a yellow solid. Recrystallization from THF gave yellow needles: mp 250 °C dec; IR (KBr) 1660, 1615, 1296 cm^{-1}; ^1H NMR (DMSO-d_6) 3.47 (CH_2, s, 2 H), 6.70 (CH=C—, s, 2 H); ^{13}C NMR (DMSO-d_6) 28.9, 133.5, 146.06, 185.1, 186.8. Anal. Calcd for $C_{28}H_{16}O_8$: C, 70.00; H, 3.36. Found: C, 69.91; H, 3.49.

From 8. The calixarene 8 (1.0 g, 1.36 mmol) was oxidized by a similar method to that described above to yield 0.37 g (57%) of 7.

From 6. The calixarene 6 was oxidized by a similar method to that described above to give 7 in an 84% yield.

Calix[4]hydroquinone (6). 7 (0.6 g, 1.25 mmol) was dispersed in 250 mL of $CHCl_3$ at 65–70 °C. To the suspension was added a solution of 3.5 g of hydrosulfite in 30 mL of water. The mixture was refluxed vigorously for 2 h at 80–90 °C. After the reaction mixture had been cooled, it was allowed to stand overnight at rt to give a white crude solid. It was recrystallized from MeOH–H_2O, which contained a small amount of $SnCl_2$ and HCl, to yield 0.57 g (93%) of 6 as white plates: mp 400–450 °C dec; IR (KBr) 3200, 1620, 1470 cm^{-1}; ^1H NMR (acetone-d_6) 3.74 (CH_2, bs, 2 H), 6.60 (Ar, s, 2 H), 7.82 (OH, s, 1 H), 9.79 (OH, s, 1 H); ^{13}C NMR (acetone-d_6) 32.7, 116.7, 130.6, 143.2, 152.9; MS m/e 489 (M + H). Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.85; H, 4.95. Found: C, 68.81; H, 4.94.

Acknowledgment. We thank Mr. Y. Akima, Mr. T. Ohyada, and Mr. S. Ohta for carring out part of the experiments. We are grateful to Dr. Y. Kai of Osaka University for providing the X-ray crystal structure analysis of compound 7 and to Dr. M. Komatsu of Osaka University for providing part of NMR and IR data. We also wish to thank Mr. S. Horiguchi, vice-president of Dainichiseika Color & Chemicals Mfg Co., Ltd., for helpful suggestions on diazo coupling reaction and his support during the course of this work.

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Comparative Studies on the Reactivity of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione, 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione, and Bicyclomycin. Examination of a Key Structural Element Necessary for Bicyclomycin-Mediated Transformations

Yeong Soo Oh and Harold Kohn*

Department of Chemistry, University of Houston, Houston, Texas 77204-5641

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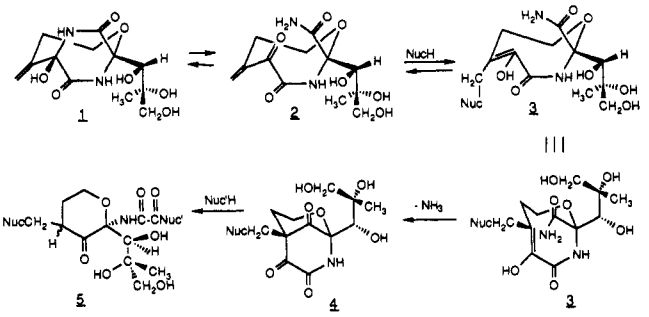
Two select mimics, 4-methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) and 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) of the structurally novel antibiotic, bicyclomycin (1), have been prepared. Comparison of the chemical reactivity of 7 versus 1 both in the presence and absence of added nucleophiles at various "pH" values has provided important new information concerning the role of key structural elements present in bicyclomycin. The product profiles determined for 7 indicated that modification of the terminal double bond proceeded through an α,β-unsaturated ring imine intermediate (i.e., **43**). Correspondingly, activation of the *exo*-methylene group in bicyclomycin is believed to occur through initial hemiaminal bond scission to give a ring-opened α,β-unsaturated carbonyl species (i.e., 2). Functionalization of the terminal double bond in 7 has been shown to proceed under milder conditions than that required for 1. These results demonstrated that incorporation of the *exo*-methylene group within the O(2)–C(3)–C(4)–C(5) bridge in 1 required that the terminal double bond activation pathway proceed by an alternative, energetically more-demanding pathway than that observed for 7. Ramifications of the decreased reactivity noted for 1 are to allow other functional groups (i.e., the C(1)-triol moiety) in the antibiotic to have important catalytic roles in the drug modification processes and to permit thiolate species (the proposed biological targets?) to effectively compete with other nucleophiles for 2.

Bicyclomycin (1) is a structurally unique antibiotic bearing no resemblance to any other known class of antibiotics.[1-3] The structure and relative configuration of 1 was determined by Tokuma and co-workers by single-crystal X-ray analysis.[4,5] Subsequently, Maag and his group established the absolute configuration through synthesis and X-ray analysis of a bicyclomycin acid-cata-

Scheme I. Proposed Pathway for the Mode of Action of Bicyclomycin (1)



(1) For an excellent review, see: Williams, R. M.; Durham, C. A. *Chem. Rev.* **1988**, *88*, 511.

(2) (a) Miyoshi, T.; Miyairi, 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.; Goto, S.; Kuwahara, S. *Ibid.* **1972**, *25*, 594.

(3) (a) Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K.; 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.

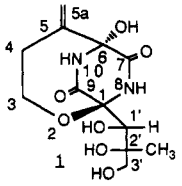
(4) Tokuma, Y.; Koda, S.; Miyoshi, T.; Morimoto, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 18.

0022-3263/92/1957-3662$03.00/0

lyzed dehydrative rearrangement product.[6]

Most proposals have suggested that bicyclomycin reacts with nucleophiles necessary for the remodeling of the peptidoglycan assembly within the bacterial cell wall and that drug binding occurs at the *exo*-methylene group in 1.[7-14] This hypothesis stems from the pioneering studies of Iseki and co-workers who demonstrated that bicyclomycin reacts with NaSMe at the terminal double bond under basic conditions (pH 12.5).[7a] Recently, the structural assignment for this methanethiolate adduct has been revised.[15] Additional information concerning the *exo*-methylene modification process was provided by the Williams group through the use of bicyclomycin model compounds.[10] These investigators have concluded that the minimum structural requirements needed in 1 for NaSMe binding under basic conditions included the presence of the following: (1) a free (N–H) amide at N(10); (2) an *exo*-methylene moiety at C(5); (3) a bridgehead hydroxyl at C(6); and (4) a C(1′) hydroxyl moiety. In a recent series of papers[12-15] focusing on the reactivity of bicyclomycin with thiols and amines under near neutral conditions, Kohn and Abuzar have advanced a novel mechanism for the activation of 1 (Scheme I). In this scenario, drug activation occurred by initial ring cleavage of the hemiaminal bond producing 2. Subsequent nucleophilic addition to the α,β-unsaturated carbonyl system generated enol 3, which undergoes an intramolecular mixed-Claisen condensation to yield 4 and NH_3. Incorporated in this hypothesis is the speculation that 4 may serve as an efficient trap for additional nucleophiles present at the drug binding site to give the disubstituted adduct 5.

These combined studies have provided clues on the mode of action of bicyclomycin. An important question that still remains unanswered is what is the biological significance for the placement of the *exo*-methylene moiety within the unique bicyclic ring system present in 1? To provide information concerning this inquiry, we have attempted to compare the reactivity of two bicyclomycin mimics, 3-hydroxy-3-(3-hydroxy-1-methylenepropyl)-2,5-piperazinedione (6) and 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7), with bicyclomycin. In 6 and 7 the



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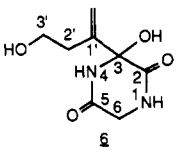

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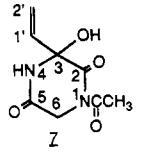
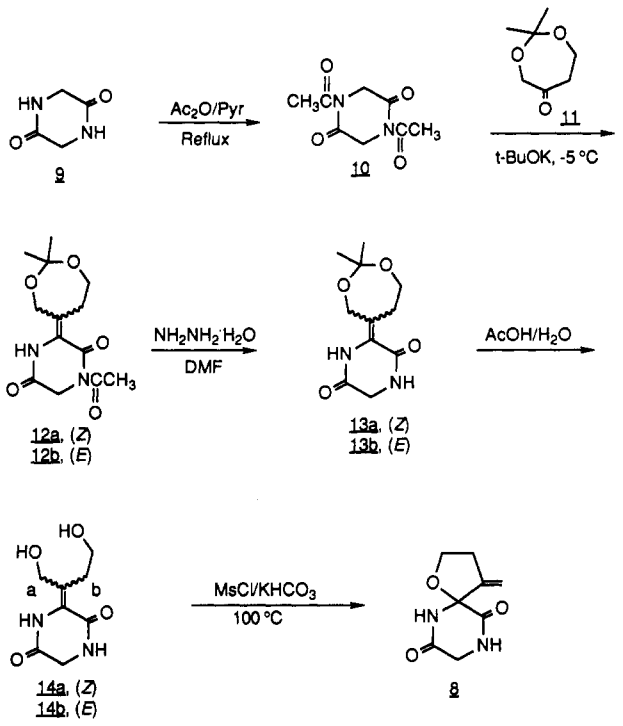


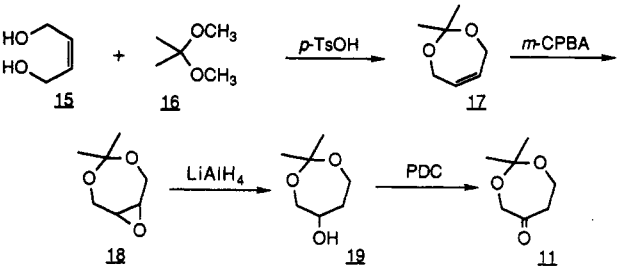
7

terminal double bond is not constrained within a bicyclic ring system. Our results indicated that alternative pathways for double-bond activation existed in these systems that are not accessible to the *exo*-methylene group in the antibiotic. The potential significance of this finding in relationship to the mode of action of the drug is discussed.

Scheme II. Synthetic Sequence for the Preparation of Model Compound 8

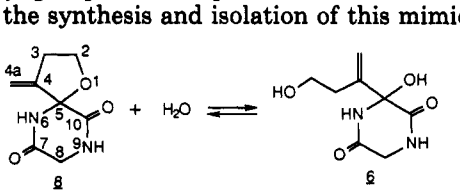


Scheme III. Synthetic Route for the Preparation of Compound 11



Results and Discussion

Selection of Compounds. 3-Hydroxy-3-(3-hydroxy-1-methylenepropyl)-2,5-piperazinedione (6) and 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) were chosen to probe the importance of the bridging unit in bicyclomycin-mediated transformations. In both model compounds this structural constraint was deleted. We had anticipated that 6 would be accessible under select hydrolytic conditions through the known 4-methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione[6] (8). In 7, the *N*(1)-acetyl group was incorporated within the molecule to aid in the synthesis and isolation of this mimic.



8 6

Synthesis of Compounds. The protected bicyclomycin model compound 8 was prepared according to the procedure of Maag and co-workers[6] with slight modification (Scheme II). Treatment of 2,5-piperazinedione (9) with Ac_2O in the presence of pyridine gave *N*,*N*′-diacetyl-2,5-piperazinedione[16] (10). Compound 10 was condensed with

(5) For a related study, see: Kohn, H.; Abuzar, S.; Korp, J. D.; Zektzer, A. S.; Martin, G. E. *J. Heterocycl. Chem.* **1988**, *25*, 1511.
(6) Maag, H.; Blount, J.; Coffen, D.; Steppe, T.; Wong, F. *J. Am. Chem. Soc.* **1978**, *100*, 6786.
(7) (a) Someya, A.; Iseki, M.; Tanaka, N. *J. Antibiot.* **1979**, *32*, 402. (b) Tanaka, N.; Iseki, M.; Miyoshi, T.; Aoki, H.; Imanaka, H. *Ibid.* **1976**, *29*, 155. (c) Someya, A.; Iseki, M.; Tanaka, N. *Ibid.* **1978**, *31*, 712.
(8) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733.
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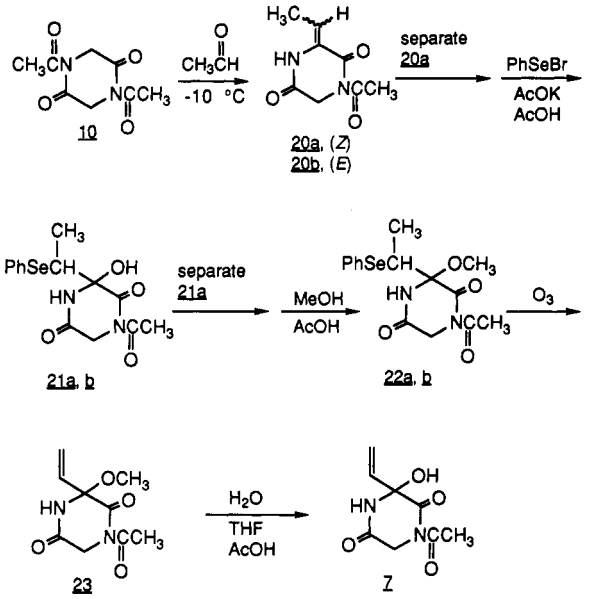
(10) (a) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 6419. (b) Williams, R. M.; Tomizawa, K.; Armstrong, R. W.; Dung, J.-S. *Ibid.* **1987**, *109*, 4028.
(11) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1988**, *110*, 4089.
(12) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1989**, *111*, 4895.
(13) Abuzar, S.; Kohn, H. *J. Org. Chem.* **1989**, *54*, 4000.
(14) Abuzar, S.; Kohn, H. *J. Am. Chem. Soc.* **1990**, *112*, 3114.
(15) Kohn, H.; Abuzar, S. *J. Am. Chem. Soc.* **1988**, *110*, 3661.

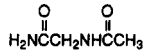
Scheme IV. Synthetic Sequence for the Preparation of Model Compound 7



4,4-dimethyl-3,5-dioxacycloheptanone[6] (**11**) (tBuOK, DMF, 0 °C) to give **12a** and **12b** as a 3:2 mixture of the (*Z*)- and (*E*)-stereoisomers, respectively. Hydrazinolysis of **12a** and **b** followed by treatment with aqueous acetic acid yielded a 1:1 mixture of diols **14a** and **14b**. Treatment of the binary mixture of **14a** and **14b** with mesyl chloride in the presence of $KHCO_3$ (100 °C) furnished the desired 4-methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (**8**) (11% overall yield from **9**). Ketone **11** was prepared in four steps from *cis*-1,4-dihydroxy-2-butene (**15**) (Scheme III, 22% overall yield). Treatment of **15** with 2,2-dimethoxypropane (**16**) in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate gave **17**.[17] Epoxidation of **17** with *m*-CPBA followed by reduction with $LiAlH_4$ produced alcohol **19**, which was oxidized with pyridinium dichromate (PDC) in the presence of catalytic amounts of pyridinium trifluoroacetate to yield **11**.

The procedure employed for the synthesis of the second bicyclomycin mimic, 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (**7**), is outlined in Scheme IV beginning with 1,4-diacetyl-2,5-piperazinedione (**10**). Addition of tBuOK in tBuOH to a DMF solution containing **10** and acetaldehyde gave a 7:1 isomeric mixture of (*Z*)-**20a** and (*E*)-**20b**, 1-acetyl-3-ethylidene-2,5-piperazinedione,[18] respectively (67% yield). Noteworthy, reduction of the temperature for this transformation from the previously suggested value of 0 °C[19,20] to −10 °C led to an improvement in the yield (37–67%) and the isolation of the minor isomeric product **20b**. Treatment of **20a**[21] with PhSeBr and KOAc[22] furnished after aqueous workup **21a** and **21b** (53% yield) as a 2.8:1 diastereomeric mixture.[23] Dissolution of the major product **21a** in methanolic AcOH generated **22a** (3%) and **22b** (39%) along with recovered starting material (47%). Introduction of the terminal double bond was accomplished by ozonolysis of **22a** and **22b** to give **23** (88% yield), which was then converted in situ to the target compound **7** by dissolution in an H_2O–AcOH–THF solution ("pH" 2.0–4.0).[24]

Attempts to isolate purified samples of **7** proved difficult. The C-3 methoxy to C-3 hydroxy interchange process (**23** → **7**) was readily monitored by ^{13}C NMR spectroscopy in a D_2O–DCl–THF-d_8 solution. Formation of **7** was accompanied by the disappearance of signals at 51.46 (C(3)-OCH_3) and 87.19 (C(3)) ppm for **23** and the appearance of new peaks at 49.26 and 82.21 ppm for CH_3OD and the C(3) signal in **7**, respectively. Correspondingly, use of a D_2O–CD_3CO_2D–THF-d_8 ("pD" 2.0) solution containing **23** led to the efficient production (rt, 16 h) of **7** (^1H and ^{13}C NMR spectroscopic analyses). High-resolution FAB mass spectral analysis of the product mixture prior to workup confirmed the formation of **7** as the monodeuterio adduct. Neutralization ("pH" 6.9) of half of the reaction mixture followed by removal of the solvent (35 °C) in vacuo led to the production of N_α-acetylglycinamide[25] (**24**). Azeotropic



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removal of the solvent from the remaining half of the reaction by the successive addition of H_2O gave a crude sample (~70% pure, ^1H and ^{13}C NMR analyses) of **7** as an oil which resisted further purification.

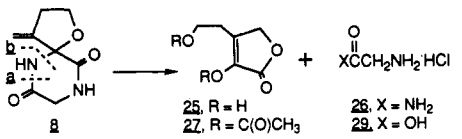
Comparative Studies on the Reactivity of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) and Bicyclomycin (1). A tacit assumption in the selection of 4-methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (**8**) as a bicyclomycin model compound was that **8** would exist in equilibrium with 3-hydroxy-3-(3-hydroxy-1-methylenepropyl)-2,5-piperazinedione (**6**) under select conditions. Information concerning this notion and the reactivity of the *exo*-methylene group in **8** was achieved by examining the reactivity of **8** and **1** in H_2O and alcoholic solutions under neutral, acidic, and basic conditions.

Treatment of **8** with EtSH ("pH" 8.0), benzyl mercaptan ("pH" 8.1), and imidazole ("pH" 9.0–11.0) at room temperature in THF–H_2O (3:1) mixtures (48–96 h) led to the recovery of starting material in all cases.[26] No evidence for product formation was detected by TLC analyses. This result differed markedly from **1** in which modification of the *exo*-methylene group by the added nucleophile was observed to occur under comparable conditions.[11-14]

Acid hydrolysis of **8** (0.1 N HCl, 100 °C, 90 min; 1 N H_2SO_4, THF–H_2O (3:1), 80 °C, 4 h) gave after workup 3-hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone[27] (**25**) and glycinamide hydrochloride[25] (**26**). Verification of the formation of **25** was accomplished by conversion of this adduct to the diacetate **27**[27] with Ac_2O and pyridine. Addition of benzyl mercaptan to an acidic ("pH" 1.0)

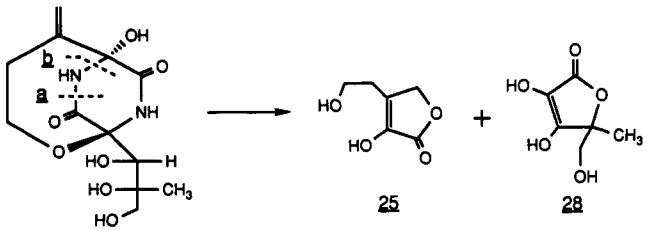
(16) Abderhalden, E.; Komm, E. *Z. Physiol. Chem.* **1924**, *139*, 181; *Chem. Abstr.* **1925**, 995[2].
(17) Elliott, W.; Fried, J. *J. Org. Chem.* **1976**, *41*, 2469.
(18) Distinctive patterns existed in the ^1H NMR spectra for **20a** and **20b** which allowed their differentiation and identification. Within this pair of compounds a significant deshielding effect (0.24–0.30 ppm) was noted for the methine and methyl protons in closest proximity to the carbonyl group. This pattern has been attributed to the anisotropic effect provided by the C(2)-carbonyl group. Similar trends were noted for comparable pairs of geometric isomers prepared in this study.
(19) Gallina, C.; Liberatori, A. *Tetrahedron Lett.* **1973**, *14*, 1135.
(20) Shin, C.; Sato, K.; Ohtsuka, A.; Kazutoshi, M.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3876.
(21) Although separation of the isomers is not necessary for the overall preparation of **7** this step was introduced to facilitate the separation of subsequent product mixtures and their spectral identification.
(22) Sharpless, K. B.; Young, M. W. *J. Org. Chem.* **1975**, *40*, 947.
(23) Treatment of **20a** with either PhSeCl or PhSeBr in the presence of silver salts (silver trifluoroacetate or silver acetate) gave (*Z*)- and (*E*)-1-acetyl-3-(1-(phenylselenenyl)ethylidene)-2,5-piperazinedione (39–42% yield).
(24) Direct ozonolysis of **21a** led to the production of an intimate mixture containing **7** which we were not able to further purify.
(25) The reaction product was compared to an authentic sample purchased from Aldrich Chemical Co.
(26) No detectable reactions (NMR analysis) of aqueous solutions of **8** were noted for aqueous solutions maintained at pD 2.3–4.4 (rt, 24 h; 100 °C, 90 min) and at pD 1.0 (rt, 24 h; 50 °C, 12 h).
(27) Kohn, H.; Abuzar, S. *J. Org. Chem.* **1988**, *53*, 2769.

THF–H_2O mixture containing **8** led only to the formation of **25** and **26**. No evidence was obtained for the formation of thiolate-substituted products (^1H NMR and TLC analyses).



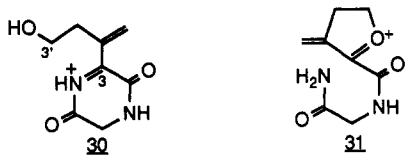
8 **25**, R = H; **27**, R = C(O)CH_3 **26**, X = NH_2; **29**, X = OH

Compound **25** has been isolated along with **28** in the acid hydrolysis (0.1–1.0 N H_2SO_4, 100 °C, 90 min) of bicyclomycin.[27] The overall pathway for the conversion of **1** to **25** and **28** was not ascertained in this previous study. Both amide (i.e., route a) and hemiaminal (i.e., route b) bond cleavage pathways were consistent with the reaction products.[27] Our observation that **26** is produced together with **25** from **8** supports the notion that in both compounds acid hydrolysis of the piperazinedione ring proceeded by an overall hemiaminal bond scission (route b), since cleavage of **8** by a pathway similar to route a in **1** would have yielded glycine hydrochloride (**29**) in place of glycinamide hydrochloride (**26**).

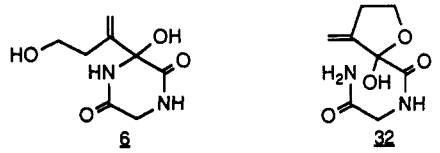


1 **25** **28**

Several pathways are consistent with the hemiaminal bond cleavage process (route b) noted for **8**. These differ principally in whether initial C(5)–O(1) or C(5)–N(6) bond scission in **8** occurs to generate **30** and **31**, respectively, and whether subsequent reaction with H_2O preferentially proceeds at the terminal end of the conjugated ions **30** and **31** to give the *exo*-methylene-functionalized alcohol or at C(3) in **30** and the corresponding carbon in **31** to give hemiaminal **6** and hemiketal **32**, respectively.

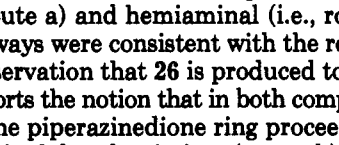


30 **31**



6 **32**

Both **6** and **32** would be expected to furnish the reactive α,β-unsaturated carbonyl intermediate **33** capable of undergoing conjugate addition by H_2O. Suggestive information that the reaction proceeded by initial C–O bond scission (i.e., **30**) was derived from the study of Maag and co-workers.[6] Treatment of **1** in aqueous H_2SO_4 gave **34a** and **34b**.[6] Similarly, dissolution of **1** in 1 N propanolic H_2SO_4 (98 °C, 90 min) led to the same diastereomeric mixture. Correspondingly, attempted methanolysis (1 N methanolic H_2SO_4, 65 °C, 90 min) or propanolysis (1 N propanolic H_2SO_4, 98 °C, 90 min) of **8** led to the recovery of starting spirofuran in both instances.



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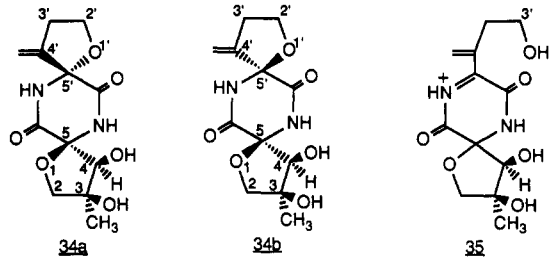
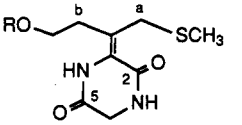


34a **34b** **35**

These cumulative results provided evidence that C–O bond scission in **1** and **8** is promoted by acid.[28] The similar product profiles obtained for **1** and **8** in acidic water and alcoholic solutions suggested that under these conditions both reactions proceeded through a ring-opened intermediate (i.e., **35**, **30**). The formation of a terminal double bond functionalized adduct (i.e., **25**) in water but not in alcoholic solvent systems from **1** and **8** has been attributed in part to the inability of methanol or propanol to effectively compete with the corresponding internal C(3′) primary alcohol group in **35** and **30** for the α,β-unsaturated iminium ion system.[29] Unfortunately, the absence of methanolic and propanolic products from **8** did not permit us to speculate on whether the *exo*-methylene functionalization process observed for this compound in strong aqueous acid proceeded by the direct addition of H_2O to **30** or through ring-opened intermediate **33**.

Our study on the chemical reactivity of 4-methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (**8**) versus bicyclomycin concluded with the treatment of **8** with NaSMe in base (3:1 THF–H_2O, "pH" 12.5, rt, 2 h). Under these conditions a single product **36** was isolated (69% yield), which has been tentatively assigned as the (*E*)-stereoisomer on the basis of the observed ^1H NMR data. In both **14b** (Scheme II) and **36** the C(b) methylene protons appeared upfield (0.18–0.24 ppm) from the corresponding protons in **14a**, suggesting that the C(b) methylene protons were trans to the C(2) ring carbonyl group. Acetylation of **36** furnished 3-(3-acetoxy-1-((methylthio)methyl)-propylidene)-2,5-piperazinedione (**37**).



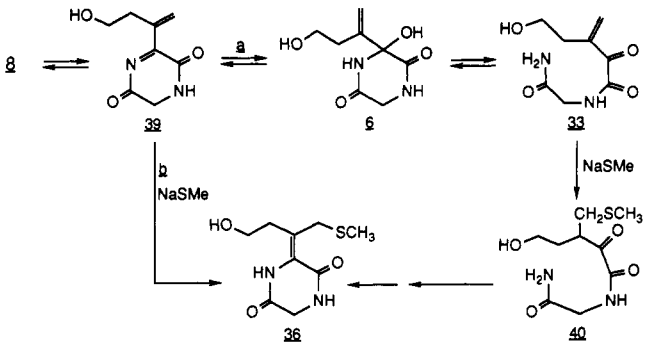
36, R = H
37, R = C(O)CH_3

The facility of the NaSMe-mediated reaction with **8** was reminiscent of the reactivity of bicyclomycin with this nucleophile under identical conditions.[7a,15] In these reactions, functionalization of the *exo*-methylene group proceeded along with cleavage of the piperazinedione ring to generate the diastereomeric C-5a-substituted product **38**. Several mechanisms for the conversion of **8** to **36** can be envisioned. Two of these are presented in Scheme V and proceed through **39**. In one route, hydration of the imine bond in **39** gives **6** which can undergo ring opening

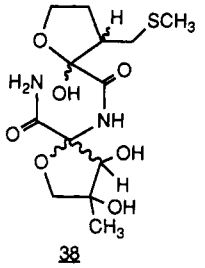
(28) A similar process is likely to have occurred in the production of **7** from **23**.

(29) For the relative nucleophilicities of H_2O versus alcohols, see: (a) Schadt, F. L.; Bentley, T. W.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1976**, *98*, 7667. (b) Bentley, T. W.; Schadt, F. L.; Schleyer, P. v. R. *Ibid.* **1972**, *94*, 993.

Scheme V. Proposed Pathway for the NaSMe-Mediated Modification of Compound 8 in Base



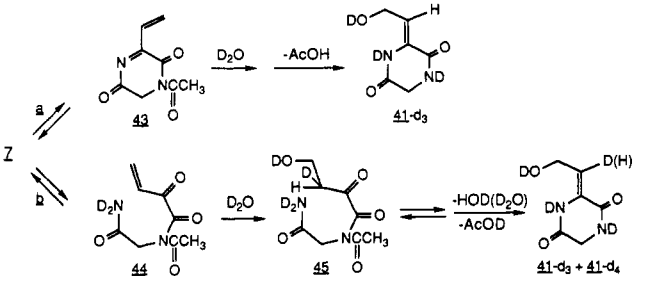
to give the α,β-unsaturated enone **33**. Subsequent thiolate addition followed by ring closure affords **36**. In the second pathway depicted, thiolate addition to the α,β-unsaturated system in **39** generates **36** without the intervention of **6** and **33**. Information related to this transformation was provided by the treatment of both **8** and **1** with NaSMe in methanolic 0.03 N NaOMe ("pH" 11.9). With **8** no reaction products were detected (rt, 24 h), while **1** furnished the diastereomeric C-5a-substituted products[15] **38a** and **38b** in 66% yield (rt, 2 h). These results suggested that under aqueous basic conditions conversion of **8** to **33** via **6** may have occurred prior to the addition of NaSMe to the *exo*-methylene group. Alternatively, decreased amounts of **39** may have been generated in the basic methanol solutions versus the corresponding aqueous solutions, thereby diminishing the likelihood of the conjugate addition of NaSMe to the α,β-imine system.[30] Attempts to gain additional information concerning these mechanistic notions by examining the reactivity of the bis-spiro bicyclomycin adducts **34a** and **34b**[6] under comparable conditions were unsuccessful. Treatment of either a 1:1 THF–H_2O mixture ("pH" 12.4, rt, 24 h) or a methanolic 0.03 N NaOMe ("pH" 11.8, rt, 24 h) solution containing **34a,b** with NaSMe (2 equiv) led to the generation of a complex mixture in both cases. Careful preparative TLC of the aqueous THF reaction permitted the isolation of the major component in the product mixture. ^1H and ^{13}C NMR analysis indicated that this compound corresponded to one of the two starting diastereomers **34a** or **34b**, suggesting that these isomers displayed differential reactivities under basic conditions.



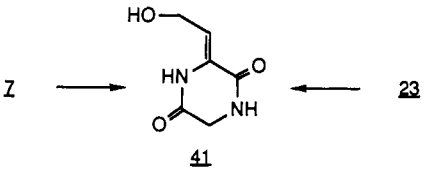
The lack of reactivity of **8** under moderate reaction conditions prevented us from addressing the chemical ramifications of incorporating the *exo*-methylene group in the constrained O(2)–C(5) bridge in bicyclomycin. Pertinent information concerning this issue was provided by the second model compound **7**.

Comparative Studies on the Reactivity of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) and Bicyclomycin (1). The inability to modify the *exo*-methylene group in **8** under moderate conditions has been attributed in part to the ease of spiro-ring formation from the corresponding α,β-unsaturated iminium ion (i.e., **30**) or imine (i.e., **39**) system. In the simplified model compound **7**, spiro-ring formation is not possible prior to conjugate addition to the terminal double bond. Furthermore, incorporation of the C(3)-hydroxy group in **7** ensured that the reaction proceeded through a hemiaminal species. These modifications permitted us to compare the reactivity of this compound with bicyclomycin at intermediate "pH" values as well as in acid and base.

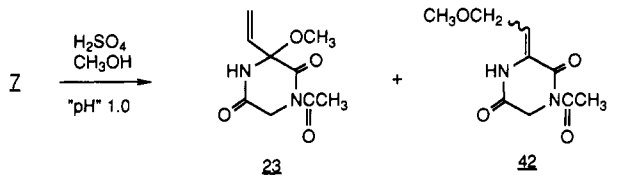
Scheme VI. Potential Mechanisms for the Conversion of Compound 7 to Compound 41 in D_2O Solutions



Compound **7** was generated from **23** in aqueous acid ("pH" 2–4). At these "pH" values **7** was stable for extended periods of time (>2 d) at room temperature. Reduction of the "pD" of the solution to 1.0 led to the formation of 3-(2-hydroxyethylidene)-2,5-piperazinedione (**41**). A similar result was observed for **23** at this "pD" value. Both transformations took approximately 16 h to complete at room temperature and proceeded with the loss of the N(1)-acetyl group.



A parallel reaction for **7** was performed in MeOH. Treatment of **7** with methanolic H_2SO_4 ("pH" 1.0, rt, 10 h) gave **23** as the major product (53% yield). A minor adduct (2% yield) was also isolated which has been tentatively assigned as the (*E*)- and (*Z*)-isomers of **42** (^1H NMR analysis).[31]



Two likely pathways for the conversion of **7** to **41** in acid are presented in Scheme VI. These routes differ in whether a ring-cleavage process occurred. Information in support of pathway a was derived from the ^1H NMR spectrum of **41** obtained from the acid hydrolysis experiment conducted in D_2O (pD 1.0), which demonstrated that no deuterium incorporation had occurred at the olefinic position (δ 5.71). This observation was reinforced by the high-resolution mass spectrum of this adduct, which displayed a prominent peak for the nondeuterated molecular ion after workup. The absence of deuterium in **41** eliminated route b in Scheme VI. This pathway required that

(30) For an example of the addition of thiols to α,β-unsaturated imines, see: Buckwell, S.; Page, M. I.; Longridge, J. L. *J. Chem. Soc., Chem. Commun.* **1986**, 1039.

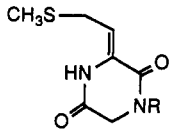
(31) Attempts to confirm the identity of **42** by preparing an authentic sample from 3-methoxypropionaldehyde and **10** with tBuOK/tBuOH were unsuccessful.

a substantial amount of deuterium be incorporated at the vinylic position.[32]

Elevation of the "pD" from the conditions employed for the preparation of 7 led to the gradual activation of the terminal double bond in the model compound. Addition of 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) to an aqueous "pD" 6.0 solution (3 h) furnished 41. Correspondingly, dissolution of 7 in a "pD" 8.8 solution (30 min) gave a mixture of 24 (45%) and 41 (13%), respectively. ^{1}H NMR analyses of 41 isolated from both of these reactions indicated that no deuterium incorporation had occurred at the olefinic site suggesting that a mechanism comparable to Scheme VI route a was operative in these transformations as well. The formation of significant amounts of N_α-acetylglycinamide (24) at "pD" 8.8 may signal that competing reaction processes are occurring at higher "pD" values.

The lability of 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) under moderate conditions contrasted to that observed for bicyclomycin (1). Dissolution of 1 at "pD" 5.7–10.2 in the absence of external nucleophiles led to little drug modification (24 h) (NMR analysis). At "pD" 5.7 in a buffered D_2O–THF-d_8 (2:1) solution no change in 1 was noted, while at "pD" 10.2 approximately 10% of bicyclomycin had been converted to an unidentified adduct(s).

Efforts to add a sulfur nucleophile to 7 under near-neutral pH conditions proved unsuccessful. Addition of NaSMe to a 3:1 THF–D_2O solution ("pD" 7.0) containing 7 gave only 41 after workup. No evidence was obtained for 3-(2-(methylthio)ethylidene)-2,5-piperazinedione (46) or the corresponding *N*-acetyl derivative 47. We suspect under these conditions the methanethiol was insufficiently ionized[33] to effectively compete with the bulk solvent for the α,β-unsaturated imine system in 43.[34]



46, R = H
47, R = C(O)CH$_3$

Conclusions

The enhanced reactivity of the terminal double bond in 1-acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) versus bicyclomycin (1) toward nucleophilic attack at moderate "pH" values has been attributed to the differential pathways for activation that exist for these compounds. In 7 nucleophilic addition is promoted by ring imine formation, which permits the terminal double bond to be in conjugation with the endocyclic imine bond (Scheme VI, route a). A similar process for 1, however, is not likely. In bicyclomycin generation of a bridgehead imine bond would be accompanied by a significant amount of ring strain.[35] Moreover, this process would not permit the imine bond to be in conjugation with the *exo*-methylene group due to the structural constaints imposed by the [4.2.2] bicyclic ring system. Accordingly, activation of the terminal double bond in bicyclomycin must proceed by an alternative, energetically more-demanding pathway than that accessible to 7. Previous studies have demonstrated that this process occurs by initial hemiaminal bond cleavage to generate the reactive ring-opened α,β-unsaturated carbonyl species 2.[11–15] Ramifications of this decreased reactivity for 1 are as follows: (1) to permit other functional groups (i.e., C(1)-triol moiety) present in the antibiotic to play important catalytic roles in the drug activation and binding processes[10–14] and (2) to necessitate that the basicity of the medium be sufficiently high to activate the *exo*-methylene group in 1. Elevation of the "pH" in this manner should permit key protein thiolate species (i.e., the biological target?) to effectively compete with nucleophiles present in the biological milieu for the presumed reactive α,β-unsaturated carbonyl intermediate 2.

Experimental Section

General Methods. The experimental procedures used in this study were identical to those employed in previous investigations.[11–15,27] Generous supplies of bicyclomycin were obtained from Fujisawa Pharamceutical Co., Ltd., Japan. *cis*-1,4-Dihydroxy-2-butene (98%) was purchased from American Tokyo Kasei, Portland, OR. THF and Et_2O were distilled from Na^0 and benzophenone, and CH_2Cl_2 was distilled from P_2O_5. DMF was fractionally distilled from $CaSO_4$ under reduced pressure. Pyridine was dried by refluxing with KOH pellets. The pD of the solution was obtained by using the following relationship: pD = pH meter reading + 0.4.[36]

Preparation of 4,4-Dimethyl-3,5-dioxacycloheptanol (19). To a suspension of $LiAlH_4$ (10.00 g, 238 mmol) in anhydrous Et_2O (500 mL) was added epoxide 18[17] (24.00 g, 164 mmol) in anhydrous Et_2O (200 mL) dropwise (3 h) at 0 °C. The mixture was allowed to warm to rt and stirred (24 h). The excess $LiAlH_4$ was destroyed by the successive addition of H_2O (10 mL), an aqueous 15% NaOH solution (10 mL), and H_2O (30 mL).[37] The resulting mixture was filtered through a Celite bed, and the filtrate was washed with an aqueous saturated NaCl solution (200 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography (50% ethyl acetate–hexane) to give 19 as a liquid: yield 16.70 g (68%); R_f 0.35 (50% ethyl acetate–hexane); IR (neat) 3380, 1385, 1375, 1215, 1155, 1100, 1080, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23, 1.25 (2 s, 6 H, $C(CH_3)_2$), 1.62–1.73 (m, 1 H, OCH_2CHH'), 1.73–1.86 (m, 1 H, OCH_2CHH'), 3.42 (br s, 1 H, OH), 3.54–3.84 (m, 5 H, CH, OCH_2CH, OCH_2CH_2); ^{13}C NMR ($CDCl_3$) 24.40 ($C(CH_3)_2$), 37.50 (OCH_2CH_2), 56.84 (OCH_2CH_2), 64.98 (OCH_2CH), 67.77 (CH), 100.95 ($C(CH_3)_2$) ppm; the ^{13}C NMR assignments were confirmed by the corresponding APT experiment; MS *m/e* (rel intensity) 146 (3), 131 (52), 115 (11), 101 (21), 75 (100).

Anal. Calcd for $C_7H_{14}O_3$: C, 57.51; H, 9.65. Found: C, 57.39; H, 9.45.

Preparation of 4,4-Dimethyl-3,5-dioxacycloheptanone (11).[6] To a CH_2Cl_2 solution (200 mL) of 19 (16.00 g, 110 mmol) were added pyridinium dichromate (84.00 g, 223 mmol) and pyridinium trifluoroacetate (8.47 g, 44 mmol), and then the suspension was mechanically stirred at rt (24 h). The solid was filtered through a silica gel bed, and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (35% ethyl acetate–hexane) to give 11 as a low-boiling liquid: yield[38] 7.38 g (47%); R_f 0.78 (50% ethyl acetate–hexane); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 6 H, $C(CH_3)_2$), 2.81 (t, J = 7.0 Hz, 2 H, OCH_2CH_2), 3.94 (t, J = 7.0 Hz, 2 H, OCH_2CH_2), 4.00 (s, 2 H, $OCH_2C{=}O$); ^{13}C NMR ($CDCl_3$) 24.84 ($C(CH_3)_2$), 41.63 (OCH_2CH_2), 56.54 (OCH_2CH_2), 68.49 ($OCH_2C{=}O$), 102.11 ($C(CH_3)_2$), 210.99 ($C{=}O$) ppm.

(32) The possibility that the deuterium incorporated at the vinylic site in 41 was lost by an enamine H/D exchange process during the chromatographic workup with 20% MeOH–$CHCl_3$ has been ruled out. Repeated PTLC separation of **20a** with 20% MeOD–$CHCl_3$ led to no detectable deuterium incorporation at the olefinic site.

(33) Crampton, M. R. In *The Chemistry of the Thiol Group*; Patai, S., Ed.; Wiley: London, 1974; pp 379–415.

(34) In the thiol addition studies reported by Page and co-workers,[30] higher thiol to imine ratios were utilized. Moreover, this study was conducted in water.

(35) Maier, W. F.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 1891 and references cited therein.

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

(37) Micovic, V. M.; Mihailovic, M. L. J. *J. Org. Chem.* **1953**, *18*, 1190.

(38) The volatility of the product did not permit the complete removal of EtOAc. The yield was estimated by ^{1}H NMR spectroscopy.

Preparation of 1-Acetyl-3-(4,4-dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (12a and 12b).[6] To a solution of **10**[16] (9.63 g, 48.6 mmol) and **11** (7.00 g, 48.6 mmol) in dry DMF (100 mL) was added a solution of tBuOK (5.46 g, 48.6 mmol) in tBuOH (100 mL) dropwise (1 h) at −10 °C. The temperature was kept at −10 °C (2 h), and then the solution was warmed to rt and stirred (18 h). The solution was poured into H_2O, neutralized with AcOH, and extracted with $CHCl_3$ (3 × 200 mL). The organic layers were combined, washed with an aqueous saturated NaCl solution (3 × 200 mL), dried, and concentrated in vacuo. The products were purified by flash chromatography (50% ethyl acetate–hexane) to give a mixture of **12a** and **12b**: yield 7.54 g (55%). The binary mixture (300 mg) was separated by PTLC (50% ethyl acetate–hexane) to give pure **12a** and **12b**.

(*Z*)-1-Acetyl-3-(4,4-dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (12a): yield 152 mg (51%); R_f 0.47 (50% ethyl acetate–hexane); IR (KBr) 1715, 1694, 1633, 1360, 1224, 1197, 1076 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 6 H, $C(CH_3)_2$), 2.58 (s, 3 H, $CH_3C{=}O$), 3.24 (t, J = 6.3 Hz, 2 H, OCH_2CH_2), 3.86 (t, J = 6.3 Hz, 2 H, OCH_2CH_2), 4.40 (s, 2 H, CH_2N), 4.41 (s, 2 H, $OCH_2C{=}C$), 9.19 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 24.31 ($C(CH_3)_2$), 26.98 ($CH_3C{=}O$), 29.44 (OCH_2CH_2), 45.13 (CH_2N), 61.18 (OCH_2CH_2 and $OCH_2C{=}C$), 102.31 ($C(CH_3)_2$), 120.03 ($CH_2C{=}C$), 141.40 ($C{=}CN$), 161.60 ($C{=}CC{=}O$), 165.87 ($CH_2C{=}O$), 172.08 ($CH_3C{=}O$) ppm; MS (EI) m/e (rel intensity) 282 (1), 264 (6), 251 (4), 240 (1), 224 (62), 182 (100); MS (+CI) m/e (rel intensity) 283 (1), 265 (4), 225 (100), 183 (8); M_r (EI) 282.121 00 (calcd for $C_{13}H_{18}N_2O_5$, 282.121 57).

Anal. Calcd for $C_{13}H_{18}N_2O_5{\cdot}H_2O$: C, 52.02; H, 6.72; N, 9.33. Found: C, 52.39; H, 6.71; N, 8.98.

(*E*)-1-Acetyl-3-(4,4-dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (12b): yield 113 mg (38%); R_f 0.42 (50% ethyl acetate–hexane); ^1H NMR ($CDCl_3$) δ 1.41 (s, 6 H, $C(CH_3)_2$), 2.57 (s, 3 H, $CH_3C{=}O$), 2.83 (t, J = 6.3 Hz, 2 H, OCH_2CH_2), 3.85 (t, J = 6.3 Hz, 2 H, OCH_2CH_2), 4.38 (s, 2 H, CH_2N), 4.74 (s, 2 H, $OCH_2C{=}C$), 9.44 (br s, 1 H, NH); ^{13}C NMR 24.29 ($C(CH_3)_2$), 26.83 ($CH_3C{=}O$), 29.91 (OCH_2CH_2), 45.16 (CH_2N), 60.28 (OCH_2CH_2), 62.97 ($OCH_2C{=}C$), 102.18 ($C(CH_3)_2$), 119.55 ($OCH_2C{=}C$), 143.04 ($C{=}CN$), 164.86 ($C{=}CC{=}O$), 166.00 ($CH_2C{=}O$), 172.11 ($CH_3C{=}O$) ppm; MS (EI), m/e (rel intensity) 282 (1), 264 (2), 251 (1), 240 (1), 224 (57), 182 (100); MS (+CI) m/e (rel intensity) 283 (9), 265 (5), 225 (100), 183 (19); M_r (EI) 282.121 00 (calcd for $C_{13}H_{18}N_2O_5$, 282.121 57).

Anal. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.32; H, 6.38; N, 9.93. Found: C, 54.97; H, 6.49; N, 9.75.

Preparation of 3-(4,4-Dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (13a and 13b).[6] To a solution of **12a** and **12b** (6.70 g, 23.8 mmol) in dry DMF (50 mL) was added $N_2H_4{\cdot}H_2O$ (2.38 g, 47.5 mmol). The solution was stirred at rt (2 h) and then poured into ice–H_2O. The solid was collected and purified by flash column chromatography (10% MeOH–$CHCl_3$) to give a 3:2 mixture of **13a** and **13b**: yield 5.22 g (92%); mp 207–209 °C. The following spectral properties have been assigned to **13a** and **13b**.

(*Z*)-3-(4,4-Dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (13a): ^1H NMR (DMSO-d_6) δ 1.29 (s, 6 H, $C(CH_3)_2$), 3.14 (t, J = 6.1 Hz, 2 H, OCH_2CH_2), 3.65 (t, J = 6.1 Hz, 2 H, OCH_2CH_2), 3.79 (s, 2 H, CH_2N), 4.28 (s, 2 H, $OCH_2C{=}C$), 8.11, 9.58 (s, 2 H, 2 × NH); ^{13}C NMR (DMSO-d_6) 24.86 ($C(CH_3)_2$), 28.44 (OCH_2CH_2), 44.44 (CH_2N), 60.58, 60.85 (OCH_2CH_2 and $OCH_2C{=}C$), 104.89 ($C(CH_3)_2$), 121.16 ($CH_2C{=}C$), 132.94 ($C{=}CN$), 162.11 ($C{=}CC{=}O$), 165.66 ($CH_2C{=}O$) ppm.

(*E*)-3-(4,4-Dimethyl-3,5-dioxacycloheptylidene)-2,5-piperazinedione (13b): ^1H NMR (DMSO-d_6) δ 1.28 (s, 6 H, $C(CH_3)_2$), 2.62 (t, J = 6.1 Hz, 2 H, OCH_2CH_2), 3.68 (t, J = 6.1 Hz, 2 H, OCH_2CH_2), 3.81 (s, 2 H, CH_2N), 4.61 (s, 2 H, $OCH_2C{=}C$), 8.06, 9.58 (s, 2 H, 2 × NH); ^{13}C NMR (DMSO-d_6) 24.86 ($C(CH_3)_2$), 29.10 (OCH_2CH_2), 44.44 (CH_2N), 60.32, 61.51 (OCH_2CH_2 and $OCH_2C{=}C$), 104.89 ($C(CH_3)_2$), 120.90 ($CH_2C{=}C$), 133.07 ($C{=}CN$), 161.84 ($C{=}CC{=}O$), 165.92 ($CH_2C{=}O$) ppm.

Preparation of 3-(3-Hydroxy-1-(hydroxymethyl)-propylidene)-2,5-piperazinedione (14a and 14b).[6] A solution of **13a** and **13b** (4.80 g, 20 mmol) in 50% aqueous AcOH (100 mL) was stirred at rt (2 h). The solvent was removed in vacuo, and the residue was purified by flash chromatography (methanol–acetone–chloroform (1:7:8)) to give a 1:1 mixture of **14a** and **14b**: yield 3.36 g (84%); mp 166–169 °C; R_f 0.22 (methanol–acetone–chloroform (1:7:8)); MS (EI) m/e (rel intensity) 200 (3), 154 (16), 139 (100), 124 (39), 111 (34). The following spectral properties have been assigned to **14a** and **14b**.

(*Z*)-3-(3-Hydroxy-1-(hydroxymethyl)propylidene)-2,5-piperazinedione (14a): ^1H NMR (DMSO-d_6) δ 2.73 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 3.61 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 3.82 (s, 2 H, CH_2N), 4.18 (s, 2 H, $HOCH_2C{=}C$), 4.65 (br s, 1 H, $HOCH_2CH_2$), 5.51 (br s, 1 H, $HOCH_2C{=}C$), 8.15 (s, 1 H, CH_2NH), 9.54 (br s, 1 H, $NHC{=}C$) [The ^1H NMR assignments were confirmed by the corresponding COSY experiment. The assignments for the amide NH and CH_2N proton signals are uncertain and may correspond to the resonances for the corresponding protons in **14b**]; ^{13}C NMR (DMSO-d_6) 32.69 (OCH_2CH_2), 44.30 (CH_2N), 60.51 (OCH_2CH_2), 61.82 ($OCH_2C{=}C$), 123.98 ($CH_2C{=}C$), 130.39 ($C{=}CN$), 161.31 ($C{=}CC{=}O$), 164.69 ($CH_2C{=}O$) ppm. The ^{13}C NMR assignments were confirmed by the ^1H–^{13}C heteronuclear chemical shift connectivity experiment. The assignments for the CH_2N, C=C, and C=O carbon signals are uncertain and may correspond to the resonances for the corresponding carbons in **14b**.

(*E*)-3-(3-Hydroxy-1-(hydroxymethyl)propylidene)-2,5-piperazinedione (14b): ^1H NMR (DMSO-d_6) δ 2.49 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 3.52 (m, 2 H, $HOCH_2CH_2$), 3.84 (s, 2 H, CH_2N), 4.50 (d, 1 H, J = 4.5 Hz, $HOCH_2C{=}C$), 4.80 (t, 1 H, J = 4.5 Hz, $HOCH_2C{=}C$), 5.12 (br s, 1 H, $HOCH_2CH_2$), 8.17 (s, 1 H, CH_2NH), 9.75 (br s, 1 H, $NHC{=}C$) [The ^1H NMR assignments were confirmed by the corresponding COSY experiment. The assignments for the amide NH and CH_2N proton signals are uncertain and may correspond to the resonances for the corresponding protons in **14a**]; ^{13}C NMR (DMSO-d_6) 31.67 (OCH_2CH_2), 44.42 (CH_2N), 59.18 ($OCH_2C{=}C$), 60.00 (OCH_2CH_2), 124.07 ($CH_2C{=}C$), 133.38 ($C{=}CN$), 161.94 ($C{=}CC{=}O$), 165.64 ($CH_2C{=}O$) ppm. The ^{13}C NMR assignments were confirmed by the ^1H–^{13}C heteronuclear connectivity experiment. The assignments for the CH_2N, C=C, and C=O carbon signals are uncertain and may correspond to the resonances for the corresponding carbons in **14a**.

Preparation of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8).[6] To a 1,4-dioxane solution (150 mL) containing **14a** and **14b** (2.50 g, 12.5 mmol) were added methanesulfonyl chloride (2.18 g, 18.9 mmol) and $KHCO_3$ (1.50 g, 15 mmol). The suspension was heated to reflux (15 h), and then the solvent was removed in vacuo, and the residue was purified by flash column chromatography (10% MeOH–$CHCl_3$) to give **8**: yield 591 mg (26%); R_f 0.53 (10% MeOH–$CHCl_3$); mp 211–213 °C; IR (KBr) 3330, 3180, 3070, 2940, 1660, 1650, 1440, 1095, 918 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.60–2.73 (m, 2 H, OCH_2CH_2), 3.75–3.94 (m, 4 H, OCH_2CH_2, CH_2N), 5.05 (s, 1 H, $C{=}CHH'$), 5.24 (s, 1 H, $C{=}CHH'$), 8.15, 8.88 (2 s, 2 H, 2 × NH); ^{13}C NMR (DMSO-d_6) 32.09 (OCH_2CH_2), 44.49 (CH_2N), 65.96 (OCH_2CH_2), 88.78 (OCN), 109.78 ($C{=}CH_2$), 148.53 ($C{=}CH_2$), 165.51 (2 × C=O) ppm; MS (EI) m/e (rel intensity) 182 (2), 154 (15), 139 (100), 125 (4), 111 (67), 98 (55), 81 (39), 67 (80), 53 (73); M_r (EI) 182.069 45 (calcd for $C_8H_{10}N_2O_3$, 182.069 14).

Preparation of 1-Acetyl-3-ethylidene-2,5-piperazinedione (20a and 20b).[18] To a solution of **10** (10.00 g, 51 mmol) and acetaldehyde (22.25 g, 510 mmol) in dry DMF (100 mL) was added a solution of tBuOK (5.67 g, 51 mmol) in tBuOH (100 mL) dropwise (1 h) at −10 °C. The temperature was kept at −10 °C (1 h), and then the solution was warmed to rt. The solution was poured into H_2O, neutralized with AcOH, and extracted with $CHCl_3$ (3 × 300 mL). The organic layers were combined, washed with an aqueous saturated NaCl solution (300 mL), dried, and concentrated in vacuo. The residue was purified by flash column chromatography (50% ethyl acetate–hexane) to give **20a** and **20b**.

(*Z*)-1-Acetyl-3-ethylidene-2,5-piperazinedione (20a): yield 5.42 g (59%); mp 184 °C (lit.[20] mp 182–184 °C); R_f 0.69 (50% ethyl acetate–hexane); ^1H NMR (DMSO-d_6) δ 1.82 (d, J = 7.5 Hz, 3 H, $CHCH_3$), 2.48 (s, 3 H, $CH_3C{=}O$), 4.27 (s, 2 H, CH_2N), 6.15 (q, J = 7.5 Hz, 1 H, $CHCH_3$), 10.25 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6) 11.81 ($CHCH_3$), 26.82 ($CH_3C{=}O$), 45.90 (CH_2N), 117.49 ($CH_3CH{=}C$), 128.51 ($CH_3CH{=}C$), 160.42 ($C{=}CC{=}O$), 163.64 ($CH_2C{=}O$), 172.13 ($CH_3C{=}O$) ppm. The ^{13}C NMR assignments were confirmed by the corresponding APT experiment.

(*E*)-1-Acetyl-3-ethylidene-2,5-piperazinedione (20b): yield 0.74 g (8%); mp 183–184 °C; R_f 0.73 (50% ethyl acetate–hexane); ^{1}H NMR (DMSO-d_6) δ 2.06 (d, J = 7.5 Hz, 3 H, CHC*H*$_3$), 2.51 (s, 3 H, CH_3C=O), 4.21 (s, 2 H, CH_2N), 5.85 (q, J = 7.5 Hz, 1 H, C*H*CH$_3$), 10.36 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6) 13.72 (CH*C*H$_3$), 26.98 (*C*H$_3$C=O), 45.71 (CH_2N), 122.32 (CH$_3$*C*H=C), 127.60 (CH$_3$CH=*C*), 162.40 (C=*C*C=O), 165.50 (CH_2*C*=O), 171.68 (CH$_3$*C*=O) ppm. The ^{13}C NMR assignments were confirmed by the corresponding APT experiment.

Preparation of 1-Acetyl-3-hydroxy-3-(1-(phenylselenenyl)ethyl)-2,5-piperazinedione (21a and 21b). To a suspension of PhSeBr (1.79 g, 7.58 mmol) and KOAc (1.00 g, 10.19 mmol) in AcOH (40 mL) was added **20a** (1.00 g, 5.49 mmol) at rt under N_2. The reaction mixture was stirred at rt (12 h) and then poured into H_2O (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were combined and washed with an aqueous saturated $NaHCO_3$ solution (3 × 100 mL) and an aqueous saturated NaCl solution (150 mL). The solvent was removed in vacuo, and the residue was purified by flash column chromatography (60% ethyl acetate–hexane) to give a diastereomeric mixture of **21a** and **21b** along with recovered starting material **20a** (0.30 g, 30%).

Compound 21a: yield 761 mg (39%); mp 148–150 °C; R_f 0.45 (5% MeOH–$CHCl_3$); IR (KBr) 3410, 3160, 1690, 1675, 1650, 1410, 1355, 1270, 1200, 785, 735 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.56 (d, J = 7.3 Hz, 3 H, SeCHC*H*$_3$), 2.31 (s, 3 H, CH_3C=O), 3.78 (q, J = 7.3 Hz, 1 H, SeC*H*CH$_3$), 4.12 ($^1/_2$ ABq, J = 17.9 Hz, 1 H, C*H*H'N), 4.34 ($^1/_2$ ABq, J = 17.9 Hz, 1 H, CH*H'*N), 7.23 (s, 1 H, OH), 7.28–7.37 (m, 3 H, ArH), 7.43–7.55 (m, 2 H, ArH), 9.09 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6) 15.59 (SeCH*C*H$_3$), 26.53 (*C*H$_3$C=O), 46.04 (CH_2N), 46.80 (Se*C*HCH$_3$), 84.32 (N*C*(OH)C(O)), 127.34, 129.00, 129.58, 132.99 (C$_6$H$_5$), 165.16, 168.30 (2 × NC=O), 171.58 (CH$_3$*C*=O) ppm; the ^{13}C NMR assignments were confirmed by the corresponding APT experiment; MS (EI) m/e (rel intensity) 358 (4), 356 (21), 354 (9), 353 (4), 352 (4), 314 (72), 312 (88), 310 (55), 257 (85), 255 (55), 199 (59), 185 (100), 183 (85), 182 (61), 181 (64), 157 (97), 155 (83), 154 (67).

Anal. Calcd for $C_{14}H_{16}N_2O_4Se$: C, 47.33; H, 4.54; N, 7.89. Found: C, 47.43; H, 4.50; N, 7.83.

Compound 21b: yield 273 mg (14%); mp 147–150 °C; R_f 0.53 (5% MeOH–$CHCl_3$); IR (KBr) 3405, 3160, 1695, 1670, 1650, 1410, 1360, 1275, 780 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.49 (d, J = 7.0 Hz, 3 H, SeCHC*H*$_3$), 2.52 (s, 3 H, CH_3C=O), 3.95 (q, J = 7.0 Hz, 1 H, SeC*H*CH$_3$), 4.31 ($^1/_2$ ABq, J = 18.3 Hz, 1 H, C*H*H'N), 4.53 ($^1/_2$ ABq, J = 18.3 Hz, 1 H, CH*H'*N), 5.22 (br s, 1 H, OH), 7.24–7.29 (m, 3 H, ArH), 7.58–7.61 (m, 2 H, ArH), 8.00 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 17.30 (SeCH*C*H$_3$), 26.82 (*C*H$_3$C=O), 46.35 (CH_2N), 46.84 (Se*C*HCH$_3$), 84.37 (N*C*(OH)C(O)), 127.17, 128.25, 129.24, 134.64 (C$_6$H$_5$), 166.66, 167.09 (2 × NC=O), 171.59 (CH$_3$*C*=O) ppm; the ^{13}C NMR assignments were confirmed by the corresponding APT experiment; MS (EI), m/e (rel intensity) 358 (12), 356 (58), 354 (25), 353 (12), 352 (10), 199 (78), 185 (86), 183 (78), 182 (65), 181 (68), 157 (100), 155 (63).

Anal. Calcd for $C_{14}H_{16}N_2O_4Se$: C, 47.33; H, 4.54; N, 7.89. Found: C, 47.41; H, 4.56; N, 7.78.

Preparation of 1-Acetyl-3-methoxy-3-(1-(phenylselenenyl)ethyl)-2,5-piperazinedione (22a and 22b). A solution of **21a** (500 mg, 1.408 mmol) in MeOH (50 mL) and AcOH (10 mL) was stirred at rt (24 h). The solvent was removed in vacuo, and the residue was poured into H_2O (50 mL) and extracted with $CHCl_3$ (5 × 50 mL). The organic layers were combined and washed with an aqueous saturated $NaHCO_3$ solution (3 × 100 mL), an aqueous saturated NaCl solution (100 mL), and dried. The solvent was evaporated in vacuo, and the residue was purified by PTLC (5% MeOH–$CHCl_3$) to give a diastereomeric mixture of **22a** and **22b** along with recovered **21a** (0.23 g, 47%).

Compound 22a: yield 13 mg as an oil (3%); R_f 0.92 (5% MeOH–$CHCl_3$); IR ($CHCl_3$) 3310, 1715, 1700, 1685, 1425, 1365, 1250, 1210, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J = 7.1 Hz, 3 H, CHC*H*$_3$), 2.61 (s, 3 H, CH_3C=O), 3.19 (s, 3 H, OCH_3), 4.12 (q, J = 7.1 Hz, 1 H, C*H*CH$_3$), 4.26 ($^1/_2$ ABq, J = 18.6 Hz, 1 H, C*H*H'N), 4.59 ($^1/_2$ ABq, J = 18.6 Hz, 1 H, CH*H'*N), 7.06 (s, 1 H, NH), 7.26–7.35 (m, 3 H, ArH), 7.62–7.65 (m, 2 H, ArH); ^{13}C NMR ($CDCl_3$) 17.02 (CH*C*H$_3$), 27.27 (*C*H$_3$C=O), 44.08 (*C*HCH$_3$), 46.43 (CH_2N), 51.73 (OCH_3), 89.68 (N*C*(OCH$_3$)C(O)), 127.40, 128.55, 129.14, 136.00 (C$_6$H$_5$), 165.37, 165.97 (2 × NC=O), 171.70 (CH$_3$*C*=O) ppm; the ^{13}C NMR assignments were confirmed by the corresponding APT experiment; MS (EI) m/e (rel intensity) 372 (3), 370 (17), 368 (7), 367 (3), 366 (3), 185 (100), 183 (51), 157 (47).

Anal. Calcd for $C_{15}H_{18}N_2O_4Se$: C, 48.79; H, 4.91; N, 7.59. Found: C, 48.68; H, 4.76; N, 7.36.

Compound 22b: yield 205 mg (39%); mp 151–152 °C; R_f 0.78 (5% MeOH–$CHCl_3$); IR (KBr) 3180, 1705, 1675, 1425, 1360, 1245, 1205, 1075, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.61 (d, J = 7.3 Hz, 3 H, CHC*H*$_3$), 2.37 (s, 3 H, CH_3C=O), 3.29 (s, 3 H, OCH_3), 3.91 (q, J = 7.3 Hz, 1 H, C*H*CH$_3$), 4.39 ($^1/_2$ ABq, J = 18.7 Hz, 1 H, C*H*H'N), 4.56 ($^1/_2$ ABq, J = 18.7 Hz, 1 H, CH*H'*N), 7.24–7.32 (m, 3 H, ArH), 7.46–7.49 (m, 2 H, ArH), 8.04 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 15.22 (CH*C*H$_3$), 27.19 (*C*H$_3$C=O), 46.10 (*C*HCH$_3$), 46.54 (CH_2N), 52.16 (OCH_3), 89.70 (N*C*(OCH$_3$)C(O)), 128.13, 128.32, 129.20, 133.95 (C$_6$H$_5$), 165.88, 166.50 (2 × NC=O), 171.98 (CH$_3$*C*=O) ppm; the ^{13}C NMR assignments were confirmed by the corresponding APT experiment; MS (EI) m/e (rel intensity) 372 (3), 370 (17), 368 (8), 367 (2), 366 (3), 185 (100), 183 (47).

Anal. Calcd for $C_{15}H_{18}N_2O_4Se$: C, 48.79; H, 4.91; N, 7.59. Found: C, 48.43; H, 4.71; N, 7.32.

Preparation of 1-Acetyl-3-methoxy-3-vinyl-2,5-piperazinedione (23). To a dry CH_2Cl_2 solution (20 mL) of **22a** and **22b** (120 mg, 0.325 mmol) was bubbled O_3 through the reaction at −20 °C until the solution became blue in color (1 min). The excess O_3 was then purged from the reaction system by bubbling O_2 through the solution (15 min). During this time the reaction was monitored for the presence of O_3 using an aqueous 2% KI indicator solution. The solvent was removed in vacuo, and the residue was purified by PTLC (5% MeOH–$CHCl_3$) to give **23** as an oil: yield 61 mg (89%); R_f 0.55 (5% MeOH–$CHCl_3$); IR (KBr) 3320, 2980, 1710, 1695, 1680, 1445, 1205, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3 H, CH_3C=O), 3.30 (s, 3 H, OCH_3), 4.22 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, C*H*H'N), 4.72 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, CH*H'*N), 5.61 (d, J = 17.7 Hz, 1 H, C*H*H'=CH), 5.69 (d, J = 10.8 Hz, 1 H, CH*H'*=CH), 6.12 (dd, J = 10.8, 17.7 Hz, 1 H, CH_2=C*H*), 8.64 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 26.97 (*C*H$_3$C=O), 45.90 (CH_2N), 50.64 (OCH_3), 85.86 (N*C*(OCH$_3$)C(O)), 120.70 (CH_2=CH), 132.15 (CH_2=*C*H), 165.35, 168.69 (2 × NC=O), 171.67 (CH$_3$*C*=O) ppm; MS (EI) m/e (rel intensity) 169 (15), 139 (40), 127 (83), 112 (100).

Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.96; H, 5.66; N, 13.21. Found: C, 50.71; H, 5.67; N, 12.92.

Use of NMR Spectroscopy To Monitor the Preparation of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) from 1-Acetyl-3-methoxy-3-vinyl-2,5-piperazinedione (23). A solution of **23** (10 mg, 0.047 mmol) in THF-d_8 (0.10 mL) and DCl–D_2O (pD 3.96, 0.50 mL) was prepared and then monitored by ^{1}H and ^{13}C NMR spectroscopy. At the onset of the reaction the following NMR signals were observed: ^{1}H NMR (D_2O–THF-d_8) δ 2.43 (s, 3 H, CH_3C=O), 3.21 (s, 3 H, OCH_3), 4.24 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, C*H*H'N), 4.36 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, CH*H'*N), 5.48 (d, J = 17.7 Hz, 1 H, C*H*H'=CH), 5.52 (d, J = 10.8 Hz, 1 H, CH*H'*=CH), 5.88 (dd, J = 10.8, 17.7 Hz, 1 H, CH_2=C*H*); ^{13}C NMR (D_2O–THF-d_8) 26.79 (*C*H$_3$C=O), 46.34 (CH_2N), 51.46 (OCH_3), 87.19 (N*C*(OCH$_3$)C(O)), 121.36 (CH_2=CH), 133.22 (CH_2=*C*H), 167.02, 169.20 (2 × NC=O), 174.18 (CH$_3$*C*=O) ppm. After 35 h, the signals corresponding to the starting material **23** were significantly reduced, and the following new set of signals attributed to **7** were observed: ^{1}H NMR (D_2O–THF-d_8) δ 2.43 (s, 3 H, CH_3C=O), 3.22 (s, 3 H, CH_3OD), 4.31 (s, CH_2N), 5.42 (d, J = 10.8 Hz, 1 H, C*H*H'=CH), 5.50 (d, J = 17.7 Hz, 1 H, CH*H'*=CH), 5.93 (dd, J = 10.8, 17.7 Hz, 1 H, CH_2=C*H*); ^{13}C NMR (D_2O–THF-d_8) 26.69 (*C*H$_3$C=O), 46.41 (CH_2N), 49.27 (CH_3OD), 82.22 (N*C*(OH)C(O)), 119.72 (CH_2=CH), 134.61 (CH_2=*C*H), 168.58, 168.74 (2 × NC=O), 174.36 (CH$_3$*C*=O) ppm. The relative intensity of the two sets of peaks (**23** versus **7**) was approximately 1:1. After 96 h, the signals corresponding to the starting material **23** completely disappeared, and only the signals which corresponded to the title compound **7** and methanol-d_1 were detected: ^{1}H NMR (D_2O–THF-d_8) δ 2.44 (s, 3 H, CH_3C=O), 3.22 (s, 3 H, CH_3OD), 4.33 (s, CH_2N), 5.49 (d, J = 10.8 Hz, 1 H, C*H*H'=CH), 5.56 (d, J = 17.7 Hz, 1 H, CH*H'*=CH), 6.01 (dd, J = 10.8, 17.7 Hz, 1 H, CH_2=C*H*); ^{13}C NMR ($CDCl_3$) 26.70 (*C*H$_3$C=O), 46.39 (CH_2N), 49.26 (CH_3OD), 82.21 (N*C*(OH)C(O)), 119.76 (CH_2=CH), 134.58 (CH_2=*C*H), 168.54, 168.75 (2 × NC=O), 174.48 (CH$_3$*C*=O) ppm.

Mass Spectral Identification and Isolation of Crude 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7). A solution of **23** (50 mg, 0.236 mmol) in a THF-d_8–D_2O–CD_3CO_2D mixture ("pD" 2.0) was prepared and permitted to stand at rt (16 h). The ^1H and ^{13}C NMR spectra indicated that the reaction was complete: ^1H NMR (THF-d_8–D_2O–CD_3CO_2D) δ 2.44 (s, 3 H, CH_3C=O), 3.36 (s, 3 H, CH_3OD), 4.31 (s, CH_2N), 5.42 (d, J = 10.5 Hz, 1 H, C*H*H'=CH), 5.50 (d, J = 17.2 Hz, 1 H, CH*H'*=CH), 5.94 (dd, J = 10.5, 17.2 Hz, 1 H, CH_2=C*H*); ^{13}C NMR (THF-d_8–D_2O–CD_3CO_2D) 26.83 (*C*H_3C=O), 46.23 (CH_2N), 49.43 (CH_3OD), 82.50 (N*C*(OH)C(O)), 119.74 (*C*H_2=CH), 134.98 (CH_2=*C*H), 168.59, 169.14 (2 × NC=O), 174.40 (CH_3C=O) ppm; MS (+FAB) 199 ([M + 1]$^+$, M = $C_8H_{10}N_2O_4$), 200 ([M + 1]$^+$, M = $C_8H_9DN_2O_4$), 201 ([M + 1]$^+$, M = $C_8H_8D_2N_2O_4$); M_r (+FAB) 200.077 86 (calcd for $C_8H_9DN_2O_4$, [M + 1]$^+$ 200.078 16). The solution was divided into two equal portions. The first portion was neutralized with an aqueous 0.01 N NaOH solution (final "pH" 6.9). The solvent was removed in vacuo (35 °C), and the residue was triturated with DMF-d_7 and filtered through glass wool. The filtrate exhibited the following NMR spectroscopic properties which have been attributed to **24**: ^1H NMR (DMF-d_7) δ 1.83 (s, 3 H, CH_3C=O), 3.60 (d, J = 5.8 Hz, 2 H, *C*H_2NH), 7.01 (br s, 1 H, *H*H'NC=O), 7.32 (br s, 1 H, H*H'*NC=O), 8.02 (t, J = 5.8 Hz, 1 H, CH_2N*H*); ^{13}C NMR (DMSO-d_6) 22.57 (*C*H_3C=O), 41.98 (CH_2N), 169.82, 171.43 (2 × NC=O) ppm. Addition of authentic **24**[25] to the NMR sample led to an increase in all the peaks attributed to **24**, and no additional peaks were detected in either the ^1H or ^{13}C NMR spectrum. To the second half of the reaction, was continuously added H_2O (10 mL), and the resulting azeotrope was evaporated in vacuo. This procedure provided a crude sample of **7** as an oil (~70% pure): ^1H NMR (DMSO-d_6) δ 2.38 (s, 3 H, CH_3C=O), 4.12 ($^1/_2$ ABq, J = 8.2 Hz, C*H*H'N), 4.31 ($^1/_2$ ABq, J = 8.2 Hz, CH*H'*N), 5.36 (d, J = 10.5 Hz, 1 H, C*H*H'=CH), 5.48 (d, J = 17.2 Hz, 1 H, CH*H'*=CH), 5.97 (dd, J = 10.5, 17.2 Hz, 1 H, CH_2=C*H*), 9.20 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6) 26.90 (*C*H_3C=O), 46.23 (CH_2N), 81.88 (N*C*(OH)C(O)), 117.39 (*C*H_2=CH), 135.78 (C-H_2=*C*H), 166.85, 167.77 (2 × NC=O), 171.96 (CH_3C=O) ppm.

Use of ^1H NMR Spectroscopy To Monitor the Hydrolysis of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8). Solutions of **8** (10 mg, 0.055 mmol) in DCl–D_2O (1 mL, initial pD 4.36, 3.11, 2.31, and 1.08) were prepared and then monitored by ^1H NMR spectroscopy. No spectral changes were noted in the pD 4.36, 3.11, and 2.31 experiments after 24 h at rt, 12 h at 50 °C, and 1.5 h at 100 °C. Correspondingly, the pD 1.08 experiment exhibited no significant changes in the ^1H NMR spectrum at rt (24 h) and at 50 °C (12 h), but after the solution was maintained at 100 °C (1.5 h), signals corresponding to **8** were no longer evident and were replaced by the following resonances: ^1H NMR (D_2O) δ 2.64 (t, J = 5.7 Hz, 2 H), 3.80 (t, J = 5.7 Hz, 2 H), 3.87 (s, 2 H). A large peak was also observed at δ 4.80 and has been assigned to the residual H_2O present in the NMR sample. The signal at δ 3.87 has been attributed to **26**. Addition of an authentic sample of **26**[25] to the pD 1.08 solution led to an increase in the δ 3.87 peak.

Hydrolysis of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8). An aqueous 0.1 N HCl solution (1 mL) of **8** (30 mg, 0.165 mmol) was heated at 100 °C (1.5 h). The reaction mixture was cooled to rt and purified by PTLC (25% MeOH–$CHCl_3$) to obtain **25**[27] (13 mg, 56%) and **26** (6 mg, 33%). Addition of an authentic sample of **26**[25] to the NMR sample containing the product led to increases in the δ 3.86 (^1H NMR) and 42.89 and 171.81 (^{13}C NMR) peaks. Correspondingly, addition of glycine led to new signals at δ 3.54 (^1H NMR) and 43.91 and 174.96 (^{13}C NMR).

3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (25):[27] R_f 0.70 (25% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 2.40 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 3.57 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 4.62 (s, 2 H, CH_2OC=O); ^{13}C NMR (DMSO-d_6) 28.73 (HOC-H_2CH_2), 59.15 ($HOCH_2CH_2$), 69.17 (*C*H_2OC=O), 129.84 (C=*C*-CH_2), 140.46 (*C*=CCH_2), 172.33 (C=O) ppm.

Glycinamide hydrochloride (26): R_f 0.15 (25% MeOH–$CHCl_3$); ^1H NMR (D_2O) δ 3.86 (s, 2 H, CH_2); ^{13}C NMR (D_2O) 42.89 (CH_2), 171.81 (C=O) ppm.

Acetylation of 3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (25). Preparation of 3-Acetoxy-4-(2-acetoxyethyl)-2(5*H*)-furanone (27).[27] A solution of **25** (10 mg, 0.069 mmol) in dry pyridine (1 mL) and Ac_2O (1 mL) was stirred at rt under N_2 (16 h). The solvent was removed in vacuo, and the crude material was purified by PTLC (three developments) to give **27** (9 mg, 58%) as an oil: R_f 0.25 ($CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.07 (s, 3 H, CH_3C=O), 2.31 (s, 3 H, CH_3C=O), 2.72 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 4.26 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 4.85 (s, 2 H, CH_2OC=O); ^{13}C NMR ($CDCl_3$) 20.24 (*C*H_3C=O), 20.79 (*C*H_3C=O), 25.44 (OCH_2CH_2), 60.60 (OCH_2CH_2), 69.31 (*C*H_2O-C=O), 135.35 (C=*C*CH_2), 146.08 (*C*=CCH_2), 166.98 (CH_3C=O), 167.10 (CH_3C=O), 170.56 (OC=O) ppm.

General Procedure for the Attempted Alcoholysis of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8). A solution of **8** (10 mg, 0.055 mmol) in anhydrous alcoholic 1 N H_2SO_4 (1 mL) was heated to reflux (1.5 h) under N_2. The solvent was removed in vacuo, and the residue was directly examined by ^1H NMR spectroscopy. Use of MeOH (65 °C) and PrOH (98 °C) in this procedure led to the recovery of only **8**.

Attempted Propanolysis of Bicyclomycin (1). A solution of **1** (50 mg, 0.166 mmol) in anhydrous 1-propanolic 1 N H_2SO_4 (5 mL) was heated to reflux (1.5 h) under N_2. The solvent was removed in vacuo, and the residue was added to H_2O (5 mL) and extracted with EtOAc (5 × 10 mL). The organic layers were combined, dried, and concentrated in vacuo. The residue was purified by PTLC (10% MeOH–$CHCl_3$) to give a 1:1 mixture[6,27] of rearranged products **34a** and **34b**: yield 12 mg (26%); mp 238–240 °C (lit.[6] mp 238–242 °C); R_f 0.50 (10% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.22 (s, 3 H, CH_3), 2.68–2.75 (m, 2 H, C(3')H_2), 3.64 and 3.67 (2d, J = 9.0 Hz, 1 H, C(2)*H*H'), 3.81 and 3.84 (2d, J = 9.0 Hz, 1 H, C(2)H*H'*), 3.93 and 3.95 (2d, J = 7.1 Hz, 1 H, C(2')*H*H'), 4.00 and 4.04 (2d, J = 7.1 Hz, 1 H, C(2')H*H'*), 4.28 and 4.31 (2d, J = 6.0 Hz, 1 H, C(4)H), 5.07 (s, 1 H, C(3)OH), 5.27 and 5.32 (2s, 2 H, C=CH_2), 5.74 and 5.77 (2 d, J = 6.0 Hz, 1 H, C(4)OH), 7.45 and 7.52 (2s, 1 H, NH), 9.10 and 9.13 (2s, 1 H, NH).

Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with Benzyl Mercaptan under Acidic Conditions. A solution of **8** (30 mg, 0.165 mmol) and benzyl mercaptan (100 mg, 0.805 mmol) in 25% THF–H_2O (5 mL, "pH" 1) was heated to reflux (4 h, 80 °C). During the reaction the concentration of benzyl mercaptan was determined at 30-min intervals with Ellman's reagent,[39] 5,5'-dithiobis(2-nitrobenzoic acid), to ensure that dimerization of benzyl mercaptan had not proceeded to an appreciable extent. After 4 h, greater than 1.7 equiv of benzyl mercaptan remained. The solvent was removed in vacuo, and the residue was purified by PTLC (10% MeOH–$CHCl_3$) to give **25** (11 mg, 46%) and glycinamide hydrochloride (**26**) (6 mg, 33%).

3-Hydroxy-4-(2-hydroxyethyl)-2(5*H*)-furanone (25):[27] R_f 0.70 (25% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 2.39 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 3.57 (t, J = 6.0 Hz, 2 H, $HOCH_2CH_2$), 4.62 (s, 2 H, CH_2OC=O).

Glycinamide hydrochloride (26): R_f 0.15 (25% MeOH–$CHCl_3$); ^1H NMR (D_2O) δ 3.87 (s, 2 H, CH_2).

Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with EtSH at Near-Neutral "pH". To a tetrahydrofuran–aqueous Tris buffer solution (3:1, 1 mL, "pH" 8.0) were added **8** (5 mg, 0.027 mmol) and EtSH (27 mg, 0.440 mmol) (final "pH" 8.1). No change in the reaction was noted by TLC analysis after stirring at rt (96 h). The solvent was evaporated in vacuo, and the residue was purified by PTLC (10% MeOH–$CHCl_3$) to recover **8** (3.6 mg, 72%): ^1H NMR (DMSO-d_6) δ 2.58–2.71 (m, 2 H, OCH_2CH_2), 3.73–3.92 (m, 4 H, OCH_2CH_2, CH_2N), 5.03 (s, 1 H, C=C*H*H'), 5.22 (s, 1 H, C=CH*H'*), 8.11, 8.86 (2s, 2 H, 2 × NH).

Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with Benzyl Mercaptan at Near-Neutral "pH". To a THF–H_2O solution (3:1, 1 mL) were added **8** (5 mg, 0.027 mmol) and benzyl mercaptan (6.8 mg, 0.055 mmol). The "pH" was adjusted from 7.7 to 8.1 with dilute aqueous NaOH,

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and then the solution was stirred at rt (48 h). No change in the reaction was noted by TLC analysis. The solvent was removed in vacuo, and the residue was purified by PTLC (10% MeOH–$CHCl_3$) to recover 8 (2.9 mg, 58%): ^1H NMR (DMSO-d_6) δ 2.60–2.73 (m, 2 H, OCH_2CH_2), 3.76–3.94 (m, 4 H, OCH_2CH_2, CH_2N), 5.05 (s, 1 H, C=CHH'), 5.24 (s, 1 H, C=CHH'), 8.15, 8.88 (2s, 2 H, 2 × NH).

General Procedure for the Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with Imidazole. Solutions of 8 (5 mg, 0.027 mmol) and imidazole (3 mg, 0.044 mmol) in THF–H_2O (3:1, 1 mL, final "pH" 9.0, 10.2, and 11.0) were prepared and then monitored by TLC analysis. No changes in these reactions were noted after stirring at rt (48 h). In each case the solvent was removed in vacuo, and the residue was purified by PTLC (10% MeOH–$CHCl_3$) to recover 8 (2.5–3.3 mg, 50–66%) and imidazole (1.5–1.9 mg, 50–63%).

Compound 8: ^1H NMR (DMSO-d_6) δ 2.61–2.74 (m, 2 H, OCH_2CH_2), 3.76–3.95 (m, 4 H, OCH_2CH_2, CH_2N), 5.05 (s, 1 H, C=CHH'), 5.25 (s, 1 H, C=CHH'), 8.17, 8.89 (2s, 2 H, 2 × NH).

Imidazole: mp 88–90 °C (lit.[40] mp 89–91 °C); R_f 0.40 (10% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.13 (s, 2 H, NCHCHN), 7.72 (s, 1 H, NCHN), 11.57 (br s, 1 H, NH).

Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with NaSMe under Basic Conditions. To a solution of 8 (40 mg, 0.220 mmol) in 25% H_2O–THF (3 mL) was added NaSMe (36 mg, 0.513 mmol) in H_2O (1 mL) (final "pH" 12.5). The solution was stirred at rt (2 h) and then neutralized ("pH" 7.3) with 0.1 N aqueous HCl. The solvent was removed in vacuo, and the residue was purified by PTLC (15% MeOH–$CHCl_3$) to give **36** as an oil: yield 35 mg (69%); R_f 0.62 (15% MeOH–$CHCl_3$); IR (KBr) 3240, 3100, 2920, 1665, 1625, 1435, 1410, 1315, 1095, 1045, 905 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.98 (s, 3 H, SCH_3), 2.55 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 3.91 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 3.93 (s, 2 H, CH_2S), 4.01 (s, 2 H, CH_2N); ^{13}C NMR (acetone-d_6) 13.67 (SCH_3), 33.40 (CH_2CH_2C=C or SCH_2), 33.82 (CH_2CH_2C=C or SCH_2), 45.32 (CH_2N), 59.76 (OCH_2CH_2), 124.26 (CH_2C=C), 132.02 (CH_2C=C), 163.43 (C=O), 165.26 (C=O) ppm; MS (EI) m/e (rel intensity) 230 (98), 212 (32), 197 (44), 183 (100), 165 (65), 151 (34); M_r (EI) 230.072 25 (calcd for $C_9H_{14}N_2O_3S$, 230.072 49), 212.062 30 (calcd for $C_9H_{12}N_2O_2S$, 212.061 95), 197.038 42 (calcd for $C_8H_9N_2O_2S$, 197.038 47), 183.076 35 (calcd for $C_8H_{11}N_2O_3$, 183.076 97), 165.065 09 (calcd for $C_8H_9N_2O_2$, 165.066 40).

Acetylation of 3-(3-Hydroxy-1-((methylthio)methyl)-propylidene)-2,5-piperazinedione (36). Preparation of 3-(3-Acetoxy-1-((methylthio)methyl)propylidene)-2,5-piperazinedione (37). A solution of **36** (23 mg, 0.100 mmol) in dry pyridine (1 mL) and Ac_2O (1 mL) was stirred at rt under N_2 (24 h). The solvent was removed in vacuo, and the residue was purified by PTLC (5% MeOH–$CHCl_3$) to give **37** as an oil: yield 16 mg (60%); R_f 0.64 (5% MeOH–$CHCl_3$); IR ($CDCl_3$) 2987, 2256, 1736, 1377, 1251, 1047, 906, 738, 653 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (s, 3 H, SCH_3), 2.13 (CH_3C=O), 2.64 (t, J = 6.0 Hz, 2 H, OCH_2CH_2), 3.97 (s, 2 H, CH_2S), 4.09 (s, 2 H, CH_2N), 4.26 (t, J = 6.0 Hz, 2 H, OCH_2CH_2); ^{13}C NMR ($CDCl_3$) 14.47 (SCH_3), 20.87 (CH_3C=O), 31.31 (OCH_2CH_2), 34.18 (CH_2S), 45.20 (CH_2N), 63.08 (OCH_2CH_2), 124.79 (CH_2C=C), 127.63 (CH_2C=C), 161.13 (C=C-O), 164.14 (CH_2C=O), 171.64 (CH_3C=O) ppm; MS (EI) m/e (rel intensity) 272 (8), 230 (9), 212 (69), 183 (13), 165 (100), 137 (56), 108 (26); M_r (EI) 272.082 95 (calcd for $C_{11}H_{16}N_2O_4S$, 272.083 08), 212.061 30 (calcd for $C_9H_{12}N_2O_2S$, 212.061 95), 165.066 18 (calcd for $C_8H_9N_2O_2$, 165.066 40), 137.071 73 (calcd for $C_7H_9N_2O$, 137.071 49).

Treatment of 4-Methylene-1-oxa-6,9-diazaspiro[4.5]decane-7,10-dione (8) with NaSMe in Basic MeOH. A solution of 8 (40 mg, 0.220 mmol) and NaSMe (36 mg, 0.513 mmol) in 0.03 N NaOMe–MeOH (final "pH" 11.9) was stirred at rt under N_2 (24 h). The solution was neutralized ("pH" 7.1) with 0.1 N methanolic H_2SO_4. The solvent was removed in vacuo, and the residue was purified by PTLC (10% MeOH–$CHCl_3$) to recover 8 (33 mg, 83%): ^1H NMR (DMSO-d_6) δ 2.60–2.74 (m, 2 H, OCH_2CH_2), 3.76–3.95 (m, 4 H, OCH_2CH_2, CH_2N), 5.05 (s, 1 H, C=CHH'), 5.25 (s, 1 H, C=CHH'), 8.18, 8.90 (2s, 2 H, 2 × NH).

Treatment of Bicyclomycin (1) with NaSMe in Basic MeOH. A solution of 1 (50 mg, 0.166 mmol) and NaSMe (36 mg, 0.513 mmol) in 0.03 N NaOMe–MeOH (final "pH" 11.9) was stirred at rt under N_2 (2 h). The solution was neutralized ("pH" 7.0) with 0.1 N methanolic H_2SO_4. The solvent was removed in vacuo, and the residue was purified by PTLC (20% MeOH–$CHCl_3$) to give a diastereomeric mixture[7a,15] of **38a** (27 mg, 46%) and **38b** (12 mg, 20%) as a semisolid and an unidentified minor product (5 mg).

Compound 38a: R_f 0.45 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.17 (s, 3 H, C(2')CH_3), 1.65–1.89 (m, 1 H, C(4)HH'), 2.01 (s, 3 H, SCH_3), 2.08–2.20 (m, 1 H, C(4)HH'), 2.21–2.40 (m, 2 H, C(5)H, C(5a)HH'), 2.57–2.73 (m, 1 H, C(5a)HH'), 3.57–3.81 (m, 4 H, C(1')H, C(3')H_2, C(3)HH'), 3.86–4.00 (m, 1 H, C(3)HH'), 4.79 (s, 1 H, C(1')OH), 6.25 (s, 1 H, C(2')OH), 6.49 (s, 1 H, C-(6)OH), 7.11, 7.20 (2 s, 2 H, N(10)H_2), 8.04 (s, 1 H, N(8)H); ^{13}C NMR (DMSO-d_6) 15.05 (SCH_3), 23.65 (C(2')CH_3), 29.73 (C(5a)), 32.96 (C(4)), 46.43 (C(5)), 66.81 (C(3)), 74.83 (C(2')), 76.02 (C(3')), 78.96 (C(1')), 88.15 (C(1)), 101.86 (C(6)), 169.49 (C(7) or C(9)), 171.89 (C(7) or C(9)) ppm; MS (+FAB) m/e (rel intensity) 373 [M + Na^+] (100), 351 [M + H^+] (71), 333 (77), 316 (40); M_r (+FAB) 373.104 05 (calcd for $C_{13}H_{22}NaN_2O_7S$, 373.104 54).

Compound 38b: R_f 0.41 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.17 (s, 3 H, C(2')CH_3), 1.65–1.89 (m, 1 H, C(4)HH'), 2.01 (s, 3 H, SCH_3), 2.07–2.20 (m, 1 H, C(4)HH'), 2.24–2.43 (m, 2 H, C(5)H, C(5a)HH'), 2.57–2.75 (m, 1 H, C(5a)HH'), 3.40–3.82 (m, 4 H, C(1')H, C(3')H_2, C(3)HH'), 3.89–4.00 (m, 1 H, C(3)HH'), 4.59 (s, 1 H, C(1')OH), 5.45 (s, 1 H, C(2')OH), 6.59 (s, 1 H, C-(6)OH), 7.60, 7.83 (2 s, 2 H, N(10)H_2), 8.71 (s, 1 H, N(8)H); ^{13}C NMR (DMSO-d_6), 15.00 (SCH_3), 23.58 (C(2')CH_3), 29.49 (C(5a)), 32.88 (C(4)), 46.41 (C(5)), 66.85 (C(3)), 74.90 (C(2')), 76.04 (C(3')), 78.93 (C(1')), 88.09 (C(1)), 101.89 (C(6)), 169.47 (C(7) or C(9)), 171.85 (C(7) or C(9)) ppm; MS (+FAB) m/e (rel intensity) 373 [M + Na^+] (100), 351 [M + H^+] (68), 333 (100); M_r (+FAB) 373.104 05 (calcd for $C_{13}H_{22}NaN_2O_7S$, 373.104 54).

Treatment of Compounds 34a and 34b with NaSMe under Basic Conditions. A THF–H_2O mixture ("pH" 12.4) containing **34a** and **34b** (40 mg, 0.14 mmol) and NaSMe (20 mg, 0.28 mmol) was stirred at rt (24 h) under Ar. The solution was neutralized with aqueous 0.1 N HCl, and the solvent was removed in vacuo. The residue was subjected to preparative TLC (15% MeOH–$CHCl_3$) to give **34a** (**34b**)[6] as the major component: R_f 0.55 (15% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.33 (s, 3 H, C(3)CH_3), 2.76–2.95 (m, 2 H, C(3')H_2), 3.82 (d, J = 9.3 Hz, 1 H, C(2)HH'), 3.95 (d, J = 9.3 Hz, 1 H, C(2)HH'), 4.05–4.15 (m, 2 H, C(2')H_2), 4.41 (s, 1 H, C(4)H), 5.24–5.26 (m, 1 H, C(4')=CHH'), 5.33–5.35 (m, 1 H, C(4')=CHH'); ^{13}C NMR (CD_3OD) 21.18 (C(3)CH_3), 33.21 (C(3')), 68.31 (C(2')), 77.56 (C(4)), 78.75 (C(3)), 78.83 (C(2)), 90.15 (C(5)), 111.62 (C(4')=CH_2), 150.43 (C(4')), 168.56 (C=O), 169.10 (C=O). The remaining quaternary carbon was not detected. Inspection of the ^{13}C spectrum indicated that the product existed as a single diastereoisomer.

Hydrolysis of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) under Acidic Conditions. Compound 7 (15 mg, 0.076 mmol) was prepared at "pD" 2.0 in situ (THF-d_8–D_2O–CD_3CO_2D), and then the "pD" of the solution was adjusted to 1.0 with 1.0 N DCl–D_2O. The resulting solution was permitted to stand at rt (16 h). TLC analysis prior to workup indicated that the reaction was complete and no other significant product was noted other than 41 that migrated beyond the origin. The solution was neutralized ("pD" 7.0), and then the solvent was removed in vacuo. The residue was purified by PTLC (20% MeOH–$CHCl_3$) to give **41**: yield 6 mg (51%); mp 239–241 °C; R_f 0.25 (20% MeOH–$CHCl_3$); IR (KBr) 3305, 3130, 2930, 1670, 1625, 1435, 1095 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.91 (s, 2 H, CH_2N), 4.17 (d, J = 5.7 Hz, 2 H, $CHCH_2O$), 5.42 (s, 1 H, OH), 5.71 (t, J = 5.7 Hz, 1 H, $CHCH_2O$), 8.15, 8.17 (2 s, 2 H, 2 × NH); ^{13}C NMR (DMSO-d_6) 46.47 (CH_2N), 56.81 ($CHCH_2O$), 115.68 (C=$CHCH_2$), 126.83 (C=$CHCH_2$), 158.48, 163.48 (2 × NC=O) ppm; MS (+CI) m/e (rel intensity) 157 (100), 139 (72), 107 (88); M_r (EI) 156.058 49 (calcd for $C_6H_8N_2O_3$, 156.053 50).

Hydrolysis of 1-Acetyl-3-methoxy-3-vinyl-2,5-piperazinedione (23) under Acidic Conditions. Compound **23** (20 mg, 0.094 mmol) was dissolved in 25% H_2O–THF (1 mL), and then the "pH" was adjusted to 1.0 with an aqueous 1.0 N HCl solution. The resulting solution was permitted to stand at rt (16 h). TLC analysis prior to workup indicated that the reaction was complete, and no other significant product was noted other than 41 that migrated beyond the origin. The solution was neutralized

("pH" 7.0), and the solvent was removed in vacuo. The residue was purified by PTLC (20% MeOH–$CHCl_3$) to give **41**: yield 5 mg (34%); mp 239–241 °C; R_f 0.25 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 3.93 (s, 2 H, CH_2N), 4.20 (d, J = 5.7 Hz, 2 H, $CHCH_2O$), 5.51 (s, 1 H, OH), 5.72 (t, J = 5.7 Hz, 1 H, $CHCH_2O$), 8.13, 8.17 (2 s, 2 H, 2 × NH); ^{13}C NMR (DMSO-d_6) 46.09 (CH_2N), 56.89 ($CHCH_2O$), 115.74 ($C{=}CHCH_2$), 126.86 ($C{=}CHCH_2$), 159.00, 163.46 (2 × NC=O) ppm.

Methanolysis of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) under Acidic Conditions. A solution of crude **7** (30 mg, 0.153 mmol) in methanolic H_2SO_4 ("pH" 1.0) was permitted to stand at rt (12 h). The solution was neutralized to "pH" 6.8. The solvent was removed in vacuo, and the residue was purified by PTLC (30% acetone–chloroform) to give **23** (17 mg, 53%) and a small amount of product (~0.6 mg, 2%) which has been tentatively assigned as a 2:1 mixture of (*Z*)-**42a** and (*E*)-**42b**, 1-acetyl-3-(2-methoxyethylidene)-2,5-piperazinedione.

1-Acetyl-3-methoxy-3-vinyl-2,5-piperazinedione (23): ^1H NMR ($CDCl_3$) δ 2.59 (s, 3 H, $CH_3C{=}O$), 3.31 (s, 3 H, OCH_3), 4.19 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, $CHH'N$), 4.71 ($^1/_2$ ABq, J = 18.0 Hz, 1 H, $CHH'N$), 5.61 (d, J = 17.7 Hz, 1 H, $CHH'{=}CH$), 5.64 (d, J = 10.8 Hz, 1 H, $CHH'{=}CH$), 6.11 (dd, J = 10.8, 17.7 Hz, 1 H, $CH_2{=}CH$), 8.69 (s, 1 H, NH).

(*Z*)-1-Acetyl-3-(2-methoxyethylidene)-2,5-piperazinedione (42a): ^1H NMR (DMSO-d_6) δ 2.50 (s, 3 H, $CH_3C{=}O$), 3.94 (s, 2 H, CH_2N), 4.09 (d, J = 6.5 Hz, 2 H, $CHCH_2O$), 5.71 (t, J = 6.5 Hz, 1 H, $CHCH_2O$), 9.95 (s, 1 H, NH).

(*E*)-1-Acetyl-3-(2-methoxyethylidene)-2,5-piperazinedione (42b): ^1H NMR (DMSO-d_6) δ 2.50 (s, 3 H, $CH_3C{=}O$), 3.87 (s, 2 H, CH_2N), 4.38 (d, J = 6.5 Hz, 2 H, $CHCH_2O$), 5.44 (t, J = 6.5 Hz, 1 H, $CHCH_2O$), 10.25 (s, 1 H, NH).

Hydrolysis of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) at "pD" 6.0. Compound **7** (10 mg, 0.051 mmol) was prepared in situ at "pD" 2.0 in THF-d_8–D_2O–CD_3CO_2D, and the "pD" was adjusted to 6.0 with 1.0 N NaOD–D_2O. The solution was permitted to stand at rt (3 h). TLC analysis prior to workup indicated that the reaction was complete, and no other significant product was noted other than **41** that migrated beyond the origin. The solution was neutralized to "pD" 7.0, and then the solvent was removed in vacuo. The residue was purified by PTLC (20% MeOH–$CHCl_3$) to give **41**: yield 3 mg (38%); mp 238–241 °C; R_f 0.25 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 3.90 (s, 2 H, CH_2N), 4.19 (d, J = 5.7 Hz, 2 H, $CHCH_2O$), 5.43 (s, 1 H, OH), 5.73 (t, J = 5.7 Hz, 1 H, $CHCH_2O$), 8.07, 8.14 (2 s, 2 H, 2 × NH).

Hydrolysis of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) at "pD" 8.8. Compound **7** (15 mg, 0.076 mmol) was prepared in situ at "pD" 2.0 in THF-d_8–D_2O–CD_3CO_2D and the "pD" was adjusted to 8.8 with 1.0 N NaOD–D_2O. The resulting solution was permitted to stand at rt (30 min). The solution was neutralized to "pD" 7.0 with DCl–D_2O. The solvent was removed in vacuo, and the residue was purified by PTLC (20% MeOH–$CHCl_3$) to give **41** (2 mg, 13%) and **24** (4 mg, 45%).

3-(2-Hydroxyethylidene)-2,5-piperazinedione (41): mp 238–241 °C; R_f 0.25 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 3.90 (s, 2 H, CH_2N), 4.19 (d, J = 5.7 Hz, 2 H, $CHCH_2O$), 5.43 (s, 1 H, OH), 5.73 (t, J = 5.7 Hz, 1 H, $CHCH_2O$), 8.07, 8.14 (2 s, 2 H, 2 × NH).

N_α-Acetylglycinamide (24): R_f 0.05 (10% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.87 (s, 3 H, $CH_3C{=}O$), 3.57 (d, J = 5.8 Hz, 2 H, CH_2NH), 7.00 (br s, 1 H, $HH'NC{=}O$), 7.36 (br s, 1 H, $HH'NC{=}O$), 8.06 (t, J = 5.8 Hz, 1 H, CH_2NH). An authentic sample of **24**[25] was added to the NMR sample, and no new additional peaks were observed.

Studies on the Stability of Bicyclomycin in Near-Neutral and Moderately Basic Solutions. Bicyclomycin (**1**) (10 mg, 0.03 mmol) was added to a buffered KD_2PO_4 (0.10 M) D_2O–THF-d_8 (2:1) solution (0.50 mL, "pD" 5.7), and then the solution was maintained at rt. The reaction was monitored by ^1H NMR spectroscopy. No detectable change was noted after 24 h.

Bicyclomycin (**1**) (10 mg, 0.03 mmol) was added to a buffered $NaDCO_3$ (0.10 M) D_2O–THF-d_8 (2:1) solution (0.50 mL, "pD" 10.2), and then the solution was maintained at rt. The reaction was monitored by ^1H NMR spectroscopy. After 24 h, approximately 10% of **1** had been converted into a new adduct as evidenced by the appearance of a new set of vinylic protons upfield from the corresponding signals in **1**.

Treatment of 1-Acetyl-3-hydroxy-3-vinyl-2,5-piperazinedione (7) with NaSMe under Near-Neutral Conditions ("pD" 7.0). Compound **7** (10 mg, 0.051 mmol) was prepared at "pD" 2.0 in THF-d_8–D_2O–CD_3CO_2D in situ, the "pD" was adjusted to 4.9 with 1.0 N NaOD–D_2O, and then NaSMe (14 mg, 0.200 mmol) in a 3:1 mixture of THF-d_8–D_2O (0.5 mL) was added. The final "pD" of the resulting solution was adjusted to 7.0 with 0.1 N NaOD–D_2O. The solution was permitted to stand at rt (24 h). TLC analysis prior to workup indicated that the reaction was complete, and no other significant product was noted other than **41** that migrated beyond the origin. The solvent was removed in vacuo, and the residue was purified by PTLC (20% MeOH–$CHCl_3$) to give **41**: yield 2 mg (19%); R_f 0.25 (20% MeOH–$CHCl_3$); ^1H NMR (DMSO-d_6) δ 3.92 (s, 2 H, CH_2N), 4.18 (d, J = 5.7 Hz, 2 H, $CHCH_2O$), 5.44 (s, 1 H, OH), 5.74 (t, J = 5.7 Hz, 1 H, $CHCH_2O$), 8.17, 8.25 (2 s, 2 H, 2 × NH).

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