Synthesis of Naturally Occurring Antitumor Agents: Stereocontrolled Synthesis of the Azabicyclic Ring System of the Azinomycins

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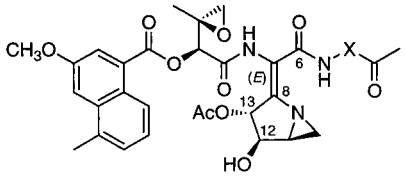
Abstract: Full details of the synthesis of the fully elaborated aziridino[1,2-*a*]pyrrolidine substructure **2** of the antitumor agents azinomycins A and B are reported. Stereoselective bromination of dehydroamino acid **4** provided control of olefin configuration in the final product, as a consequence of a stereospecific cyclization of aziridine **3** onto the proximal β-bromoacrylate, which effected pyrrolidine ring introduction. Dehydroamino acid **4** was constructed by olefination of aldehyde **5** with a glycine-based phosphonate. Two complementary synthetic routes to **2** are presented. In the first route, the selectively protected C12/C13 diol system of the targets was introduced into starting structure **6** in a stereocontrolled manner using Brown's (γ-alkoxyallyl)-diisopinocampheylborane reagent system. Transient protection of the C12 hydroxyl group of **48** as the trimethylsilyl ether was used to prevent C13 acetate migration prior to cyclization. Deprotection of the C12 hydroxyl following cyclization to the azabicyclic system afforded the extremely unstable core substructure **44**, which could not be isolated, but was characterized in situ. In the second route, the racemic γ-alkoxystannane **8** was added in a chelation-controlled manner to serinal derivative **9** under conditions of kinetic resolution for introduction of the C12 and C13 stereogenic centers of the target. Phenylacetate and methoxyacetate esters were used for C12 hydroxyl protection. This work represents the first synthesis of the intact core substructure **44** of this novel class of natural products.

Introduction

The antitumor agents azinomycins A (**1a**) and B (**1b**) were isolated in 1986 from cultures of *Streptomyces griseofuscus* S42227.[1] These agents possess an intricately functionalized structure containing the unusual aziridino[1,2-*a*]pyrrolidine system as part of a dehydroamino acid. This heterocyclic system presents the most significant synthetic challenge of these natural products since it possesses a significant proportion of the stereogenic elements and contains highly reactive functional groups that require careful protection and delicate timing of introduction.



Azinomycin A (X = CH_2) **1a**
Azinomycin B (X = C=CHOH) **1b**

The azinomycins exhibit potent in vitro cytotoxic activity and significant in vivo antitumor activity against P388 leukemia in mice.[2] However, detailed biological evaluation of these agents has been hampered by chemical instability and poor availability from natural sources. The presence of electrophilic epoxide and aziridine rings suggests that the azinomycins act by covalent alkylation and cross-linking of DNA, in a manner similar to that of mitomycin C.[3] Lown and Majumdar[4] demonstrated that azinomycin B (née carzinophilin)[5] covalently cross-links native DNA without prior activation. Studies on azinomycin/oligonucleotide interactions by Armstrong and co-workers[6] were interpreted to show cross-link formation between the agent and N7 of G and N7 of G or A within the major groove of DNA, and these results were confirmed recently by Saito and co-workers.[7] To date, the mechanism of action of these agents remains incompletely defined.[8]

The intricate and unusual structure, complex molecular mechanism of action, and effective antitumor activity make the azinomycins particularly attractive targets for synthetic efforts. An additional compelling rationale lies in the construction of structurally and functionally related agents for elucidation of the details of covalent interaction of these agents with oligonucleotides.[9] This is made particularly urgent because of the poor availability and instability of the agents.

(1) Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527. Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554.

(2) Ishizeki, S.; Ohtsuka, M.; Irinoda, K.; Kukita, K.; Nagaoka, K.; Nakashima, T. *J. Antibiot.* **1987**, *40*, 60. In vitro cytotoxicity: $IC_{50} = 0.07$ μg/mL (**1a**) and 0.11 μg/mL (**1b**) against L5178Y cells. In vivo antitumor activity: 193% ILS at 16 μg/kg **1b** (3/7 survivors) against P388 leukemia; 161% ILS at 32 μg/kg **1b** (5/8 survivors) against Erlich carcinoma. In the same system, mitomycin C exhibited a 204% ILS at 1 mg/kg against P388 leukemia.

(3) Tomasz, M.; Lipman, R.; McGuinness, B. F.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892 and references therein. For a recent review, see: Tomasz, M. *Chem. Biol.* **1995**, *2*, 575.

(4) Lown, J. W.; Majumdar, K. C. *Can. J. Biochem.* **1977**, *55*, 630.

(5) Azinomycin B is apparently identical to carzinophilin A, an antitumor agent isolated in 1954 from *S. sahachiroi*: Hata, T.; Koga, F.; Sano, Y.; Kanamori, K.; Matsumae, A.; Sugawara, R.; Hoshi, T.; Shimi, T.; Ito, S.; Tomizawa, S. *J. Antibiot. Ser. A.* **1954**, *7*, 107.

(6) Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144.

(7) Fujiwara, T.; Saito, I.; Sugiyama, H. *Tetrahedron Lett.* **1999**, *40*, 315.

(8) For a recent review of DNA cross-linking agents, see: Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.

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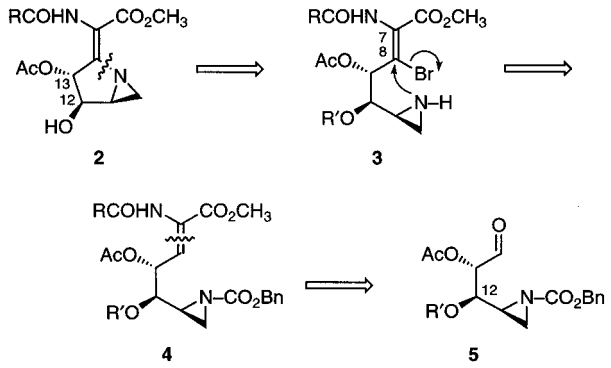
While there has been a significant amount of synthetic activity in the area,[10−15] to date no total synthesis of these agents has been reported, largely due to difficulties surrounding the selectively acylated C12/C13 diol system. With the exception of our work,[11] there are no reports of azabicyclic ring systems containing a differentiated C12/C13 diol system, nor are there reports of systems containing a free C12 hydroxyl group. Recently, Terashima et al.[8j] reported the synthesis of the C12/C13 bis-benzyl ether of the natural products, although these workers were unsuccessful in effecting either differentiation or deprotection of the diol.

We recently reported the successful development of two conceptually different routes to **2** that were based on the crotylstannylation of L-serinal[12] and on the use of a (γ-alkoxyallyl)diisopinocampheylborane reagent system[13] for introduction of the selectively acylated diol at the C12 and C13 stereogenic centers. Herein, we present full details of our syntheses of the fully elaborated aziridino[1,2-*a*]pyrrolidine substructure of the azinomycins that deals successfully with all structural features of this system, including the first reported introduction of the selectively protected 1,2-diol of the agents.[12,13] In the course of our studies on this substructure, we have uncovered a potential origin of the instability associated with the natural agents.

Synthetic Strategy

Synthetic challenges presented by these apparently simple natural products and specifically by substructure **2** include: (1) diastereocontrol in the introduction of the tetrasubstituted C7−C8 *E*-double bond, (2) incorporation of the differentially acetylated C12−C13 *vic*-diol from a suitably protected precursor, and (3) general difficulties surrounding the highly electrophilic aziridine ring, particularly as part of the larger, densely functionalized system.

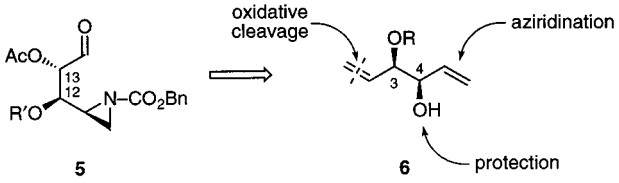
Our strategy for the synthesis of the 1-azabicyclo[3.1.0]hexane substructure is based on the cyclization of the aziridine of **3** onto a proximal (*E*)-β-bromoacrylate to form the pyrrolidine ring of target **2**.[14,15] Our original synthesis[14] used D-glucosamine as a chiral starting material for introduction of the three stereogenic centers of **2**, but we were unsuccessful at installing a removable C12 hydroxyl-protecting group using this route. Both *tert*-butyldimethylsilyl and benzyl ethers proved recalcitrant in attempts to remove them from late intermediates. This alcohol-protecting group must be carefully chosen to allow selective removal at a late stage of the synthesis of the aziridino[1,2-*a*]pyrrolidine ring system, in the presence of a diverse and congested assemblage of other functional groups.



In common with our previous work, a key synthetic intermediate en route to azabicyclic system **2** is dehydroamino acid **4**, which serves as a direct precursor to vinyl bromide **3**. In studies on the diastereoselective bromination of dehydroamino acids related to **4**, we demonstrated effective stereocontrol in the transformation of **4** to the desired *E*-vinyl bromide **3**.[16] This proved to be a critical transformation for achieving introduction of the C7−C8 tetrasubstituted *E*-olefin of the target molecules since the cyclization of **3** → **2** was found to be stereospecific and to occur with complete stereoselectivity. Aldehyde **5** serves as a fully elaborated precursor to the dehydroamino acid **4** via Wadsworth−Horner−Emmons olefination. Aldehyde **5** possesses the three stereogenic centers and selectively acetylated diol of the target.

The basis of the first synthetic plan described herein was the recog-

nition that an alkene could serve as a precursor to both the aldehyde and aziridine of **5**. Retrosynthetically, this gives rise to pseudosymmetrical diene **6**, wherein differentiation of the *syn*-diol serves to permit the introduction of the appropriate acylation pattern of the natural products and to provide a means for differentiation of the two double bonds. 1,5-Hexadien-3,4-diol **6** is available in enantiomerically pure form using Brown's (γ-alkoxyallyl)diisopinocampheylborane system.[17]



Since Brown's methodology produces **6** with an alkyl ether on the C3 hydroxyl group (R = CH_2OCH_3) and with the C4 hydroxyl group unprotected, it was ideally suited for our purposes. By virtue of its selectivity for allylic alcohols, the Sharpless asymmetric epoxidation reaction was the perfect accompaniment to the Brown chemistry and was used for differentiation of the two double bonds of **6**. The resulting 5,6-epoxide would then serve indirectly as a precursor to the aziridine of **5**. This meant that the C3 ether of **6** would be

(9) For molecular modeling work on the azinomycins relevant to their mechanism of DNA binding, see: Alcaro, S.; Coleman, R. S. *J. Org. Chem.* **1998**, *63*, 4620.

(10) (a) Bryant, H. J.; Dardonville, C. H.; Hodgkinson, T. J.; Shipman, M.; Slawin, A. M. *Synlett* **1996**, *10*, 973. (b) Bryant, H. J.; Dardonville, C. Y.; Hodgkinson, T. J.; Hursthouse, M. B.; Malik, K. M. A.; Shipman, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1249. (c) Armstrong, R. W.; Tellew, J. E.; Moran, E. J. *Tetrahedron Lett.* **1996**, *37*, 447. (d) Moran, E. J.; Tellew, J. E.; Zhao, Z.; Armstrong, R. W. *J. Org. Chem.* **1993**, *58*, 7848. (e) Armstrong, R. W.; Moran, E. J. *J. Am. Chem. Soc.* **1992**, *114*, 371. (f) Combs, A. P.; Armstrong, R. W. *Tetrahedron Lett.* **1992**, *33*, 6419. (g) Armstrong, R. W.; Tellew, J. E.; Moran, E. J. *J. Org. Chem.* **1992**, *57*, 2208. (h) Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 3807. (i) England, P.; Chun, K. H.; Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1990**, *31*, 2669. (j) Hashimoto, M.; Terashima, S. *Heterocycles* **1998**, *47*, 59. (k) Hashimoto, M.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 9409. (l) Hashimoto, M.; Terashima, S. *Chem. Lett.* **1994**, *6*, 1001. (m) Hashimoto, M.; Matsumoto, M.; Yamada, K.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 2207. (n) Hashimoto, M.; Yamada, K.; Terashima, S. *Chem. Lett.* **1992**, *6*, 975. (o) Konda, Y.; Machida, T.; Sasaki, T.; Takeda, K.; Takayanagi, H.; Harigaya, Y. *Chem. Pharm. Bull.* **1994**, *42*, 285. (p) Ando, K.; Yamada, T.; Shibuya, M. *Heterocycles* **1989**, *29*, 2209. (q) Shishido, K.; Omodani, T.; Shibuya, M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2053. (r) Shibuya, M.; Terauchi, H. *Tetrahedron Lett.* **1987**, *28*, 2619. (s) Shibuya, M. *Tetrahedron Lett.* **1983**, *24*, 1175.

(11) Coleman, R. S. *Synlett* **1998**, 1031.

(12) Coleman, R. S.; Richardson, T. E.; Carpenter, A. J. *J. Org. Chem.* **1998**, *63*, 5738.

(13) Coleman, R. S.; Kong, J.-S. *J. Am. Chem. Soc.* **1998**, *120*, 3538.

(14) Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 5813.

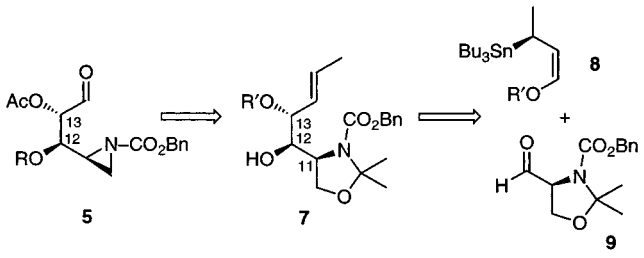
(15) Coleman, R. S.; Carpenter, A. J. *Tetrahedron* **1997**, *53*, 16313.

(16) Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1993**, *58*, 4452.

(17) Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535.

transformed to the C13 acetate ester of **5** and that a suitable protecting group would have to be installed at the free C4 hydroxyl group of **6**, which becomes C12 of **5**. We found that the a *p*-methoxybenzyl ether was an effective solution for protection of the C12 hydroxyl group of **2**, a seemingly trivial problem whose solution had escaped us to this point. In the end, we uncovered a significant degree of instability associated with the free C12 hydroxyl group that may explain prior difficulties we had in deprotecting the hydroxyl group at this position.

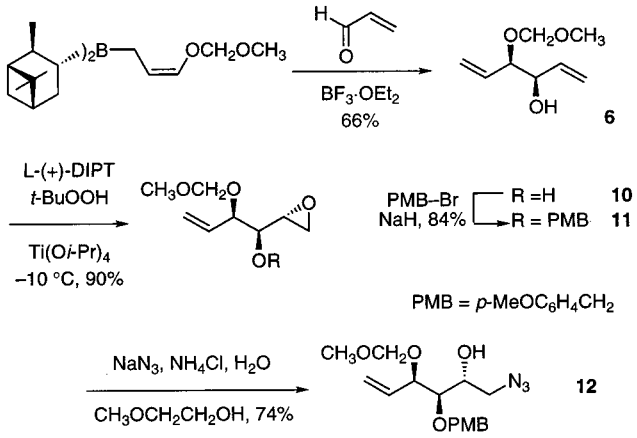
The second synthetic approach to the core aziridino[1,2-*a*]-pyrrolidine system was based on chelation-controlled addition of a γ-alkoxycrotylstannane to serinal for the key C12−C13 bond construction. Marshall and co-workers[18] demonstrated that γ-alkoxycrotylstannanes (**8**, R′ = $SiMe_2t$-Bu, CH_2OCH_3) undergo Lewis-acid promoted addition to α-aminoaldehydes with *syn*-stereoselectivity at the newly formed bond.[19] In the context of the azinomycins, this strategy would produce **7** with the C12 position *unprotected* and would thereby permit the divergent introduction of C12 hydroxyl-protecting groups (azinomycin numbering throughout). In practice, we used phenylacetyl and methoxyacetyl esters as C12 hydroxyl-protecting groups. Appropriate stereoselection for the C11−C12 *syn*/C12−C13 *syn*-diastereomer **7** in the addition of stannane **8** to aldehyde **9** is a consequence of a chelated aldehyde (C11−C12 bond) and the *anti*-S_E' transition state for crotylstannane addition (C12−C13 bond).



Results

Hexadienediol Route. The sequence of transformations from diol **6**, prepared as shown in 66% yield (>95% ee) following Brown et al.,[17] to the key aldehyde **5**, proceeded in greater than 35% yield for the eight-step conversion. The two olefins of **6** were easily differentiated by virtue of the allylically disposed hydroxyl group using a Sharpless asymmetric epoxidation.[20] Under standard conditions with the L-(+)-diisopropyl tartrate catalyst, epoxide **10** was obtained uneventfully in 90% yield and ≥98% enantiomeric excess. Preventing this reaction from going totally to completion served to increase the enantiomeric purity of the system by virtue of the kinetic resolution that can occur during asymmetric epoxidations.[21]

At this juncture we were faced with the choice of a C12 hydroxyl-protecting group. We had gained considerable experience in this matter from our earlier studies, albeit without discovery of a viable protection scheme for the 1,2-diol. We had unsuccessfully examined the overly stable *tert*-butyldimethylsilyl and benzyl ethers at this position, and a triethylsilyl group was found to be too labile.[15] After considering deprotection conditions that would be orthogonal with the reactivity patterns of various late synthetic intermediates and anticipating compatibility with pending transformations, we opted for the *p*-methoxybenzyl (PMB) ether, which can be removed under neutral, mildly oxidizing conditions. In addition, an ether-protecting group was complementary with the concurrent crotylstannane based synthesis (vida infra).



Alkylation of the remaining alcohol of **10** with sodium hydride and *p*-methoxybenzyl bromide, afforded **11** (84%) and occurred without rearrangement of the epoxide. Addition of azide[22] to the terminal carbon of the epoxide **11** provided a 74% yield of primary azide **12**. On larger scales this reaction was difficult to force to completion and we would typically isolate unreacted starting epoxide, which was resubjected to the reaction conditions. Transformation of the azide to the amine by reduction with triphenylphosphine in a toluene/water mixture[23] and N-acylation of the resulting primary amine **13** with benzyl chloroformate and triethylamine afforded carbamate **14** in quantitative yield over the two-step procedure. Manipulation of the hydroxyl-protecting and activating groups proceeded by acylation of the free secondary alcohol of **14** with methanesulfonyl chloride in the presence of triethylamine to afford mesylate **15** (96%), cleavage of the methoxymethyl acetal with methanolic HCl (74%), and introduction of the azinomycin C13 acetate by standard acylation with acetic anhydride and pyridine (99%) to afford **16**. Acid-catalyzed cleavage of the acetal of **15** was accompanied by a sometimes significant amount of *p*-methoxybenzyl ether cleavage, which could be minimized by carefully monitoring the reaction as it progressed. Cyclization of **16** to the aziridine **17** occurred upon low-temperature deprotonation of the carbamate of **16** with potassium *tert*-butoxide and effectively provided the pivotal intermediate **17** (100%) in an overall yield of >35% from **6**. This compound possesses all of the functionality and protecting groups for elaboration to the azinomycin core, including the essential C13 acetate ester and a readily removable *p*-methoxybenzyl ether at the emergent C12 position.

Crotylstannane Route. Aldehyde (*S*)-**9**[24] was complexed with magnesium bromide etherate at −20 °C followed by the slow addition of crotylstannane (*S*)-**8**.[25] Upon warming the reaction mixture to 25 °C, stannane addition to the aldehyde occurred to afford the selectively protected diol **7** in near quan-

(18) Marshall, J. A.; Seletsky, B. M.; Coan, P. S. *J. Org. Chem.* **1994**, *59*, 5139.

(19) For the *syn*-selective addition of a vinylzinc reagent to an α-aminoaldehyde, see: Coleman, R. S.; Carpenter, A. J. *Tetrahedron Lett.* **1992**, *33*, 1697.

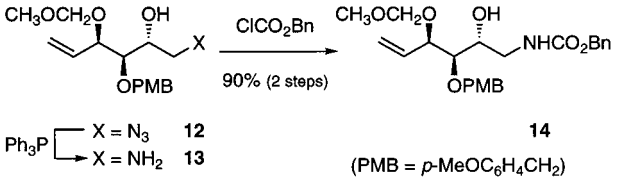
(20) Johnson, R. A.; Sharpless, K. B. In *Comprehensive Organic Synthesis*; Trost, B. M., Ley, S. V., Eds.; Pergamon Press: Elsmford, NY, 1991; Vol. 7, p 389.

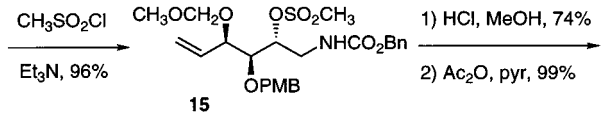
(21) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237.

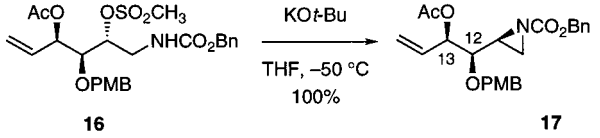
(22) Behrens, C. H.; Ko, S. Y.; Sharpless, K. B.; Walker, F. J. *J. Org. Chem.* **1985**, *50*, 5687.

(23) Knouzi, N.; Vaultier, M.; Carrie, R. J. *Bull. Soc. Chim. Fr.* **1985**, 815.

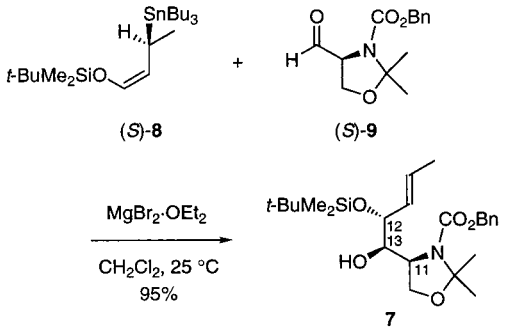
(24) Preparation of aldehyde **6** proceeded smoothly, following the published protocol: Garner, P.; Park, J. M. *Org. Synth.* **1992**, *70*, 18.







titative yield with >10:1 selectivity for the *syn*-diastereomer. The only minor isomer that could be detected was the corresponding *Z*-olefin, which was inconsequential to our purposes, as we planned to cleave the double bond by ozonolysis.



Performing the addition to (*S*)-**9** with 2.3 equiv of the racemic stannane *rac*-**8** effected useful levels of kinetic resolution (>10:1 *S*/*R*) and this obviated the tedious and expensive preparation of the enantiomerically pure γ-alkoxystannane (*S*)-**8**.[26] Separation of the addition product from unreacted stannane **8** could be achieved in subsequent chromatographic purifications. The atom-economy based on consumed tin was a concern because we were throwing away half of *rac*-**8** in the kinetic resolution. In the conversion of **8** + **9** → **7**, comparing the racemic versus enantiomerically pure γ-alkoxycrotylstannane **8**, the overall atom economy based on total *n*-Bu_3SnH consumed is 6.3 mol of Sn per mol of **7** for the racemic stannane compared to 14.4 mol of Sn per mol of **7** for the enantiomerically pure stannane. Thus, the kinetic resolution is more than *twice* as efficient, based on total tin consumed. In addition, the expense of the reagents necessary for the synthesis of enantiomerically pure (*S*)-**8** further increased the economic advantage of performing this reaction in the kinetic reaction mode, particularly considering that this was the first synthetic step in the total synthesis effort.

Following installation of the two stereogenic centers at the emergent C12 and C13 positions, we needed to introduce a C12-protecting group that could be removed at a later stage of the synthesis in the presence of a considerable number and variety of other functional groups, particularly in the presence of the C13 acetate. For this duty we chose an ester group for protection, for the following reasons: (1) the ester linkage was compatible with all projected transformations en route to the final product; (2) there is a great deal of flexibility possible within the ester family, particularly with respect to mechanism of and reagents for cleavage; (3) alkyl ethers were impossible to install at C12 of **7** and later intermediates due to a competing cyclization of the alkoxide onto the proximal benzyl carbamate to form the corresponding oxazolidinone.

For C12 hydroxyl protection we selected an ester that could be removed selectively as a consequence of a differential in the rate of hydrolysis compared to an acetate ester, either enzymatically or chemically. The phenylacetyl group was selected from among several alternatives (e.g., hydrocinnamyl, valeryl), because the benzyl group of a phenylacetyl group is recognized by penicillin G acylase.[27] A methoxyacetate was selected as the alternate in anticipation of its heightened lability relative to a simple acetate ester.[28]

The sterically encumbered C12 hydroxyl group of **7** was unreactive toward forcing acylation conditions with acid chlorides or anhydrides, so the *N*,*O*-acetonide was removed prior to acylation. An additional motivation for early cleavage of the *N*-acyl oxazolidine ring of **7** comes from the fact that these systems exist as a mixture of slowly interconverting rotamers, which seriously complicated analysis by NMR spectroscopy.

Cleavage of the oxazolidine ring of **7** occurred upon warming in THF in the presence of ethylene glycol and camphorsulfonic acid. The diol **18** was acylated at the primary hydroxyl group with methanesulfonyl chloride and triethylamine to afford **19**. The secondary hydroxyl group was protected as the ester (R^2 = CH_2Ph or CH_2OCH_3) by treatment with the corresponding carboxylic acid in the presence of dicyclohexylcarbodiimide (DCC) and (dimethylamino)pyridine (DMAP) to afford **20** and **21**. Interchange of C13 hydroxyl-protecting groups was accomplished by removal of the silyl group from **21** or **22** with hydrofluoric acid and acylation of the resulting alcohol with acetic anhydride and triethylamine to afford **22** and **23**. In six high-yielding steps (57% overall yield), the advanced intermediates **22** and **23** were generated with complete control of absolute stereochemistry and introduction of suitable protecting groups. Final aziridine installation was achieved by treatment of methanesulfonate **22** or iodide **24** with potassium *tert*-butoxide at −43 °C afforded **25** and **26**, respectively, in high yields. Iodide **24** was obtained from **23** with sodium iodide; methanesulfonate **23** could not be induced to cyclize effectively.

Dehydroamino Acids. With the important aldehyde precursors in hand and readily available in multigram quantities, we proceeded along the established plan for elaboration to the azinomycin core substructure. Installation of the dehydroamino acid system was preceded by oxidative cleavage of the terminal olefin of **17** using ozone with dimethyl sulfide workup to afford in high yield the aldehyde **27**. While not particularly labile, this aldehyde was carried into the subsequent Wadsworth-Horner-Emmons olefination without purification[29] using the

(25) Marshall, J. A.; Welmaker, G. S.; Gung, B. W. *J. Am. Chem. Soc.* **1991**, *113*, 647.

(26) The preparation of enantiomerically pure γ-alkoxycrotylstannanes requires the reduction of an unstable intermediate acylstannane with BINAL-H, and the optimal reagent for preparation of the acylstannane from the α-hydroxystannane was expensive azocarbonyl dipiperidine (ADD.; $13/mmol).

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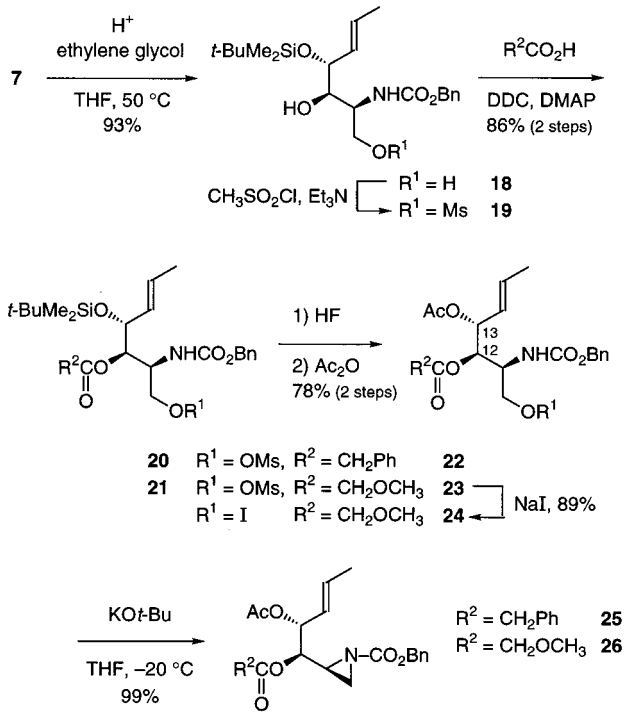
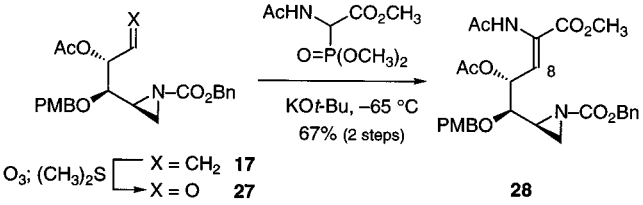
(28) Reese, C. B.; Stewart, J. C. M. *Tetrahedron Lett.* **1968**, 4273. Reese, C. B.; Stewart, J. C. M.; van Boom, J. H.; de Leeuw, H. P. M.; Nagel, J.; de Rooy, J. F. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 934.



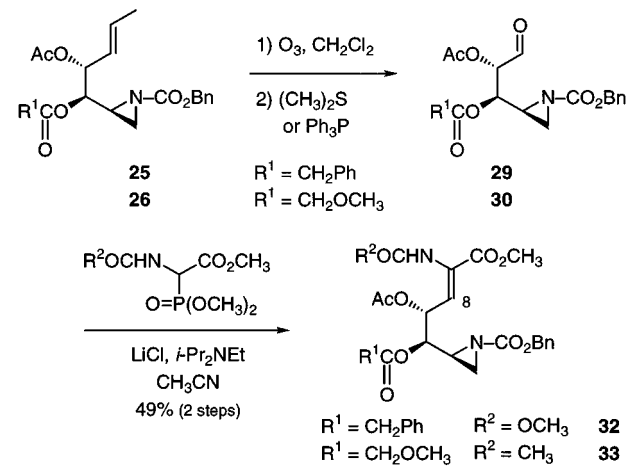
N-acetyl glycine phosphonate[30] and potassium *tert*-butoxide, to afford olefin **28** as a >4:1 mixture of Z/E isomers in 60−70% yield.

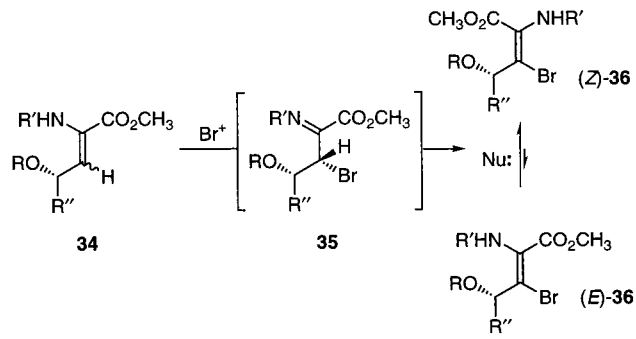


Similarly, ozonolysis of the double bond of **25** and **26** afforded aldehydes **29** and **30**, respectively. Olefination with the *N*-methoxycarbonyl or *N*-acetyl glycine phosphonates[31] afforded dehydroamino acids **32** and **33**, respectively, as a mixture of Z/E isomers (typically 2.5:1) in modest yields under carefully optimized reaction conditions with diisopropylethylamine and lithium chloride.[32] The ratio of olefin stereoisomers is irrelevant, since both isomers converge to the same mixture of stereoisomeric vinyl bromides in the subsequent bromination reaction sequence. The use of potassium *tert*-butoxide in THF or CH_2Cl_2 was less successful, and provided the olefin **32** in yields typically below 25%.

These olefination reactions were particularly difficult because of the presence of the proximal acetoxy group at C13. Under carefully controlled reaction conditions the isolated yields were more than satisfactory, and were justified given the delicacy of C13-protecting group manipulations on more highly elaborate systems. We had established quite definitively in earlier studies the undesirability of manipulating diol-protecting groups once the dehydroamino acid double bond had been installed.

Dehydroamino Acid Bromination. Defined reaction conditions had been developed earlier for stereocontrolled installation of a C8-vinyl bromide into dehydroamino acids.[16] We had observed that systems of general structure **34** undergo reaction with *N*-bromosuccinimide (NBS) at room temperature to produce the intermediate α-bromoimines **35**, which undergo tautomerization upon treatment with base to the diastereomeric (*E*)- and (*Z*)-*β*-bromo-α,*β*-dehydroamino acids (*E*)-**36** and (*Z*)-**36**, respectively. Both the *Z*- and *E*-isomers of dehydroamino acids **34** afforded the same ratio of isomeric vinyl bromides.



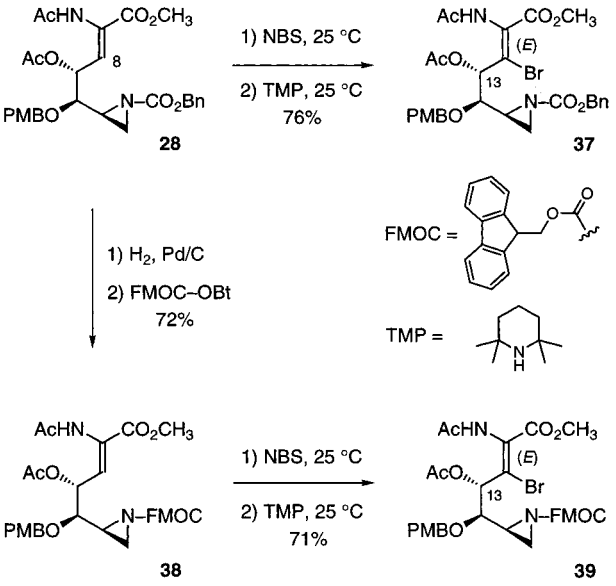


Stereocontrol in this transformation was important since our previous studies had demonstrated that the cyclization used for pyrrolidine introduction was stereospecific.[12−15] By setting olefin stereochemistry at the stage of (*E*)-**36**, we were assured the formation of the correct *E*-isomer of the final bicyclic product. The undesired *Z*-vinyl bromides (*Z*)-**36** are the thermodynamically more stable isomers, and are typically formed from the kinetic *E*-isomers (*E*)-**36** by nucleophile-induced isomerization, for example with 1,8-diazabicyclo[2.2.2]octane (DABCO). High levels of stereoselectivity for the kinetic products (*E*)-**36** were obtained by treatment of the intermediate α-bromoimines **35** with sterically hindered bases such as 2,2,6,6-tetramethylpiperidine (TMP) or potassium *tert*-butoxide. In our earlier studies, we demonstrated reaction conditions for the *stereodivergent* preparation of both *E*- and *Z*-vinyl bromide products **36** from either diastereomeric *E*- or *Z*-olefin starting materials **34** with acceptably high levels of diastereoselectivity.

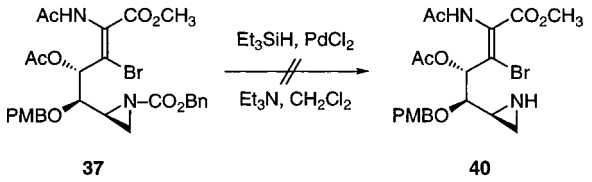
When olefin **28** was treated with 1 equiv of *N*-bromosuccinimide (NBS) in $CHCl_3$ at room temperature, a mixture of the stereoisomeric α-bromoimines was obtained. Treatment of the α-bromoimines with 2,2,6,6-tetramethylpiperidine effected tautomerization to the desired vinyl bromide (*E*)-**37** with >5:1 E/Z stereoselectivity as measured by ^{1}NMR analysis of the crude reaction mixture. Demonstration of stereochemistry was made by nuclear Overhauser enhancement between the NH and C13−H protons of (*E*)-**37**, and the lack of a similar enhancement

(29) Lieberknecht, A.; Schmidt, J.; Stezowski, J. J. *Tetrahedron Lett.* **1991**, *32*, 2113.
(30) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53.
(31) Zoller, U.; Ben-Ishai, D. *Tetrahedron* **1975**, *31*, 863.
(32) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.

with the corresponding *Z*-isomer, which was isolated in minor quantities from the bromination/tautomerization reaction of **28**. In addition, we made use of an extensive set of chemical shift correlations generated during our previous work. In this correlation, the allylic (C13) proton of the *E*-bromides consistently resonated upfield of the same proton in the *Z*-isomer.[15]

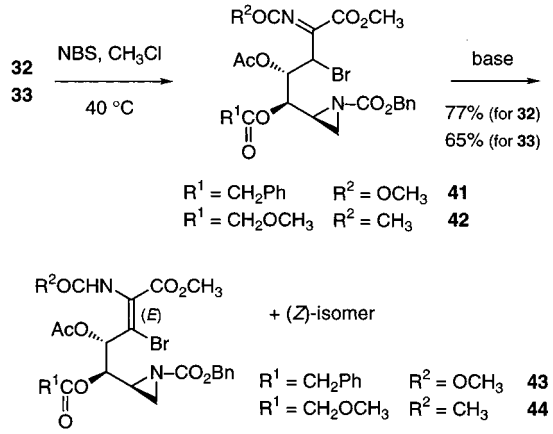


Normally, aziridine N−CO_2Bn-protecting groups can be easily removed using triethylsilane and a palladium chloride catalyst,[33,34] but for nonobvious reasons the benzyl carbamate of **37** was resistant to these reaction conditions, and under forcing conditions consumption of starting material was observed without the production of isolable product(s). At best, free aziridine **40** was produced as a minor product under these conditions.

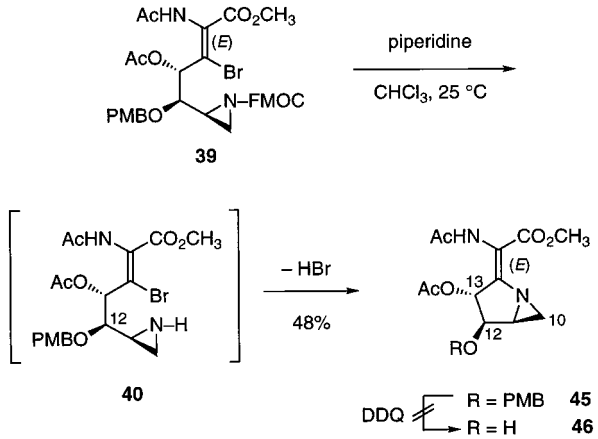


For this reason, we opted to proceed in the synthesis using a different aziridine-protecting group. The 9-fluorenylmethoxycarbonyl (FMOC)-protecting group[35] seemed potentially ideal, being removed rapidly by treatment with a secondary amine base. Retreating one step to intermediate **28**, we could interchange the CO_2Bn for an FMOC group by hydrogenolysis followed by acylation of the aziridine nitrogen with FMOC−OBt[36] (Bt = benzotriazolyl) to afford **38** in good yields. Bromination of **38** proceeded without problem to afford (*E*)-**39** with 12:1 diastereoselection.

In contrast to the *p*-methoxybenzyl-protected system, in the bromination of phenylacetyl and methoxyacetyl-protected **32** and **33**, we found that the intermediate α-bromoimines **41** and **42** produced by treatment of either (*Z*)- or (*E*)-**32** or **33** with *N*-bromosuccinimide underwent only a modestly stereoselective base-promoted tautomerization using potassium *tert*-butoxide. The desired bromides (*E*)-**43** and (*E*)-**44** was obtained in at best a 3.5:1 ratio to the *Z*-isomer, but more typically 1:1. This is a notable divergence from the other system, wherein we obtained ≥10:1 *E*-selectivity when sterically bulky bases were used. The origin of this divergence is not known, but the C12 ester is obvious to implicate. In practice, the undesired isomer (*Z*)-**43** and (*Z*)-**44** was used to develop subsequent synthetic steps, and so was not entirely useless.



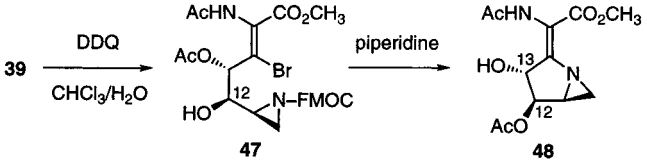
Azabicyclic Systems and C12 Hydroxyl Group Deprotections. The 9-fluorenylmethoxy carbamate was removed easily from **39** by treatment with piperidine to afford the intermediate aziridine **40**, which cyclized at room temperature by displacement of the vinylic bromide to afford aziridino[1,2-*a*]pyrrolidine **45** in good yield. We had previously demonstrated this cyclization reaction to be stereospecific,[11] and the *E*-stereochemistry of olefin **45** was confirmed by ^{1}H NMR spectroscopy by observation of a strong reciprocal NOE between the C13−H and proximal NH, and by the characteristic chemical shift of the C13−H and NH protons.[11] A strong five-bond *W*-coupling (J_5 = 1.1 Hz) was observed across the bicyclic system between the C13−H and C10−H_{endo} of the aziridine. Unfortunately, we could not remove the C12-PMB ether from **45** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[37] without causing concomitant destruction of the aziridine ring system.



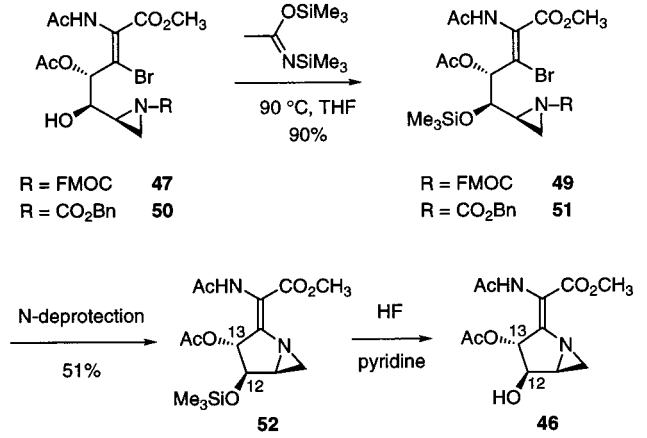
The C12 *p*-methoxybenzyl-protecting group could be removed at the stage of **39** to afford the corresponding free alcohol **47** in good yield, using standard literature conditions with DDQ in a mixed aqueous/$CHCl_3$ solvent system. Alcohol **47** was surprisingly unstable to silica gel chromatography even over triethylamine-deactivated silica, but it could be partially purified by chromatography over Sephadex. Deprotection of the FMOC

(33) Birkofer, L.; Bierwirth, E.; Ritter, A. *Chem. Ber.* **1961**, *94*, 821.
(34) Coleman, R. S.; Shah, J. A. *Synthesis* **1999**, 1399.
(35) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
(36) Paquet, A. *Can. J. Chem.* **1982**, *60*, 976.
(37) Nakajima, N.; Abe, R.; Yonemitsu, O. *Chem. Pharm. Bull.* **1988**, *36*, 4244.

carbamate of **45** with piperidine afforded an intermediate free aziridine, which cyclized under the deprotection reaction conditions to afford an aziridino[1,2-*a*]pyrrolidine system, the structure of which was assigned as **48** where the C13 acetate had *migrated* to the free C12 alcohol. This was apparent in the ^{1}H NMR spectrum of the product, which showed the diagnostic C13−H upfield from its expected chemical shift. Presumably this migration occurred prior to cyclization, under the basic conditions used to remove the FMOC carbamate, as it seems unlikely that acetate migration could occur across the *trans*-diol system of the rigid five-membered ring. It was surprising to obtain only a single regioisomeric acetate from this reaction, but considering the instability of systems containing the free C12 hydroxyl group (vida infra), it may be that **48** is the only stable product of the mixture produced in the reaction.

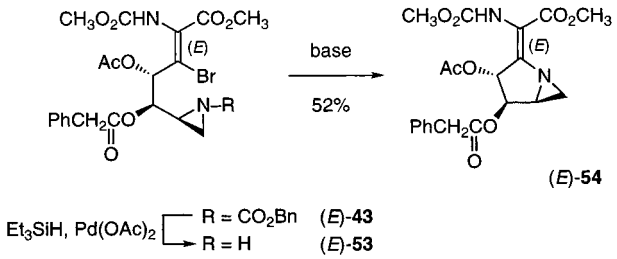


This migration problem was solved by a transient protection of the C12 alcohol of **47** as the labile trimethylsilyl ether **49**, which was formed by treatment of **47** with neat *N*,*O*-bis-trimethylsilylacetamide at 90 °C. Similarly, the corresponding N−CO_2Bn-protected system **50** (prepared from **37** by DDQ oxidation) could be silylated to afford **51**. Removal of the FMOC carbamate from **49** with piperidine, or the benzyl carbamate from **51** with triethylsilane and palladium chloride (this reaction now worked well in the presence of a C12-trimethylsilyl ether), and cyclization afforded the aziridino[1,2-*a*]pyrrolidine system **52**, as a moderately stable, although isolable intermediate.

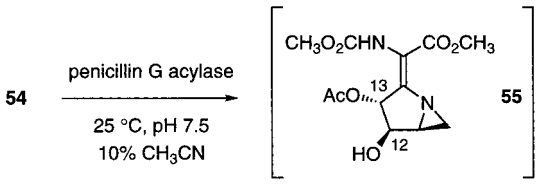


Mild deprotection of the C12 silyl ether of **52** with commercial 70% HF·pyridine in THF afforded the target substructure **46**, which could not be isolated, but was characterized by ^{1}H NMR and high resolution mass spectroscopy. It was surprising that the presence of a free hydroxyl group introduced such a significant degree of instability into the system, especially considering the stable nature of preceding intermediates. Repeated attempts to isolate **46** under extremely mild conditions were unsuccessful, leading completely to unidentifiable products. Even removing the reaction media in vacuo was sufficient to cause significant decomposition. In the end, we were able to obtain sufficient data from in situ ^{1}H NMR in THF-d_8 to provide characterization of **46**. The chemical shifts of the protons on the azabicyclic ring agreed well with those reported for the azinomycins.[1]

From these studies, we learned that a free C12 hydroxyl group would participate as the receiving partner in a thermodynamically driven acetate migration reaction from the proximal C13 ester prior to cyclization, and that C12 ester deprotection must await final closure of the pyrrolidine ring. In the ester-protected system, triethylsilane mediated removal of the aziridine N−CO_2-Bn group of **43** cleanly afforded the corresponding free aziridine **53**, which underwent cyclization upon warming in the presence of *N*-methylmorpholine to afford the pyrrolidine **54**. This product was accompanied by a significant amount of byproduct tentatively identified as the product formed from opening of the aziridine ring by bromide. This byproduct could be suppressed by performing the reaction in the presence of Dowex anion-exchange resin in the carbonate form, which served both as a base and bromide ion scavenger. The cyclization was found to be stereospecific.



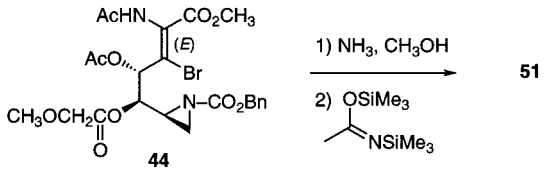
Deprotection of the C12 phenylacetyl ester of **54** could be achieved by treatment with polymer-supported penicillin G acylase in a mixed solvent system of acetonitrile/aqueous buffer. Since systems such as **55** where the C12 hydroxyl group is unprotected are not sufficiently stable to permit isolation, we attempted to characterize **55** formed in situ using ^{1}H NMR (500 MHz, 9:1 D_2O/CD_3CN). Under carefully optimized conditions, the phenylacetate ester of **54** could be removed with an approximate half-life of 2 h, and **55** could be observed as one of several products of this reaction. Since neither isolation nor further characterization of **55** could be achieved, we concluded from the data that **55** was undergoing further reaction at rates similar to its enzymatic production from **54**.



We were unsuccessful at removing the C12 phenylacetate ester prior to cyclization, which may be the result of the sterically crowded environment around the ester carbonyl group. A number of systems related to **43** were completely unreactive even upon extended exposure to penicillin G acylase, whereas the very early intermediate **24** was smoothly hydrolyzed to the corresponding alcohol under these conditions (data not shown).

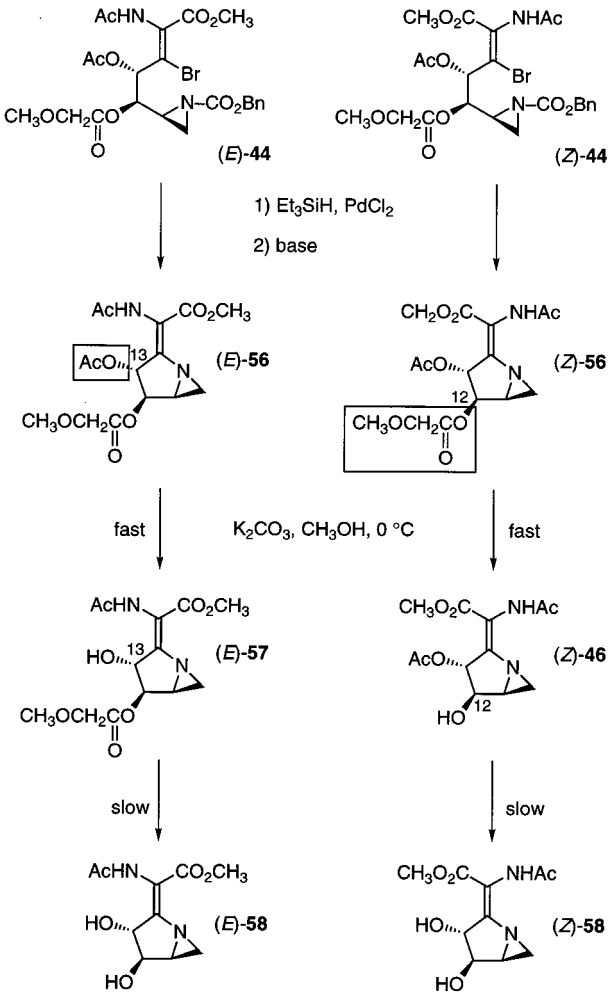
In contrast, the labile methoxyacetate ester of uncyclized (*E*)-**44** could be removed selectively by virtue of its enhanced susceptibility to saponification using K_2CO_3 in methanol at 0 °C. Subsequent silylation of the (previously characterized) C12 alcohol with *N*,*O*-bis-trimethylsilylacetamide afforded **51**, thereby junctioning the two synthetic routes.

With the aim of removing a C12 methoxyacetate as the final synthetic operation, cyclization of the methoxyacetate-protected system (*Z*)- and (*E*)-**44** was achieved uneventfully by deprotection with triethylsilane and palladium chloride to afford the



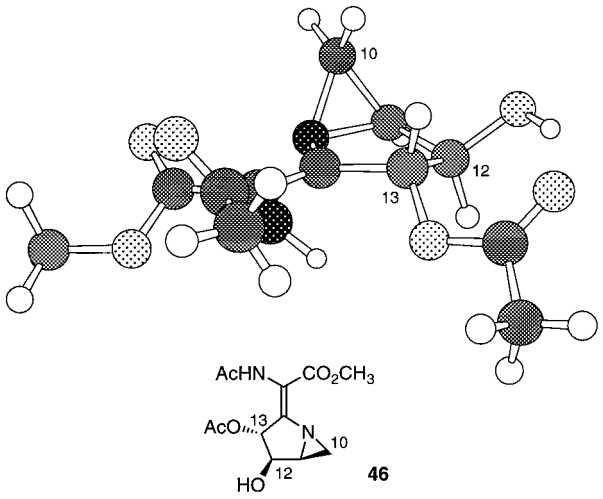
corresponding free aziridines. Cyclization as before to the bicyclic system (*E*)- and (*Z*)-**56** occurred with complete stereospecificity in modest yields.

To our disappointment, the behavior of the two isomers of **56** was remarkably different toward hydrolytic conditions. We could achieve selective saponification of the more labile C12 methoxyacetate ester on the *wrong Z*-isomer using K_2CO_3 in methanol at 0 °C, to afford (*Z*)-**46**, isomeric with the bicyclic system of the natural products. Upon more extended exposure to these reaction conditions, the C13 acetate ester was hydrolyzed to afford unstable diol (*Z*)-**58**. In contrast, with the *correct E*-isomer of **56**, the C13 acetate ester was slightly more labile toward saponification, and the initial product formed was the C13 alcohol (*E*)-**57**, which underwent a less rapid saponification of the C12 methoxyacetate ester to afford diol (*E*)-**58** over the course of 1 h. In neither case was there a useful difference in the rates of saponification of the two esters. It seemed that the electronic factors favoring selective hydrolysis of the methoxyacetate were overriden by biases resident in the densely functionalized bicyclic system.



Conclusions

Our observations on the instability of the azinomycin core substructure **46** and **55** may provide at least a partial explanation of the unstable character of the natural products. While we would not have conjectured a priori that the C12 hydroxyl group would have played a major role in stability issues, it seems clear from the studies described herein that this functional group is to a significant degree the cause. However, without clear evidence on the reaction pathway by which the C12 hydroxyl reacts, which we have been unable to obtain, we can offer no rationale at this time as to why this group effects the stability of the agents. The stability of more advanced systems remains to be demonstrated.



With respect to the rather extensive amount in effort directed toward protecting group issues for the 1,2-diol of the natural products, we have been unable to devise a protecting group scheme that permitted either the chemo- or regioselective deprotection of the C12 hydroxyl group on an azabicyclic system. In recourse, we turned to the labile trimethylsilyl ether that was installed immediately prior to cyclization, and which could be removed selectively as the final synthetic step to afford the native azabicyclic system of the azinomycins.

We have described the first synthesis of the fully elaborated core substructures **46** and **55** of the azinomycins, including a description of a protecting group strategy for the selectively acylated C12/C13 diol of the natural products. This synthesis of **46** proceeds in 15 steps from diene **6** or 11−12 steps from crotylstannane **8** and aldehyde **9** in a modest overall yield that is reflective of the high degree of complexity and instability of the target system. We have constructed, for the first time, a system containing a free C12 hydroxyl group, and we have placed this functional group in a key position in defining the origin of the instability of the natural agents.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (CA-65875). We thank Dr. Andrew J. Carpenter for his important contributions to the early stages of the work on the stannane-based route[12] and to earlier work as cited. During the course of this work, R.S.C. was the recipient of a Dreyfus Foundation Distinguished New Faculty Award (1989-94), an American Cancer Society Junior Faculty Research Award (1990-93), the American Cyanamid Young Faculty Award (1993-96), and an Alfred P. Sloan Foundation Research Fellowship (1995-98).

Supporting Information Available: Detailed experimental protocols and characterization of synthetic intermediates (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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