X-ray crystallographic data, a description of the structure determination, and tables of atomic coordinates and isotropic thermal parameters, bond lengths and angles, anisotropic thermal parameters, and refined and calculated hydrogen atom coordinates with the Cambridge Crystallographic Data Centre. The data can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.