in context with the biosynthetic studies on the kinamycins[32] and on metabolites of the toromycin/gilvocarcin group (e.g., chrysomycin B[33]) an angucyclinone-type intermediate was proven and assumed, respectively, which undergoes a rearrangement leading to the found structures. Thus, a strong similarity of the polyketide synthases of these antibiotic groups with the angucycline-PKS is or seems to be evident.

Such similarities of the polyketides synthases may have implications in future biosynthetic studies on the different types of multicyclic polyketides which cannot be carried out without the tools of genetic engineering and/or mutagenesis. This also justifies further biosynthetic studies on the angucyclines, which will be focused on in early biosynthetic steps, since these may be also relevant for the clinically important tetracyclines and anthracyclines.

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The Synthesis and Reactivity of [N(8)–C(3′)]-Cyclized Bicyclomycin. Evidence of the Role of the C(1)-Triol Group in Bicyclomycin-Mediated Processes

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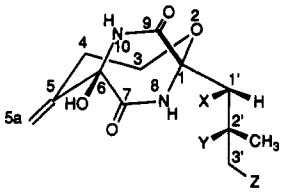
Received December 18, 1991 (Revised Manuscript Received June 3, 1992)

The C(1) triol group in the antibiotic, bicyclomycin (1) has been proposed to play an integral role in the bonding of key protein nucleophiles to the distal C(5)–C(5a) terminal double bond in the drug. Evidence in support of this concept has been provided by the comparison of the reactivities of bicyclomycin (1), the [N(8)–C(3′)]-cyclized bicyclomycin adduct 3, 2′,3′-bicyclomycin acetonide (17), and the acetonide derivative of 3, 18, with sodium ethanethiolate. Significantly, 3 displayed enhanced reactivity versus 1, 17, and 18 in this transformation. The controlling factors for the increased reactivity of 3 have been discerned and the importance of the C(1′) hydroxyl group delineated. Key kinetic parameters are reported for the treatment of both 3 and 17 with 2-mercaptopyridine. Structural details are provided for both C(5a) thiolate and amine adducts of 3. The importance of these findings in relation to the mode of action of bicyclomycin are briefly discussed.

Bicyclomycin (1) is a structurally unique antibiotic possessing a diverse spectrum of biological activity.[1-4] Important architectural features in 1 include the bicyclic [4.2.2] ring structure, the C(5)–C(5a) exomethylene group, and the C(1) triol moiety. Most mechanistic proposals concerning the mode of action of 1 suggest that nucleophilic residues present in key proteins involved in bacterial cell wall growth irreversibly bind to the terminal double bond at C(5).[5-8] The role of the appended C(1) triol group in these transformations is unclear. This information remains an important objective in the elucidation of the biological pathway of this commercial antibiotic.

Several studies pertinent to this issue have appeared. First, all structural modifications of the C(1) triol moiety in bicyclomycin led to a pronounced reduction in the biological activity of the drug candidates.[6,9,10] Second,



1 X = Y = Z = OH
4 X = Y = OH; Z = $OS(O)_2CH_3$
14 X = OH; Y, Z = O
15 X = Y = OH; Z = SCH_2CH_3
16 X = Y = OH; Z = $OC(O)NHCH_2CH_3$
17 X = OH; Y, Z = $OC(CH_3)_2O$

Williams and co-workers reported that thiolate addition to the bicyclomycin mimic 2 at "pH" 12.5 in tetrahydrofuran (THF)–water (3:1) mixtures was promoted by intramolecular transfer of a proton from the C(1′) hydroxyl group to the C(9) carbonyl moiety.[10] Third, Kohn and Abuzar demonstrated that modification of the C(1) triol moiety in 1 both impeded the functionalization of the exomethylene group and prevented the formation of bicyclomycin-derived piperidinedione-type adducts at near neutral "pH" values.[8d] In this paper, we report that reaction at the exomethylene group in the annelated bicyclomycin adduct 3[9,11] with thiolate species proceeded *more rapidly* than the corresponding process with 1. Analysis of the structural factors responsible for the enhanced reactivity of 3 versus 1 provides evidence for the

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(6) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733.
(7) Pisabarro, A. G.; Canada, F. J.; Vasquez, D.; Arriaga, P.; Rodriguez-Tebar, A. *J. Antibiot.* **1986**, *34*, 914.
(8) (a) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089. (b) Abuzar, S.; Kohn, H. *J. Org. Chem.* **1989**, *54*, 4000. (c) Kohn, H.; Abuzar, S. *J. Am. Chem. Soc.* **1988**, *110*, 3661. (d) Abuzar, S.; Kohn, H. *Ibid.* **1990**, *112*, 3114.
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(11) The following uninverted Chemical Abstracts Index name for 3 modified by current IUPAC guidelines has been kindly provided by Dr. P. M. Giles (Chemical Abstract Services): (5*R*,9*R*,10*S*,10a*S*)-hexahydro-5,9,10-trihydroxy-9-methyl-4-methylene-8*H*-5,10a-(iminomethano)-6*H*-pyrrolo[2,1-*b*][1,3]oxazocine-6,11-dione.

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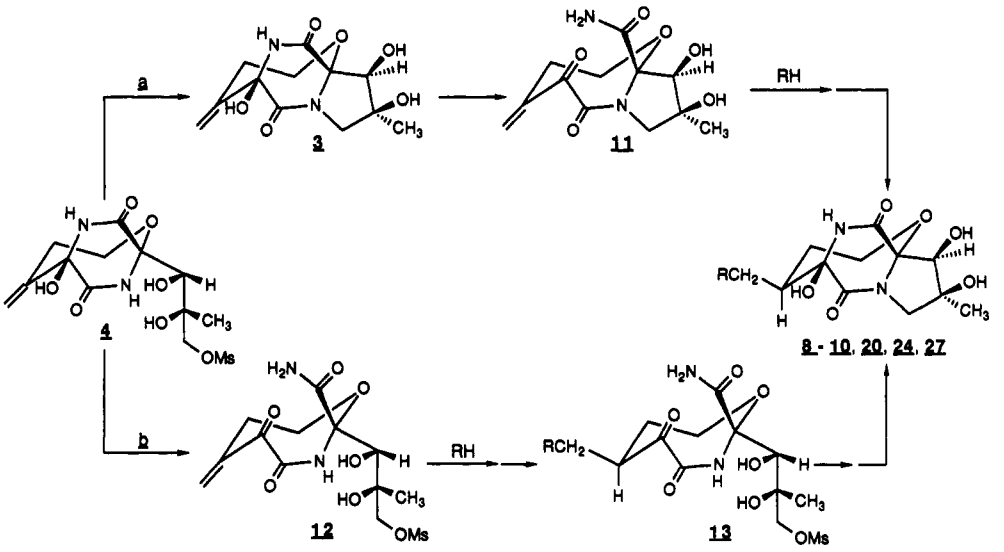
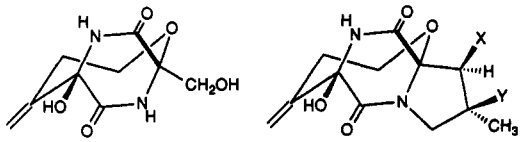
Scheme I. Potential Pathways for the Formation of Compounds 8–10, 20, 24, and 27



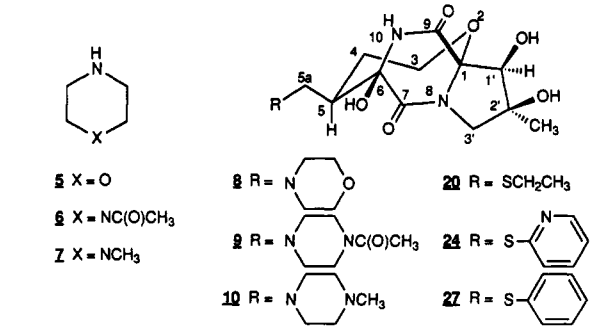
importance of the C(1) triol moiety in activating the distal exomethylene group in bicyclomycin at moderate "pH" values.



2 3 X = Y = OH 18 X,Y = $OC(CH_3)_2O$

Results and Discussion

Our studies began with the observation that treatment of the known bicyclomycin-3′-*O*-methanesulfonate[9] (4) with 2 equiv of the heterocyclic amines, morpholine (5), *N*-acetylpiperazine (6), and *N*-methylpiperazine (7), led to the efficient production of 8–10, respectively.[12] Inspection of the ^1H and ^{13}C NMR spectra for these adducts indicated that functionalization of the terminal double bond had occurred along with tetrahydropyrrole ring formation.



5 X = O
6 X = $NC(O)CH_3$
7 X = NCH_3

8 R = morpholino 20 R = SCH_2CH_3
9 R = 4-$NC(O)CH_3$-piperazino 24 R = 2-S-pyridyl
10 R = 4-NCH_3-piperazino 27 R = S-phenyl

Key ^1H and ^{13}C NMR spectral properties observed that supported the proposed structural assignments for 8–10 are listed in Table I. Among these was the upfield shift noted for the C(3′) methylene protons and carbon resonances in 8–10 versus 4, the downfield shift of the C(1) carbon resonances in 8–10 versus 1[13] and 4, and the distinctive pattern noted for the C(5a) methylene protons in 8–10 in the ^1H NMR spectra. Interestingly, the ^{13}C NMR spectra for 8–10 revealed only a single set of signals in each case indicating that a single stereoisomer was present in the final product mixture. Verification of the proposed structural assignments for 8–10 was accomplished from the long-range heteronuclear multiple quantum chemical shift correlation (HMBC) NMR spectrum[14] and X-ray crystallographic analysis of 8. In particular, several long-range proton–carbon connectivities (i.e., C(5a)HH′–C(6), C(3′)-HH′–C(1)) were observed in the HMBC experiment consistent with the proposed molecular framework depicted for 8 (see supplementary material, Figure 1). The X-ray structure demonstrated that the ring N(8) and N(10) nitrogens were essentially planar and that an intramolecular hydrogen bond existed between the C(6) hydroxyl group and the morpholine nitrogen atom (see supplementary material, Figure 2).

The facility of this transformation prompted our inquiry into the origin of these products. Two distinct pathways are conceivable, which differ in the relative sequence of the tetrahydropyrrole ring formation process versus the exomethylene functionalization step (Scheme I). Information concerning the preferred pathway for these transformations was deduced by preparing the known [N-(8)–C(3′)]-cyclized bicyclomycin adduct 3.[9] This compound was synthesized from epoxide 14[9] and NaI or by directly treating the methanesulfonate 4 with a saturated NH_3–THF solution. The NMR spectral properties of 3 mirrored those detected for 8–10 except for the resonances associated with the C(5)–C(5a) region within the molecule (Table I). The HMBC NMR spectrum for 3 once again displayed several informative long-range proton–carbon connectivities consistent with the assigned molecular framework (see supplementary material, Figure 3).

Addition of morpholine (5) to a THF–H_2O (3:1) mixture containing 3 led to the efficient production of 8 (8 h) and suggested that 3 may have served as an intermediate in the production of 8–10 from methanesulfonate 4 and heterocyclic amines 5–7. This notion was reinforced by our monitoring the reaction of 4 with morpholine (5) as a function of time. TLC analysis of the reaction mixture during the early stages of this transformation (0–2 h) indicated only the presence of starting material and cyclized

(12) The following uninverted Chemical Abstracts Index name for 8 modified by current IUPAC guidelines has been kindly provided by Dr. P. M. Giles (Chemical Abstract Services): (4*R*,5*R*,9*R*,10*S*,10a*S*)-hexahydro-5,9,10-trihydroxy-9-methyl-4-(4-morpholinylmethyl)-8*H*-5,10a-(iminomethano)-6*H*-pyrrolo[2,1-*b*][1,3]oxazocine-6,11-dione.

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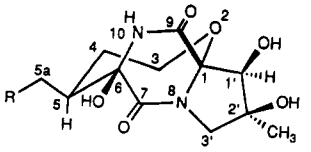
Table I. Key ^1H and ^{13}C NMR Spectral Properties for Bicyclomycin and Select Cyclized Bicyclomycin Derivatives[a]



	^1H NMR[b]						^{13}C NMR[c]					
compd	C(5)H	C(5a)*H*H′	C(5a)H*H*′	C(1′)H	C(3′)*H*H′	C(3′)H*H*′	C(1)	C(6)	C(1′)	C(2′)	C(3′)	C(5a)
1	–	5.13 (s)	5.55 (s)	4.07 (s)	3.50 (d, J = 11.4 Hz)	3.66 (d, J = 11.4 Hz)	89.56	82.99	72.25	78.17	68.51	116.88
3	–	5.11 (s)	5.55 (s)	3.85 (s)	3.48 (d, J = 12.1 Hz)	3.74 (d, J = 12.1 Hz)	94.70	84.27	81.80	75.20	58.41	117.07
4	–	5.14 (s)	5.56 (s)	4.07 (s)	4.25 (d, J = 9.9 Hz)	4.31 (d, J = 9.9 Hz)	89.71	82.96	71.41	76.93	75.74	116.96
8	2.40–2.70 (m)	2.28 (dd, J = 4.0, 13.0 Hz)	2.88 (dd, J = 11.6, 13.0 Hz)	3.85 (s)	3.50 (d, J = 12.2 Hz)	3.62–3.78 (m)	93.73	86.75	81.78	75.21	58.44	59.90
9	2.46–2.70 (m)	2.20 (dd, J = 3.9, 12.6 Hz)	2.90 (dd, J = 11.1, 12.6 Hz)	3.84 (s)	3.51 (d, J = 12.3 Hz)	3.70 (d, J = 12.3 Hz)	93.79	86.57	81.77	75.22	58.38	59.08
10	2.41–2.70 (m)	2.25–2.35 (m)	2.90 (dd, J = 11.1, 12.9 Hz)	3.85 (s)	3.51 (d, J = 12.3 Hz)	3.72 (d, J = 12.3 Hz)	93.73	86.77	81.64	75.20	58.44	59.40
20	2.12–2.22 (m)	2.36 (dd, J = 11.4, 13.2 Hz)	3.18 (dd, J = 2.1, 13.2 Hz)	3.83 (s)	3.52 (d, J = 12.6 Hz)	3.68 (d, J = 12.6 Hz)	94.35	84.90	82.21	75.19	57.69	30.17
24	2.23–2.30 (m)	3.13 (dd, J = 11.1, 13.8 Hz)	3.65–3.77 (m)	3.84 (s)	3.52 (d, J = 12.0 Hz)	3.65–3.77 (m)	94.37	85.18	82.34	75.19	57.69	28.88
27	2.22–2.27 (m)	2.74 (dd, J = 11.4, 13.8 Hz)	3.62–3.72 (m)	3.83 (s)	3.50 (d, J = 12.3 Hz)	3.62–3.72 (m)	94.31	84.90	82.28	75.20	57.74	30.14

[a] All spectra were recorded in CD_3OD. [b] 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. ^1H NMR spectra were recorded at 300 MHz. [c] ^{13}C NMR spectra were obtained at 75 MHz.

bicyclomycin **3**, while subsequent chromatograms confirmed the presence of **8** along with **4** and **3**. Finally, we noted that treatment of epoxide **14** with **5** in THF–H_2O mixtures (3:1) furnished **8** as the major product.

Repetition of the morpholine-mediated functionalization of **3** in THF–D_2O (3:1) mixtures led to the selective incorporation of a single nonexchangeable deuterium at C(5) to give **8**-d_1. In agreement with this structural assignment, both of the doublet of doublets observed for the diastereotopic C(5a) methylene protons (i.e., δ 2.28 (J = 4.0, 13.0 Hz), 2.88 (J = 11.6, 13.0 Hz)) appeared as doublets in **8**-d_1 (i.e., δ 2.28 (J = 13.2 Hz), 2.87 (J = 13.2 Hz)), and the multiplet patterns for the C(4) hydrogens in **8** were simplified in **8**-d_1. Attempts to interchange the C(5) proton for deuterium in **8** proved unsuccessful. Dissolution of **8** in THF–D_2O mixtures ("pD" 8.0, 9.5) containing morpholine led to the recovery of starting material.

Additional data concerning the ease of the terminal double bond functionalization process in cyclized bicyclomycin **3** was derived from a series of competition experiments. Previously, we showed that modification of the C(3′)-substituent in **1** (i.e., **15**, **16**) diminished the reactivity of the C(5)–C(5a) exomethylene group toward thiols at near-neutral "pH" values.[8d] For example, treatment of a binary mixture of bicyclomcyin (**1**) and 3′-*S*-ethylbicyclomycin (**15**) with 1 equiv of *N*-acetyl-L-cysteine *N*′-methylamide at "pH" 7.4 (THF–H_2O (3:1)) led to the sole functionalization of **1**, while at "pH" 10.2 (18 h) the reaction was less selective with modification of **1** proceeding to a greater extent than **15**. This series of competition experiments was extended by treating in pairs equimolar amounts of bicyclomycin (**1**) and the bicyclomycin derivatives **3**, **17**,[15] and **18**[16] with sodium ethanethiolate (**19**) (1–2 equiv) in buffered THF–H_2O (3:1) mixtures at "pH" 9.1

$$Na^{+-}SCH_2CH_3$$
19

Acetonide **18** was prepared from **3** and 2,2-dimethoxypropane in DMF containing *p*-toluenesulfonic acid.[19] The

Table II. Competition Experiments for Sodium Ethanethiolate (19) Addition to Bicyclomycin Derivatives[a]

entry	reactants	obsd products
1	**1** + **3**	**20**[b]
2	**3** + **17**	**20**[b]
3	**3** + **18**	**20**[b]
4	**1** + **17**	**21** + **22**[c]
5[d]	**1** + **18**	**21**
6	**17** + **18**	**22**

[a] Reactions were performed in buffered THF–H_2O (3:1) mixtures (0.1 M Tris·HCl, 0.5 mL, "pH" 9.1) containing equimolar amounts (~0.007 mmol) of the bicyclomycin-derived substrates and 1 equiv of sodium ethanethiolate (**19**) unless otherwise specified. The reactions were allowed to proceed at room temperature (48 h) and then the reaction products identified (TLC analysis), isolated, and confirmed by ^1H NMR analysis. [b] A trace amount of **3** was observed (TLC analysis). [c] Noticeable amounts of **1** and **17** were observed at the conclusion of the reaction. [d] Two equiv of sodium ethanethiolate (**19**) were utilized.

(15) Kamiya, T.; Maeno, S.; Kitaura, Y. Belgium Patent 847 475.

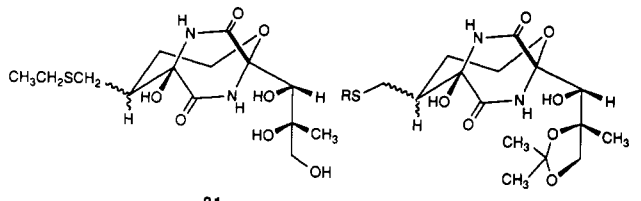
(16) Attempts to prepare the corresponding C(2′)-methyl ether of **3** by treatment with MeI, Ag_2CO_3, $AgClO_4$ in THF[17] or MeI, KF–alumina[18] in acetonitrile led to the recovery of starting material in both cases.

(17) Ramsay, M. V. J.; Roberts, S. M.; Russell, J. C.; Shingler, A. H.; Slawin, A. M. Z.; Sutherland, D. R.; Tiley, E. P.; Williams, D. J. *Tetrahedron Lett.* **1987**, *28*, 5353.

(18) Ando, T.; Yamawaki, J.; Kawate, T.; Sumi, S.; Hanafusa, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2504.

(19) Dissolution of **18** in DCl–D_2O mixtures (pD ~1.0) at 50 °C (3 h) led to the regeneration of **3**.

experiments performed are listed in Table II. Each reaction was run at room temperature for 48 h, and then the products were identified and isolated. In the case of 1 and 17, functionalization of the C(5) terminal bond furnished the direct substituted adducts 21[8d] and 22, respectively. The data obtained indicated that the order of reactivity for these four substrates at this "pH" value was 3 > 1 ~ 17 > 18. The finding that 18 was the least reactive substrate in this series helped remove the notion that the enhanced reactivity of 3 versus 1 primarily stemmed from an overall increase in the strain energy of this compound created by the fusion of the tetrahydropyrrole ring to the central piperazinedione unit in the molecule.



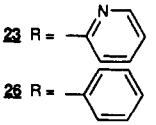
21 22 R = CH_2CH_3 25 R =

The competitive experiments involving bicyclomycin and bicyclomycin derivatives with sodium ethanethiolate provided useful information on the role of key structural elements within the drug in the activation of the distal C(5)–exomethylene group. Additional data on the mechanism of this process was deduced from a kinetic study of 3 and 17 with 2-mercaptopyridine[20] (23) using HPLC analysis. Compounds 3 and 17 were selected for further examination since only the C(1′) hydroxyl group in these substrates can formally facilitate the bonding of the thiolate species to the terminal double bond. Several factors led to our selection of 2-mercaptopyridine (23) as the thiol reagent. First, we required that the solutions remain homogeneous throughout the reaction. Second, we preferred a reagent that provided a distinctive UV signal in the HPLC traces permitting detection and quantification of both the starting thiol and the bicyclomycin-derived thiol products. Third, we required that the retention times in the HPLC chromatograms for the bicyclomycin-derived products 24 and 25, the starting thiol (i.e., 23), and the internal standard to be as close as possible to increase the precision of the analysis.[21] The relative rates for the functionalization of both compounds with 23 (10 equiv) were determined in buffered THF–H_2O ("pH" 9.1) and THF–D_2O ("pD" 9.1) mixtures. Both 3 and 17 were cleanly converted to 24 and 25, respectively. Supportive evidence that C(5)–C(5a) modification in 3 by 23 proceeded at the sulfur terminus of the bifunctional nucleophile rather than at the nitrogen end was provided by the corresponding reaction of 3 with thiophenol (26). Treatment of 3 with 26 led to the efficient production of 27. The ^1H and ^{13}C NMR spectral properties of both 24 and 27 were similar (Table I). In particular, we noted that the C(5a) residues in 24 (δ 3.13, 3.71) compared favorably to those observed for 27 (δ 2.74, 3.67) and that a similar correspondence in values existed for the C(5a) ^{13}C NMR chemical shift values (i.e., 24: δ 28.88; 27: δ 30.14). A kinetic study was also attempted for acetonide 18 with 23. At 26 °C, no reaction was observed after 7 days in buffered THF–H_2O ("pH" 9.1) mixtures.[22] A similar result was observed at 36 °C after 48 h.

Table III. Rate Constants for the Reaction of Bicyclomycin Derivatives 3 and 17 with 2-Mercaptopyridine (24) in THF–H_2O (D_2O) Mixtures (3:1) at "pH" 9.1 ("pD" 9.1) at Various Temperatures

substrate	solvent	temp (°C)	$k_2 \times 10^{-2}$ (L/mol-min)
3	THF–H_2O	6	0.29
	THF–H_2O	16	1.07
	THF–H_2O	26	3.11
	THF–D_2O	26	0.79
	THF–H_2O	36	8.87
	THF–H_2O	46	17.5
17	THF–H_2O	16	0.54
	THF–H_2O	26	0.76
	THF–D_2O	26	0.29
	THF–H_2O	36	2.29
	THF–H_2O	46	7.03

RSH

23 R = 

The kinetics for 2-mercaptopyridine-mediated reactions were monitored over several half-lives at various temperatures. The determined second-order rate constants are listed in Table III when 10 equiv of 23 (0.19 M) were utilized. Several key kinetic parameters were discerned for these transformations. Significantly, we observed that 3 reacted approximately four times faster than 17. Moreover, both compounds displayed comparable energies of activation (i.e., 3: E^{act} = 18.3 ± 1.0 kcal/mol; 17: E^{act} = 15.9 ± 1.0 kcal/mol) and solvent isotope effects (i.e., 3: k_{H_2O}/k_{D_2O} = 3.89; 17: k_{H_2O}/k_{D_2O} = 2.62) suggesting that the mechanism for both thiolate bonding processes was similar. Reasonable fits to second-order kinetics were observed at lower (i.e., 0.05 M, 3 equiv) and higher (i.e., 0.69 M, 50 equiv) concentrations of 2-mercaptopyridine. A plot of the initial pseudo first-order rate constants for the appearance of 24 versus total thiol concentration (i.e., [RSH]) demonstrated that the rate of production of 24 increased with increasing 2-mercaptopyridine concentrations but with curvature. Accordingly, when [RSH] = 0.05 M, k_{obs} is 2.0 × 10^{-3} min^{-1}, when [RSH] = 0.18 M, k_{obs} is 4.5 × 10^{-3} min^{-1}, and when [RSH] = 0.69 M, k_{obs} is 8.2 × 10^{-3} min^{-1}. This finding shows a non-zero order dependence on thiol in the rate law, but the curvature may be indicative of a change-over in the rate-determining step with increasing concentrations of the thiol.[23]

The differential reactivities of the bicyclomycin-derived compounds 1, 3, 17, and 18 provided direct evidence that the C(1′) hydroxyl group played an important role in the activation of the exomethylene group in the antibiotic. In particular, we note the enhanced reactivity of 3 versus 1 toward sodium ethanethiolate and the pronounced diminution of reactivity of 3 when the C(1′) and C(2′) hydroxyl groups in this derivative were protected (i.e., 18). Several mechanisms can be presented that are consistent with this structure–reactivity relationship, the observed bimolecular kinetics, and the solvent isotope effects secured for 3 and 17. In one scenario (Scheme II) tautom-

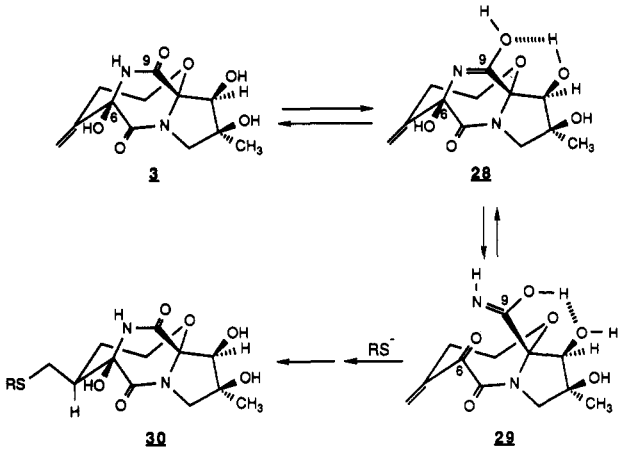
(20) The pK_a of 2-mercaptopyridine has been determined to be 9.94; see: Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solutions*; Butterworth: London, 1965; p 164.

(21) Snyder, L. R.; Kirland, J. J. *Introduction to Modern Liquid Chromatography*; Wiley-Interscience: New York, 1974; pp 440–441.

(22) Deletion of the buffer did not lead to any noticeable consumption of 18.

(23) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; Chapter 11.

Scheme II. Proposed Pathway for the Modification of the Exomethylene Group in Bicyclomycin Derivative 3

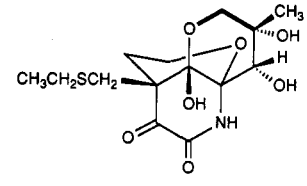


erization of the C(9) amide system in **3** to **28** precedes the ring opening of the piperazinedione-type system to give **29**. Hemiaminal bond cleavage is envisaged to be facilitated by intramolecular transfer of a proton from the C(1′) hydroxyl group to the C(9) amide system,[24] possibly through the agency of a bridging water molecule. Preequilibrium formation of **29** then permits the rate-limiting addition of the thiolate species at low thiol concentration to take place leading to the eventual production of **30**.[25] This mechanism is supported by the non-zero-order dependency in **23** observed for the formation of **24**. We suspect that the deuterium solvent isotope effect detected for **3** and **17** largely reflects the combination of the decrease in acidity[26] of the weak acid **23**[20] in D_2O versus H_2O and the inhibitory equilibrium solvent isotope effect on the ring opening of **28** (i.e., bond strengths of C(6)O–H of **28** versus C(9)N–H of **29**) upon replacement of the H_2O by the D_2O.

The pathway outlined in Scheme II is similar to the mechanism projected by Williams and co-workers for the reaction of **2** with the more reactive nucleophile, sodium methanethiolate, at "pH" 12.5 in THF–H_2O mixtures.[10] These investigators suggested that the rate-limiting step was the ring opening of the piperazinedione system to give the α,β-unsaturated enone in which ring cleavage is facilitated by a bridging water molecule. Support for this mechanism was derived from the observed solvent isotope effect. This study did not examine the effect of varying methanethiolate concentration on the rate even though the kinetic data were derived using a second-order kinetic treatment that is first-order in methanethiol. We have, however, provided an alternative explanation for the solvent isotope effect that is not exclusively dependent on the rate-limiting hemiaminal bond cleavage of the piperazinedione ring system. In addition, we have observed a non-zero-order dependency in 2-mercaptopyridine in the rate law for the formation of **24** from **3** which approaches a first-order dependency at low thiol concentrations (i.e., 0.05–0.19 M). Accordingly, we favor the mechanism depicted in Scheme II. The observed deviation at higher thiol concentrations suggests that a mixed kinetic dependency exists for this reaction and that a complete change-over in the rate-determining step may take place in the case of more reactive thiolates.

Conclusions

The enhanced reactivity of **1** and **17** versus **18** provided evidence that the C(1′) hydroxyl group in bicyclomycin derivatives played a key role in the activation of the C-(5)–C(5a) exomethylene group toward nucleophilic attack by thiolate species. The special reactivity observed for **3** versus **1** and **18** is consistent with the notion that localization of the C(1′) hydroxyl group in close proximity to the C(9) amide bond system facilitates the conversion of **3** to **30** presumably by an intramolecular proton transfer process. Our findings that the C(5)–C(5a) thiolate bonding process in **3** is facilitated by this moiety underscores the importance of elucidating the role of the C(1) triol group in other bicyclomycin transformations including the generation and subsequent modification of C(5a)-substituted piperidinediones (i.e., **31**)[8] and the binding of the antibiotic to the biological receptor.



31

Experimental Section

General Methods. The experimental procedure used in this study were identical to those employed in previous investigations.[8] Generous supplies of bicyclomycin were obtained from Fujisawa Pharmaceutical Co., Ltd., Japan. THF was distilled from Na^0 and benzophenone. Long-range heteronuclear multiple quantum chemical shift correlation (HMBC) experiments were conducted at Rice University on a Bruker AMX-500-MHz NMR instrument by Dr. Garry King. Mass spectral determinations were conducted at the Baylor College of Medicine on VG ZAB-SEQ and VG JS250 instruments by Dr. Simon Gaskell, Ms. Odile Burlet, and Mr. Ralph Orkiszewski. "pH" measurements of aqueous organic mixtures were determined on either a Radiometer pHM84 or a pHM26 meter using a Radiometer G202 glass electrode.

Bicyclomycin-3′-*O*-methanesulfonate (4).[9] The title compound was prepared according to the procedure reported by Muller and co-workers[9] with slight modification. Bicyclomycin (150 mg, 0.50 mmol) was dissolved in anhydrous pyridine (2.0 mL), and the temperature of the solution was lowered to −10 °C. CH_3SO_2Cl (148 mg, 1.30 mmol) was then added, and the temperature was maintained at 0 °C (2 h). The reaction mixture was filtered, and the solvent was removed in vacuo. The residue was subjected to flash chromatography on SiO_2 (10% MeOH–$CHCl_3$). A pale-yellow solid was obtained after drying overnight under vacuum: yield 117 mg (61%); mp 138–142 °C dec (lit.[9] mp 151–153 °C); R_f 0.55 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.40 (s, 3 H, C(2′)CH_3), 2.58–2.68 (m, 2 H, C(4)H_2), 3.08 (s, 3 H, CH_3SO_3), 3.80–3.90 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 4.25 (d, 1 H, C(3′)*H*H′, J = 9.9 Hz), 4.32 (d, 1 H, C(3′)H*H*′, J = 9.9 Hz), 5.13 (s, 1 H, C(5a)*H*H′), 5.56 (s, 1 H, C(5a)H*H*′); ^{13}C NMR (CD_3OD) 23.67 (C(2′)CH_3), 36.66 (C(4)), 37.30 (CH_3SO_3), 65.73 (C(3)), 71.41 (C(1′)), 75.74 (C(3′)), 76.93 (C(2′)), 82.96 (C(6)), 89.71 (C(1)), 116.96 (C(5a)), 149.51 (C(5)), 168.35 (C(7) or C(9)), 172.48 ((C9) or C(7)) ppm. The ^{13}C NMR assignments were confirmed by the APT and the heteronuclear correlation experiments.

Reaction of Methanesulfonate 4 with Saturated NH_3 in THF. Preparation of 3. In a freshly prepared saturated NH_3 solution in THF (10 mL) was suspended compound **4** (100 mg, 0.26 mmol), and the mixture was stirred at rt (24 h) and then filtered. TLC analysis of the precipitate indicated the presence of **3** as the major product along with several other unidentified reaction adducts. The yellow precipitate was dissolved in a minimum amount of MeOH and then purified by preparative TLC (25% MeOH–CH_2Cl_2) to give compound **3** as an amorphous

(24) Cox, J. P. L.; Nicholls, I. A.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1991**, 1295.

(25) For a study of the Michael addition of *n*-propyl mercaptan to cyclopentenones, see: Wilson, S. R.; Chen, H.-T. *Bioorg. Chem.* **1980**, *9*, 212.

(26) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; 3rd ed.; Harper and Row: New York, 1987; p 243.

off-white solid: yield 26 mg (36%); mp 127–130 °C (lit.[9] mp 120 °C); R_f 0.70 (25% MeOH–CH_2Cl_2); FT-IR (KBr) 1690 (sh), 1678 cm^{-1}; ^1H NMR (CD_3OD) δ 1.45 (s, 3 H, C(2′)CH_3), 2.58–2.64 (m, 2 H, C(4)H_2), 3.48 (d, 1 H, C(3′)*H*H′, J = 12.2 Hz), 3.52–3.62 (m, 1 H, C(3)*H*H′), 3.74 (d, 1 H, C(3′)H*H′*, J = 12.2 Hz), 3.85 (s, 1 H, C(1′)H), 3.90–4.00 (m, 1 H, C(3)H*H′*), 5.11 (s, 1 H, C(5a)*H*H′), 5.55 (s, 1 H, C(5a)H*H′*); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 26.78 (C(2′)*C*H_3), 36.50 (C(4)), 58.41 (C(3′)), 66.36 (C(3)), 75.20 (C(2′)), 81.80 (C(1′)), 84.27 (C(6)), 94.70 (C(1)), 117.07 (C(5a)), 149.59 (C(5)), 167.56 (C(7) or C(9)), 170.11 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 285 [M + 1]$^+$; M_r (+FAB) 285.108 21 [M + 1]$^+$ (calcd for $C_{12}H_{17}N_2O_6$ 285.108 71).

General Procedure for Reaction of Methanesulfonate 4 with Heterocyclic Amines. To a solution of compound **4** (50 mg, 0.13 mmol) in a THF–H_2O (3:1) mixture (1 mL) was added the corresponding amine (0.26 mmol). The reaction was stirred at rt (24 h), during which time two liquid phases formed and the "pH" of the heterogeneous mixture dropped from 8.7–9.4 to 7.1–8.4. The solvents were removed in vacuo, and the residue was dissolved in a minimum amount of MeOH and then purified by preparative TLC. TLC analysis indicated the complete consumption of the starting material and formation of the corresponding adducts (i.e., **8–10**) as the major product along with several other unidentified, more polar adducts.

Reaction of Methanesulfonate 4 with Morpholine (5). Preparative TLC (10% MeOH–$CHCl_3$, three developments) and recrystallization from MeOH afforded white crystals of adduct **8**: yield 12 mg (25%); mp 149–151 °C; R_f 0.60 (20% MeOH–$CHCl_3$); $[\alpha]^{25}_D$ = +37.2° (c = 0.01, MeOH); IR (KBr) 1672 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.46 (s, 3 H, C(2′)CH_3), 1.48–1.54 (m, 1 H, C(4)*H*H′), 1.71–1.82 (m, 1 H, C(4) H*H′*), 2.28 (dd, 1 H, C(5a)*H*H, J = 4.0, 13.0 Hz), 2.40–2.70 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(5)H), 2.88 (dd, 1 H, C(5a)H*H′*, J = 11.6, 13.0 Hz), 3.45–3.55 (m, 2 H, C(3′)*H*H′, C(3)*H*H′), 3.62–3.78 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(3′)H*H′*), 3.85 (s, 1 H, C(1′)H), 3.86–3.95 (m, 1 H, C(3)H*H′*); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 27.05 (C(2′)CH_3), 32.06 (C(4)), 44.08 (C(5)), 54.22 (N(*C*H_2*C*H_2)$_2$O), 58.44 (C(3′)), 59.90 (C(5a)), 63.68 (C(3)), 67.80 (N(*C*H_2*C*H_2)$_2$O), 75.21 (C(2′)), 81.78 (C(1′)), 86.75 (C(6)), 93.73 (C(1)), 167.87 (C(7) or C(9)), 171.53 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT and the ^{13}C–^1H coupled experiments; MS (+FAB) 372 [M + 1]$^+$; M_r (+FAB) 372.177 36 [M + 1]$^+$ (calcd for $C_{16}H_{28}N_3O_7$ 372.177 07).

Reaction of Methanesulfonate 4 with *N*-Acetylpiperazine (6). Preparative TLC (20% MeOH–CH_2Cl_2, two developments) gave adduct **9** as a semisolid: yield 9 mg (17%); R_f 0.30 (20% MeOH–$CHCl_3$); IR (KBr) 1675 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.48 (s, 3 H, C(2′)CH_3), 1.49–1.56 (m, 1 H, C(4)*H*H′), 1.75–1.85 (m, 1 H, C(4)H*H′*), 2.09 (s, 3 H, CH_3CO), 2.20 (dd, 1 H, C(5a)*H*H′, J = 3.9, 12.6 Hz), 2.46–2.70 (m, 5 H, N(*C*H_2*C*H_2)$_2$NAc, C(5)H), 2.90 (dd, 1 H, C(5a)H*H′*, J = 11.1, 12.6 Hz), 3.51 (d, 1 H, C(3′)*H*H′, J = 12.3 Hz), 3.49–3.65 (m, 5 H, N(*C*H_2*C*H_2)$_2$NAc, C(3)*H*H′), 3.70 (d, 1 H, C(3′)H*H′*, J = 12.3 Hz), 3.84 (s, 1 H, C(1′)H), 3.85–3.94 (m, 1 H, C(3)H*H′*); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 21.05 (*C*H_3CO), 26.99 (C(2′)CH_3), 31.92 (C(4)), 42.57 (AcN[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]N or AcN[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]N), 44.62 (C(5)), 47.19 (AcN-[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]N or AcN[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]N), 53.46 (N[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]NAc or N[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]NAc), 53.76 (N[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]NAc or N[(*C*H_2*C*H_2)(*C*H_2*C*H_2)]-NAc), 58.38 (C(3′)), 59.08 (C(5a)), 63.66 (C(3)), 75.22 (C(2′)), 81.77 (C(1′)), 86.57 (C(6)), 93.79 (C(1)), 167.79 (C(7) or C(9) or *C*OMe), 171.51 (C(9) or C(7) or *C*OMe), 171.65 (*C*OMe or C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 413 [M + 1]$^+$; M_r (+FAB) 413.203 66 [M + 1]$^+$ (calcd for $C_{18}H_{29}N_4O_7$ 413.203 62).

Reaction of Methanesulfonate 4 with *N*-Methylpiperazine (7). Preparative TLC (30% MeOH–$CHCl_3$, two developments) afforded adduct **10** as a semisolid. Further purification of this material was accomplished by preparative TLC (30% MeOH–$CHCl_3$, two developments) to give the title compound: yield 3.1 mg (7%); R_f 0.25 (30% MeOH–$CHCl_3$); IR (KBr) 1675 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.45 (s, 3 H, C(2′)CH_3), 1.45–1.57 (m, 1 H, C(4)*H*H′), 1.72–1.85 (m, 1 H, C(4)H*H′*), 2.25–2.35 (m, 1 H, C-(5a)*H*H′), 2.40 (s, 3 H, NCH_3), 2.41–2.70 (m, 9 H, N-(*C*H_2*C*H_2)$_2$]NMe, C(5)H), 2.90 (dd, 1 H, C(5a)H*H′*, J = 11.1, 12.9 Hz), 3.51 (d, 1 H, C(3′)*H*H′, J = 12.3 Hz), 3.48–3.60 (m, 1 H, C(3)*H*H′), 3.72 (d, 1 H, C(3′)H*H′*, J = 12.3 Hz), 3.85 (s, 1 H, C(1′)H), 3.85–3.92 (m, 1 H, C(3)H*H′*); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 27.07 (C(2′)*C*H_3), 32.12 (C(4)), 44.19 (C(5)), 45.89 (N*C*H_3), 55.77 (N(*C*H_2*C*H_2)$_2$NMe), 58.44 (C(3′)), 59.40 (C(5a)), 63.62 (C(3)), 75.20 (C(2′)), 81.64 (C(1′)), 86.77 (C(6)), 93.73 (C(1)), 167.96 (C(7) or C(9)), 171.51 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 385 [M + 1]$^+$; M_r (+FAB) 385.208 54 [M + 1]$^+$ (calcd for $C_{17}H_{29}N_4O_6$ 385.208 71).

Reaction of 3 with Morpholine (5). A solution of compounds **3** (6 mg, 0.02 mmol) and **5** (1.91 mg, 0.02 mmol) in a THF–H_2O (3:1) mixture (0.2 mL) was stirred at rt (6 h). The "pH" of the solution dropped from 9.3 to 8.8 during this time interval. TLC analysis indicated the formation of adduct **8** as the major product along with several other unidentified minor adducts. The solvents were removed in vacuo, and the residue was dissolved in MeOH and purified by preparative TLC (20% MeOH–$CHCl_3$) to afford adduct **8**: yield 3.3 mg (42%); R_f 0.60 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.48 (s, 3 H, C(2′)CH_3), 1.50–1.60 (m, 1 H, C(4)*H*H′), 1.72–1.83 (m, 1 H, C(4)H*H′*), 2.30 (dd, 1 H, C(5a)*H*H′, J = 4.0, 13.1 Hz), 2.45–2.75 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(5)H), 2.89 (dd, 1 H, C(5a)H*H′*, J = 11.6, 13.1 Hz), 3.48–3.58 (m, 2 H, C-(3′)*H*H′, C(3)*H*H′), 3.68–3.78 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(3′)H*H′*), 3.85 (s, 1 H, C(1′)H), 3.86–3.95 (m, 1 H, C(3)H*H′*). The identity of this product was verified by cospotting with an authentic sample on a TLC plate.

Bicyclomycin Epoxide 14.[9] The title compound was prepared according to the procedure reported by Muller and co-workers[9] with slight modification. A solution of **4** (50 mg, 0.13 mmol) and triethylamine (61 mg, 0.6 mmol) in anhydrous MeOH (2 mL) was stirred at rt (3 h). The solvent was removed in vacuo, and the residue was dissolved in a minimum amount of MeOH. Preparative TLC (20% MeOH–$CHCl_3$) gave **14**: yield 18 mg (48%); R_f 0.50 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.42 (s, 3 H, C(2′)CH_3), 2.57–2.67 (m, 2 H, C(4)H_2), 2.71 (d, 1 H, C(3′)*H*H′, J = 4.5 Hz), 3.27 (d, 1 H, C(3′)H*H′*, J = 4.5 Hz), 3.75–3.85 (m, 2 H, C(3)H_2), 4.31 (s, 1 H, C(1′)H), 5.12 (s, 1 H, C(5a)*H*H′), 5.56 (s, 1 H, C(5a)H*H′*); ^{13}C NMR (CD_3OD) 21.06 (C(2′)*C*H_3), 36.59 (C(4)), 53.84 (C(3′)), 59.74 (C(2′)), 65.94 (C(3)), 71.73 (C(1′)), 83.16 (C(6)), 87.97 (C(1)), 116.94 (C(5a)), 149.41 (C(5)), 164.33 (C(7) or C(9)), 168.94 (C(9) or C(7)) ppm.

Reaction of 14 with Morpholine (5). A solution of compounds **14** (5.0 mg, 0.02 mmol) and **5** (1.5 mg, 0.02 mmol) in a THF–H_2O (3:1) mixture (0.2 mL) was stirred at rt (24 h). TLC analysis indicated the formation of **8** as the major product along with several other unidentified minor adducts. The solvents were removed in vacuo and the residue dissolved in MeOH and then purified by preparative TLC to give compound **8**: yield 2 mg (27%); R_f 0.60 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.48 (s, 3 H, C(2′)CH_3), 1.46–1.54 (m, 1 H, C(4)*H*H′), 1.71–1.83 (m, 1 H, C(4)H*H′*), 2.30 (dd, 1 H, C(5a)*H*H′, J = 4.0, 13.0 Hz), 2.45–2.70 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(5)H), 2.88 (dd, 1 H, C(5a)H*H′*, J = 11.6, 13.0 Hz), 3.45–3.55 (m, 2 H, C(3′)*H*H′, C(3)*H*H′), 3.60–3.78 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(3′)H*H′*), 3.82 (s, 1 H, C(1′)H), 3.85–3.95 (m, 1 H, C(3)H*H′*). The identity of this product was verified by cospotting with an authentic sample on a TLC plate.

Reaction of Methanesulfonate 4 with Morpholine (5) in THF–D_2O (3:1). The preceding protocol was used except D_2O was used in place of H_2O. The initial "pD" value[27] of 9.2 dropped to 8.3 (36 h). The product was separated using preparative TLC (10% MeOH–$CHCl_3$, three developments) and then further purified by preparative TLC (20% MeOH–$CHCl_3$) to give **8**-d_1: yield 8.3 mg (17%); R_f 0.60 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.47 (s, 3 H, C(2′)CH_3), 1.48–1.54 (m, 1 H, C(4)*H*H′), 1.76 (dd, 1 H, C(4) H*H′*, J = 7.7, 16.3 Hz), 2.28 (d, 1 H, C(5a)*H*H′, J = 13.2 Hz), 2.44–2.67 (m, 4 H, N(*C*H_2*C*H_2)$_2$O), 2.87 (d, 1 H, C(5a)H*H′*, J = 13.2 Hz), 3.47–3.55 (m, 2 H, C(3′)*H*H′, C(3)*H*H′), 3.65–3.76 (m, 5 H, N(*C*H_2*C*H_2)$_2$O, C(3′)H*H′*), 3.83 (s, 1 H, C(1′)H), 3.86–3.93 (m, 1 H, C(3)H*H′*).

(27) Bates, R. G. *Determination of pH: Theory and Practice*, 2nd ed.; Wiley: New York, 1973; pp 375–376.

Equilibration of 8 with Morpholine (5) in 3:1 THF–D_2O Solutions. THF–D_2O (3:1) solutions (0.5 mL, "pD" 8.0, 9.5) of **8** (5.3 mg, 0.014 mmol) and **5** (1.3 mg, 0.014 mmol) were stirred at rt (24 h). The solvents were removed in vacuo, and the residues were dissolved in CD_3OD. The ^1H NMR spectra indicated that no incorporation of deuterium had occurred.

Reaction of 3 with Ethyl Mercaptan. Compound **3** (5 mg, 0.02 mmol) and EtSH (20.8 μL, 0.28 mmol) were dissolved in a THF–H_2O (3:1) mixture (0.2 mL) and then was degassed with Ar (5 min), capped, and stirred at rt (36 h). The "pH" (7.9) of the solution remained unchanged during the reaction. TLC analysis indicated the complete consumption of the starting material and the formation of **20** as the major product along with several other unidentified minor adducts. The solvents were removed in vacuo, and the residue was dissolved in MeOH and then purified by preparative TLC (20% MeOH–$CHCl_3$) to afford **20** as a semisolid: yield 2.1 mg (34%); IR (KBr) 1672 (br) cm^{-1}; R_f 0.60 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.24 (t, 3 H, SCH_2CH_3, J = 7.3 Hz), 1.51 (s, 3 H, C(2′)CH_3), 1.95–2.08 (m, 2 H, C(4)H_2), 2.12–2.22 (m, 1 H, C(5)H), 2.36 (dd, 1 H, C(5a)*H*H′, J = 11.4, 13.2 Hz), 2.45–2.56 (m, 2 H, SCH_2CH_3), 3.18 (dd, 1 H, C(5a)H*H*′, J = 2.1, 13.2 Hz), 3.52 (d, 1 H, C(3′)*H*H′, J = 12.6 Hz), 3.68 (d, 1 H, C(3′)H*H*′, J = 12.6 Hz), 3.69–3.79 (m, 1 H, C(3)*H*H′), 3.83 (s, 1 H, C(1′)H), 3.91–4.01 (m, 1 H, C(3)H*H*′); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 15.13 (SCH_2CH_3), 26.58 (C(2′)CH_3), 26.98 (SCH_2CH_3), 30.17 (C(4)), 30.17 (C(5a)), 50.52 (C(5)), 57.69 (C(3′)), 63.78 (C(3)), 75.19 (C(2′)), 82.21 (C(1′)), 84.90 (C(6)), 94.35 (C(1)), 166.47 (C(7) or C(9)), 171.37 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 347 $[M + 1]^+$; M_r [+FAB] 347.127 31 (calcd for $C_{14}H_{23}N_2O_6S$ 347.127 68).

Reaction of 3 with 2-Mercaptopyridine (23). A solution of **3** (4.0 mg, 0.01 mmol) and **23** (3.1 mg, 0.03 mmol) in a THF–H_2O (3:1) mixture (0.5 mL) was stirred at rt (24 h) under Ar at a "pH" value of 9.1. The "pH" was initially adjusted with a dilute NaOH solution. During the course of the reaction the "pH" value dropped to 8.5. The solvents were removed in vacuo, and the residue was subjected to preparative TLC (10% MeOH–$CHCl_3$) to give **24**: yield 3.0 mg (54%); R_f 0.35 (10% MeOH–$CHCl_3$); IR (KBr) 1675 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.50 (s, 3 H, C(2′)CH_3), 1.95–2.03 (m, 2 H, C(4)H_2), 2.23–2.30 (m, 1 H, C(5)H), 3.13 (dd, 1 H, C(5a)*H*H′, J = 11.1, 13.8 Hz), 3.52 (d, 1 H, C(3′)*H*H′, J = 12.0 Hz), 3.65–3.77 (m, 3 H, C(5a)H*H*′, C(3′H*H*′, C(3)*H*H′), 3.84 (s, 1 H, C(1′)H), 4.04–4.11 (m, 1 H, C(3)H*H*′), 7.07–7.11 (m, 1 H, ArH), 7.32–7.35 (m, 1 H, ArH), 7.62–7.65 (m, 1 H, ArH), 8.36–8.38 (m, 1 H, ArH); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 26.51 (C(2′)CH_3), 28.88 (C(5a)), 30.40 (C(4)), 51.15 (C(5)), 57.69 (C(3′)), 64.03 (C(3)), 75.19 (C(2′)), 82.34 (C(1′)), 85.18 (C(6)), 94.37 (C(1)), 121.05, 123.35, 137.99, 150.28, 160.20 (C_5H_4N), 166.39 (C(7) or C(9)), 171.16 (C(9) or C(7)) ppm; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 396 $[M + 1]^+$; M_r [+FAB] 395.115 11 $[M]^+$ (calcd for $C_{17}H_{21}N_3O_6S$ 395.114 60).

Reaction of 17 with 2-Mercaptopyridine (23). A solution of **17** (5.0 mg, 0.015 mmol) and **23** (4.9 mg, 0.044 mmol) in a THF–H_2O (3:1) mixture (0.5 mL) was stirred at rt (24 h) under Ar at a "pH" value of 9.5. The "pH" was adjusted using a dilute aqueous NaOH solution. During the course of the reaction the "pH" value dropped to 8.5. The solvents were removed in vacuo, and the residue was subjected to preparative TLC (5% MeOH–$CHCl_3$, two developments) to give **25**: yield 2.8 mg (42%); R_f 0.35 (10% MeOH–$CHCl_3$); IR (KBr) 1670 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.36, 1.44, 1.46, 1.47 (4 s, 9 H, C(2′)CH_3, $C(CH_3)_2$), 1.90–2.40 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.90–3.05 (m, 1 H, C(5a)H*H*′), 3.70–3.80 (m, 2 H, C(3)*H*H′, C(3′)*H*H′), 4.05–4.15 (m, 2 H, C(3)H*H*′, C(1′)H), 4.42–4.50 (m, 1 H, C(3′)H*H*′), 7.06–7.11 (m, 1 H, ArH), 7.32–7.35 (m, 1 H, ArH), 7.59–7.64 (m, 1 H, ArH), 8.32–8.37 (m, 1 H, ArH); ^{13}C NMR (CD_3OD) 24.84 (C(2′)CH_3), 26.84, 28.22 ($C(CH_3)_2$), 30.18, 30.60 (C(4), C(5a)), 53.27 (C(5)), 63.66, 63.88 (C(3)), 73.30, 73.41, 73.49 (C(1′), C(3′)), 83.90 (C(6)), 86.69 (C(2′)), 88.72 (C(1)), 111.69 ($C(CH_3)_2$), 121.00, 123.17, 138.04, 149.95, 160.00 (C_5H_4NS), 168.40 (C(7) or C(9)), 171.45 (C(9) or C(7)) ppm; ^{13}C NMR analysis indicated that the product existed as a 1.5:1 diastereomeric mixture; MS (+FAB) 454 $[M + 1]^+$; M_r [+FAB] 454.165 47 (calcd for $C_{20}H_{28}N_3O_7S$ 454.164 80).

Reaction of 3 with Thiophenol (26). A solution of **3** (3.0 mg, 0.01 mmol) and **26** (3.0 mg, 0.01 mmol) in a THF–H_2O (3:1) mixture (1 mL) was stirred at rt (24 h) under Ar at a "pH" value of 8.0. The "pH" was initially adjusted with a dilute KOH solution. During the course of the reaction the "pH" value dropped to 7.6. The solvents were removed in vacuo, and the residue was subjected to preparative TLC (20% MeOH–$CHCl_3$) to give **27**: yield 3.1 mg (51%); R_f 0.70 (20% MeOH–$CHCl_3$); IR (KBr) 1675 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.49 (s, 3 H, C(2′)CH_3), 1.95–2.06 (m, 2 H, C(4)H_2), 2.22–2.27 (m, 1 H, C(5)H), 2.74 (dd, 1 H, C(5a)*H*H′, J = 11.4, 13.8 Hz), 3.50 (d, 1 H, C(3′)*H*H′, J = 12.3 Hz), 3.62–3.72 (m, 3 H, C(3′)H*H*′, C(3)*H*H′, C(5a)H*H*′), 3.83 (s, 1 H, C(1′)H), 3.92–4.00 (m, 1 H, C(3)H*H*′), 7.13–7.40 (m, 5 H, C_6H_5); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 26.52 (C(2′)CH_3), 30.14 (C(5a)), 32.34 (C(4)), 50.16 (C(5)), 57.74 (C(3′)), 63.69 (C(3)), 75.20 (C(2′)), 82.28 (C(1′)), 84.90 (C(6)), 94.31 (C(1)), 127.11, 130.11, 137.41 (C_6H_5), 166.38 (C(7) or C(9)), 171.13 (C(9) or C(7)) ppm; the signal at 130.11 ppm was considerably larger than nearby peaks; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 395 $[M + 1]^+$; M_r [+FAB] 395.127 03 (calcd for $C_{18}H_{23}N_2O_6S$ 395.127 79).

Preparation of Cyclized Bicyclomycin Acetonide 18. A solution of **3** (10 mg, 0.035 mmol), 2,2-dimethoxypropane (183 mg, 1.76 mmol), and a few crystals of *p*-toluenesulfonic acid in dry DMF (1.5 mL) was heated at 80 °C (2 h) under Ar. The solvent was removed in vacuo, and the residue was taken up in EtOAc (10 mL), successively washed with saturated $NaHCO_3$ (3 × 10 mL) and saturated brine (2 × 10 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure and dried overnight under vacuum. Compound **18** was obtained as a yellow semisolid: yield 3.4 mg (31%); R_f 0.60 (10% MeOH–$CHCl_3$); IR (KBr) 1675 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.35 (s, 6 H, $C(CH_3)_2$), 1.56 (s, 3 H, C(2′)CH_3), 2.55–2.64 (m, 2 H, C(4)H_2), 3.32 (d, 1 H, C(3′)*H*H′, J = 12.9 Hz), 3.45–3.52 (m, 1 H, C(3)*H*H′), 3.96–4.05 (m, 1 H, C(3)H*H*′), 4.03 (d, 1 H, C(3′)H*H*′, J = 12.9 Hz), 4.39 (s, 1 H, C(1′)H), 5.14 (s, 1 H, C(5a)*H*H′), 5.58 (s, 1 H, C(5a)H*H*′); the ^1H NMR assignments were confirmed using the COSY experiment; ^{13}C NMR (CD_3OD) 25.45 ($C(CH_3)_2$ or C(2′)CH_3), 26.93 ($C(CH_3)_2$ or C(2′)CH_3), 27.94 (C(2′)CH_3 or $C(CH_3)_2$), 36.27 (C(4)), 58.55 (C(3′)), 67.06 (C(3)), 86.30 (C(6) or C(2′)), 86.37 (C(2′) or C(6)), 90.66 (C(1′)), 94.35 (C(1)), 113.21 ($C(CH_3)_2$), 117.28 (C(5a)), 149.96 (C(5)) ppm; the two carbonyl carbon resonances were not detected; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 325 $[M + 1]^+$; M_r [+FAB] 325.139 34 (calcd for $C_{15}H_{21}N_2O_6$ 325.139 96).

General Procedure for the Competition Experiments of Bicyclomycin Derivatives with Sodium Ethanethiolate (19) in THF–H_2O Mixtures at "pH" 9.1. Each reaction was performed in buffered THF–H_2O (3:1) mixtures (0.1 M Tris-HCl, 0.5 mL, "pH" 9.1) containing equimolar amounts (~0.007 mmol) of the bicyclomycin substrates and 1 equiv of sodium ethanethiolate (**19**) unless otherwise specified. The solution was degassed with Ar, capped, and stirred at rt (48 h). The reaction was analyzed by TLC, and all compounds were verified by cospotting the reaction mixture with authentic samples. The solvents were removed in vauco, and the residue was triturated with MeOH and filtered. The filtrate was concentrated and subjected to preparative TLC. The identities of the individual reaction components were confirmed by ^1H NMR analysis.

Reaction of 1 vs 3. Use of **1** (2.10 mg, 0.007 mmol), **3** (2.00 mg, 0.007 mmol), and **19** (0.70 mg, 0.007 mmol) followed by preparative TLC (20% MeOH–$CHCl_3$) led to the recovery of **1** (0.8 mg, 38%) [R_f 0.40 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.35 (s, 3 H, C(2′)CH_3), 2.58–2.65 (m, 2 H, C(4)H_2), 3.50 (d, 1 H, C(3′)*H*H′, J = 11.4 Hz), 3.67 (d, 1 H, C(3′)H*H*′, J = 11.4 Hz), 3.75–3.96 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 5.13 (s, 1 H, C(5a)*H*H′), 5.55 (s, 1 H, C(5a)H*H*′)] and **20** (0.7 mg, 30%) [R_f 0.60 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.24 (t, 3 H, SCH_2CH_3, J = 7.3 Hz), 1.51 (s, 3 H, C(2′)CH_3), 1.95–2.08 (m, 2 H, C(4)H_2), 2.12–2.23 (m, 1 H, C(5)H), 2.36 (dd, 1 H, C(5a)*H*H′, J = 11.4, 13.2 Hz), 2.46–2.56 (m, 2 H, SCH_2CH_3), 3.18 (dd, 1 H, C(5a)H*H*′, J = 2.1, 13.2 Hz), 3.52 (d, 1 H, C(3′)*H*H′, J = 12.6 Hz), 3.68 (d, 1 H, C(3′)H*H*′, J = 12.6 Hz), 3.69–3.78 (m, 1 H, C(3)*H*H′), 3.82 (s, 1 H, C(1′)H), 3.91–4.01 (m, 1 H, C(3)H*H*′)].

Reaction of 3 vs 17. Use of **3** (2.00 mg, 0.007 mmol), **17** (2.40 mg, 0.007 mmol), and **19** (0.70 mg, 0.007 mmol) followed by preparative TLC (10% MeOH–$CHCl_3$) led to the recovery of **17** (0.9 mg, 37%) [R_f 0.55 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.36, 1.42, 1.45 (3 s, 9 H, C(2′)CH_3, and C$(CH_3)_2$), 2.60–2.68 (m, 2 H, C(4)H_2), 3.73 (d, 1 H, C(3′)*H*H′, J = 8.1 Hz), 3.90–4.10 (m, 2 H, C(3)H_2), 4.15 (s, 1 H, C(1′)H), 4.45 (d, 1 H, C(3′)H*H*′, J = 8.1 Hz), 5.14 (s, 1 H, C(5a)*H*H′), 5.59 (s, 1 H, C(5a)H*H*′)] and **20** (1.0 mg, 42%) [R_f 0.50 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.24 (t, 3 H, SCH_2CH_3, J = 7.3 Hz), 1.51 (s, 3 H, C(2′)CH_3), 1.98–2.06 (m, 2 H, C(4)H_2), 2.12–2.22 (m, 1 H, C(5)H), 2.37 (dd, 1 H, C(5a)*H*H′, J = 11.1, 13.2 Hz), 2.48–2.55 (m, 2 H, SCH_2CH_3), 3.19 (dd, 1 H, C(5a)H*H*′, J = 2.0, 13.2 Hz), 3.52 (d, 1 H, C(3′)*H*H′, J = 12.6 Hz), 3.68 (d, 1 H, C(3′)H*H*′, J = 12.6 Hz), 3.70–3.78 (m, 1 H, C(3)*H*H′), 3.82 (s, 1 H, C(1′)H), 3.92–4.00 (m, 1 H, C(3)H*H*′)].

Reaction of 3 vs 18. Use of **3** (2.00 mg, 0.007 mmol), **18** (2.30 mg, 0.007 mmol), and **19** (0.70 mg, 0.007 mmol) followed by preparative TLC (10% MeOH–$CHCl_3$) led to the recovery of **18** (1.1 mg, 48%) [R_f 0.60 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.36 (s, 6 H, C$(CH_3)_2$), 1.56 (s, 3 H, C(2′)CH_3), 2.56–2.65 (m, 2 H, C(4)H_2), 3.33 (d, 1 H, C(3′)*H*H′, J = 12.8 Hz), 3.45–3.54 (m, 1 H, C(3)*H*H′), 3.95–4.05 (m, 1 H, C(3)H*H*′), 4.05 (d, 1 H, C(3′)H*H*′, J = 12.8 Hz), 4.40 (s, 1 H, C(1′)H), 5.15 (s, 1 H, C(5a)*H*H′), 5.59 (s, 1 H, C(5a)H*H*′)] and **20** (0.8 mg, 33%) [R_f 0.50 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.25 (t, 3 H, SCH_2CH_3, J = 7.3 Hz), 1.51 (s, 3 H, C(2′)CH_3), 1.98–2.09 (m, 2 H, C(4)H_2), 2.12–2.23 (m, 1 H, C(5)H), 2.37 (dd, 1 H, C(5a)*H*H′, J = 11.4, 13.2 Hz), 2.49–2.58 (m, 2 H, SCH_2CH_3), 3.20 (dd, 1 H, C(5a)H*H*′, J = 2.1, 13.2 Hz), 3.53 (d, 1 H, C(3′)*H*H′, J = 12.6 Hz), 3.68 (d, 1 H, C(3′)H*H*′, J = 12.6 Hz), 3.69–3.78 (m, 1 H, C(3)*H*H′), 3.82 (s, 1 H, C(1′)H), 3.92–4.02 (m, 1 H, C(3)H*H*′)].

Reaction of 1 vs 17. Use of **1** (2.10 mg, 0.007 mmol), **17** (2.40 mg, 0.007 mmol), and **19** (0.70 mg, 0.007 mmol) followed by preparative TLC (10% MeOH–$CHCl_3$) afforded three fractions: unreacted **17** (0.7 mg, 29%) [R_f 0.55 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.39, 1.43, 1.47 (3 s, 9 H, C(2′)CH_3 and C$(CH_3)_2$), 2.60–2.68 (m, 2 H, C(4)H_2), 3.73 (d, 1 H, C(3′)*H*H′, J = 8.1 Hz), 3.91–4.03 (m, 2 H, C(3)H_2), 4.16 (s, 1 H, C(1′)H), 4.46 (d, 1 H, C(3′)H*H*′, J = 8.1 Hz), 5.11 (s, 1 H, C(5a)*H*H′), 5.58 (s, 1 H, C(5a)H*H*′)], **22** (0.7 mg, 25%) [R_f 0.65 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.25 (t, 3 H, SCH_2CH_3, J = 7.1 Hz), 1.36 (s, 3 H, C(2′)CH_3), 1.50 (s, 6 H, C$(CH_3)_2$), 2.05–2.25 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.45–2.55 (m, 2 H, SCH_2CH_3), 3.15–3.26 (m, 1 H, C(5a)H*H*′), 3.70 (d, 1 H, C(3′)*H*H′, J = 8.2 Hz), 3.80–4.00 (m, 2 H, C(3)H_2), 4.08 (s, 1 H, C(1′)H), 4.45 (d, 1 H, C(3′)H*H*′, J = 8.2 Hz)], and a fraction (1.10 mg) containing both **1** and **21**[8d] (approximate ratio by ^{1}H NMR is 2:1) [R_f 0.30–0.35 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.20–1.30 (m, SCH_2CH_3), 1.32, 1.33 (2 s, C(2′)CH_3), 2.05–2.30 (m, C(4)H_2, C(5)H, C(5a)*H*H′), 2.45–2.65 (m, SCH_2CH_3, C(4)H_2), 3.15 (d, C(5a)*H*H′, J = 11.6 Hz), 3.45–3.55 (m, C(3′)*H*H′), 3.65–3.75 (m, C(3′)H*H*′), 3.75–3.95 (m, C(3)H_2), 4.03, 4.05 (m, C(1′)H), 5.16 (s, C(5a)*H*H′), 5.57 (s, C(5a)H*H*′)].

Reaction of 1 vs 18. Use of **1** (2.10 mg, 0.007 mmol), **18** (2.30 mg, 0.007 mmol), and **19** (1.30 mg, 0.014 mmol) followed by preparative TLC (10% MeOH–$CHCl_3$) afforded unreacted **18** (0.9 mg, 39%) [R_f 0.60 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.35 (s, 6 H, C$(CH_3)_2$), 1.55 (s, 3 H, C(2′)CH_3), 2.55–2.65 (m, 2 H, C(4)H_2), 3.34 (d, 1 H, C(3′)*H*H′, J = 12.6 Hz), 3.44–3.54 (m, 1 H, C(3)*H*H′), 3.95–4.04 (m, 1 H, C(3)H*H*′), 4.05 (d, 1 H, C(3′)H*H*′, J = 12.6 Hz), 4.39 (s, 1 H, C(1′)H), 5.15 (s, 1 H, C(5a)*H*H′), 5.58 (s, 1 H, C(5a)H*H*′)] and **21**[8d] (1.0 mg, 39%) [R_f 0.30 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.20–1.32 (m, 3 H, SCH_2CH_3), 1.35 (s, 3 H, C(2′)CH_3), 2.05–2.30 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.48–2.58 (m, 2 H, SCH_2CH_3), 3.15 (d, 1 H, C(5a)H*H*′, J = 11.6 Hz), 3.50 (d, 1 H, C(3′)*H*H′, J = 11.4 Hz), 3.60–3.70 (m, 1 H, C(3′)H*H*′), 3.70–3.80 (m, 1 H, C(3)*H*H′), 3.90–4.00 (m, 1 H, C(3)H*H*′), 4.05 (s, 1 H, C(1′)H)].

Reaction of 17 vs 18. Use of **17** (2.40 mg, 0.007 mmol), **18** (2.30 mg, 0.007 mmol), and **19** (0.70 mg, 0.007 mmol) followed by preparative TLC (10% MeOH–$CHCl_3$) led to the recovery of **18** (1.0 mg, 43%) [R_f 0.60 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.35 (s, 6 H, C$(CH_3)_2$), 1.56 (s, 3 H, C(2′)CH_3), 2.57–2.65 (m, 2 H, C(4)H_2), 3.33 (d, 1 H, C(3′)*H*H′, J = 12.8 Hz), 3.45–3.54 (m, 1 H, C(3)*H*H′), 3.96–4.05 (m, 1 H, C(3)H*H*′), 4.05 (d, 1 H, C(3′)H*H*′, J = 12.8 Hz), 4.39 (s, 1 H, C(1′)H), 5.15 (s, 1 H, C(5a)*H*H′), 5.58 (s, 1 H, C(5a)H*H*′)] and **22** (1.0 mg, 36%) [R_f 0.65 (10% MeOH–$CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.25 (t, 3 H, SCH_2CH_3, J = 7.1 Hz), 1.35 (s, 3 H, C(2′)CH_3), 1.48 (s, 6 H, C$(CH_3)_2$), 2.05–2.25 (m, 4 H, C(4)H_2, C(5)H, C(5a)*H*H′), 2.44–2.56 (m, 2 H, SCH_2CH_3), 3.13–3.21 (m, 1 H, C(5a)H*H*′), 3.72 (d, 1 H, C(3′)*H*H′, J = 8.1 Hz), 3.80–4.00 (m, 2 H, C(3)H_2), 4.10 (s, 1 H, C(1′)H), 4.45 (d, 1 H, C(3′)H*H*′, J = 8.1 Hz). The ^{1}H NMR assignments were confirmed using the COSY experiment].

Repetition of this experiment on a larger scale using **17** (6.8 mg, 0.02 mmol) and **18** (7.0 mg, 0.02 mmol) led to the recovery of sufficient samples (3.1 mg, 38%) of **22** for IR, ^{13}C NMR, and mass spectral analyses: IR (KBr) 1672 (br) cm^{-1}; ^{13}C NMR (CD_3OD) 15.12 (SCH_2CH_3), 24.72 (C(2′)CH_3), 26.84 (C$(CH_3)_2$), 26.97 (SCH_2CH_3), 28.17 (C$(CH_3)_2$), 30.56 (C(5a)), 31.80 (C(4)), 52.72 (C(5)), 63.60 (C(3)), 73.37 (C(3′)), 73.79 (C(1′)), 83.80 (C(6)), 86.27 (C(2′)), 88.59 (C(1)), 111.68 (*C*$(CH_3)_2$) ppm; the two carbonyl carbon resonances were not detected; the ^{13}C NMR assignments were confirmed using the APT experiment; MS (+FAB) 405 [M + 1]$^+$; M_r [+FAB] 405.168 82 (calcd for $C_{17}H_{29}N_2O_7S$ 405.169 55).

Kinetic Studies of 3 and 17 with 2-Mercaptopyridine (23). The kinetic experiments reported in Table III were run under O_2-free Ar in a degassed 0.1 N Tris·HCl buffer THF–H_2O (3:1) solution (0.5 mL, "pH" 9.1) containing the bicyclomycin substrate (~0.014 M, 1 equiv), **23** (~0.14 M, 10 equiv), and benzyl alcohol (~0.056 M, 4 equiv) as an internal standard. The temperature of the kinetic experiments ranged from 6 to 46 °C (±1 °C). For the experiments performed in THF–D_2O mixtures, Tris-d_5 was made from Tris and D_2O and the "pD" of the solution determined from the observed pH meter reading by using the relationship[27] pD = pH meter reading + 0.4. Aliquots (30 μL) were removed at various time intervals, neutralized with aqueous 1 N HCl, and then analyzed by HPLC (5-μL injections) using the following Waters Associates units: 510A pump, 510B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The following linear gradient conditions were employed: C_{18} μBondapak (SS) column 3.9 × 300 mm, from 100% A (3 mM triethylammonium phosphate, pH 4.7), 0% B (3 mM triethylamine in CH_3CN) to 50% A, 50% B in 25 min. The column was fitted with a μBondapak C_{18} guardpak. A flow rate of 1.0 mL/min was used, and the detection wavelengths employed were 255, 260, and 280 nm.

Each transformation was monitored for at least 2 half-lives, and at least five aliquots were assessed during this time interval. The reaction endpoint was determined after 72–98 h (~$8t_{1/2}$). Verification of the product peaks (i.e., **24**, **25**) was conducted by coinjection of authentic samples with the reaction mixture in the HPLC. The relative amounts of **23** and product at each time interval were calculated from the HPLC chromatograms by comparing the relative absorbances at 255 nm of these compounds versus the absorption of the internal standard, benzyl alcohol. The concentrations of the reactants and products at specific times were then calculated using the values determined for the ratio of product versus the internal standard at the end of the reaction and the initial substrate concentrations. The concentration of **23** was adjusted at each time interval to account for the small amount of 2-dipyridyl disulfide[28] formed (<10%) during the reaction. Standard data plots of ln $[b_0(a_0 - x)/a_0(b_0 - x)]$ versus time where a_0 and b_0 were the starting concentrations of **23** and the bicyclomycin substrate (i.e., **3**, **17**), respectively, yielded linear slopes from which the second-order rate constants (k_2, L/mol-min) were calculated. Duplicate kinetic runs at each temperature were performed and the results averaged.

Acknowledgment. We express our deep appreciation to Professor J. Paul Street of this department for his valued thoughts provided throughout this investigation. We thank the National Institutes of Health (Grant No. GM37934) and the Robert A. Welch Foundation (Grant No. E-607) for their support of this research. Special thanks is given to Dr. Simon Gaskell, Ms. Odile Burlet, and Ralph Orkiszewski (Baylor College of Medicine) for obtaining mass spectral results and Dr. James D. Korp for

(28) Aldrich Chemical Co.

carrying out the X-ray crystallographic study of 8. We also express our appreciation to Dr. K. Inokuchi and the Fujisawa Pharmaceutical Co., Ltd., Japan, for providing us with a gift of bicyclomycin.

Supplementary Material Available: Experimental procedure for the X-ray analysis of 8, ORTEP drawing of 8 with atom labeling scheme (Figure 2), Table 4 listing the final cell constants, as well as other information pertinent to data collection and refinement, and Tables 5-9 giving a complete listing of atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, and hydrogen-bonding parameters, select long-range proton-carbon connectivities observed in the proton-detected long-range heteronuclear multiple quantum chemical shift correlation (HMBC) experiments for 8 (Figure 1) and 3 (Figure 3) and ^1H and/or ^{13}C NMR spectra for all new compounds (42 pages). This material is contained in many 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.

Separation of Remote Diol and Triol Stereoisomers by Enzyme-Catalyzed Esterification in Organic Media or Hydrolysis in Aqueous Media

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Received April 30, 1992

The separation of symmetric, remote, secondary diol stereoisomers by stereoselective enzyme-catalyzed acetylation with acetic anhydride in anhydrous, low polarity organic solvents or by stereoselective enzyme-catalyzed hydrolysis of the corresponding peracetate in aqueous media is described. Whether or not an alcohol is acetylated or an acetate is hydrolyzed is determined solely by its own stereochemical arrangement and not by the stereochemistry at any other stereogenic center. Since the enzyme used, Amano P lipoprotein lipase from *Pseudomonas* species, acetylates secondary alcohol stereogenic centers of the (*R*)-configuration, an (*R*,*R*)-diol is converted to its diacetate, a meso-diol is converted to the monoacetate at its (*R*)-stereogenic center, and an (*S*,*S*)-diol is left unchanged. Similarly, when hydrolysis is used, (*R*)-stereogenic centers are hydrolyzed so that the (*R*,*R*)-stereoisomer is converted to the corresponding diol while the (*S*,*S*)-stereoisomer remains a diacetate. The resulting mixture is separated, and the remaining acetates are removed by hydrolysis to give diols and triols of high stereochemical purity. Diols successively separated by esterification include α,α'-dimethyl-1,4-benzenedimethanol, **1**, α,α'-dimethyl-1,3-benzenedimethanol, **4**, α,α'-dimethyl-2,6-pyridinedimethanol, **5**, and α,α'-dimethyl-4,4'-biphenylenedimethanol, **6**. For two cases, α,α'-dimethyl-2,6-pyridinedimethanol, **5**, and α,α',α''-trimethyl-1,3,5-benzenetrimethanol, **7**, the separation was achieved using the hydrolysis procedure. The stereochemical purity of each of the separated diol stereoisomers was determined by evaluating the NMR spectrum of its bis-MTPA ester. In most cases, it was possible to establish both the stereochemical purity of the fraction and the amount of each contaminating stereoisomer that was present. The diol products are expected to be of value for preparing optically active polymers and optically active crown ethers.

Diols are valuable intermediates in the preparation of polymers, acetals, and crown ethers, and optically active diols have been widely used for stereochemical control in homochiral syntheses. Unfortunately, the number of optically active diols, other than those associated with carbohydrates, is quite small. Thus, a general source could provide valuable new building blocks for many structures. Most techniques for the preparation of optically active diols focus on the stereospecific synthesis of a single enantiomer.[1,2] The chemicals for preparing both enantiomers via such a procedure are not always available. In many cases, the stereochemistry at the second stereogenic center is determined by that at the first, limiting the allowable distance between the two. Finally, a completely different approach is generally required for preparing the meso stereoisomer.

As a result of our recent activity in the synthesis of optically active $[AA-BB]_x$ polyesters,[3] the importance of finding an efficient approach to the preparation of all possible stereoisomers of symmetric, secondary diol monomers in a highly purified form became apparent. Having all three isomers allows, for example, the synthesis of an all (*R*), an all (*S*), or the "pseudo-syndiotactic" (*R*,*S*)-polymer as well as a polymer containing any combination of the above stereochemistries. Moreover, since our interest lay in the use of enzymes to effect polycondensations, preparing diols free of any meso material became particularly important. While such separations can be achieved by VPC,[4] it seems unlikely they will be useful on a preparative scale. Upon consideration of possible alternative methods for reaching this goal, we concluded that a combination of enzymatic and chemical methods should allow a synthetic mixture of symmetric diol stereoisomers to be separated most easily. The most important feature of such a separation is that it would depend only on the ability of an enzyme to distinguish the chemistry at each stereogenic center in the diol, and would be independent of any interaction between the stereogenic centers.

The specificity of hydrolase enzymes for diol stereochemistry has been exploited for some time. However, until recently, their use has been limited to modification of one stereogenic center in a meso diol (or diacylated meso diol)[5] or modification of a specific hydroxyl (or esterified hydroxyl) in a diol bearing a prochiral center.[6] Early

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