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Bicyclomycin Fluorescent Probes: Synthesis and Biochemical, Biophysical, and Biological Properties

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Bicyclomycin (**1**) is a commercially available antibiotic whose primary site of action in *Escherichia coli* is the transcription termination factor rho. Key aspects of the **1**·rho interaction—K_d, stoichiometry for **1**·rho binding, and whether **1** and ATP binding induce conformational changes in rho—remain unknown. In this study, the design, synthesis, and characterization of a series of bicyclomycin fluorescent probes (BFP) constructed to sense the **1**·rho interaction are described and their use documented. We show that dihydrobicyclomycins with medium-to-large C(5a)-substituents afforded excellent inhibitory activities exceeding those of **1** in the poly(C)-dependent ATPase assay. The utility of BFP in bicyclomycin−rho binding studies was documented through the use of 5a-(phenazin-2-ylmethylsulfanyl)dihydrobicyclomycin (**15**). Excitation (290 nm) of W381 in wild-type rho in the presence of **15** and ATP led to fluorescence resonance energy transfer (FRET) and gave a K_d (**15**) of 9.9 μM. Using ADP in place of ATP or excluding nucleotide did not result in energy transfer, which suggests that ATP binding induced a conformational change in rho. FRET measurements provided an approximate weighted average distance (23 Å) between W381 and **15** in the presence of bound ATP. The K_d value for **15**·rho was correlated with ATP binding at the 3 tight ATP binding (K_d(ATP) = 95 nM) sites in wild-type rho.

The *Escherichia coli* transcription termination factor rho is a hexameric protein consisting of identical 47-kDa protomers.[1] Rho plays an integral role in processing gene products in *E. coli* and many other Gram-negative organisms and without rho cell death results.[2−5]

Rho binds to specific *rut* sites on newly synthesized RNA and then tracks toward the stalled polymerase powered by ATP hydrolysis.[1] Interaction of rho with the polymerase leads to mRNA release and transcript termination. Rho has resisted crystallization, and thus, detailed structural information is lacking. Rho shows significant homology with F_1-ATP synthase, another hexameric protein, permitting a structural model of rho to be generated from sequence comparison with the β-subunit of F_1-ATP synthase and the X-ray crystallographic structures of bovine F_1-ATP synthase.[6−10] These models are in agreement with experimental results that have identified the ATP[11,12] and the RNA tracking sites.[13,14]

The commercially available antibiotic bicyclomycin (**1**)[15] is the only known natural product inhibitor of the *E. coli* rho transcription termination factor.[16] We have provided information concerning the mechanism of the **1**·rho interaction.[17−19] Kinetic studies showed that **1** is a noncompetitive inhibitor with respect to ATP[17] and a mixed inhibitor with respect to short ribonucleotides (i.e., ribo$(C)_{10}$)[18] believed to mimic the RNA that rho traverses.

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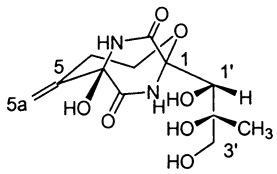
Use of bicyclomycin affinity labels[10,20,21] and rho mutational studies[9,22] permitted us[23] and the Richardson group[22] to project the site of **1** binding in rho. We place bicyclomycin binding between the B and C helices with the C(1)-triol group in **1** directed toward the outside of rho and the terminal C(5)−C(5a)-exomethylene unit pointed toward the ATP terminal γ-phosphate and the P-loop (residues 175−183) located near the central hole.



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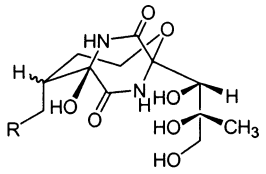

BFP 2-16

Our findings that bicyclomycin disrupts rho-mediated ATP hydrolysis and rho-RNA binding[17−19] have contributed to a working model for **1** inhibition of the rho transcription termination process.[10] However, much remains to be learned before this pathway can be verified. For example, we know the I_{50} and K_i values for **1** when rho is stimulated under in vitro conditions[17] but do not know the inherent rho binding constant(s) (K_d) for **1**. Inactivation of one to two subunits in hexameric rho with bicyclomycin affinity labels is sufficient to halt ATP hydrolysis,[21] but we do not know if **1** binds equally to all six subunits or selectively to a subset of the six. ATP, by comparison, binds tightly to only three of the six protomers,[24−27] and a similar situation exists in F_1-ATP synthase.[28] Finally, RNA binding to rho induces a conformational change in rho seen by altered trypsin digestion, but this technique is too insensitive to monitor conformational changes upon binding **1** or ATP.[18,29,30]

In this study, we report the design, synthesis, and characterization of a series of bicyclomycin fluorescent probes (BFP) constructed to sense the interaction of **1** with rho. We document that BFP possess useful biochemical and photophysical properties amenable to mechanistic investigations and demonstrate the utility of one of these compounds.

Results and Discussion

Choice of Compounds. There are no known BFP, so we selectively incorporated a fluorescent tag to **1**. Previous bicyclomycin structure−activity relationship (SAR) studies demonstrated that modification of either the **1** C(1)-triol[31] or the [4.2.2]piperazinedione ring system[32] led to derivatives with little or no inhibitory activity in rho functional assays. Correspondingly, placement of small- and medium-size substituents at the C(5a)-position in **1** provided compounds that inhibited rho-dependent hydrolysis of ATP at levels that matched **1**.[23,33] Accordingly, we decided to incorporate a fluorophore at the C(5a)-exomethylene position in **1** and to link the fluorophore moiety to **1** through a sulfur atom. Our choice of sulfur was governed by the ease with which thiols add to the C(5a) site of bicyclomycin derivatives to give the corresponding C(5a)-substituted dihydobicyclomycins and the excellent inhibitory activities found for select C(5a)-sulfur substituted dihydrobicyclomycins (see ref 33 for more details). We began with 5a-phenylsulfanyldihydrobicyclomycin (**2**), a compound we previously reported[33] that functions as an efficient rho inhibitor ($I_{50} = 75\ \mu$M), and progressively increased the size of the appended aromatic ring, changed the site of aromatic substitution, and altered the ring composition. We knew from the outset that several of the compounds slated for synthesis and evaluation would not have photophysical properties suitable for biochemical measurements, but we were uncertain if C(5a)-substituents larger than a six-membered ring would efficiently bind to rho and retain sufficient solubility in aqueous buffer solutions.

Two classes of compounds were prepared. The first (Table 1, group A, compounds **2**−**10**) contained a thiol directly attached to the aromatic unit. The second set (Table 1, group B, compounds **11**−**16**) contained a methylene spacer placed between the thiol unit and the aromatic moiety. Both series included monocyclic (**2**−**6**, **11**), bicyclic (**7**−**9**, **12**, **13**), and tricyclic (**10**, **14**−**16**) C(5a)-substituents. We included in these series dihydrobicyclomycin C(5a)-moieties expected either to undergo excitation and fluorescence emission or to serve as probes in FRET experiments.[34,35]

Synthesis. The thiol-containing aromatic substituent was introduced by Michael addition[33] to bicyclomycin 2′,3′-acetonide (**17**)[36] (Scheme 1). The Michael acceptor was the enone formed upon hemiaminal ring opening at C(6) of **17** under basic conditions. The reactions proceeded in moderate yields (46−73%) to give diastereomeric mixtures (∼3:1) of the C(5)−C(5a)-dihydrobicyclomycin adducts (**18**−**31**). The diastereomeric mixtures were not readily separable and were used directly in the functional assays, as described in Biochemical and Biological Properties and BFP−Rho Binding. We observed higher yields for group B BFP than for group A derivatives, a finding we expected given the likely increased nucleophilicity for the thiolate anions used in these syntheses.[37] Subsequent

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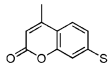
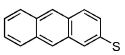
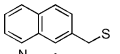
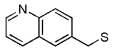
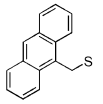
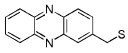
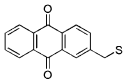
TABLE 1. Spectral, Biochemical and Biological Properties for BFP 2−16

		^{1}H NMR[a]		^{13}C NMR[b]	UV[c]	Fluorescence[d]			Inhibitory[e]	
Cpd No.	R	C(5a)*H*H′	C(5a)H*H*′	C(5a)	λ_{max} (nm) (ε, M^{-1} cm^{-1})	$\lambda_{max,exc}$[f] (nm)	$\lambda_{max,em}$[g] (nm)	Φ[h]	I_{50}[i] (μM) (Bcm)[k]	MIC[j] (mg/mL) (Bcm)[k]
Group A:										
2		2.56 (dd)[l] *2.73 (dd)*[l]	3.45–4.07 (m)[l]	29.9[l] *30.5*[l]	nd[m]	nd	nd	nd	75 (60)[l]	>32 (0.32)[l]
3		2.65 (dd) *2.79 (dd)*	3.70–4.11 (m)	30.5 *30.9*	263 (1.39×10^4)	nf[n]	nf	nf	55 (60)	16 (0.89)
4		2.97 (dd) *3.12 (dd)*	3.65–4.14 (m)	30.3 *30.8*	288 (4.91×10^3)	nf	nf	nf	110 (60)	>32 (0.89)
5		2.46 (dd) *2.66 (dd)*	3.43 (d)	29.9 *30.5*	298 (7.93×10^2)	nf	nf	nf	>400 (60)	>32 (0.89)
6		2.53 (dd) *2.72 (dd)*	3.60–4.06 (m)	29.8 *30.4*	255 (9.76×10^3)	nf	nf	nf	100 (60)	>32 (0.89)
7		2.69 (dd) *2.86 (dd)*	3.71–4.06 (m)	30.2 *30.7*	301 (6.75×10^3)	301	384	0.07	110 (60)	>32 (0.89)
8		2.66 (dd) *2.86 (dd)*	3.64–4.09 (m)	30.0 *30.5*	283 (5.99×10^3)	285	383	0.03	45 (60)	>32 (0.89)
9		2.66 (dd) *2.84 (dd)*	3.65–4.10 (m)	30.3 *30.7*	333 (1.69×10^4)	339	426	0.49	75 (60)	>32 (0.24)
10		2.71 (dd) *2.90 (dd)*	3.63–4.13 (m)	30.2 *30.7*	257 (1.76×10^4) 362 (1.52×10^3) 378 (1.83×10^3) 403 (2.23×10^3)	257 360 383	444	0.19	50 (60)	>32 (0.24)
Group B:										
11		1.90–2.27 (m) *2.28 (dd)*	3.13 (d) *3.10 (d)*	30.1 *30.4*	nm[o]	nf	nf	nf	75 (60)	>32 (0.24)
12		1.87–2.35 (m)	3.15 (d)	30.1 *30.2*	276 (4.34×10^3)	278	336	0.07	25 (55)	>32 (0.24)
13		1.90–2.32 (m)	3.11 (d)	30.1 *30.7*	319 (5.38×10^3)	nf	nf	nf	35 (55)	>32 (0.24)
14		2.33–2.54 (m)	3.58-3.95 (m)	28.9 *28.7*	ps[p]	ps	ps	ps	ps	>32 (0.24)
15		1.90–2.41 (m)	3.12 (d)	30.2 *30.4*	377 (1.76×10^4)	nf	nf	nf	25 (55)	>32 (0.24)
16		1.98–2.41 (m)	3.10 (d)	30.2 *30.8*	261 (2.99×10^4) 337 (4.30×10^3)	nf	nf	nf	55 (55)	>32 (0.24)

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to TMS, followed by the multiplicity of the signal. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD (4:1 CD_3OD−DMSO-d_6 was used for **14**). The entries 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 TMS. All spectra were recorded at 75 MHz, and the solvent used was CD_3OD (DMSO-d_6 was used for **14**). The entries in italics correspond to the signals detected for the minor diastereomer observed in the product mixture. [c] The number in each entry corresponds to an emission maxima wavelength. The value in parentheses corresponds to the molar extinction coefficient at that maxima. Measurements were performed in water at 25 °C. [d] Fluorescent measurements were performed in water at 25 °C. [e] Inhibitory properties, poly(C)-dependent ATPase assay[42] and filter disk assay.[43] [f] Wavelength of fluorescence excitation (exc) maximum. [g] Wavelength of fluorescence emission (em) maximum. [h] Quantum yield, determined using tryptophan or quinine sulfate as standards.[35] [i] Inhibitory activity measured using the rho poly (C)-dependent ATPase assay.[42] 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. [j] MIC value is the minimum inhibitory concentration of the compound determined from duplicate tests using W3550 *E. coli* in a filter disk assay.[43] The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses. [k] Bcm = bicyclomycin. [l] Value is from Park et al.[33] [m] Value not determined. [n] Compound is not fluorescent. [o] Not measured. [p] Partially soluble in water; value not determined.

TFA removal of the 2′,3′-acetonide group in **18**−**31** provided the desired **3**−**16**, respectively, in 55−99% yields. In most cases, we isolated and characterized the intermediate dihydrobicyclomycin acetonide, but for **18**, **19**, **24**, **25**, **29**, and **31** we found it more expeditious to directly deprotect the crude acetonide to give **3**, **4**, **9**, **10**, **14**, and **16**, respectively.

Two general procedures were used for the Michael addition step. We either added thiol[38] directly to **17** (Scheme 1, method 1) or mixed the corresponding thioacetate derivative with **17** (Scheme 1, method 2) knowing

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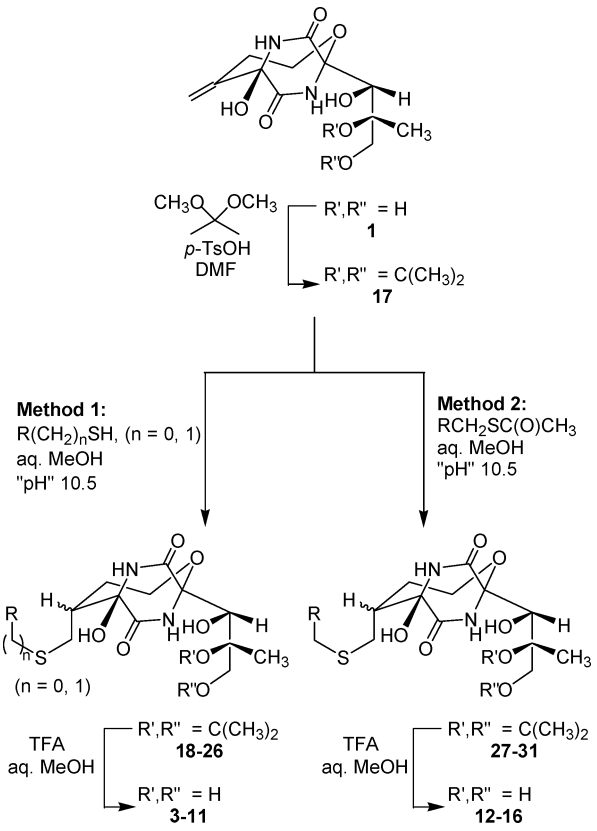
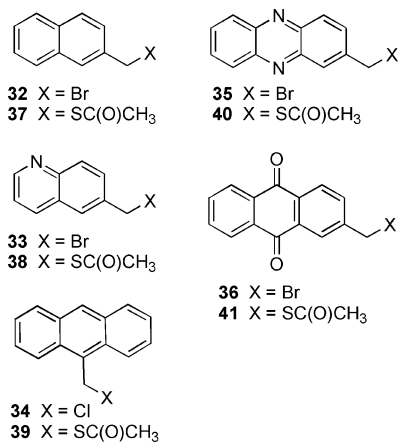
SCHEME 1. Preparation of BFP 2−16



that the thioacetyl unit would be removed under the basic conditions[39] employed in the Michael addition reaction. The arylmethylthioacetates **37**−**41** were prepared from the corresponding halides[40] by displacement with potassium thioacetate in acetone.[41]



Characterization of BFP. Satisfactory spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) were obtained for all new compounds. Diagnostic signals for the diastereotopic 5a-methylene protons (δ 1.87−3.12, 3.10−4.14) and 5a-carbon (δ 28.7−30.8) were observed in the ^{1}H and ^{13}C NMR spectra, respectively, documenting sulfur substitution at this site[33] (Table 1).

Biochemical and Biological Properties: Structure−Activity Trends. Dihydrobicyclomycin derivatives **2**−**16** were evaluated in the rho poly(C)-dependent ATPase assay (Table 1).[42] This test has been shown to be a reliable assay and has permitted the identification of bicyclomycin derivatives that inhibit rho.[23,31−33] Each compound was evaluated in duplicate assays using His-tagged wild-type rho[9] and compared with a concurrently tested **1** sample.

We found that many of the compounds exhibited inhibitory activity comparable with or better than **1**. The most active compounds were **12**, **13**, and **15**, which were approximately twice as potent as **1**.[17] These compounds are among the most effective bicyclomycin inhibitors in the rho poly(C)-dependent ATPase assay reported to date. The number of compounds in this study (15) did not permit us to deduce definitive SAR patterns. Nonetheless, the following may provide useful guidelines for future studies: (1) The size of the C(5a)-aromatic substituent did not affect the dihydrobicyclomycin inhibitory activity in the poly(C)-dependent ATPase assay. In series A, we observed few differences in activity when proceeding from bicyclomycin derivatives with monocyclic to bicyclic to tricyclic ring substituents (i.e., **2**, **8**, and **10**). In series B, excellent activity was also observed for all compounds except the sparingly soluble **14**, irrespective of the size of the ring system. Interestingly, the data set did not define the maximum size of the C(5a)-substituent that still retained potent inhibitory activity. This is an interesting topological issue since the C(5a)-substituent is directed toward the approximate 40 Å wide hole within the rho toroid, the site of RNA translocation. We will explore this aspect in future studies but indicate here that larger C(5a)-substituents will require the use of appended hydrophilic groups to solubilize the bicyclomycin derivative in water. (2) In select cases, inclusion of heteroatoms (O, N) within the aromatic ring system of the C(5a)-substituent did not alter the inhibitory properties of the bicyclomycin derivative. For example, we found that **2** (I_{50} = 75 μM) and **3** (I_{50} = 55 μM) displayed comparable inhibitory activities. Similarly, **12** (I_{50} = 25 μM) and **13** (I_{50} = 35 μM) were both effective rho inhibitors. (3) The aromatic ring site of attachment to the C(5a)-dihydrobicyclomycin may affect rho inhibition. Diminished activities were found when the point of attachment was near an aromatic fusion site (i.e., I_{50} value: **7**, 110 μM) compared with a peripheral aromatic locus (i.e., I_{50} values (μM): **8**, 45; **12**, 25; **13**, 35; **15**, 25; **16**, 55). This finding may reflect the structural requirements for the C(5a)-substituent for binding to rho. Together, these findings indicated that medium-to-large C(5a)-substituted dihydrobicyclomycins serve as excellent inhibitors of rho poly(C)-dependent ATPase activity and that the site of aromatic substitution is more important than the ring size and composition.

The antimicrobial minimal inhibitory concentration (MIC) values of dihydrobicyclomycins **2**−**16** were determined against W3350 *E. coli* in the filter disk assay[43]

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(Table 1). None of the compounds exhibited significant activity (≥16 mg/mL). Similar findings have been observed for other C(5a)-substituted dihydrobicyclomycins.[33]

Photophysical Properties. The UV−vis and fluorescent properties of **3**−**16** were assessed (Table 1). We did not resynthesize and evaluate **2** since **6** did not exhibit useful photophysical properties amenable to fluorescence-based studies. As expected, we observed in the UV−vis spectra for **3**−**16** a progressive increase in the λ_{max} for the highest wavelength absorption as we proceeded from C(5a)-substituents with an appended monocyclic ring to those with tricyclic substituents.[44] The longest λ_{max} observed was for **10** (403 nm). The extinction coefficients for **3**−**16** were graphically determined from a set of serially diluted samples and followed Beer's law. We found that compounds **7**−**10** and **12** displayed fluorescent properties. The λ_{max} for fluorescent excitation ranged from 257 nm (**10**) to 383 nm (**10**) while the λ_{max} for fluorescent emission was from 336 nm (**12**) to 444 nm (**10**). The quantum yield (Φ) for fluorescent emission ranged from 0.03 to 0.49 with **9** being the most efficient and **8** the least.

BFP−**Rho Binding.** We demonstrated the utility of BFP in **1**·rho binding studies. Compound **15** was employed in the initial studies, and we asked if the fluorescence emission generated upon excitation of W381 (λ_{exc} = 290 nm) in rho resulted in fluorescence resonance energy transfer (FRET) by bound **15**. The UV−vis absorption spectrum (λ_{max} = 377 nm) for **15** overlaps the W381 fluorescence emission spectrum ($\lambda_{em,max}$ = 345 nm).[45] W381 is the only tryptophan residue in wild-type rho and is predicted to be in a region in the C terminus where the structure is less similar to F_1-ATP synthase. The sequence correspondence of rho and F_1-ATP synthase in this sector is not high, and thus, the projected coordinates and spatial orientation for W381 are uncertain. Nonetheless, the estimated distance between bicyclomycin and W381 on each subunit (21 Å and 35 Å) is within the range of FRET processes.[35] Significantly, our homology model was built using the F_1-ATP synthase β-subunit with a bound ATP mimic.[21b] This is important since in F_1-ATP synthase the ATP hydrolysis hinge opens in the absence of ATP and closes with nucleotide binding.[28] The interaction between the β subunit and the rotating γ subunit can result in a movement of approximately 20 Å on the C terminus of the β subunit.[28a] The net effect is that the W381−**15** distance may be shorter in the presence of ATP and longer in its absence.

We measured the binding of **15** (0−40 μM) to wild-type rho[46,47] (1 μM based on the monomer) at 25 °C in the absence of nucleotide and in the presence of either ADP (400 μM) or ATP (250 μM). Previous studies have shown that under these conditions rho exists as a hexamer (data not shown).[45] We observed that in the absence of nucleotide or in the presence of ADP no energy transfer occurred upon excitation of W381 in the presence of **15**. A modest reduction (e.g., 40 μM **15**: ≈15%) in fluorescence emission was detected with increasing **15** concentration, an observation attributed to inner filter effects.[35,48] A similar effect was found using a 1 μM solution of free tryptophan and **15** (0−40 μM) (data not shown).[48] Inclusion of ATP (250 μM) in the reaction solution containing wild-type rho and **15** led to a pronounced loss of the W381 fluorescence emission when excited at 290 nm that depended upon **15** concentration (Figure 1A,B). Adding **1** (400 μM) at two different **15** concentrations (20, 40 μM) led to significant gains in fluorescence emission (20 μM **15**: 93%; 40 μM **15**: 85%), indicating that **15** and **1** share a common binding site in rho. After correcting for inner filter effects,[48] we observed a single dissociation constant for **15** with hexameric rho by Scatchard analysis[49] (K_d = 9.9 μM) (Figure 1C).

The FRET studies indicated that the rho W381-**15** distance was ATP dependent and demonstrated that ATP induced a conformational change in rho that permitted energy transfer provided that **15** binds to rho in the absence of nucleotide or in the presence of ADP. Previous attempts to detect an ATP-induced rho conformational change by limited tryptic digestion were inconclusive.[18,29,30] Addition of ATP led to diminished levels of trypsin cleavage near residue 128.[29] Similar findings were also observed for the adenine nucleotides ADP and AMPPNP. Our homology model indicated that W381 on adjacent subunits could potentially contribute to the observed FRET. Thus, the FRET distance calculations[35,50,51] for the ATP-mediated W381−**15** energy transfer revealed that the weighted average W381−**15** distances is approximately 23 Å. Similarly, the lower limit for W381−**15** (if bound) in the presence of ADP or in the absence of nucleotide is ≥46 Å.[35,50,51] These distance estimates rest on several assumptions that require validation in future studies. Among these are that nucleotide (ATP, ADP) binding to hexameric rho does not markedly influence **15** complexation to the protein. We know that **1** lowered the K_d for ATP binding by approximately 50% at the three tight sites and only marginally affects ATP binding at the three loose sites,[45] but the reverse may not be true. In addition, we have assumed that nucleotide (ATP, ADP) binding does not affect the spatial orientation of the dihydrobicyclomycin C(5a)-phenazin-2-ylmethylsulfanyl moiety. We have done so because **1** is a reversible, noncompetitive inhibitor with respect to ATP[17] and C(5a)-substituted bicyclomycin reductive amination probes efficiently modify rho in the presence of ATP.[20,21] Repeated attempts to resolve the **15** diastereomeric mixture by TLC using different solvent systems (10% MeOH−$CHCl_3$, EtOAc) did not separate the mixture. HPLC analysis showed two closely spaced peaks (t_R 75, 76 min) indicating that preparative HPLC may permit separation of the two diastereomers. Future efforts will be directed to provide purified samples of each

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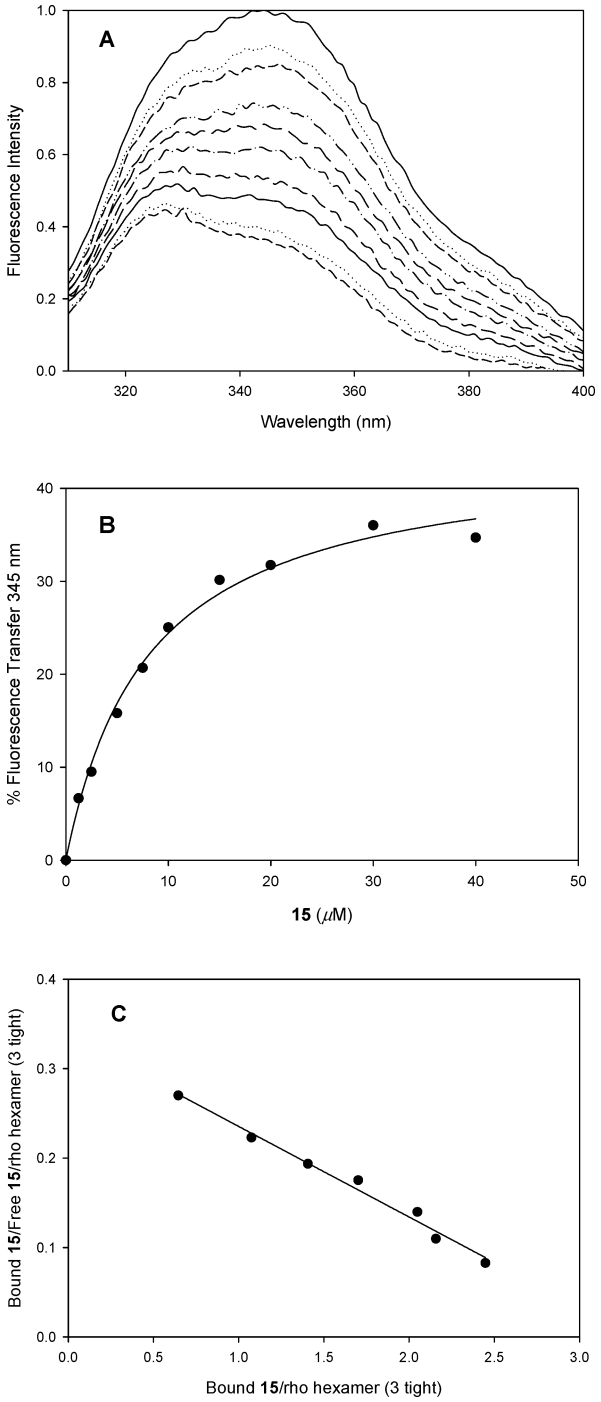

FIGURE 1. Fluorescence resonance energy transfer between wild-type rho and **15** in the presence of ATP (250 μM). (A) Emission spectra at varying **15** concentrations measured at 25 °C. BFP **15** concentrations were as follows: 0, 1.3, 2.5, 5.0, 7.5, 10, 15, 20, 30, 40 μM. The emission spectra were measured at an exciting wavelength of 290 nm. (B) Saturation concentration curve showing percent fluorescence energy transfer from wild-type rho (W381) to **15** as a function of **15** concentration. (C) Scatchard analysis of **15** binding to wild-type rho.

isomer to learn if the stereochemistry at the C(5)-position affects the I_{50}, K_d, and FRET distances upon **15** binding to wild-type rho.

Only a single dissociation constant for **15** with hexameric rho was observed by Scatchard analysis (Figure 1C). This finding does not permit us to determine if there

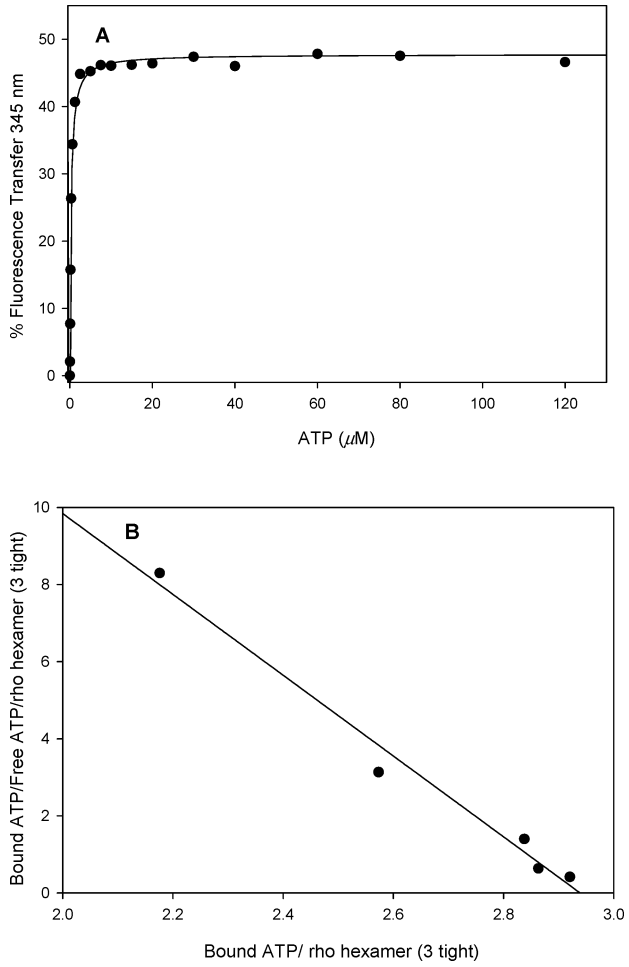

FIGURE 2. Effect of ATP on fluorescence resonance energy transfer between wild-type rho and **15** (μM). (A) The percent fluorescence transfer as a function of ATP concentration (0−120 μM) measured at 25 °C. No significant change in the plateau was observed for the maximum ATP concentration (240 μM) used (data not shown). (B) Scatchard analysis of ATP binding to **15**·rho complex.

are six equal binding sites or a subset of six (e.g., three). Accordingly, we measured the extent of energy transfer (345 nm) upon excitation (290 nm) of a solution containing wild-type rho and **15** (60 μM) as a function of ATP concentration (0−240 μM) (Figure 2A). We found that low amounts of ATP (2 μM) led to maximal levels of energy transfer, and Scatchard analysis indicated a $K_d = 95$ nM for ATP binding (Figure 2B). This value is in agreement with the estimated (see below) value for the tight ATP binding site in wild-type rho in the presence of **15**. In a recent study,[45] we showed that in the absence of poly(C), the rho mutant, F355W, has three tight ($K_{d1} = 3$ μM) and three loose ($K_{d2} = 58$ μM) ATP binding sites and that adding **1** strengthened the ATP binding at the tight site ($K_{d1} = 1.4$ μM), while only modestly affecting the loose ATP binding site ($K_{d2} = 51$ μM). Furthermore, we observed that the K_{d1} and K_{d2} values for ATP binding to F355W rho were higher (weaker) than those reported for wild-type rho where $K_{d1} \approx 0.33$ μM and $K_{d2} \approx 10$ μM.[25] The I_{50} value for **15** was approximately one-half that of **1** in the poly(C)-dependent ATPase assay (Table 1), indicating that **15** binds tighter to rho than **1**. Accordingly, we predict that the K_d value for ATP binding in

the presence of **15** will be approximately 4-fold lower (tighter) than 0.33 μM. Significantly, the **15**-mediated FRET measurements at a constant ATP concentration (250 μM) (Figure 1) correlated with ATP binding to the three tight sites. Thus, the measured K_d value for **15** (9.9 μM) (Figure 1B) corresponded to only these three ATP binding sites. Since ATP induced a conformational change in rho that allowed for the observed energy transfer at only three of the six sites, we do not know the extent of **15** binding to the remaining three subunits in hexameric rho, which are governed by loose ATP binding and the site of **15** occupancy that led to the observed FRET measurements.

Conclusions

Synthetic procedures were developed to permit incorporation of fluorescent labels at the C(5a)-site in dihydrobicyclomycins. SAR studies for BFP showed that incorporation of medium-to-large C(5a)-substituents provided dihydrobicyclomycin derivatives with excellent inhibitory activities in the poly(C)-dependent ATPase assay that exceeded **1**. The utility of BFP in bicyclomycin−rho binding studies was documented through the use of **15**. FRET studies with **15** and wild-type rho showed that K_d = 9.9 μM in the presence of ATP. There was no energy transfer when ADP was used in place of ATP or when nucleotide was excluded, revealing preliminary information that ATP binding induced a rho conformational change, provided that **15** binds to rho in the absence of nucleotide. The FRET measurements for **15** were correlated with ATP binding to the three tight sites in wild-type rho.

Experimental Section

General Methods. See the Supporting Information and ref 23.

General Procedure 1. Preparation of 5a-Substituted Dihydrobicyclomycin 2′,3′-Acetonides (Methods 1 and 2). To a methanolic solution (3−10 mL) of **17** under Ar was added the desired nucleophile or substituted thioacetate (2−10 equiv) and then "pH" was adjusted to 10.5 with aqueous 1.0 M NaOH. The mixture was stirred at room temperature (2−24 h) until no starting material remained (TLC analysis). The "pH" of the mixture was adjusted to 7.0 with aqueous 0.1−1.0 M HCl. The solvent was removed in vacuo, and the residue was redissolved in MeOH and filtered when applicable. The solvent was removed in vacuo, and the residue was purified by preparative TLC (5−10% MeOH−$CHCl_3$) to afford the desired product.

General Procedure 2. Preparation of 5a-Substituted Dihydrobicyclomycins. To a 50% aqueous methanolic solution (unless otherwise indicated) (2−10 mL) containing the 5a-substituted dihydrobicyclomycin 2′,3′-acetonide was added TFA (2−10 drops), and then the solution was stirred at room temperature (4−48 h) until no starting material remained (TLC analysis). The solvent was removed in vacuo and the residue was purified by preparative TLC (10−20% MeOH−$CHCl_3$) to afford the desired product.

General Procedure 3. Preparation of Precursor Arylmethylthioacetates 37−41. To an acetone solution (10−20 mL) of the desired aromatic methyl halide (**32**−**36**) under Ar was added potassium thioacetate (1.2 equiv). The mixture was heated to reflux (1−3 h) until no starting material remained (TLC analysis) and H_2O (15 mL) added. The reaction mixture was extracted with Et_2O (3 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo. The product was purified by column chromatography using 10−50% EtOAc−hexanes.

5a-(2-Aminophenylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (20). Using general procedure 1, **17** (33 mg, 0.10 mmol) and 2-aminothiophenol (60 mg, 0.48 mmol) gave **20** as a mixture of diastereomers (∼3:1): yield 48 mg (67%); R_f 0.71 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3312 (br), 2960 (br), 1690, 1392, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3 H, C(2′)CH_3), 1.44 (s, 6 H, C(CH_3)$_2$), 1.95−2.33 (m, 3 H, C(4)*H*H′, C(4)H*H′*, C(5)H), 2.43 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.47 (dd, J = 2.7, 11.5 Hz, 1 H, C(5a)H*H′*), 3.70 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.72−4.03 (m, 2 H, C(3)*H*H′, C(3)H*H′*), 4.09 (s, 1 H, C(1′)H), 4.44 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), 6.57−6.65 (m, 1 H, C(5″)H), 6.74 (dd, J = 1.6, 8.2 Hz, 1 H, C(6″)H), 7.01−7.09 (m, 1 H, C(4″)H), 7.29 (dd, J = 1.6, 8.2 Hz, 1 H, C(3″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.36 (s, 3 H, C(2′)CH_3), 1.43 (s, 6 H, C(CH_3)$_2$), 2.66 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 4.10 (s, 1 H, C(1′)H), 4.45 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), 6.76 (dd, J = 1.6, 8.2 Hz, 1 H, C(6″)H), 7.30 (dd, J = 1.6, 8.2 Hz, 1 H, C(3″)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.9, 28.3, 30.4, 34.8, 52.4, 63.6, 73.3, 73.4, 83.8, 86.4, 88.7, 111.7, 116.3, 118.4, 119.1, 130.5, 136.2, 150.2, 168.2, 171.7 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 28.4, 29.8, 33.2, 50.3, 63.6, 89.0, 119.2, 130.7, 136.3, 167.2, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 468 [M + 1]$^+$; M_r (+CI) 468.180 70 [M + 1]$^+$ (calcd for $C_{21}H_{30}N_3O_7S$ 468.180 45).

5a-(4-*tert*-Butylphenylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (21). Using general procedure 1, **17** (45 mg, 0.13 mmol) and 4-*tert*-butylbenzenethiol (153 mg, 0.92 mmol) gave **21** as a mixture of diastereomers (∼3:1): yield 48 mg (72%); R_f 0.71 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3307 (br), 2962 (br), 1691, 1397, 1050 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.29 (s, 9 H, C(CH_3)$_3$), 1.36 (s, 3 H, C(2′)CH_3), 1.44 (s, 6 H, C(CH_3)$_2$), 1.90−2.33 (m, 3 H, C(4)*H*H′, C(4)H*H′*, C(5)H), 2.49 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.61−4.08 (m, 4 H, C(3′)*H*H′, C(3)*H*H′, C(3)H*H′*, C(5a)H*H′*), 4.10 (s, 1 H, C(1′)H), 4.44 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), 7.27−7.38 (m, 4 H, 2 C(2″)H, 2 C(3″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 2.74 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 4.10 (s, 1 H, C(1′)H), 4.45 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.8, 26.9, 28.3, 30.3, 31.7 (3 C), 34.0, 35.3, 51.9, 63.6, 73.3, 73.4, 83.8, 86.3, 88.7, 111.7, 127.1 (2 C), 130.4 (2 C), 133.6, 150.7, 168.1, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer 28.4, 29.7, 32.2, 49.8, 63.5, 73.5, 83.7, 89.0, 111.7, 127.2 (2 C), 150.6, 167.3, 173.5 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 509 [M + 1]$^+$; M_r (+CI) 509.230 97 [M + 1]$^+$ (calcd for $C_{25}H_{37}N_2O_7S$ 509.232 15).

5a-(Naphth-1-ylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (22). Using general procedure 1, **17** (47 mg, 0.13 mmol) and 1-naphthalenethiol (154 mg, 0.96 mmol) gave **22** as a mixture of diastereomers (∼3:1): yield 46 mg (67%); R_f 0.65 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3296 (br), 2985, 2934, 1689, 1385, 1047 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3 H, C(2′)CH_3), 1.43 (s, 6 H, C(CH_3)$_2$), 1.96−2.38 (m, 3 H, C(4)*H*H′, C(4)H*H′*, C(5)H), 2.66 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.69 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.72−4.06 (m, 3 H, C(3)*H*H′, C(3)H*H′*, C(5a)H*H′*), 4.09 (s, 1 H, C(1′)H), 4.43 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), 7.37−7.57 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.68 (d, J = 7.1 Hz, 1 H, C(2″)H), 7.73 (d, J = 8.8 Hz, 1 H, C(4″)H), 7.85 (d, J = 9.3 Hz, 1 H, C(5″)H), 8.31 (d, J = 6.6 Hz, 1 H, C(8″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.44 (s, 6 H, C(CH_3)$_2$), 2.89 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 4.10 (s, 1 H, C(1′)H), 4.45 (d, J = 8.8 Hz, 1 H, C(3′)H*H′*), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H-^{1}H

COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.8, 26.9, 28.2, 30.6, 34.3, 52.0, 63.6, 73.3, 73.5, 83.8, 86.3, 88.7, 111.7, 125.7, 126.8, 127.3, 127.4, 128.2, 128.9, 129.7, 134.1, 134.3, 135.5, 168.1, 171.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 28.3, 30.1, 32.7, 63.5, 88.9, 128.4, 129.0, 167.3, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 503 $[M + 1]^+$; M_r (+CI) 503.184 37 $[M + 1]^+$ (calcd for $C_{25}H_{31}N_2O_7S$ 503.185 20).

5a-(Naphth-2-ylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (23). Using general procedure 1, **17** (52 mg, 0.15 mmol) and 2-naphthalenethiol (122 mg, 0.76 mmol) gave **23** as a mixture of diastereomers (~3:1): yield 35 mg (46%); R_f 0.56 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3421 (br), 2984 (br), 1690, 1387, 1053 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3 H, C(2′)CH_3), 1.45 (s, 6 H, C(CH_3)$_2$), 1.90−2.40 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.63 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.70 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.72−4.08 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.10 (s, 1 H, C(1′)H), 4.44 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 7.38−7.49 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.71−7.82 (m, 3 H, C(4″)H, C(5″)H, C(8″)H), 7.85 (d, J = 1.6 Hz, 1 H, C(1″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 1.43 (s, 6 H, C(CH_3)$_2$), 2.88 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)-*H*H′), 3.69 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 4.11 (s, 1 H, C(1′)H), 4.45 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.9, 28.3, 30.5, 33.3, 51.9, 63.6, 73.3, 73.4, 83.8, 86.4, 88.7, 111.7, 126.7, 127.4, 127.6, 128.0, 128.1, 128.7, 129.6, 133.2, 134.9, 135.4, 168.1, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 29.9, 31.8, 50.7, 89.0, 167.1, 173.5 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 503 $[M + 1]^+$; M_r (+CI) 503.177 14 $[M + 1]^+$ (calcd for $C_{25}H_{31}N_2O_7S$ 503.177 37).

5a-Benzylsulfanyldihydrobicyclomycin 2′,3′-Acetonide (26). Using general procedure 1, **17** (40 mg, 0.12 mmol) and benzyl mercaptan (101 mg, 0.82 mmol) gave **26** as a mixture of diastereomers (~3:1): yield 40 mg (73%); R_f 0.55 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3303 (br), 2958 (br), 1688, 1392, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3 H, C(2′)CH_3), 1.41 (s, 3 H, C(CH_3)$_2$), 1.44 (s, 3 H, C(CH_3)$_2$), 1.82−2.26 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)-*H*H′), 3.18 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 3.63−3.93 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(3′)*H*H′), 3.68 (s, 2 H, SCH_2Ph), 4.08 (s, 1 H, C(1′)H), 4.42 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 7.16−7.38 (m, 5 H, Ph); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.45 (s, 3 H, C(CH_3)$_2$), 2.28 (dd, J = 11.5, 15.7 Hz, 1 H, C(5a)-*H*H′), 3.16 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 4.07 (s, 1 H, C(1′)H), 4.44 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.9, 28.2, 30.4, 31.7, 37.4, 52.4, 63.4, 73.3, 73.4, 83.8, 86.4, 88.7, 111.7, 128.0, 129.5 (2 C), 130.1 (2 C), 140.1 168.3, 171.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.9, 28.4, 29.9, 50.1, 63.6, 73.3, 73.6, 83.7, 88.9, 167.2, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 467 $[M + 1]^+$; M_r (+CI) 467.185 79 $[M + 1]^+$ (calcd for $C_{22}H_{31}N_2O_7S$ 467.185 20).

5a-(Naphth-2-ylmethylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (27). Using general procedure 1, **17** (24 mg, 0.07 mmol) and **37** (106 mg, 0.49 mmol) gave **27** as a mixture of diastereomers (~3:1): yield 26 mg (72%); R_f 0.58 (5% MeOH−$CHCl_3$); FT-IR (KBr) 3283 (br), 2928 (br), 1687, 1383, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.30 (s, 3 H, C(2′)CH_3), 1.33 (s, 3 H, C(CH_3)$_2$), 1.41 (s, 3 H, C(CH_3)$_2$), 1.83−2.25 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)-*H*H′), 3.18 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 3.27−3.82 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(3′)*H*H′), 3.85 (s, 2 H, SCH_2Ar), 4.06 (s, 1 H, C(1′)H), 4.37 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 7.39−7.52 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.74 (d, J = 1.1 Hz, 1 H, C(1″)H), 7.76−7.83 (m, 3 H, C(4″)H, C(5″)H, C(8″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.44 (s, 3 H, C(CH_3)$_2$), 2.30 (dd, J = 11.5, 15.7 Hz, 1 H, C(5a)*H*H′), 3.57 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 4.05 (s, 1 H, C(1′)H), 4.43 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H-^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.8, 28.2, 30.4, 31.5, 37.6, 52.4, 63.3, 73.2, 73.4, 83.8, 86.4, 88.7, 111.6, 126.8, 127.2, 128.3, 128.6, 128.7, 128.8, 129.3, 134.1, 134.8, 137.3, 168.3, 171.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.9, 28.4, 37.5, 50.1, 63.6, 73.5, 83.7, 89.0, 111.7, 128.3, 128.7 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 517.202 04 $[M + 1]^+$ (calcd for $C_{26}H_{33}N_2O_7S$ 517.200 85).

5a-(Quinolin-6-ylmethylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (28). Using general procedure 1, **17** (51 mg, 0.15 mmol) and **38** (106 mg, 0.50 mmol) gave **28** as a mixture of diastereomers (~3:1): yield 43 mg (56%); mp 125−132 °C; R_f 0.35 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3360 (br), 2984, 2928, 1689, 1384, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 1.34 (s, 3 H, C(CH_3)$_2$), 1.41 (s, 3 H, C(CH_3)$_2$), 1.83−2.36 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.14 (d, J = 11.5 Hz, 1 H, C(5a)-H*H*′), 3.68 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.65−3.97 (m, 2 H, C(3)*H*H′, C(3)H*H*′), 3.89 (s, 2 H, SCH_2Ar), 4.07 (s, 1 H, C(1′)H), 4.39 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 7.52 (dd, J = 4.4, 8.5 Hz, 1 H, C(3″)H), 7.75−7.92, (m, 2 H, C(5″)H, C(7″)H), 7.98 (br d, J = 8.5 Hz, 1 H, C(4″)H), 8.32 (d, J = 8.2 Hz, 1 H, C(8″)H), 8.80 (dd, J = 1.6, 4.4 Hz, 1 H, C(2″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.45 (s, 3 H, C(CH_3)$_2$), 4.07 (s, 1 H, C(1′)H), 4.44 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H-^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.8, 28.2, 30.5, 31.5, 37.0, 52.3, 63.4, 73.2, 73.4, 83.8, 86.3, 88.7, 111.6, 122.8, 128.8, 129.4, 129.6, 132.6, 138.2, 138.9, 148.0, 151.0, 168.3, 171.5 ppm; ^{13}C NMR (CD_3-OD) for the minor diastereomer, 26.9, 28.3, 36.8, 63.6, 73.5, 83.6, 88.9, 111.7, 128.9, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 518 $[M + 1]^+$; M_r (+CI) 518.196 04 $[M + 1]^+$ (calcd for $C_{25}H_{32}N_3O_7S$ 518.196 10).

5a-(Phenazin-2-ylmethylsulfanyl)dihydrobicyclomycin 2′,3′-Acetonide (30). Using general procedure 1, **17** (52 mg, 0.15 mmol) and **40** (82 mg, 0.30 mmol) gave **30** as a mixture of diastereomers (~3:1): yield 48 mg (56%); mp 134−140 °C; R_f 0.58 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3283 (br), 2982, 2928, 1687, 1379, 1044 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.24 (s, 3 H, C(2′)CH_3), 1.32 (s, 3 H, C(CH_3)$_2$), 1.36 (s, 3 H, C(CH_3)$_2$), 1.83−2.43 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.15 (d, J = 11.5 Hz, 1 H, C(5a)-H*H*′), 3.65 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.67−4.10 (m, 2 H, C(3)*H*H′, C(3)H*H*′), 3.96 (d, J = 5.1 Hz, 2 H, SCH_2Ar), 4.07 (s, 1 H, C(1′)H), 4.36 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 7.82−7.96 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 8.02−8.21 (m, 4 H, C(1″)H, C(4″)H, C(5″)H, C(8″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.44 (s, 3 H, C(CH_3)$_2$), 4.43 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.8, 26.7, 28.2, 30.5, 31.4, 37.2, 52.3, 63.5, 73.2, 73.4, 83.7, 86.3, 88.7, 111.6, 128.9, 130.1, 130.2, 130.4, 131.9, 132.1, 134.0, 143.5, 143.8, 144.2, 144.3, 144.5, 168.1, 171.4 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.9, 28.3, 37.3, 50.2, 73.5, 83.6, 88.9, 111.7, 128.8, 143.6, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 569 $[M + 1]^+$; M_r (+CI) 569.207 43 $[M + 1]^+$ (calcd for $C_{28}H_{33}N_4O_7S$ 569.207 00).

5a-(2-Aminophenylsulfanyl)dihydrobicyclomycin (5). Using general procedure 2, **20** (30 mg, 0.06 mmol) gave **5** as a mixture of diastereomers (~3:1): yield 24 mg (89%); mp 140−145 °C; R_f 0.71 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3296 (br), 2936 (br), 1687, 1403, 1038 cm^{-1}; UV−vis (H_2O) 298 nm (ϵ 793); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 2.08−2.25 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.46 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.43 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 3.52 (d, J = 8.8 Hz, 1 H, C(3′)*H*H′), 3.65 (d, J = 8.8 Hz, 1 H, C(3′)H*H*′), 3.66−4.06 (m, 2 H, C(3)*H*H′, C(3)H*H*′), 4.01 (s, 1 H, C(1′)H), 6.57−6.65 (m, 1 H, C(5″)H), 6.75 (dd, J = 1.6, 8.2 Hz, 1 H, C(6″)H), 7.01−7.09 (m, 1 H, C(4″)H), 7.29 (dd, J = 1.6, 8.2 Hz, 1 H, C(3″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.66 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 4.03 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 29.9, 34.5, 52.3, 62.1, 68.5, 72.3, 78.1, 83.7, 89.4, 116.3, 118.4, 119.1, 130.5, 136.1, 150.2, 168.8, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.5, 33.6, 50.4, 63.3, 72.4, 83.6, 89.6, 118.7, 119.2, 130.6, 167.6, 174.0 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 428.149 29 $[M + 1]^+$ (calcd for $C_{18}H_{26}N_3O_7S$ 428.149 15).

5a-(4-*tert*-Butyl-phenylsulfanyl)dihydrobicyclomycin (6). Using general procedure 2, **21** (55 mg, 0.07 mmol) gave **6** as a mixture of diastereomers (~3:1): yield 41 mg (81%); mp 128−132 °C; R_f 0.31 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3338 (br), 2958 (br), 1691, 1401, 1045 cm^{-1}; UV−vis (H_2O) 255 nm (ϵ 9760); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.29 (s, 9 H, C(CH_3)$_3$), 1.33 (s, 3 H, C(2′)CH_3), 1.92−2.32 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.53 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.52 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.60−4.06 (m, 4 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.02 (s, 1 H, C(1′)H), 7.26−7.36 (m, 4 H, 2 C(2″)H, 2 C(3″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.72 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.55 (d, J = 11.6 Hz, 1 H, C(3′)*H*H′), 4.04 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 29.8, 31.7 (3 C), 33.5, 35.3, 51.8, 62.1, 68.5, 72.2, 78.1, 83.7, 89.4, 127.1 (2 C), 130.3 (2 C), 133.6, 150.5, 168.7, 172.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.4, 32.6, 63.2, 72.4, 83.6, 89.6, 127.1 (2 C), 130.2 (2 C), 133.8, 167.7, 174.0 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 469.200 97 $[M + 1]^+$ (calcd for $C_{22}H_{33}N_2O_7S$ 469.200 85).

5a-(Naphth-1-ylsulfanyl)dihydrobicyclomycin (7). Using general procedure 2, **22** (42 mg, 0.07 mmol) gave **7** as a mixture of diastereomers (~3:1): yield 27 mg (59%); mp 130−135 °C; R_f 0.53 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3249 (br), 2934 (br), 1687, 1394, 1039 cm^{-1}; UV−vis (H_2O) 301 nm (ϵ 6750); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 2.00−2.38 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.69 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.53 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.67 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)H*H*′), 3.71−4.06 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.02 (s, 1 H, C(1′)H), 7.38−7.57 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.68 (d, J = 7.1 Hz, 1 H, C(2″)H), 7.73 (d, J = 8.8 Hz, 1 H, C(4″)H), 7.85 (d, J = 9.3 Hz, 1 H, C(5″)H), 8.31 (d, J = 6.6 Hz, 1 H, C(8″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.86 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.54 (d J = 11.6 Hz, 1 H, C(3′)*H*H′), 4.04 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.2, 34.0, 51.9, 62.2, 68.5, 72.4, 78.1, 83.8, 89.4, 125.7, 126.8, 127.3, 127.4, 128.2, 128.8, 129.7, 134.1, 134.3, 135.5, 168.7, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.7, 33.0, 50.0, 63.2, 72.5, 83.6, 89.6, 125.7, 126.8, 128.3, 128.9, 134.1, 134.4 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 463 $[M + 1]^+$; M_r (+CI) 463.154 04 $[M + 1]^+$ (calcd for $C_{22}H_{27}N_2O_7S$ 463.153 90).

5a-(Naphth-2-ylsulfanyl)dihydrobicyclomycin (8). Using general procedure 2, **23** (35 mg, 0.07 mmol) gave **8** as a mixture of diastereomers (~3:1): yield 19 mg (59%); mp 125−130 °C; R_f 0.50 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3259 (br), 2935 (br), 1686, 1402, 1038 cm^{-1}; UV−vis (H_2O) 283 nm (ϵ 5990); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 1.99−2.40 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.66 (dd, J = 11.5 Hz, 14.3 Hz, 1 H, C(5a)*H*H′), 3.53 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 3.64−4.09 (m, 4 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.03 (s, 1 H, C(1′)H), 7.37−7.50 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.72−7.82 (m, 3 H, C(4″)H, C(5″)H, C(8″)H), 7.86 (d, J = 1.6 Hz, 1 H, C(1″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.86 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.69 (d, J = 11.3 Hz, 1 H, C(3′)*H*H′), 4.05 (s, 1 H, C(1′)H), 7.84 (d, J = 1.6 Hz, 1 H, C(1″)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.0, 32.9, 51.8, 62.1, 68.6, 72.3, 78.2, 83.7, 89.4, 126.7, 127.3, 127.6, 128.0, 128.1, 128.7, 129.5, 133.2, 134.9, 135.4, 168.7, 172.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.5, 32.1, 72.4, 83.8, 89.7, 127.5, 135.1, 167.7 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 463 $[M + 1]^+$; M_r (+CI) 463.154 91 $[M + 1]^+$ (calcd for $C_{22}H_{27}N_2O_7S$ 463.153 90).

5a-Benzylsulfanyldihydrobicyclomycin (11). Using general procedure 2, **26** (39 mg, 0.08 mmol) gave **11** as a mixture of diastereomers (~3:1): yield 29 mg (81%); mp 114−119 °C; R_f 0.52 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3398 (br), 2928 (br), 1687, 1405, 1038 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 1.90−2.27 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.13 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 3.50 (d, J = 12.6 Hz, 1 H, C(3′)*H*H′), 3.64−3.91 (m, 3 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′), 3.67 (s, 2 H, SCH_2Ar), 4.01 (s, 1 H, C(1′)H), 7.16−7.39, (m, 5 H, Ar); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.28 (dd, J = 11.5, 15.7 Hz, 1 H, C(5a)*H*H′), 3.10 (d, J = 11.5 Hz, 1 H, C(5a)H*H*′), 3.54 (d, J = 12.6 Hz, 1 H, C(3′)*H*H′), 4.02 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.1, 31.6, 37.4, 52.3, 62.0, 68.5, 72.4, 78.1, 83.8, 89.4, 128.0, 129.5 (2 C), 130.1 (2 C), 140.1, 168.8, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.4, 30.6, 37.5, 50.2, 63.3, 72.5, 83.6, 89.6, 167.6, 173.7 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 427 $[M + 1]^+$; M_r (+CI) 427.152 66 $[M + 1]^+$ (calcd for $C_{19}H_{27}N_2O_7S$ 427.153 90).

5a-(Naphth-2-ylmethylsulfanyl)dihydrobicyclomycin (12). Using general procedure 2, **27** (35 mg, 0.07 mmol) gave **12** as a mixture of diastereomers (~3:1): yield 32 mg (99%); mp 122−128 °C; R_f 0.52 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3238 (br), 2926 (br), 1687, 1395, 1032 cm^{-1}; UV−vis (H_2O) 276 nm (ϵ 4340); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.31 (s, 3 H, C(2′)CH_3), 1.87−2.35 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)H*H*′), 3.15 (d, J = 11.6 Hz, 1 H, C(5a)H*H*′), 3.53 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), 3.42−3.88 (m, 3 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′), 3.84 (s, 2 H, SCH_2Ar), 4.00 (s, 1 H, C(1′)H), 7.37−7.52 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 7.73−7.83 (m, 4 H, C(1″)H, C(4″)H, C(5″)H, C(8″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.28 (s, 3 H, C(2′)CH_3), 4.01 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.3, 30.1, 31.4, 37.6, 52.3, 62.0, 68.5, 72.4, 78.1, 83.8, 89.3, 126.8, 127.2, 128.3, 128.6, 128.7, 128.8, 129.3, 134.1, 134.8, 137.4, 168.8, 172.1 ppm; ^{13}C NMR (CD_3OD) for the minor

diastereomer, 30.2, 30.7, 50.2, 63.3, 72.5, 78.1, 83.6, 89.6, 127.2, 128.7 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 477.171 33 $[M + 1]^+$ (calcd for $C_{23}H_{29}N_2O_7S$ 477.169 55).

5a-(Quinolin-6-ylmethylsulfanyl)dihydrobicyclomycin (13). Using general procedure 2, **28** (39 mg, 0.08 mmol) gave **13** as a mixture of diastereomers (~3:1) after the "pH" was adjusted to 8 with aqueous 0.1 M NaOH: yield 30 mg (83%); mp 133−140 °C; R_f 0.52 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3337 (br), 2930 (br), 1686, 1400, 1039 cm^{-1}; UV−vis (H_2O) 319 nm (ϵ 5380); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.31 (s, 3 H, C(2′)CH_3), 1.90−2.32 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.11 (d, J = 11.6 Hz, 1 H, C(5a)H*H*′), 3.47 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), 3.56−3.95 (m, 3 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′), 3.88 (s, 2 H, SCH_2Ar), 4.01 (s, 1 H, C(1′)H), 7.52 (dd, J = 4.4, 8.5 Hz, 1 H, C(3″)H), 7.75−7.92, (m, 2 H, C(5″)H, C(7″)H), 7.98 (br d, J = 8.5 Hz, 1 H, C(4″)H), 8.32 (d, J = 8.2 Hz, 1 H, C(8″)H), 8.80 (dd, J = 1.6, 4.4 Hz, 1 H, C(2″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 4.02 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.1, 31.3, 37.0, 52.2, 62.0, 68.5, 72.3, 78.1, 83.7, 89.3, 122.8, 128.8, 129.4, 129.7, 132.7, 138.2, 139.0, 148.1, 151.0, 168.8, 172.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.7, 50.1, 63.3, 72.5, 83.6, 89.6, 128.9, 167.6, 174.1 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 478 $[M + 1]^+$; M_r (+CI) 478.164 27 $[M + 1]^+$ (calcd for $C_{22}H_{28}N_3O_7S$ 478.164 80).

5a-(Phenazin-2-ylmethylsulfanyl)dihydrobicyclomycin (15). Using general procedure 2, **30** (42 mg, 0.07 mmol) gave **15** as a mixture of diastereomers (~3:1) after the "pH" was adjusted to 8 with aqueous 0.1 M NaOH: yield 30 mg (92%); mp 139−145 °C; R_f 0.56 (20% MeOH−$CHCl_3$); HPLC t_R, 75, 76 min; FT-IR (KBr) 3303 (br), 2931 (br), 1686, 1401, 1037 cm^{-1}; UV−vis (H_2O) 377 nm (ϵ 17 600); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.30 (s, 3 H, C(2′)CH_3), 1.90−2.41 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.12 (d, J = 11.6 Hz, 1 H, C(5a)H*H*′), 3.46 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), 3.61 (d, J = 10.5 Hz, 1 H, C(3′)H*H*′), 3.64−3.98 (m, 2 H, C(3)*H*H′, C(3)H*H*′), 3.96 (d, J = 5.1 Hz, 2 H, SCH_2Ar), 4.01 (s, 1 H, C(1′)H), 7.82−7.96 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 8.02−8.21 (m, 4 H, C(1″)H, C(4″)H, C(5″)H, C(8″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.32 (s, 3 H, C(2′)CH_3), 3.54 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.2, 31.4, 37.2, 52.2, 62.2, 68.5, 72.4, 78.1, 83.7, 89.4, 128.9, 130.1, 130.2, 130.4, 131.9, 132.2, 134.1, 143.6, 143.9, 144.2, 144.3, 144.5, 168.7, 171.9 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.4, 30.9, 37.4, 50.4, 63.3, 72.5, 83.5, 89.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 529 $[M + 1]^+$; M_r (+CI) 529.174 10 $[M + 1]^+$ (calcd for $C_{25}H_{29}N_4O_7S$ 529.175 70).

5a-(Pyridin-4-ylsulfanyl)dihydrobicyclomycin (3). Using general procedure 1, **17** (53 mg, 0.155 mmol) and 4-mercaptopyridine (86 mg, 0.775 mmol) gave **18** as a crude mixture (R_f 0.33 (10% MeOH−$CHCl_3$)). The reaction was not purified but immediately deprotected using general procedure 2 to give **3** as a mixture of diastereomers (~4:1) after the "pH" was adjusted to 8 with aqueous 0.1 M NaOH: yield 23 mg (37%); mp 130−140 °C dec; R_f 0.32 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3336 (br), 2935 (br), 1689, 1407, 1038 cm^{-1}; UV−vis (H_2O) 263 nm (ϵ 13 900); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.33 (s, 3 H, C(2′)CH_3), 1.93−2.39 (m, 3 H, C(4)*H*H′, C(4)-H*H*′, C(5)H), 2.65 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.53 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.69 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)H*H*′), 3.70−4.11 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(5a)-H*H*′), 4.04 (s, 1 H, C(1′)H), 7.40 (d, J = 4.9 Hz, 2 H, 2 C(3″)H), 8.29 (d, J = 4.9 Hz, 2 H, 2 C(2″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.29 (s, 3 H, C(2′)CH_3), 2.79 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.54 (d, J = 11.6 Hz, 1 H, C(3′)-*H*H′), 4.06 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.5, 30.6, 51.5, 62.3, 68.5, 72.2, 78.2, 83.7, 89.5, 122.2 (2 C), 149.5 (2 C), 151.9, 168.5, 171.9 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 29.6, 30.9, 63.2, 72.3, 83.6, 89.7, 122.1, 150.0 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 414.133 62 $[M + 1]^+$ (calcd for $C_{17}H_{24}N_3O_7S$ 414.133 50).

5a-(Pyridin-2-ylsulfanyl)dihydrobicyclomycin (4). Using general procedure 1, **17** (53 mg, 0.16 mmol) and 2-mercaptopyridine (120 mg, 1.08 mmol) gave **19** as a crude mixture (R_f 0.58 (10% MeOH−$CHCl_3$)). The reaction was not purified but immediately deprotected using general procedure 2 to give **4** as a mixture of diastereomers (~3:1) after the "pH" was adjusted to 8 with aqueous 0.1 M NaOH: yield 36 mg (56%); mp 115−120 °C; R_f 0.32 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3311 (br), 2938 (br), 1688, 1412, 1044 cm^{-1}; UV−vis (H_2O) 288 nm (ϵ 4910); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.34 (s, 3 H, C(2′)CH_3), 1.89−2.41 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.97 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.54 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.68 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)H*H*′), 3.65−4.14 (m, 3 H, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.04 (s, 1 H, C(1′)H), 7.05−7.12 (m, 1 H, C(5″)H), 7.35 (d, J = 7.7 Hz, 1 H, C(3″)H), 7.58−7.66 (m, 1 H, C(4″)H), 8.35 (d, J = 4.9 Hz, 1 H, C(6″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.12 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.56 (d, J = 11.6 Hz, 1 H, C(3′)*H*H′), 4.06 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.3, 30.0, 30.3, 53.3, 62.3, 68.6, 72.4, 78.2, 83.9, 89.4, 121.0, 123.2, 138.1, 150.2, 160.4, 168.9, 171.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 28.9, 30.8, 51.0, 63.6, 72.6, 89.7, 121.4, 123.3, 150.3 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 414 $[M + 1]^+$; M_r (+CI) 414.132 71 $[M + 1]^+$ (calcd for $C_{17}H_{24}N_3O_7S$ 414.133 50).

5a-(4-Methylcoumarin-7-ylsulfanyl)dihydrobicyclomycin (9). Using general procedure 1, **17** (50 mg, 0.15 mmol) and 7-mercapto-4-methylcoumarin (197 mg, 1.02 mmol) gave **24** as a crude mixture (R_f 0.58 (10% MeOH−$CHCl_3$)). The reaction was not purified but immediately deprotected using general procedure 2 to give **9** as a mixture of diastereomers (~3:1): yield 9 mg (13%); mp 140−150 °C dec; R_f 0.21 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3327 (br), 2936 (br), 1692, 1601, 1398, 1048 cm^{-1}; UV−vis (H_2O) 333 nm (ϵ 16 900); ^1H NMR (CD_3OD) for the major diastereomer, δ 1.34 (s, 3 H, C(2′)CH_3), 1.94−2.38 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.45 (s, 3 H, C(4″)CH_3), 2.66 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.53 (d, 1 H, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.65−4.10 (m, 4 H, C(3′)-H*H*′, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.04 (s, 1 H, C(1′)H), 6.25 (s, 1 H, C(3″)H), 7.28−7.35 (m, 1 H, C(6″)H), 7.38 (d, J = 1.6 Hz, 1 H, C(8″)H), 7.65 (d, J = 7.7 Hz, 1 H, C(5″)H); ^1H NMR (CD_3OD) for the minor diastereomer, δ 2.84 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.55 (d J = 11.6 Hz, 1 H, C(3′)*H*H′), 4.06 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the $^1H-^1H$ COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 18.6, 24.2, 30.3, 31.8, 51.7, 62.2, 68.5, 72.2, 78.2, 83.7, 89.5, 114.2, 114.9, 118.5, 124.3, 126.4, 144.4, 155.2, 155.3, 162.8, 168.6, 172.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.7, 31.1, 63.2, 72.4, 83.6, 89.7, 114.3, 115.1, 118.6, 126.5, 173.8 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 495.143 17 $[M + 1]^+$ (calcd for $C_{22}H_{27}N_2O_9S$ 495.143 73).

5a-(Anthracen-2-ylsulfanyl)dihydrobicyclomycin (10). To a mixture of NaH (60% dispersion on mineral oil, 90 mg, 3.76 mmol) in anhydrous 1-methyl-2-pyrrolidinone (7 mL) was added 1-butanethiol (338 mg, 3.76 mmol), and then the mixture was stirred (10 min) under Ar at room temperature.[38] To this mixture was added 2-chloroanthracene (200 mg, 0.94 mmol), and the mixture was heated to reflux (18 h). The mixture was cooled (0 °C), and then H_2O (5 mL) was added and the solution "pH" adjusted to 2 with aqueous 1.0 M HCl. The mixture was extracted with Et_2O (3 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo to obtain 2-mercaptoanthracene as a crude yellow solid (R_f 0.51 (10% EtOAc−hexanes)). The crude product was not purified but used directly in general procedure 1 with **17** (22 mg, 0.06 mmol) to provide **25** as a crude mixture (R_f 0.47 (10% MeOH−$CHCl_3$)). The product was then deprotected using general procedure 2 (solvent: 25% methanolic H_2O) to give **10** as a mixture of diastereomers (∼3:1): yield 8 mg (24%); mp 145−150 °C; R_f 0.65 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3259 (br), 2928 (br), 1686, 1403, 1044 cm^{-1}; UV−vis (H_2O) 257 (ϵ 17 600), 362 (ϵ 1520), 378 (ϵ 1830), 403 (ϵ 2230); ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.34 (s, 3 H, C(2′)CH_3), 1.95−2.49 (m, 3 H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.71 (dd, J = 11.5, 14.3 Hz, 1 H, C(5a)*H*H′), 3.53 (d, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.63−4.13 (m, 4 H, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′, C(5a)H*H*′), 4.04 (s, 1 H, C(1′)H), 7.36 (dd, J = 2.2, 9.3 Hz, 1 H, C(3″)H), 7.42−7.49 (m, 2 H, C(6″)H, C(7″)H), 7.89−8.05 (m, 4 H, C(1″)H, C(4″)H, C(5″)H, C(8″)H), 8.32 (s, 1 H, C(9″)H or C(10″)H), 8.38 (s, 1 H, C(10″)H or C(9″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.28 (s, 3 H, C(2′)CH_3), 2.90 (dd, J = 11.5 Hz, 14.3 Hz, 1 H, C(5a)*H*H′), 3.61 (d, J = 11.6 Hz, 1 H, C(3′)*H*H′), 4.06 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.3, 30.2, 32.6, 51.8, 62.1, 68.6, 72.3, 78.2, 83.9, 89.4, 126.0, 126.3, 126.4, 126.8, 127.2, 127.6, 129.1, 129.3, 129.8, 131.5, 133.1, 133.4, 133.7, 134.6, 168.8, 172.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.7, 31.7, 63.3, 72.4, 83.7, 126.1, 126.7, 127.5, 129.9 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (+CI) 512 [M]$^+$; M_r (+CI) 512.162 68 [M]$^+$ (calcd for $C_{26}H_{28}N_2O_7S$ 512.161 72).

5a-(Anthracen-9-ylmethylsulfanyl)dihydrobicyclomycin (14). Using general procedure 1, **17** (40 mg, 0.12 mmol) and **39** (156 mg, 0.59 mmol) gave **29** as a crude mixture (R_f 0.39 (5% MeOH−$CHCl_3$)). The reaction was not purified but immediately deprotected using general procedure 2 to give **14** as a mixture of diastereomers (∼3:1): yield 17 mg (28%); mp 115−120 °C; R_f 0.51 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3312 (br), 2927 (br), 1686, 1400, 1029 cm^{-1}; ^{1}H NMR (4:1 CD_3OD−DMSO-d_6) for the major diastereomer, δ 1.30 (s, 3 H, C(2′)CH_3), 2.02−2.15 (m, 2 H, C(4)*H*H′, C(4)H*H*′), 2.33−2.54 (m, 2 H, C(5)H, C(5a)*H*H′), 3.48 (d, J = 11.6 Hz, 1 H, C(3′)*H*H′), 3.58−3.95 (m, 4 H, C(3)*H*H′, C(3)H*H*′, C(3′)H*H*′, C(5a)H*H*′), 3.99 (s, 1 H, C(1′)H), 4.75 (d, 1 H, J = 11.3 Hz, 1 H, C*H*H′Ar), 4.81 (d, 1 H, J = 11.3 Hz, 1 H, CH*H*′Ar), 7.47−7.62 (m, 4 H, C(2″)H, C(3″)H, C(6″)H, C(7″)H), 8.06 (d, J = 8.8 Hz, 2 H, C(1″)H, C(8″)H or C(4″)H, C(5″)H), 8.36 (d, J = 8.8 Hz, 2 H, C(4″)H, C(5″)H or C(1″)H, C(8″)H), 8.48 (s, 1 H, C(10″)H); ^{1}H NMR (4:1 CD_3OD-DMSO-d_6) for the minor diastereomer, δ 4.01 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (DMSO-d_6) for the major diastereomer, 23.7, 28.9, 32.3, 35.6, 51.2, 57.9, 66.6, 70.5, 76.8, 81.9, 87.4, 124.2 (2 C), 125.1 (2 C), 126.0 (2 C), 126.7 (2 C), 128.8 (2 C), 129.1, 129.4, 130.9 (2 C), 168.9, 171.2 ppm; ^{13}C NMR (DMSO-d_6) for the minor diastereomer, 28.7, 81.8, 87.6 ppm, the remaining peaks overlapped with nearby signals and were not resolved; M_r (+CI) 527.185 26 [M + 1]$^+$ (calcd for $C_{27}H_{31}N_2O_7S$ 527.185 20).

5a-(Anthraquinon-2-ylmethylsulfanyl)dihydrobicyclomycin (16). Using general procedure 1, **17** (40 mg, 0.12 mmol) and **41** (125 mg, 0.42 mmol) gave **31** as a crude mixture (R_f 0.60 (10% MeOH−$CHCl_3$)). The reaction was not purified but immediately deprotected using general procedure 2 to give **16** as a mixture of diastereomers (∼3:1): yield 15 mg (20%); mp 143−155 °C; R_f 0.57 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3320 (br), 2934 (br), 1692, 1398, 1044 cm^{-1}; UV−vis (H_2O) 261 (ϵ 29 900), 377 (ϵ 4300); ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.31 (s, 3 H, C(2′)CH_3), 1.98−2.41 (m, 4 H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.10 (m, 1 H, C(5a)H*H*′), 3.48 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), 3.63 (d, J = 10.5 Hz, 1 H, C(3′)H*H*′), 3.67−4.03 (m, 4 H, C(3)*H*H′, C(3)H*H*′, SCH_2Ar), 4.01 (s, 1 H, C(1′)H), 7.79−7.86 (m, 3 H, C(3″)H, C(6″)H, C(7″)H), 8.14−8.26 (m, 4 H, C(1″)H, C(4″)H, C(5″)H, C(8″)H); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.34 (s, 3 H, C(2′)CH_3), 3.54 (d, J = 10.5 Hz, 1 H, C(3′)*H*H′), the remaining peaks overlapped with nearby signals and were not resolved; the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 30.2, 31.6, 36.8, 52.2, 62.2, 68.5, 72.4, 78.1, 83.7, 89.4, 128.0, 128.1, 128.5, 133.6, 134.8, 134.9, 135.4, 135.5, 136.0, 147.6, 168.7, 172.0, 184.0, 184.1 ppm, the intensity of the signals at 128.5 and 134.8 ppm were approximately twice that of nearby peaks; ^{13}C NMR (CD_3OD) for the minor diastereomer, 30.8, 63.3, 72.5, 83.5 ppm, the remaining peaks overlapped with nearby signals and were not resolved; MS (−CI) 556 [M]$^-$; M_r (+CI) 557.159 02 [M + 1]$^+$ (calcd for $C_{27}H_{29}N_2O_9S$ 557.159 38).

2-Acetylsulfanylmethylnaphthalene (37). Using general procedure 3, **32** (500 mg, 2.26 mmol) and potassium thioacetate (309 mg, 2.71 mmol) gave **37**: yield 375 mg (77%); mp 29−30 °C; R_f 0.55 (10% EtOAc−hexanes); FT-IR (KBr) 3374, 2948, 1687, 1129, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3 H, C(O)CH_3), 4.19 (s, 2 H, SCH_2Ar), 7.29 (d, J = 8.2 Hz, 1 H, C(3)H), 7.32−7.40 (m, 2 H, C(6)H, C(7)H), 7.61−7.72 (m, 4 H, C(1)H, C(4)H, C(5)H, C(8)H); the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR ($CDCl_3$) 30.0, 33.4, 125.7, 126.0, 126.6, 127.2, 127.4, 127.5, 128.2, 132.3, 133.1, 134.8, 194.5 ppm; MS (+CI) 217 [M + 1]$^+$; M_r (+CI) 217.068 10 [M + 1]$^+$ (calcd for $C_{13}H_{13}OS$ 217.068 71). Anal. Calcd for $C_{13}H_{12}OS$: C, 72.19; H, 5.59; S, 14.82. Found: C, 71.90; H, 5.64; S, 14.71.

6-Acetylsulfanylmethylquinoline (38). To a CCl_4 solution of 6-methylquinoline (2.00 g, 14.0 mmol) was added NBS (freshly recrystallized from H_2O, 2.50 g, 14.00 mmol) and a catalytic amount of benzoyl peroxide (≈50 mg).[40a] The mixture was heated to reflux (2 h) until coagulants started to form (TLC analysis indicated that some starting material remained). The mixture was cooled (rt), filtered, and concentrated in vacuo to give **33**[40b] as a crude mixture. The crude product (R_f 0.43 (50% EtOAc−hexanes)) was not purified but used immediately in general procedure 3 with potassium thioacetate (1.92 g, 16.8 mmol) to provide **38**: yield 2.20 g (72%); R_f 0.47 (50% EtOAc−hexanes); FT-IR (neat) 1691, 1588, 1500, 1358, 1126 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3 H, C(O)CH_3), 4.28 (s, 2 H, SCH_2Ar), 7.36 (dd, J = 3.8, 8.2 Hz, 1 H, C(3)H), 7.61 (dd, J = 2.2, 8.8 Hz, 1 H, C(7)H), 7.72 (s, 1 H, C(5)H), 8.02−8.10 (m, 2 H, C(4)H, C(8)H), 8.87 (dd, J = 1.6, 3.8 Hz, 1 H, C(2)H); the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR ($CDCl_3$) 30.2, 33.2, 121.2, 127.1, 127.9, 129.8, 130.3, 135.6, 135.9, 147.4, 150.2, 194.6 ppm; MS (+CI) 218 [M + 1]$^+$; M_r (+CI) 218.064 08 [M + 1]$^+$ (calcd for $C_{12}H_{12}NOS$ 218.063 96). Anal. Calcd for $C_{12}H_{11}NOS$: C, 66.33; H, 5.10; N, 6.45; S, 14.76. Found: C, 66.53; H, 5.32; N, 6.21; S, 14.82.

9-Acetylsulfanylmethylanthracene (39). Using general procedure 3, **34** (500 mg, 2.20 mmol) and potassium thioacetate (301 mg, 2.64 mmol) gave **39**: yield 525 mg (90%); mp 100−102 °C; R_f 0.48 (10% EtOAc−hexanes); FT-IR (KBr) 1682, 1433, 1344, 1125 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H, C(O)CH_3), 5.09 (s, 2 H, SCH_2Ar), 7.37−7.42 (m, 2 H, C(2)H, C(7)H or C(3)H, C(6)H), 7.46−7.51 (m, 2 H, C(3)H, C(6)H or C(2)H, C(7)H), 7.89 (d, J = 8.8 Hz, 2 H, C(1)H, C(8)H or C(4)H, C(5)H),

8.14 (d, J = 8.8 Hz, 2 H, C(4)H, C(5)H or C(1)H, C(8)H), 8.27 (s, 1 H, C(10)H); the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR ($CDCl_3$) 26.3, 30.1, 123.8 (2 C), 125.0 (2 C), 126.3 (2 C), 127.4, 127.7 (2 C), 129.1 (2 C), 129.9 (2 C), 131.3, 195.5 ppm; MS (+CI) 267 [M + 1]$^+$; M_r (+CI) 266.077 14 [M]$^+$ (calcd for $C_{17}H_{14}OS$ 266.076 54). Anal. Calcd for $C_{17}H_{14}OS \cdot 0.05\ (CH_3)_2SO$: C, 76.01; H, 5.33; S, 12.46. Found: C, 75.76; H, 5.41; S, 12.42.

2-Acetylsulfanylmethylphenazine (40). Using general procedure 3, **35**[40a] (570 mg, 2.09 mmol) and potassium thioacetate (286 mg, 2.51 mmol) gave **40**: yield 302 mg (54%); mp 113−115 °C; R_f 0.23 (20% EtOAc−hexanes); FT-IR (KBr) 1680, 1508, 1355, 1116 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3 H, C(O)-CH_3), 4.33 (s, 2 H, SCH_2Ar), 7.66 (d, J = 9.3 Hz, 1 H, C(3)H), 7.71−7.79 (m, 2 H, C(6)H, C(7)H), 8.01−8.20 (m, 4 H, C(1)H, C(4)H, C(5)H, C(8)H); the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR ($CDCl_3$) 30.1, 33.3, 128.2, 129.3, 129.4, 129.7, 130.0, 130.1, 131.4, 140.0, 142.4, 142.9, 143.1, 143.3, 193.9 ppm; MS (+CI) 269 [M + 1]$^+$; M_r (+CI) 269.074 89 [M + 1]$^+$ (calcd for $C_{15}H_{13}N_2OS$ 269.074 86). Anal. Calcd for $C_{15}H_{12}N_2OS \cdot 0.2\ CH_3OH$: C, 66.45; H, 4.66; N, 10.20; S, 11.67. Found: C, 66.32; H, 4.65; N, 9.84; S, 11.72.

2-Acetylsulfanylmethylanthraquinone (41). Using general procedure 3, **36** (325 mg, 1.08 mmol) and potassium thioacetate (148 mg, 1.30 mmol) gave **41**: yield 302 mg (94%); mp 139−141 °C; R_f 0.79 (50% EtOAc−hexanes); FT-IR (KBr) 1678, 1588, 1297, 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3 H, $C(O)CH_3$), 4.24 (s, 2 H, SCH_2Ar), 7.72 (dd, J = 1.6, 8.2 Hz, 1 H, C(3)H), 7.75−7.82 (m, 2 H, C(6)H, C(7)H), 8.18 (d, J = 1.6 Hz, 1 H, C(1)H), 8.22 (d, J = 8.2 Hz, 1 H, C(4)H), 8.24−8.31 (m, 2 H, C(5)H, C(8)H); the structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment; ^{13}C NMR ($CDCl_3$) 30.2, 33.1, 127.1, 127.2, 127.3, 127.7, 132.4, 133.4, 134.0, 134.1, 134.4, 144.7, 182.6, 182.8, 194.2 ppm, the intensity of the signals at 133.4 and 134.0 ppm were approximately twice that of nearby peaks; MS (+CI) 297 [M + 1]$^+$; M_r (+CI) 297.058 12 [M + 1]$^+$ (calcd for $C_{17}H_{13}O_3S$ 297.058 54). Anal. Calcd for $C_{17}H_{12}O_3S$: C, 68.90; H, 4.08; S, 10.82. Found: C, 68.86; H, 4.35; S, 10.52.

Inhibitory Properties of Bicyclomycin and Bicyclomycin Derivatives in the Poly(C)-Dependent ATPase Assay.[42] The ability of His-tagged wild-type rho[9] 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 $MgCl_2$), 250 μM ATP, 0.5 μCi of [γ-^{32}P] ATP, 300 nM poly(C), and 200 nM rho based on monomer. Reactions were preincubated at 32 °C for 90 s prior to the addition of ATP. Aliquots (1 μL) were removed at various times (15, 30, 45, 60, 75 s) during the reaction and spotted onto PEI-TLC plates. [γ-^{32}P] ATP and $^{32}P_i$ were separated by chromatography on PEI-TLC plates using 0.75 M KH_2PO_4, pH 3.5, as the mobile phase. The developed PEI-TLC plates were used to expose PhosphorImager Plates (Fuji and Molecular Dynamics) (3 h), scanned on a Storm 860 PC PhosphorImager and analyzed using Molecular Dynamics's ImageQuant 5.0. 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. Each assay was performed in duplicate, and the results were averaged.

Inhibitory Properties of Bicyclomycin and Bicyclomycin Derivatives in the Antimicrobial Assay.[43] Aliquots (200 μL) from suspensions of overnight LB broth cultures (*E. coli* W3350) were diluted into LB broth (2 mL). The suspension was poured onto 15 mL volume LB agar plates. The solution was gently rocked to distribute the cells evenly over the plates surface, and any excess cell solution was removed by pipet. The plates were incubated at 37 °C (30 min), and an antibiotic-assay disk (0.25 in. diameter) containing 20 μL to the test compound (1, 2, 4, 8, 16, 32 mg/mL in 50−100% DMSO) 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. Each assay was performed in duplicate, and the results were averaged.

General Fluorescence Procedure. Binding Measurements Using Fluorescence Resonance Energy Transfer (FRET). The intrinsic tryptophan fluorescence of wild-type rho (1 μM based on monomer) was measured in fluorescence buffer (40 mM Tris−HCl, pH 7.9, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT) at 25 °C with spectral bandwiths of 4 nm (excitation) and 16 nm (emission). Rho was equilibrated in fluorescence buffer (60 min) before fluorescence measurements. Native rho showed an excitation maximum of 282 nm and an emission maximum of 345 nm. The excitation wavelength used was 290 nm to minimize inner filter effects from nucleotides and selectively excite W381. FRET due to binding of **15** and ATP was measured as a decrease in fluorescence intensity at 345 nm. To measure percent energy transfer at 345 nm at each concentration of **15** or ATP the following equation was used

$$\%\ \text{transfer}_{345} = \left(1 - \frac{F_{345}}{F_{\text{max}345}}\right)100 \qquad (1)$$

where $F_{\text{max}345}$ is the corrected fluorescence emission at 345 nm in the absence of **15** or ATP, F_{345} is the corrected fluorescence emission at each concentration of **15** or ATP. The maximum % transfer_{345} was used to indicate complete (observed) ligand binding binding for Scatchard analysis based on the observation of only tight ATP binding in our system.

Binding of 5a-(Phenazin-2-ylmethylsulfanyl)dihydrobicyclomycin (15) to Wild-Type Rho Using Fluorescence Resonance Energy Transfer (FRET) at a Constant ATP Concentration. The general fluorescence procedure was used, and each sample contained fluorescence buffer, rho (1 μM based on monomer), ATP (250 μM), and **15** (0−40 μM). Inner filter effects were corrected for by subtracting the fluorescence intensity decrease at each concentration of **15** with rho (1 μM based on monomer) in the absence of ATP.[48]

Binding of ATP to Wild-Type Rho Using Fluorescence Resonance Energy Transfer (FRET) at a Constant 15 Concentration. The general fluorescence procedure was used, and each sample contained fluorescence buffer, rho (1 μM based on monomer), **15** (60 μM), ATP (0−240 μM). No correction for inner filter effects was performed since **15** was held constant throughout the experiment and adenosine nucleotides had little affect on the inner filter effects at the excitation and emission wavelengths used.

Distance Measurement Using Fluorescence Resonance Energy Transfer (FRET). The distance between a donor/acceptor pair can be measured by Förster energy transfer.[34,35] The fluorescence intensity of the donor (W381 in rho) was measured in the presence (F_{Da}) and in the absence (F_{D}) of acceptor (**15**), and the resonance energy transfer efficiency (E) was determined from the equation

$$E = 1 - \frac{F_{\text{Da}}}{F_{\text{D}}} \qquad (2)$$

The distance (R) between the donor (W381 in rho) and the acceptor (**15**) was determined by

$$R = R_o(E^{-1} - 1)^{1/6}\ \text{in Å} \qquad (3)$$

where R_o is the Förster distance at which the transfer efficiency is equal to 50%.[50] The distance R_o was calculated by the equation[51]

$$R_o = 0.211[\kappa^2 n^{-4} \Phi J(\lambda)]^{1/6}\ \text{in Å} \qquad (4)$$

where n is the refractive index of the buffer, assumed to be 1.4 for biomolecules in aqueous solution;[35] Φ is the quantum yield of the donor (W381 in rho); κ^2 is the orientation factor, a value of 0.67 was used assuming that donor and acceptor groups rotate freely in a short time relative to the excited-state lifetime of the donor;[52] and $J(\lambda)$ is the spectral overlap integral in M^{-1} cm^{-1} nm^4 given by the following equation

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\epsilon_A(\lambda)\lambda^4\, d\lambda}{\int_0^\infty F_D(\lambda)\, d\lambda} \quad (5)$$

where $F_D(\lambda)$ is the corrected fluorescence (F_D) of the donor (rho W381) in the absence of acceptor (**15**) at λ, $\epsilon_A(\lambda)$ is the extinction coefficient of the acceptor (**15**) at λ in M^{-1} cm^{-1}, and λ is in nanometers. $J(\lambda)$ was numerically integrated at 1-nm intervals.

The fluorescence intensity (λ_{exc} = 290 nm) of a solution containing fluorescence buffer, rho (1 μM based on monomer) and **15** (40 μM) was used for F_D. This solution was also used to determine Φ of the donor (W381 in rho, Φ = 0.08) based on a tryptophan standard[35] to correct for inner filter effects.[48] The fluorescence intensity (λ_{exc} = 290 nm) of a solution containing fluorescence buffer, rho (1 μM based on monomer), **15** (40 μM) and ATP (50 μM) was used for F_{Da}. ATP was varied in F_D and F_{Da} at a constant **15** (40 μM) concentration to correct for inner filter effects.[48]

(52) Eftlink, M. E. *Methods Biochem. Anal.* **1991**, *35*, 127−205.

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Supporting Information Available: General methods along with ^1H 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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