which transfers positive charge to three rather than two L-O bonds.

It is instructive to apply this idea to an analysis of the isotope effect on the ionization of *tert*-butylmalononitrile determined by measuring rates of tritium exchange,[5] represented schematically by eq 11. Application of fractionation factor theory gives

$$RT + (L_2O)_4 \rightarrow [\overset{\delta-}{R}\cdots T\text{—}\overset{\delta+}{(OL_2)_4}]^{\ddagger} \quad (11)$$

$k_{H_2O}/k_{D_2O} = 1/\Phi_{R^-}{}^{\ddagger} l^{3\beta_i}$, in which $\Phi_{R^-}{}^{\ddagger}$ refers to isotopic fractionation in the solvation shell of the developing cyanocarbanion and β_i is an isotopic exponent that measures the portion of a full positive charge transferred to the hydron-receiving water cluster at the transition state. A value of $\Phi_{R^-}{}^{\ddagger}$ may be estimated from the isotope effect determined for the analogous reaction of *tert*-butylmalononitrile with acetate ion, eq 12; for that system $k_{H_2O}/k_{D_2O} = \Phi_{OAc^-}/\Phi_{R^-}{}^{\ddagger}$, and, with $k_{H_2O}/k_{D_2O} = 1.12$[5] and $\Phi_{OAc^-} = 0.90$,[31] $\Phi_{R^-}{}^{\ddagger} = 0.80$. This result, coupled with $k_{H_2O}/k_{D_2O} = 3.5$ for the process of eq 11[5] and $l = 0.69$, leads to $\beta_i = 0.92$; this is a reasonable value for a process in which proton transfer is almost but not quite complete at the rate-determining transition state.

$$RT + OAc^- \rightarrow [\overset{\delta-}{R}\cdots T\text{—}\overset{\delta-}{OAc}]^{\ddagger} \quad (12)$$

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council of Canada and the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for financial support of this work.

Registry No. H_2, 1333-74-0; $(CN)_2CH^-$, 41470-37-5; Br_3^-, 14522-80-6; Br_2, 7726-95-6; T_2, 10028-17-8; *tert*-butylmalononitrile, 4210-60-0; malononitrile, 109-77-3.

(31) Gold, V.; Lowe, B. M. *J. Chem. Soc. A.* **1968**, 1923-1932. Albery, W. J. In *Proton Transfer Reactions*; Caldin, E. F., Gold, V., Eds.; Chapman and Hall: London, 1975; p 283.

Mechanism, Biological Relevance, and Structural Requirements for Thiolate Additions to Bicyclomycin and Analogues: A Unique Latent Michael Acceptor System

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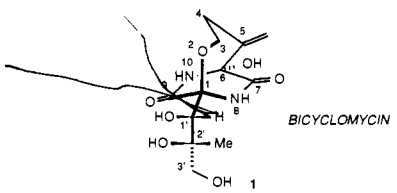
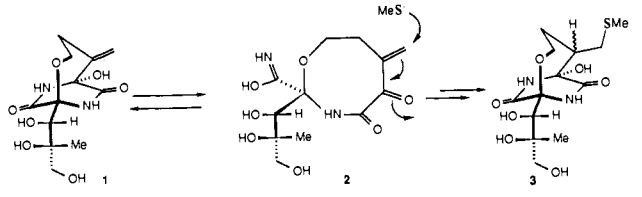
Abstract: Several bicyclomycin analogues based on the 8,10-diaza-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione ring system have been synthesized and examined for thiolate addition to the C-5 *exo*-methylene group. The results indicate that the minimum structural requirements for thiolate addition at pH 12.5 include the following: (1) obligate partnership of the C-5 *exo*-methylene and C-6-bridgehead hydroxyl groups; (2) secondary or unsubstituted (-NH-) amide at N-10; and (3) a C-1′-OH to activate the C-9 carbonyl for tautomeric ring opening to a reactive α,β-unsaturated ketone. Kinetics for conversion of **18** → **19a,b** indicate that a proton transfer from solvent is involved in the rate-limiting step: $K_{H_2O}/K_{D_2O} = 2.4$; $\Delta G^{\ddagger} = 19$ kcal/mol; $\Delta H^{\ddagger} = 17.5$ kcal/mol; $\Delta S^{\ddagger} = -5$ eu. The reaction of **18** with NaSMe to form sulfide adducts **19a,b** is irreversible as evidenced by ^{18}O incorporation and H/D exchange experiments. The results are discussed in the context of a recently proposed mechanism of action for bicyclomycin. It is shown that there is not a simple correlation between the capacity for structures to react with NaSMe and a capacity for antimicrobial activity.

Bicyclomycin (**1**) is a commercially important antibiotic that is being produced from the fermentation harvest of *Streptomyces sapporonensis* at the Fujisawa Pharmaceutical Co. (Japan) where the natural product was originally isolated and identified.[1,2] This structurally unique bicyclic dipeptide is biosynthetically derived[3] from the amino acids leucine and isoleucine and constitutes both a mechanistically and a structurally new class of antibiotics. The low toxicity of bicyclomycin[1] coupled with the efficiency of the fermentation process has resulted in the introduction of bicyclomycin[4] on both the European and Japanese markets as an effective agent against nonspecific diarrhea in humans and bacterial diarrhea in livestock, respectively.[5]



Bicyclomycin is a weak antibiotic displaying activity[1] against Gram-negative organisms such as *Escherichia coli*, *Klebsiella*, *Shigella*, *Salmonella*, *Citrobacter*, *Enterobacter cloacae*, and *Neisseria* but is inactive toward *Proteus*, *Pseudomonas aeruginosa*, and Gram-positive bacteria. The mechanism of action of bi-

Scheme I



‡Fellow of the Alfred P. Sloan Foundation 1986-1988. NIH Research Career Development Awardee 1984-1989. Eli Lilly Grantee 1986-1988.

(1) (a) Miyoshi, T.; Miyari, N.; Aoki, H.; Kohsaka, M.; Sakai, H.; Imanaka, H. *J. Antibiot.* **1972**, *25*, 569. (b) Kamiya, T.; Maeno, S.; Hashimoto, M.; Mine, Y. *Ibid.* **1972**, *25*, 576. (c) Nishida, M.; Mine, Y.; Matsubara, T. *Ibid.* **1972**, *25*, 582. (d) Nishida, M.; Mine, Y.; Matsubara, T.; Goto, S.; Kuwahara, S. *Ibid.* **1972**, *25*, 594.

(2) Bicyclomycin (Aizumycin) was simultaneously isolated from *Streptomyces aizunensis*: (a) Miyamura, S.; Ogasawara, N.; Otsuka, N.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao; Matsuki, K.; Hoshino, T. *J. Antibiot.* **1972**, *25*, 610. (b) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H. *Ibid.* **1973**, *26*, 479.

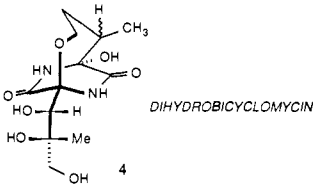
(3) Miyoshi, T.; Iseki, M.; Konomi, T.; Imanaka, H. *J. Antibiot.* **1980**, *33*, 480, 488.

(4) The commercial synonym "bicozamycin" is the name liscensed to Fujisawa Pharmaceutical Co., Ltd., Japan, see: Merck Index, 10th ed.; Merck: Rahway, NJ, 1984; No. 1213.

0002-7863/87/1509-4028$01.50/0

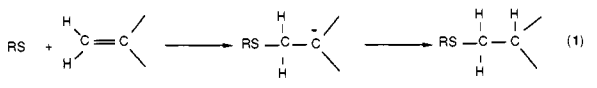
cyclomycin[6] seems to be distinct from the other known classes of antibiotics, but it induces morphological[7] changes in *E. coli* that are similar to those induced by the β-lactam antibiotics. However, Iseki[8] has previously shown that bicyclomycin binds irreversibly and covalently to inner-membrane proteins (BBPs) of *E. coli* that were shown to be distinct from the penicillin-binding proteins (PBPs). The Fujisawa group also showed that bicyclomycin inhibited the synthesis of envelope proteins, particularly the free and bound forms of lipoprotein.[6,8] Neither nucleic acid synthesis nor ribosomal-directed protein synthesis was affected by bicyclomycin. Later work by Hirota[9] and Iseki[8] showed that murein-lipoprotein inhibition was a secondary effect and not the primary lethal site of antimicrobial action since an *E. coli* mutant lacking murein-lipoprotein could grow normally under a variety of conditions. The morphological changes induced by bicyclomycin[7] include the formation of blebs on the cell surface, a highly undulated outer membrane, and the production of filamentous cells and eventual lysis indicative of disruption of the final stages of peptidoglycan assembly. Iseki[8] further showed that the stoichiometry of the bicyclomycin-BBP complexes is 1:1 and the binding is inhibited by the addition of thiols. The structure and function of the bicyclomycin-binding proteins (BBPs) remains to be determined and the nature of the bicyclomycin-protein (BBPs) interaction(s) remains unknown.

In 1979, Iseki[10] and co-workers at the Fujisawa Pharmaceutical Co. reported on the regiospecific addition of sodium methane thiolate to the C-5 *exo*-methylene moiety of bicyclomycin at pH 12.5 resulting in the sulfide adduct **3**. This reaction was proposed[10] to be biologically significant since saturation of the C-5 olefinic residue (**4**) results in a biologically inactive species.[11] In addition,



4

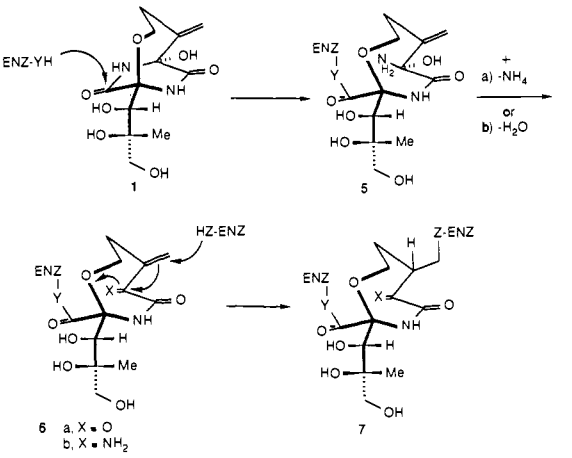
the semisynthetic bicyclomycin derivatives prepared by Muller et al.[12] that retained biological activity also contained an unsaturated system at C-5. Accordingly, it has been suggested[10] that "...the terminal olefinic group reacts with the sulfhydryl groups of the inner-membrane proteins and covalent bonds are formed. Thus the olefinic double bond seems to be the reactive site or function site of bicyclomycin.... The thiol group or thiolate anion may attack the terminal olefinic group of bicyclomycin to form an enolate anion, which may then be protonated." These workers[10] included the following scheme to accompany these suggestions (eq 1); no additional mechanistic or structural requirements were delineated.



(1)

Careful inspection of the bicyclomycin structure and consideration of the regiochemistry of the mercatan addition led us to suggest[13] the mechanistic pathway depicted in Scheme I. Base-

Scheme II



catalyzed tautomeric ring-opening of the C-6 carbinolamide furnished the monocyclic eight-membered ring α,β-unsaturated ketone **2** which should function as a reactive Michael-type acceptor. Such a "latent Michael-acceptor" mechanism readily accounts for the regiospecificity of the mercaptan adduct **3**, reported by Iseki et al.[10]

Intuitively, the significance of the nucleophilic thiolate addition to **1** in the context of a chemical mechanism of action seems tenuous[13] when one considers the extremely low toxicity of bicyclomycin[1] ($L.D._{50} > 4$ g/kg (mice)); i.e., if this structure readily underwent this reaction under physiological conditions, it would be expected to indiscriminately alkylate various biological nucleophiles. The pharmacological studies[1] clearly show that this is not the case. On the other hand, a similar and specific enzyme-catalyzed tautomeric ring-opening sequence cannot be excluded. With these uncertainties in mind, we have proposed[13] an alternative mechanism of action for the covalent modification of the BBPs by bicyclomycin. Our hypothesis[13] (depicted in Scheme II) is based on the assumption that the crucial BBP has proteolytic activity. Being itself a peptide, bicyclomycin could be recognized as a substrate by a protease that functions by catalytically cleaving important peptide bonds during the biosynthesis of the bacterial cell envelope. Cleavage of the 9,10-amide bond by the protein produces acyl enzyme derivative **5**. The amide-derived NH_2 (at C-6, **5**) is now part of a hemi-amino hemi-ketal (**5**) that should be unstable and rapidly expel either NH_4^+ or H_2O (dependent upon the local pH environment) to generate the reactive α,β-unsaturated ketone or iminium species (**6a** and **6b**), respectively. Conjugate addition to either **6a** or **6b** (or perhaps a concerted allylic displacement from **5**) would result in the covalent adduct **7** and the suicide inhibition of the enzyme.

Support for this hypothesis has very recently been contributed by Vasquez and co-workers,[14] who found that a significant increase in the diaminopimelic acid–diaminopimelic acid bridge (DAP–DAP) occurs in *E. coli* cells grown in the presence of bicyclomycin. These workers suggested a DAP–DAP diketopiperazine bridge as a hypothetical cell structure resembling bicyclomycin that must be proteolytically removed in the late stages of peptidoglycan assembly to allow normal cell growth. The relevent question raised by the Vazquez findings[14] that relates to our proposal[13] (Scheme II) is the following: are the BBPs diketopiperazineases (or *cis*-amidases)? In this article, we disclose the details of our studies[15] on the mechanism of nucleophilic addition of thiols to bicyclomycin and analogues in the context of the possible relevance of this reaction to the chemical mechanism of action of this unique antibiotic.

Our objectives in understanding the process represented in Scheme I included the following: (1) elucidation of the minimum

(5) Private communication, Fujisawa Pharmaceutical Co., Ltd. Japan.
(6) Tanaka, N.; Iseki, M.; Miyoshi, T.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1976**, *29*, 155.
(7) Someya, A.; Tanaka, A.; Tanaka, N. *Antimicrob. Agents Chemother.* **1979**, *16*, 87.
(8) Someya, A.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1978**, *31*, 712.
(9) Hirota, Y.; Suzuki, H.; Nishimura, I. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1417.
(10) Someya, A.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402.
(11) The dihydro derivative **4** is also a natural product that can be isolated from the same fermentation broths that produce **1** (private communication, M. Iseki, Fujisawa Co., Ltd., Japan).
(12) Muller, B. W.; Zak, O.; Kump, W.; Tosch, W.; Wacker, O. *J. Antibiot.* **1979**, *32*, 689.
(13) Williams, R. M.; Armstrong, R. W.; Dung, J. S. *J. Med. Chem.* **1985**, *28*, 733.
(14) Pisabarro, A. G.; Canada, F. J.; Vazquez, D.; Arriaga, P.; Rodriguez-Tebar, A. *J. Antibiot.* **1986**, *34*, 914.
(15) For a preliminary account of this ork, see: Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 6419.

Scheme III

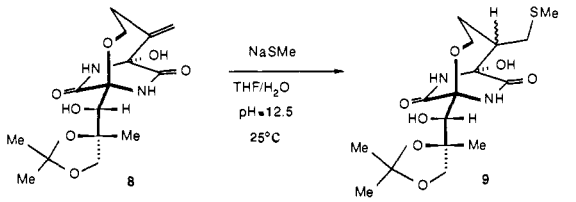
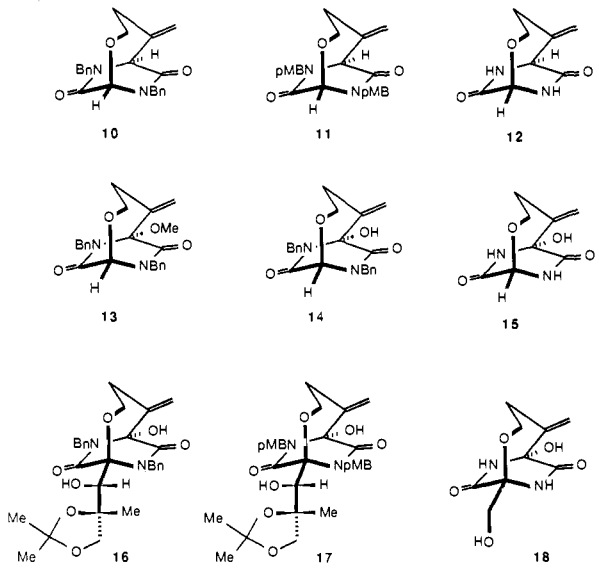


Chart I



structural requirements of the bicyclo[4.2.2] nucleus that allows for sulfide formation; (2) demonstrate the intermediacy of the ring-opened, monocyclic eight-membered ring α,β-unsaturated ketone, such as **2**; and (3) establish whether or not a correlation exists between the capacity for structures to undergo the addition of thiols at C-5 and the corresponding capacity of such reactive structures to display biological activity.

Results

All thiolate reactions were carried out in homogeneous solutions of 0.2 M $NaSCH_3$ in 3:1 THF/H_2O (adjusted to pH 12.5) at 25 °C. As a reactivity standard to titrate the freshly prepared $NaSCH_3$ solutions, the naturally derived acetonide derivative **8**[16] was used and run concomitantly alongside the analogues discussed below. The diol **8** rapidly consumed 1 equiv of methyl mercaptan under these conditions to furnish a single diastereomeric sulfide adduct **9**. The relative stereochemistry at C-5 was not determined.

Subjecting the totally synthetic[17] C-6 desoxy derivatives **10**, **11**, and **12** to these conditions resulted in no detectable sulfide adduct and efficient recovery of the unchanged starting materials.[18] In a parallel series, the C-6 oxygenated derivatives **13**, **14**, and **15** were subjected to the NaSMe solution and were similarly recovered unchanged. The *N,N'*-dibenzyl and *N,N'*-di-*p*-methoxybenzyl analogues (**16** and **17**) of control **8** were also surprisingly stable and unreactive to sulfide formation under these conditions. It is very significant to emphasize that **16** and **17** are identical structures to **8** with the exception of the corresponding alkyl groups on the amide nitrogens. The relative stereochemistry of the C-1'-C-3' polyoxo side chains is the same as that for **8**.[17] The lack of reactivity of **15**, **16**, and **17** when compared to **8** suggested that free (secondary N-H) amides and a C-1' hydroxyalkyl residue play a critical structural role in facilitating sulfide formation at C-5. This suggested that the simplest system that might react with NaSMe should be the hydroxy methyl derivative **18**.

Indeed, reaction of **18** with NaSMe in THF/H_2O (adjusted to pH 12.5) at room temperature resulted in the rapid formation of the sulfide adducts **19a,b** (stereoisomeric at C-5, 2:1 ratio, 46%; relative stereochemistry not assigned). The structures of **19a,b** were confirmed by subjecting olefin **18** to catalytic hydrogenolysis to furnish a mixture of dihydro derivatives **20a,b** (6:1 ratio). The same two dihydro derivatives (**20a,b**) were produced upon Raney nickel reduction of the sulfides **19a,b**.[19]

Kinetics for the reaction of **18** with NaSMe were measured over several half-lives ($t_{1/2} \sim 8$ min at 25 °C) with use of 1 molar equiv of **18** and 2.5 molar equiv of NaSMe at $\sim 3.8 \times 10^{-3}$ and $\sim 9.8 \times 10^{-3}$ M, respectively, at various temperatures (Table I). The reaction displayed a significant temperature dependence (Figure 1) from which the apparent Arrhenius activation parameters were calculated. From this data was obtained an apparent $E_a = 18.1 \pm 0.6$ kcal/mol, $\Delta H^{\ddagger} = 17.5 \pm 0.6$ kcal/mol, $\ln A = 28$, $\Delta S^{\ddagger} = -5$ eu $\pm$ 4 cal(mol·deg), and $\Delta G^{\ddagger} = 19 \pm 1.0$ kcal/mol. Performing the reaction kinetics several times in THF:D_2O at 7 °C[20] (pD = 12.5) furnished a solvent deuterium isotope effect $K_{H_2O}/K_{D_2O} \sim 2.4$; this is indicative of a proton transfer from solvent in the rate-limiting step. The sulfide adducts **19a,b** that were recovered from the reactions performed in D_2O

Scheme IV

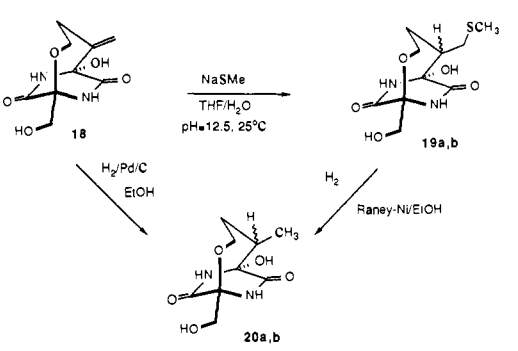


Table I. Rate Constants for the Reaction of **18** with NaSMe in Aqueous THF (pH 12.5) at Various Temperatures

temp, °C	T^{-1}, 10^3	rate constant (k), $M^{-1}\ s^{-1}$	ln k
0	3.663	1.29×10^{-2}	-4.351
7	3.571	3.15×10^{-2}	-3.458
15	3.472	8.09×10^{-2}	-2.515
25	3.356	1.95×10^{-1}	-1.635

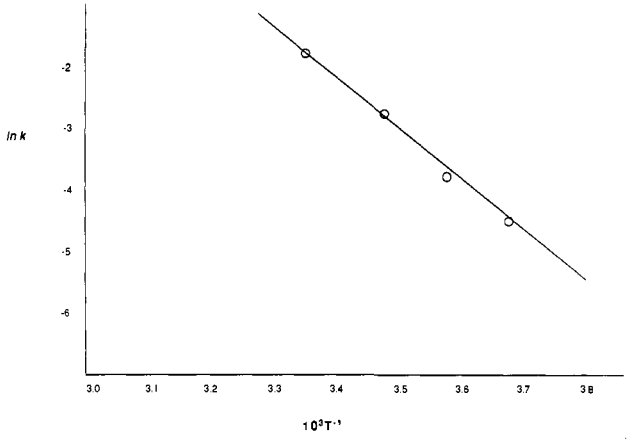


Figure 1. Plot of rate constant (ln k) vs. reciprocal temperature for the conversion of **18** → **19a,b** at 0, 7, 15, 25 °C; with $[18]_0 = 0.0039$ M and $[NaSMe]_0 = 0.0098$ M.

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

(17) (a) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 3253. (b) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1984**, *106*, 5748.

(18) In each case the control **8** or **18** was reacted with the *same* freshly prepared NaSMe solution and the expected sulfide adducts were isolated and identified as an "internal" control.

(19) The major diastereoisomer of **20** resulting from **19** corresponded to the minor diastereomer obtained from reduction of **18**.

(20) The runs performed at 7 °C provided the most reliable data due to half-life, solubility, and minimum volatile loss of NaSMe.

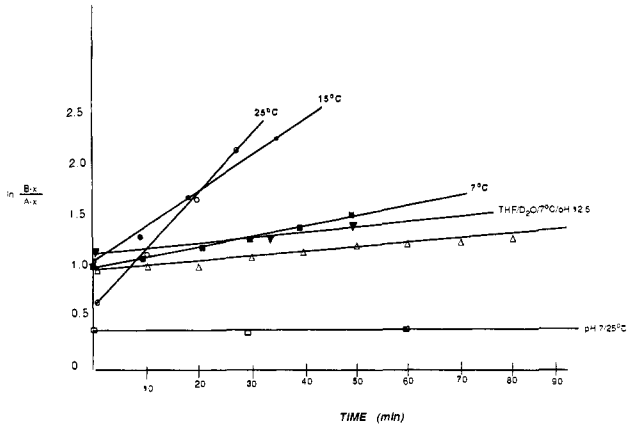
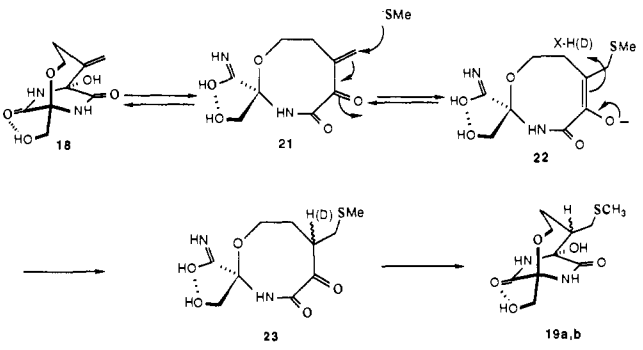


Figure 2. Kinetics of the reaction of **18** with NaSMe: (○) THF–H_2O, pH 12.5, 25 °C, [**18**] = 5.03 × 10^{-3} M, [NaSMe] = 9.80 × 10^{-3} M; (●) THF–H_2O, pH 12.5, 15 °C, [**18**] = 3.48 × 10^{-3} M, [NaSMe] = 9.80 × 10^{-3} M; (■) THF–H_2O, pH 12.5, 7 °C, [**18**] = 3.70 × 10^{-3} M, [NaSMe] = 9.80 × 10^{-3} M; (△) THF–H_2O, pH 12.5, 0 °C, [**18**] = 3.93 × 10^{-3} M, [NaSMe] = 9.80 × 10^{-3} M; (▼) THF–D_2O, pH 12.5, 7 °C, [**18**] = 3.09 × 10^{-3} M, [NaSMe] = 9.80 × 10^{-3} M; (□) THF–H_2O, pH 7.0, 25 °C, [**18**] = 5.41 × 10^{-3} M, [HSMe] = 8.07 × 10^{-3} M. A = $[\mathbf{18}]_0$; B = $[NaSMe]_0$; x = consumed [**18**].

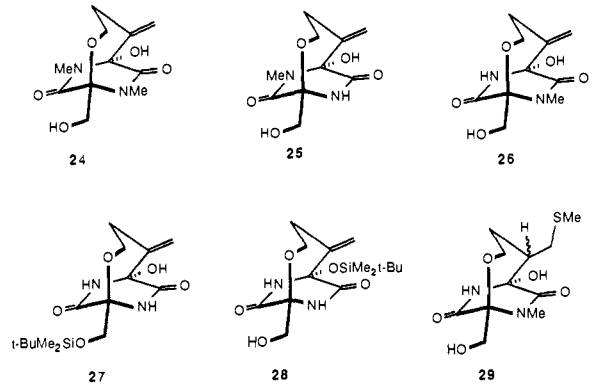
Scheme V



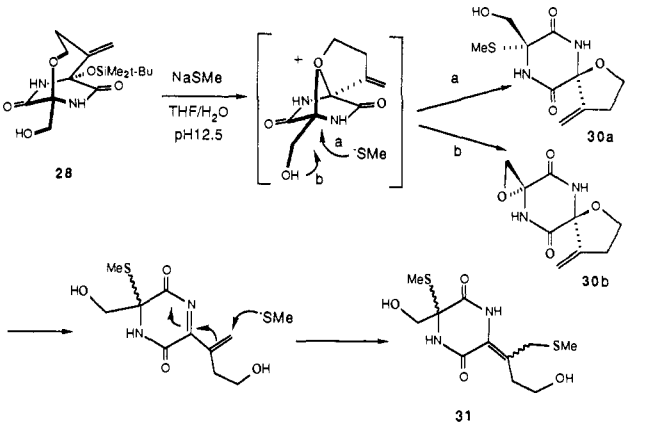
incorporaed a deuterium atom at C-5 (~100% D incorporation by MS). In an effort to assess whether the reaction was subject to specific or general acid/base catalysis, a buffer system with a pK_a value close to HSMe was desired. The CF_3CH_2OH/$CF_3CH_2O^-$ buffer seemed best suited for this purpose.[21] Varying the buffer concentration at 7 °C showed an initial rate enhancement between 0 and 10 min, but this rapidly slowed down after 10 min. Analysis of the reaction indicated that NaSMe *reacts* with trifluoroethanol at pH 12.5 at a rate that precludes a rigorous evaluation of the kinetics over several half-lives in this buffer system. However, the initial burst in rate would seem to indicate that the reaction is subject to *general* acid/base catalysis. The rate at pH 7 (25 °C, K = 3.2 × 10^{-4} M^{-1} s^{-1}) is ca. 600 times slower than that at pH 12.5 (25 °C, K = 1.95 × 10^{-1} M^{-1} s^{-1}; Figure 2). Below pH 7, the reaction slows down considerably, and at pH 3.5 there is no observable formation of sulfide adduct and only slow decomposition of **18**. The relatively rapid rates above pH 7 merely reflect the relative concentration of the highly nucleophilic thiolate anion.

Attempts at running this reaction in *anhydrous* THF containing 2.5 equiv of NaSMe (or a large molar excess) resulted in no reaction. We were quite surprised to observe that under the same conditions in scrupulously dried dimethyl sulfoxide the sulfide adducts (**19a,b**, 1:1 ratio) were obtained in >53% yield.[22] Curiously, substituting Me_2SO-d_6 (>98% atom D) for the same

Chart II



Scheme VI



reaction resulted in ca. 50% incorporation of deuterium at C-5 in the sulfide adducts **19a,b** (91% isolated yield). Perdeuteriation of the four exchangeable hydrogen atoms of **18** in D_2O, evaporation, and running the reaction in dimethyl sulfoxide resulted in sulfide adducts (**19**) with no incorporation of deuterium at C-5. This result implies that Me_2SO is capable of both protonating the incipient enolate **22** at C-5 and facilitating the ring-opening of **18** to the reactive ketone **21** (Scheme V). The fact that no reaction occurs in dry THF and the kinetics in H_2O indicate a solvent deuterium isotope effect suggests that proton transfer *intermolecularly* is an obligate feature of this reaction. Performing the reaction in anhydrous formamide with 2.5 equiv of NaSMe resulted in rapid formation of **19a,b** in 25% isolated yield.[22] On the basis of these results, it is reasonable to conclude that the highly polar solvents H_2O, Me_2SO, and $HCONH_2$ are capable of solvating the charged species **22** *and* protonating this species at *carbon-5* which allows the reaction to proceed (irreversibly) to **19a,b**. The dilemma posed above regarding the lack of reactivity of **14**, **15**, **16**, and **17** still needed to be resolved. In particular, the structural roles played by the C-1′ hydroxy alkyl moiety and amide substitutions needed clarification.

The following substituted derivatives of **18** were prepared: the *N,N′*-dimethyl species **24**; both mono-*N*-methyl derivatives **25** and **26**; and both *O-tert*-butyldimethyl silyl derivatives **27** and **28** (Chart II). Compounds **24–26** were prepared by careful N-methylation (NaH, MeI, DMF, THF) of **18**, and **27**/**28** were prepared by nonselective silylation of **18** (Me_2Bu^+SiCl, DMAP, Et_3N,CH_2Cl_2) and separation. It is also interesting to note that the major product from this reaction is the *tertiary* silyl ether **28** (ratio of **27**:**28** = 1:2.2); this can be ascribed to the lower pK_a and attendant increased nucleophilicity of the C-6 OH vs. the primary hydroxyl. The structures of **24**, **27**, and **28** were readily determined by examination of their ^1H NMR spectra. The assignment of regiochemistry to the methyl groups of the two mono-*N*-methyl derivatives **25** and **26** required careful examination of chemical shift differences of the amide N–H protons, the C-6 hydroxyl, the hydroxy methyl substituent and the methyl groups.[23]

(21) Trifluoroethanol has a pK_a value of ~12.4; see: Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1959**, *81*, 1050.

(22) By TLC, the conversion of **18** to **19** appears quantiative. However, upon evaporation of the high boiling solvent which requires warming, decomposition of **19** accompanies the isolation procedure; the yields refer to isolated final product.

Scheme VII

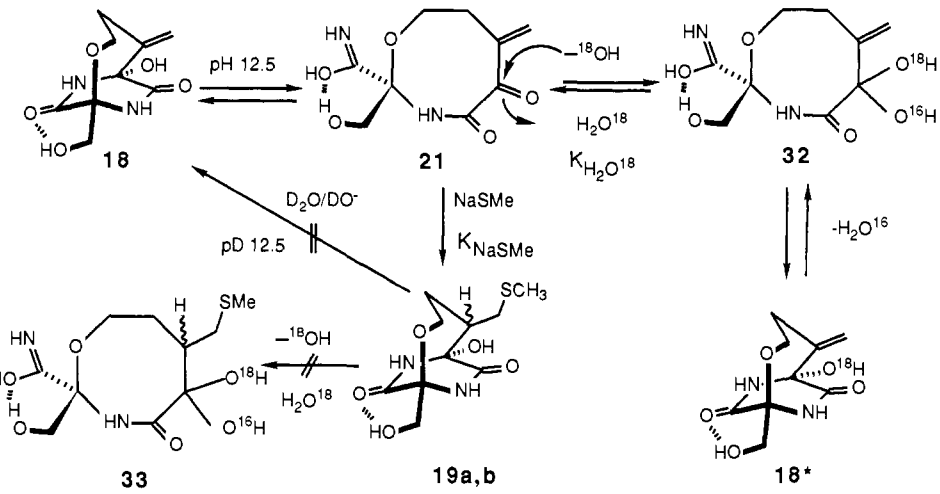


Table II. UV Spectra of Bicyclomycin Homologues

compd	EtOH λ_{max}, nm	ϵ	pH 12.5[a] λ_{max}, nm	ϵ	pH 7[a] λ_{max}, nm	ϵ
1	211	3800	226	2400		
18	212	2900	226	3400	213	3000
28	208	5700	226	5300		
27	212	3800	226	3700		
24	212	5700	226	3400		
glycine anhydride	212	1200	224	300		

[a] Aqueous solution.

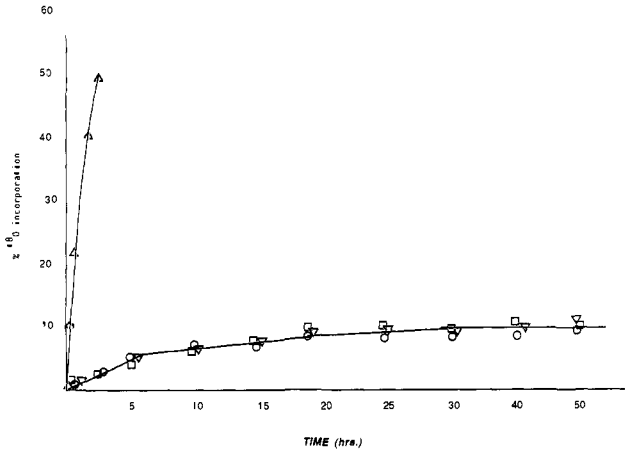
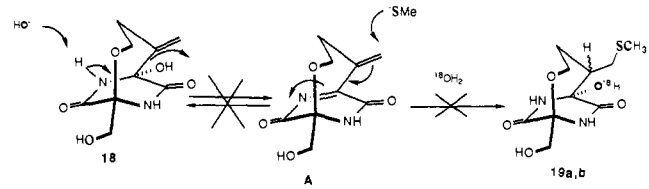


Figure 3. ^{18}O incorporation of **18** (△), **24** (▽), **27** (□), and **28** (○) at pH 12.5, in THF/$^{18}OH_2$ (98% ^{18}O) at 25 °C.

When compounds **24–28** were subjected to the NaSMe/THF/H_2O (pH 12.5) conditions, the following results were obtained. Compounds **24**, **25**, and **27** were unreactive and were recovered unchanged from the reaction medium. Compound **28** cleanly and rapidly reacted with NaSMe to afford the sulfide adduct **29** in 6% isolated yield.[24] The only anomaly in this series occurred when **28** was allowed to react under these conditions. Unexpectedly, **28** consumed 2 molar equiv of NaSMe to furnish an optically active product whose spectral properties are consistent with those of structure **31**. We attribute this to a precedented[25] proclivity for expulsion of the C-6 oxygen substituent (in this case a good leaving group, $^-OSiMe_2Bu^+$) to furnish spiro structures such as **30a,b**. Amide-assisted ring-opening of the spiro moiety followed by Michael-type addition of thiolate furnishes the α,β-unsaturated sulfide moiety. The fact that the product maintains optical activity ($[\alpha]_D^{25}$ +4.5°) would indicate that an incipient epoxide moiety (**30b**) that is nucleophilicly opened by NaSMe or direct attack by NaSMe on the tricyclic oxonium ion provides the adduct **31**. It is also possible that both mechanisms are operative or that a planar intermediate is involved, giving partially racemized material. The percent ee of **31** was not determined. Importantly, the above results clearly indicate that the amide adjacent to the C-6 hydroxyl be *unsubstituted and* the C-1′ hydroxy alkyl moiety not be blocked for sulfide formation to occur.

Scheme VIII



It remained at this juncture to demonstrate experimentally the possible intermediacy of the ring-opened ketone, such as **21**, as a viable reactive intermediate. Examination of a host of bicyclic materials by UV under various conditions did not show significant differences nor was there any indication that a significant concentration of ketone (such as **21**) was present due to the lack of absorptions above 226 nm (Table II).

However, incubation of **18** in $^{18}OH_2$ at pH 12.5, removal of aliquots, and analysis by mass spectroscopy revealed between 40% and 50% ^{18}O incorporation at the C-6 position after 30 min at 25 °C; at pH 7 and 3, there was no ^{18}O incorporation after 48 h. In marked contrast, the derivatives **24**, **27**, and **28** incorporated less than 10% ^{18}O after as long as 47 h under the same conditions (Figure 3). When the reaction of **18** with NaSMe was carried out in $^{18}OH_2$/THF (pH 12.5, 25 °C) the products **19a,b** revealed no ^{18}O incorporation. This surprising and very significant result has several implications. First, it demonstrates that the reactive substrate **18** is undergoing tautomeric ring-opening[26] to **21** (Scheme VII); hydration of the putative C-6-ketone (**32**) and loss of H_2O^{16} by reversible mass action furnishes the isotopically labeled

(23) The chemical shift of the N-10 methyl residue for **25** (δ 3.15) is downfield of the N-8 methyl residue (δ 2.92) of **26** as expected since N-10 is part of a carbinolamide moiety. In addition, the N-10-H for **26** (δ 7.65) is downfield of the N-8-H (δ 7.47) of **25**, consistent with the expected effect of the carbinolamide on N-10 relative to N-8.

(24) As in the case of **18**, this reaction is very clean by TLC analysis, but significant decomposition of the labile product accompanies the isolation procedure. Curiously, **26** undergoes scrambling of the *N*-methyl residue to furnish a mixture of **25** (26%) and **24** (trace). This could be independently verified by subjecting **26** to the reaction conditions (pH 12.5); both **25** and **24** are produced from **26**. The low yield of **29** obtained partially reflects the rearrangement of **26** to the unreactive **25**.

(25) (a) Maag, H.; Blount, J. F.; Coffen, D. L.; Steppe, T. V.; Wong, F. *J. Am. Chem. Soc.* **1978**, *100*, 6786. See also: (b) Williams, R. M.; Anderson, O. P.; Armstrong, R. W.; Josey, J.; Meyers, H.; Eriksson, C. *J. Am. Chem. Soc.* **1982**, *104*, 6092. (c) Williams, R. M.; Dung, J.-S. *Tetrahedron Lett.* **1985**, *26*, 37.

(26) ^{18}O exchange is routinely used for detection of keto tautomers; see, for example: Sue, J. M.; Knowles, J. R. *Biochemistry* **1978**, *17*, 4041 and references cited therein.

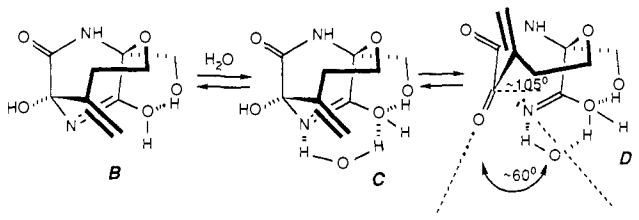
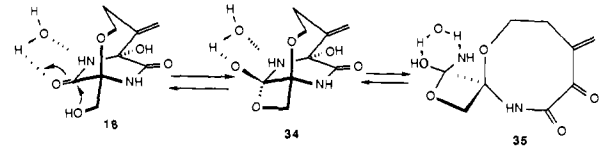


Figure 4.

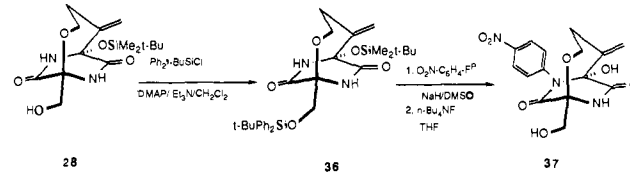
18. The fact that the sulfide adducts (**19a,b**) *do not* incorporate ^{18}O clearly indicates that the rate of thiolate addition to **21** (K_{NaSMe}) is much faster than the rate of hydration and exchange of **21** via **32** ($K_{H_2{}^{18}O}$ or $K_{NaSMe} \gg K_{H_2}{}^{18}O$). Most importantly, this result rigorously *excludes* the base-promoted expulsion of the C-6 OH forming a C-6/N-10 amidine (A) as a possible reactive electrophilic intermediate, since such an intermediate would *necessarily* incorporate a significant amount of ^{18}O from the solvent (98% $^{18}OH_2$) at C-6 in forming **19a,b**. Exclusion of this pathway is important as it directly relates to one conceptual function of a secondary (–N–H–) amide at N-10 that can now be rigorously eliminated from further consideration. These observations also indicate that once formed, **19a,b** *does not* reopen (**33**) to a keto tautomer[26] that could hydrate and exchange as the olefin (**18**) clearly does. Further evidence of the irreversibility of the reaction was provided by incubating **19a,b** in (98%) D_2O/DO^- at pD 12.5 at 25 °C. Under these conditions, there was no trace of retro-Michael reaction back to **18** nor was there any H/D exchange at C-5 (mass spectroscopic analysis). From these results, it can be concluded that the olefin **18** is capable of ring-opening to **21** at pH 12.5; at pH 7 and 3, there is no detectable ^{18}O exchange on the same substrate. The analogues (**24**, **27**, and **28**) and the sulfide adduct are not capable of undergoing a similar tautomeric ring-opening at pH 12.5 as evidenced by the lack of ^{18}O incorporation (vide supra). In order to rationalize the marked differences between the unreactive N-alkylated derivatives (**14**, **16**, **17**, **24**, and **25**) and their reactive counterparts (**1**, **8**, **18**, and **26**) the kinetic results point to a crucial proton transfer from solvent in the rate-limiting step. It is well-known that secondary amides hydrogen bond to H_2O as depicted in Figure 4, structure C. Since the C-6/N-10 bond that is cleaved in the transition state (**18** → **21**) is *orthogonal* to the C-9/N-10 amide system (Figure 5), the N-10 nitrogen has to carry the entire burden of the lone electron pair (D, Figure 4) that is displaced upon ring-opening. Thus concomitant protonation of N-10 is a reasonable obligate occurrence to lower the activation energy of increasing the electron density on this nitrogen atom in the transition state; concerted proton transfer from H_2O through the imino alcohol tautomer C readily accomplishes this. Obviously, the N-alkylated derivatives do not have access to such a tautomer, nor are they expected to hydrogen bond to solvent as in the case of the secondary amides (C). Inspection of Dreiding stereomodels for the *reverse reaction* (i.e., closure of ketone D → C) very clearly shows that this eight-membered ring cannot readily achieve the Dunitz vector[27] of ~105°; a "relaxed" vector cone of ca. 60° is defined by the rigidity of the peptide bonds (D, Figure 4). Therefore, significant distortion of the eight-membered ring must accompany reclosure of D → C via the minimum energy approach vector; by the principle of microscopic reversibility, the ring-opening or "exit" vector[27] is equally poor.

What, then, is the role of C-1′ hydroxy alkyl group? ^1H NMR studies of **1**, **8**, **16**, **17**, **18**, **24**, **25**, and **26** clearly show that both the C-1′ OH and C-6 OH[28] are tightly H bonded to the C-9 and C-7 carbonyl oxygen atoms, respectively. It is reasonable, then, that *intramolecular* hydrogen bonding to the C-9 carbonyl by the C-1′ OH additionally catalyzes the tautomerization of C → D. An alternative possibility that cannot be excluded a priori involves potential anchimeric assistance by the C-1′ OH to give the oxetane hemi-amino hemi-ortho ester **34** (Scheme IX). Such internal ortho esters are known to exist in the ergot alkaloids. However, in this particular case, the strain energy associated with the formation of the oxetane in addition to the loss of amide resonance by such interactions seems tenuous. Furthermore, it is reasonable that if such an interaction were important, the C-9 amide would be very susceptible to hydrolysis and ^{18}O exchange; neither effect seems to be operative (vide infra). On the basis of the data, we prefer the interpretation that *both intermolecular* proton transfer (from solvent) and an *intramolecular* hydrogen bond (from the C-1′ OH) are obligate requirements for ring-opening.

Scheme IX



Scheme X



In an effort to assess whether a good electron-withdrawing substituent on N-10 could facilitate ring-opening, the *N-p*-nitrophenyl substrate **37** was prepared from **18** (Scheme IX). As with the other N-10-alkylated derivatives, compound **37** was *unreactive* toward NaSMe in THF/H_2O at pH 12.5. This is surprising since the *p*-nitrophenyl group is capable of stabilizing electron density on the N-10 amide nitrogen through resonance delocalization *and* inductive withdrawal. The lack of reactivity of this substrate emphasizes the importance of the secondary amide system interacting with the solvent to allow the intermolecular proton transfer.

Perhaps the most puzzling observation concerns the recalcitrance of the sulfide adducts **19a,b** to display reversible ring-opening behavior (as evidenced by lack of ^{18}O incorporation, retro-Michael and H/D exchange at C-5). It is reasonable to expect that upon tautomeric ring-opening of the *olefinic* substrates (**1**, **8**, **18**) the newly formed ketone at C-6 enjoys some (albeit modest) resonance stabilization (perhaps a few kcal/mol) being α to the existing C-5 *exo*-methylene moiety. No such resonance delocalization exists for the sulfide adducts **19a,b**. At present then, it seems that this additional and subtle structural requirement for ring-opening is an obligate feature of this complex process.

In summary then, our data are consistent with the interpretation that (1) a free (N–H) amide must be present at N-10, (2) an *exo*-methylene moiety must exist at C-5, (3) a bridgehead hydroxyl must exist at C-6, and (4) a C-1′ hydroxylalkyl moiety must be present to allow for tautomeric ring-opening to an eight-membered-ring ketone. The kinetics indicate that the ring-opening step is rate-limiting, involving proton transfer from solvent to furnish a small concentration of highly electrophilic and reactive α,β-unsaturated ketone that rapidly suffers irreversible addition of thiolate. The strain energy involved in attaining the correct geometry for ring-opening (vide supra) is compensated by general proton catalysis. The experiments alluded to above that were run in Me_2SO and formamide imply that other good hydrogen-bonding solvents (besides H_2O) also satisfy the requirements for cleavage of the C-6/N-10 bond with concomitant proton transfer to the amide system.

Biological Relevance

One of our stated initial objectives was to see if a direct correlation exists between the capacity of structures to undergo

(27) (a) Burgi, H. B.; Dunitz, J. D.; Lehn, J. M.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563. (b) Burgi, H. B.; Dunitz, J. D.; Shefler, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065. See also: (c) Storm, D. R.; Koshland, D. E. *J. Am. Chem. Soc.* **1972**, *94*, 5805, 5815. (d) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734.

(28) It is estimated that the pK_a of the C-6-OH is <10 and exists as the sodium alkoxide at pH 12.5, see: Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705.

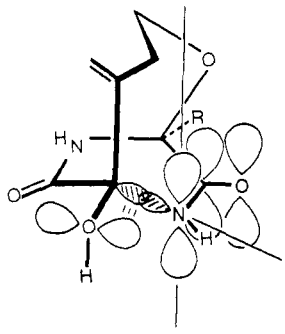


Figure 5.

thiolate addition and the complementary capacity to display biological (antimicrobial) activity. Many of the compounds reported in this study[12,13] have been evaluated for antimicrobial activity. Only compounds **1** and **14** displayed antimicrobial activity; compound **18** was reported as being biologically inactive and compound **14** is unreactive toward NaSMe as reported herein. The lack of correlation between simple thiolate susceptibility and antimicrobial activity indicates that this interesting and complex reaction *alone* cannot be used as the biomechanistic template. It is also recognized that evaluation of gross antimicrobial activity is not necessarily a reliable measure of *intrinsic* enzyme inhibitory properties due to solubility, stability, and penetration differences of substrates toward the test organism.

We are actively pursuing the alternative, related mechanism outlined above involving suicide inactivation of membrane-bound proteases. The recent findings of Vazquez[14] have stimulated additional impetus to elucidate the chemical mechanism by which compounds of the structural class represented by **1** arrest bacterial cell division.

Experimental Section

Materials and Methods. Natural bicyclomycin was kindly provided by Fujisawa Pharmaceutical Co., Ltd., Japan. The acetonide derivative **8** was prepared according to German Patent 2647322, April 28, 1977; see also ref 16. Compounds **10**, **11**, **14**, **16**, and **17** were prepared as fully described in ref 17; compounds **12** and **15** are prepared as described in ref 13. Compound **18** was prepared from natural bicyclomycin as described in ref 12.

Kinetic studies were performed by analyzing the reaction mixtures by reverse-phase (C_{18}) HPLC with 20% aqueous acetonitrile and L-alanine as an internal standard. Generally, the NaSMe reaction was performed with [substrate] $\sim 3.8 \times 10^{-3}$ M, 1.0 equiv, and [NaSMe] $\sim 9.8 \times 10^{-3}$ M, 2.5 equiv. The NaSMe was freshly prepared from HSMe in degassed THF buffer solution (pH adjusted to 12.5, buffered with 0.2 M KCl and 0.2 M NaOH). Aliquots were removed (0.1 mL) and added to 0.1 mL of 0.1 N HCl to neutralize the aqueous phase, evaporated, redissolved in a known volume of 20% aqueous acetonitrile, and directly injected into the HPLC. In some cases, the aliquot (0.1 mL) was added to 1 mL of CH_3CN, filtered through a short silica gel column (eluted with THF), evaporated, and analyzed by HPLC. ^1H NMR data are reported in δ values (ppm) downfield from Me_4Si unless otherwise stated.

In most cases, the products are obtained as amorphous hygroscopic powders and glasses that were recalcitrant to crystallization and rigorous analytical purification.

NaSMe Addition to Bicyclomycin-2′,3′-acetonide 8 (9). To a stirred solution of **8**[16] (8 mg, 0.023 mmol, 1.0 equiv) in THF (0.1 mL) at room temperature was added MeSNa (0.1 mL, 0.0208 mmol, 0.88 equiv, pH 12.5 ca. 0.208 M), the mixture was stirred for 30 min and diluted with CH_3OH, anhydrous sodium sulfate was added, and the solution was filtered, concentrated, and separated by PTLC silica gel (eluted with 10% $MeOH/CH_2Cl_2$) to afford 7 mg (76%) of **9** as a glass.

^1H NMR (270 MHz)(CD_3OD) δ (Me_4Si) 1.38 (3 H, s), 1.40 (3 H, s), 1.50 (3 H, s), 2.10 (3 H, s),, 2.10–2.40 (5 H, m), 3.39 (1 H, s), 3.79 (1 H, d, J = 9.20 Hz), 3.90–4.20 (3 H, m). IR (NaCl, neat) 3600–3200, 1640 cm^{-1}.

8,10-Diaza-6-(*tert*-butyldimethylsiloxy)-1-(hydroxymethyl)-5-methylene-2-oxabicyclo[4.2.2]decan-7,9-dione (28) and 8,10-Diaza-1-(*tert*-butyldimethylsiloxy)-6-hydroxy-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (27). To a suspended mixture of **18** (36 mg, 0.158 mmol, 1.0 equiv), *tert*-butyldimethylsilyl chloride (95 mg, 0.63 mmol, 4.0 equiv), and DMAP (29 mg, 0.237 mmol, 1.5 equiv) in 10 mL of THF was added Et_3N (0.110 mL, 0.79 mmol, 5.0 equiv) under N_2 at 25 °C. The mixture was stirred for 20 h at 25 °C. The reaction was quenched by addition of 20 mL of H_2O, and extracted with CH_2Cl_2 (50 mL × 3), and the combined extract was dried over Na_2SO_4 for 3 h, filtered, evaporated, and separated by PTLC silica gel with 10% MeOH–90% CH_2Cl_2 as eluent; **28** (white solid) and **27** (white solid) were obtained in yields of 26.2 mg (48.5%) and 11.7 mg (21.7%), respectively. **28**. ^1H NMR (270 MHz)($CDCl_3$) δ 0.12 (3 H, s), 0.287 (3 H, s), 0.94 (9 H, s), 2.50 (1 H, m), 2.63 (2 H, m), 3.76 (2 H, m), 3.96 (2 H, m), 5.13 (1 H, s), 5.59 (1 H, s), 6.24 (1 H, s), 7.00 (1 H, s); IR (KBr disk) 3440, 3240, 2960, 2930, 2860, 1705, 1695, 1690, 1400, 1260, 1210, 1170, 1125, 1070, 910, 860, 840, 780 cm^{-1}; mass spectrum, m/e (%) (NH_3/CI) 343 (55, M^+ + 1), 195 (16), 179 (12), 177 (12), 132 (13), 91 (100). **27**: ^1H NMR (270 MHz)($CDCl_3$) δ 0.09 (3 H, s), 0.11 (3 H, s), 0.90 (9 H, s), 2.66 (2 H, m), 3.76 (1 H, m), 3.83 (1 H, d, J = 11 Hz), 3.98 (1 H, m), 4.05 (1 H, d, J = 11 Hz), 4.81 (1 H, s), 5.18 (1 H, s), 5.59 (1 H, s), 6.53 (1 H, s), 6.98 (1 H, s); IR (KBr disk) 3420, 3380, 2950, 2925, 2850, 1695, 1690, 1685, 1677, 1125, 830, 775 cm^{-1}; mass spectrum, m/e (%) (NH_3/CI) 343 (100, M^+ + 1), 285 (5), 144 (5), 116 (12), 92 (14), 91 (16).

***N*,*N*′-Dimethyl-8,10-diaza-6-hydroxy-1-(hydroxymethyl)-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (24).** To a stirred solution of **18** (23.5 mg, 0.10 mmol, 1.0 equiv) in 2 mL of DMF–THF (1/1) was added NaH (2.2 mg, 0.09 mmol, 0.9 equiv, washed with hexane, dried) under N_2 at 25 °C. The solution was stirred for 1 h at 25 °C. The solution was added to a solution of CH_3I (0.1 mL, 1.61 mmol, 15 equiv) in 1 mL of DMF–THF (1/1) under N_2 at 0 °C dropwise. The mixture was stirred for 2 h at 0 °C, quenched by addition of 1 mL of H_2O, evaporated, and separated by PTLC silica gel (10% MeOH–90% CH_2Cl_2): white solid (18.2 mg, 69%); ^1H NMR (270 MHz) ($CDCl_3$) δ 2.30 (1 H, dd), 2.38 (1 H, t), 2.55 (1 H, dd), 2.94 (3 H, s), 3.18 (3 H, s), 3.43 (1 H, dd), 3.77 (1 H, dd), 3.88 (1 H, dd), 4.43 (1 H, dd), 4.89 (1 H, s), 5.21 (1 H, s), 5.65 (1 H, s); IR (KBr disk) 3440, 3280, 1685, 1675, 1660, 1635, 1385, 1115, 1080, 1055, 990 cm^{-1}; mass spectrum, m/e (%) (NH_3/CI) 257 (100, M^+ + 1), 239 (11), 225 (15), 223 (29), 197 (10), 160 (36), 142 (42), 140 (36), 116 (100), 99 (16).

***N*-10-Methyl-8,10-diaza-6-hydroxy-1-(hydroxymethyl)-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (25) and *N*-8-Methyl-8,10-diaza-6-hydroxy-1-(hydroxymethyl)-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (26).** To a stirred solution of **18** (100 mg, 0.436 mmol, 1.0 equiv) in 5 mL of DMF–THF (1/1) was added NaH (60% oil dispersion, 16.6 mg, 0.42 mmol, 0.95 equiv) under N_2 at 25 °C. The mixture was stirred for 10 min. CH_3I (41 μL, 0.654 mmol, 1.5 equiv) was added to the mixture under N_2 at 25 °C. The solution was stirred for 24 h and the reaction was quenched by addition of 2 mL of H_2O, evaporated, and separated by PTLC silica gel (10% MeOH–90% CH_2Cl_2). **24** (17.8 mg, 15.9%) and a mixture of **25** and **26** (32.8 mg) were obtained. The pure isomers **25** and **26** were obtained in yields of 11.2% (11.8 mg) and 13.3% (14.0 mg), respectively, by repeated PTLC silica gel (10% MeOH–90% CH_2Cl_2). **25**: ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$ 2.54 (1 H, dd), 2.62 (1 H, dd), 3.15 (3 H, s), 3.30 (1 H, t), 3.45 (1 H, m), 3.95 (2 H, m), 4.44 (1 H, dd), 5.13 (1 H, s), 5.33 (1 H, s), 5.61 (1 H, s), 7.47 (1 H, s); IR (KBr disk) 3500–3350, 3300–3200, 1680, 1665, 1425, 1380 cm^{-1}; mass spectrum, m/e (EI, %) 242 (M^+, 0.5), 224 (1.4), 211 (6), 167 (23), 154 (25), 140 (15), 139 (22), 127 (24), 126 (20), 99 (35), 98 (53), 96 (30), 72 (43), 55 (68), 42 (100). **26**: ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$ 2.28 (1 H, dd), 2.60 (1 H, dd), 2.88 (3 H, s), 3.49 (1 H, t), 3.72–3.95 (3 H, m), 4.07 (1 H, m), 5.26 (1 H, s), 5.54 (1 H, s), 5.70 (1 H, s), 7.65 (1 H, s); IR (KBr, disk) 3500–3350, 3350–3250, 1690, 1670, 1430, 1390 cm^{-1}; mass spectrum, m/e (EI, %) 242 (M^+, 0.4), 224 (1.3), 211 (5), 167 (4); 154 (13), 140 (16), 126 (27), 99 (24), 72 (23), 68 (100).

The Reaction of 18 with NaSMe in Aqueous THF at pH 12.5 (19a,b). To a stirred solution of **18** (21.2 mg, 0.093 mmol, 1.0 equiv) was added aqueous NaSMe (0.28 mL, 0.240 mmol, 2.5 equiv) at 25 °C. The mixture was stirred for 1 h, evaporated, and separated by PTLC silica gel (15% MeOH–85% CH_2Cl_2); white solid (**19a,b**, 12.0 mg, 46.2%) (unstable on silica gel) was obtained. **19a** (major isomer): ^1H NMR (270 MHz) (Me_2SO-d_6) δ 1.87 (2 H, m), 2.00 (3 H, s), 2.95 (1 H, m), 3.15 (2 H, d), 3.6–3.8 (3 H, m), 4.12 (1 H, dd), 4.88 (1 H, t), 6.75 (1 H, s), 8.68 (1 H, s), 8.72 (1 H, s); MS, m/e (%) (EI) 276 (1, M^+), 229 (4), 184 (9), 146 (9), 99 (13), 98 (25), 68 (55), 61 (60), 48 (81), 47 (100); (NH_3Cl) 277 (73 M^+ + 1), 216 (12), 192 (16), 165 (10), 164 (100), 147 (32), 144 (37), 128 (25), 118 (16), 87 (53); IR (KBr disk) 3300–3450, 2920, 1740, 1690, 1680, 1400, 1050 cm^{-1}. **19b** (minor isomer): ^1H NMR (270 MHz) (Me_2SO-d_6) δ 1.87 (2 H, m), 2.00 (3 H, s), 2.95 (1 H, m), 3.15 (2 H, d), 3.6–3.8 (3 H, m), 4.12 (1 H, dd), 4.86 (1 H, t), 6.66 (1 H, s), 8.75 (1 H, s), 8.80 (1 H, s). Kinetic runs were carried out as described in the Materials and Methods section at various temperatures.

The Reaction of 18 with HSMe in Aqueous THF at pH 7. To a stirred solution of **18** (11.5 mg, 0.05 mmol, 1.0 equiv) in 0.5 mL of THF–H_2O (3/1) was added HSMe (7 μL, 0.125 mmol, 2.5 equiv) under N_2 at 0 °C. The mixture was stirred in a sealed tube at 25 °C for 24 h during

which time aliquots were removed, evaporated, and separated by PTLC. **19a,b** was obtained in a yield of 31.2% (4.3 mg).

The Reaction of 28 with NaSMe in Aqueous THF at pH 12.5 (31). To a stirred solution of **28** (20.3 mg, 0.059 mmol, 1.0 equiv) in 0.8 mL of THF–H_2O (3/1) was added aqueous NaSMe (0.37 mL, 0.148 mmol, 2.5 equiv) at 25 °C. The mixture was stirred for 2 h at 25 °C (one spot by TLC), filtered through a short silica gel column (2 mL) in order to neutralize the solution, evaporated, and separated by PTLC silica gel (10% MeOH–90% CH_2Cl_2). **31** (very unstable) was isolated in a yield of 19.7% (3.6 mg). ^{1}H NMR (270 MHz) (Me_2SO-d_6) δ 1.93 (3 H, s), 2.06 (3 H, s), 2.07 (2 H, m), 3.55 (2 H, m), 3.75 (1 H, d, J = 12.6 Hz), 3.90 (2 H, m), 4.15 (1 H, d, J = 12.6 Hz), 5.22 (1 H, t), 5.26 (1 H, t), 8.69 (1 H, s), 9.79 (1 H, s); IR (NaCl, neat) 3200–3400, 2860, 2820, 1670, 1380, 1040, 1015, 690 cm^{-1}; mass spectrum, m/e (%) (NH_3/CI) 307 (2, M^+ + 1), 241 (19)8 212 (11), 211 (34), 195 (18), 174 (57), 126 (16), 102 (50), 85 (100). $[\alpha]_D^{25}$ +4.5 (2.2 mg/1 mL = +0.10). UV λ_{max} = 276 nm, $E \sim 1800$.

The Reaction of 26 with NaSMe in Aqueous THF at pH 12.5 (29). To a stirred solution of **26** (7.2 mg, 0.030 mmol, 1.0 equiv) in 1 mL of THF–H_2O (3/1) was added aqueous NaSMe (90 μL, 0.075 mmol, 2.5 equiv) at 25 °C. The mixture was stirred for 2 h at 25 °C, evaporated, and separated by PTLC silica gel (10% MeOH–90% CH_2Cl_2). **25** (1.9 mg, 26.4%) and **29** (0.5 mg, 5.8%) were isolated. **25** was identified by ^{1}H NMR and MS. **29**: ^{1}H NMR (270 MHz) ($CDCl_3$) δ 1.87 (2 H, m), 2.12 (3 H, s), 2.60 (2 H, d), 2.88 (3 H, s), 3.50 (2 H, m), 3.70–4.05 (3 H, m), 4.25 (1 H, m), 7.35 (1 H, s); mass spectrum, m/e (%) (EI) 243 (M^+ – SMe), (NH_3/CI) 291 (M^+ + 1). A trace amount of **24** was detected by TLC.

^{18}O Incorporation Experiment at pH 12.5. To a stirred solution of 1.2 mL of THF–$H_2{}^{18}O$ (3/1) were added the substrate (ca. 5 mg, 0.02 mmol) and NaH (0.32 mg, 0.0134 mmol) under N_2 at 25 °C. An aliquot (0.15 mL) was filtered through Dowex 50W-X8 (H^+ Form) and evaporated. White solid obtained was analyzed by MS (NH_3/CI).

^{18}O Incorporation Experiment of 18 at pH 7. To a stirred solution of 0.6 mL of THF–$H_2{}^{18}O$ (3/1) was added **18** (4.6 mg, 0.020 mmol) under N_2 at 25 °C. An aliquot (0.75 mL) was evaporated. The white solid obtained was analyzed by MS. No ^{18}O incorporation was observed by MS after 48 h.

^{18}O Incorporation Experiment of 18 at pH 3. To a stirred solution of 0.6 mL of THF–$H_2{}^{18}O$ (3/1) were added **18** (4.5 mg, 0.02 mmol) and concentrated HCl (1.5 μL) under N_2 at 25 °C. An aliquot (0.75 mL) was evaporated. The white solid obtained was analyzed by the above method. No ^{18}O incorporation was observed after 48 h.

The Reaction of 18 with NaSMe in THF–$H_2{}^{18}O$ (pH 12.5) at 25 °C. To a solution of 1 mL of THF–$H_2{}^{18}O$ (3/1) were added NaH (4.3 mg, 0.179 mmol, washed with hexane three times, dried) and then HSMe (5.0 μL, 0.116 mmol, 2.6 equiv) under N_2 at 0 °C. The mixture was stirred for 10 min under N_2 at room temperature. **18** (9.6 mg, 0.043 mmol, 1.0 equiv) was added to the solution of NaSMe under N_2 at 25 °C. The solution was evaporated after 1.5 h and separated by PTLC (15% MeOH–85% CH_2Cl_2); white solid (5.6 mg, 47.1%) was identified as **19a,b** by ^{1}H NMR. The product **19a,b** showed no ^{18}O incorporation by mass spectroscopic analysis.

Kinetics of the Reaction of 18 with NaSMe in THF–H_2O. The rate studies were carried out with HPLC (with a C-18 column) equipped with a differential refractometer as a detector. Aqueous MeCN (20%) as an eluent and L-alanine as an internal standard were used. Generally, the reaction was done with the concentration of **18** ca. 3.8×10^{-3} M (1.0 equiv) and that of NaSMe ca. 9.8×10^{-3} M (2.5 equiv) in 5 mL of THF–buffer solution (pH 12.5, the solution prepared from 25 mL of 0.2 M KCl and 20.4 mL of 0.2 M NaOH) (3/1). The results at various temperatures are shown in Table I.

Kinetics of the Reaction of 18 with NaSMe in THF–D_2O (pH 12.5) at 7 °C. To a stirred solution of **18** (2.767×10^{-3} M) in 5 mL of THF–buffer (the solution prepared from 12.5 mL of 0.2 M KCl in D_2O and 10.2 M NaOH in D_2O) (3/1) was added NaSMe–D_2O (9.802×10^{-3} M) at 7 °C. The rate study was done by two methods: (a) an aliquote (0.1 mL) was added to 0.1 mL of 0.1 N HCl to quench the reaction, evaporated, and analyzed by HPLC; and (b) an aliquot (0.1 mL) was added to 1 mL of MeCN, filtered through a short silica gel column, eluted with THF, evaporated, and analyzed by HPLC.

8,10-Diaza-5-methyl-6-hydroxy-1-(hydroxymethyl)-2-oxabicyclo-[4.2.2]decane-7,9-dione (20a,b) from Hydrogenation of 18. To a stirred solution of **18** (9.8 mg, 0.043 mmol, 1.0 equiv) in 2 mL of EtOH was added 10% Pd/C (10.7 mg, 0.010 mmol, 0.2 equiv) under H_2 (1 atm) at room temperature. The mixture was stirred for 20 h, filtered through a Celite pad, evaporated, and separated by PTLC silica gel (15% MeOH, CH_2Cl_2); a white solid was obtained (**20b,a**, 6.8 mg, 68.8%). **20b,a** has two isomers. **20b**, major isomer: ^{1}H NMR (270 MHz) (Me_2SO-d_6) δ 0.93 (3 H, d), 1.5–2.1 (3 H, m), 3.29 (1 H, dd), 3.76 (1 H, dd), 3.6–3.9 (2 H, m), 4.85 (1 H, t), 6.45 (1 H, s), 8.59 (1 H, s), 8.67 (1 H, s). **20a**, minor isomer: ^{1}H NMR (270 MHz) (Me_2SO-d_6) δ 0.93 (3 H, d), 1.5–2.1 (3 H, m), 3.29 (1 H, dd), 3.76 (1 H, dd), 3.6–3.9 (2 H, m), 4.85 (1 H, t), 6.40 (1 H, s), 8.63 (1 H, s), 8.71 (1 H, s).

20a,b from Raney Nickel Reduction of 19a,b. To a stirred solution of **19a,b** (10.8 mg, 0.039 mmol, 1.0 equiv) in 2 mL of EtOH was added an excess of Raney Ni in *i*-PrOH at room temperature. The mixture was stirred under reflux for 24 h. After being cooled to 25 °C, the mixture was filtered through Celite 545 to remove Raney Ni, evaporated, and separated by PTLC silica gel (15% MeOH–85% CH_2Cl_2); a white solid (**20a,b**, 2.4 mg, 26.7%) was obtained with an isomer ratio **20a**/**20b** = 15/1. These were shown to be identical with **20a,b** obtained from hydrogenation of **18**.

8,10-Diaza-6-(*tert*-butyldimethylsiloxy)-1-[(*tert*-butyldiphenylsiloxy)methyl]-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (36). To a stirred solution of **18** (228 mg, 1.0 mmol, 1.0 equiv) in 25 mL of THF were added *t*-$BuMe_2SiCl$ (603 mg, 4.0 mmol, 4.0 equiv), DMAP (183 mg, 1.5 mmol, 1.5 equive, and Et_3N (506 mg, 5.0 mmol, 5.0 equiv) at room temperature under N_2. The mixture was stirred for 4 h at room temperature, poured into H_2O, extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, filtered, evaporated, and separated by silica gel column (eluted with 5% MeOH–95% CH_2Cl_2) to give the C-6 silylated product **28** in 63.2% yield (216.0 mg) and the C-1 silylated product **27** in 1.2% yield (4.0 mg). The major product was directly subjected to the subsequent silylation reaction. (Data for both compounds were reported above.)

To a stirred solution of **28** (55.0 mg, 0.16 mmol, 1.0 equiv) in 3 mL of CH_2Cl_2 were added *t*-$BuPh_2SiCl$ (110 mg, 0.4 mmol, 2.5 equiv), DMAP (19.5 mg, 0.16 mmol, 1.0 equiv), and Et_3N (32 mg, 0.32 mmol, 2.0 equiv) at room temperature. The mixture was stirred for 24 h at room temperature, poured into H_2O, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and separated by silica gel column (eluted with THF/hexanes, 1:1) to give **36**: mp 74–75 °C; 87.7% yield (81.8 mg); ^{1}H NMR (270 MHz) ($CDCl_3$) δ 0.14 (3 H, s), 0.30 (3 H, s), 0.94 (9 H, s), 1.06 (9 H, s), 2.62 (2 H, m), 3.78 (1 H, m), 3.86 (1 H, 1/2 ABq, J = 10.8 Hz), 3.90 (1 H, m), 4.03 (1 H, 1/2 ABq, J = 10.8 Hz), 5.12 (1 H, s), 5.60 (1 H, s), 6.02 (1 H, s), 6.61 (1 H, s), 7.40 (6 H, m), 7.67 (4 H, m); IR (KBr, disk) 3370, 3250–3150, 2950, 2930, 2855, 1725, 1695, 1425, 1390 cm^{-1}; mass spectrum, m/e (EI, %) 523 (M^+ – C_4H_9, 5.0), 445 (4.7), 213 (8.1), 199 (100), 75 (100).

8,10-Diaza-6-hydroxy-1-(hydroxymethyl)-10-(*p*-nitrophenyl)-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (37). To a stirred solution of the disilyl ether **36** (60 mg, 0.09 mmol, 1.0 equiv) in 5 mL of Me_2SO was added NaH (50% oil dispersion, 5.2 mg, 0.11 mmol, 1.2 equiv) at room temperature. The mixture was heated at 80 °C for 1 h. To this mixture was added *p*-fluoronitrobenzene (25 mg, 0.18 mmol, 2.09 equiv) at 80 °C. The mixture was stirred for 15 h at 80 °C, poured into H_2O, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and separated by PTLC silica gel (eluted with 30% THF/hexanes) to give the *N*-10-*p*-nitrophenyl derivative in 9.9% yield (7.0 mg): ^{1}H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$ –0.22 (3 H, s), –0.01 (3 H, s), 0.85 (9 H, s), 1.09 (9 H, s), 2.54 (2 H, m), 3.75 (1 H, 1/2 ABq, J = 10.7 Hz), 3.90 (2 H, m), 4.19 (1 H, 1/2 ABq, J = 10.7 Hz), 5.13 (1 H, s), 5.67 (1 H, s), 6.30 (1 H, s), 7.27 (2 H, d, J = 8.9 Hz), 7.40 (6 H, m), 7.66 (4 H, m), 8.26 (2 H, d, J = 8.9 Hz).

To a stirred solution of the product obtained above (7.0 mg, 9×10^{-3} mmol, 1.0 equiv) in 1 mL of THF was added *n*-$Bu_4NF{\cdot}3H_2O$ (7.2 mg, 2.3×10^{-2} mmol, 2.5 equiv) at 0 °C. The mixture was stirred for 5 min at 0 °C and for 1 h at room temperature, evaporated, and separated by PTLC silica gel (eluted with 15% MeOH–85%, CH_2Cl_2) to give **37** in 85.7% (3.0 mg): ^{1}H NMR (270 MHz) ($CDCl_3$) δ 2.1 (1 H, s), 2.61 (2 H, m), 3.84–4.24 (4 H, m), 4.34 (1 H, s), 5.21 (1 H, s), 5.66 (1 H, s), 6.85 (1 H, s), 7.37 (2 H, d, J = 9.2 Hz), 8.28 (2 H, d, J = 9.2 Hz); IR (KBr, disk) 3500–3350, 3300–3200, 1680, 1660, 1520, 1345 cm^{-1}; mass spectrum, m/e (EI, %) 331 (0.4), 318 (0.5), 306 (0.6), 303 (0.6), 279 (3), 199 (4), 167 (12), 149 (34), 123 (10), 86 (29), 85 (21), 71 (58), 70 (25), 57 (95), 28 (100).

To a stirred solution of **37** (1.5 mg, 4.3×10^{-3} mmol, 1.0 equiv) in 0.5 mL of THF–H_2O (3/1) at pH 12.5 was added a NaSMe solution (1.1×10^{-2} mol, 2.5 equiv) at room temperature. The mixture was stirred for 2 h at room temperature. The TLC showed that the addition of NaSMe to **37** did not occur. The mixture was evaporated and separated by PTLC to give the unchanged starting material **37** (1.0 mg).

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