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The Development of Strategies for Construction of the Aziridine Core of the Antitumor Agents Azinomycins A and B

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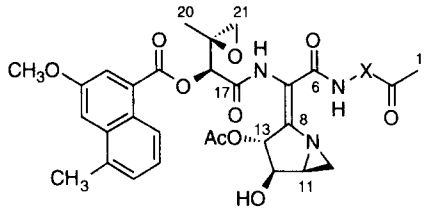
Abstract: The synthesis of the C6-C13 aziridino[1,2*a*]pyrrolidine core substructure of the antitumor agents azinomycins A (**1a**) and B (**1b**) is described. Key synthetic steps included Wadsworth-Horner-Emmons olefination for formation of the C7-C8 double bond, an E-selective electrophilic bromination of the C8 position, and a stereospecific intramolecular addition-elimination reaction sequence for formation of the N-C8 pyrrolidine bond.

We have been involved for several years with a total synthesis of the structurally unique antitumor agents azinomycins A (**1a**) and B (**1b**).[1] During the course of our work, we have uncovered a number of complexities exhibited by these deceptively simple natural products. As with any synthetic target, the azinomycins presented a number of obvious and not so obvious hurdles, but perhaps unappreciated at the start of our work were the additional problems inherent in these small, densely functionalized molecules. In response, we have developed carefully crafted synthetic strategies to circumvent problems encountered en route. Herein, we describe full details of our studies on the construction of the aziridino[1,2-*a*]pyrrolidine ring system of these agents.

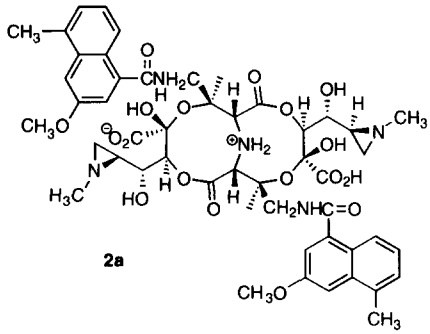


Azinomycin A (1a) X = CH_2
Azinomycin B (1b) X = CH=CHOH

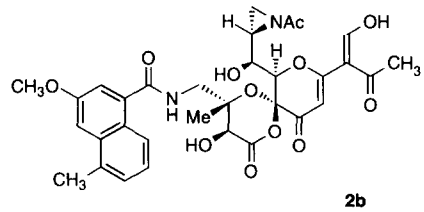
INTRODUCTION

Azinomycins A (**1a**) and B (**1b**) are antitumor antibiotics isolated from the culture broth of strain *Streptomyces griseofuscus* S42227 by Nagaoka *et al.* in 1986.[2] The absolute configuration at the C18 and C19 stereogenic centers were assigned by Shibuya and Terauchi through partial synthesis from D-glucose.[3] Azinomycin B has been suggested to be identical to the previously described antitumor agent carzinophilin A,[4]

whose structure has been the subject of considerable debate since its isolation in 1954 from cultures of *Streptomyces sahachiroi*.[5] It was not until nearly three decades later that Lown proposed incorrectly that carzinophilin existed as dimeric structure **2a**, based on inaccurate molecular weight data.[6] Lown's structure was later revised by Onda and co-workers,[7] who incorrectly reported carzinophilin to correspond to **2b**. It is now apparent that these natural products are identical.



2a



2b

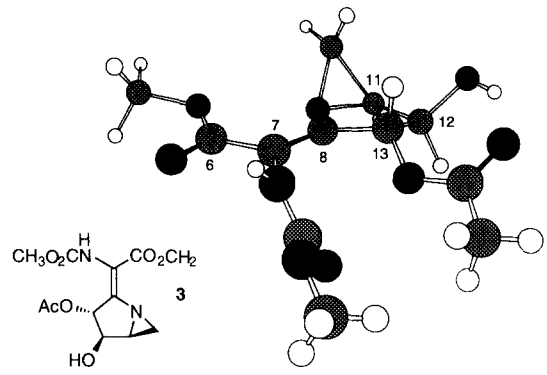
Azinomycins A and B possess potent *in vitro* cytotoxicities (IC_{50} = 0.07 μg/mL and 0.11 μg/mL, respectively) against L5178Y cells in tissue culture and *in vivo* antitumor activity measured using various murine transplantable tumors in mice.[8] Administration of azinomycin B (32 μg/kg/d) resulted in 57% survivors at 45 d and a 193% ILS against P388 leukemia. In the same assay mitomycin C exhibited 57% survivors and 204% ILS, but at a higher dose (1 mg/kg/d). Azinomycin A appeared less effective than azinomycin B in these tests. The LD_{50} of azinomycin B by a single intraperitoneal injection was 190 μg/kg.

The structures of the azinomycins suggests the possibility for bis-alkylation of double-helical DNA through nucleophilic attack at the epoxide and the aziridine ring of the 1-azabicyclo[3.1.0]hex-2-ylidene system, in a manner vaguely reminiscent of the mitomycins. A number of years ago, Lown and Majumdar demonstrated that carzinophilin A (azinomycin B) produces covalent cross-links in duplex DNA,[9] and these workers proposed that alkylation occurs within the *minor* groove at dG residues. More recently, Armstrong and co-workers have also reported that azinomycin B (carzinophilin A) effectively cross-links duplex DNA[10] and their results were interpreted to show interstrand cross-link formation between the agent and N7 positions of dG and dA within the *major* groove of DNA. The mechanism of action of the azinomycins presumably is the result of an initial intercalation into double-stranded DNA via the naphthoate moiety followed by formation of a covalent interstrand cross-link.

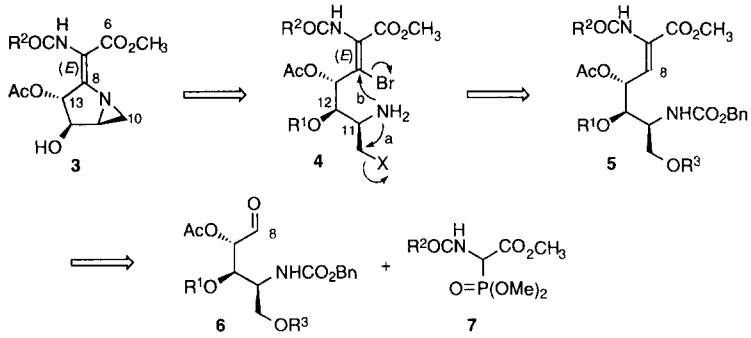
No total synthesis of the azinomycins has been disclosed to date, although several syntheses of the left hand segment of the azinomycins have been accomplished[3,4,11] and studies on the aziridino[1,2-*a*]pyrrolidine core have been reported.[12] These complex natural products possess the unique C6-C13 1-azabicyclo[3.1.0]-hex-2-ylidene system. Central to the molecule is a tetrasubstituted C7-C8 olefin possessing an E-configuration. In addition, three contiguous stereogenic centers are situated on the pyrrolidine portion of the bicyclic structure, two of which (C13 and C12) comprise a differentially protected *trans*-1,2-diol moiety and the third (C11) occupies the ring juncture position.

SYNTHETIC PLANNING

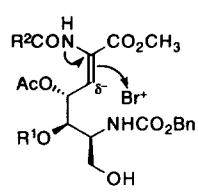
Substructure **3** contains the unique azabicyclic ring system as well as points of attachment (the amino and carboxylate groups) for connection of the upper left and right hand segments of the natural products. Because of its central position within the molecule, substructure **3**, or a closely related compound, is the key intermediate around which the remainder of the synthesis of the azinomycins will pivot. It was this substructure that we targeted at the initiation of our synthetic studies.



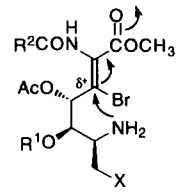
In a conceptually simple and direct strategy, the pyrrolidine ring of **3** was envisioned to arise by an intramolecular Michael addition-elimination reaction sequence[13] between a C11-amine and the electrophilic β-bromoacrylate of acyclic precursor **4** (process b), whereas the fused aziridine ring could be introduced conceptually by cyclization of the amine onto a C10 electrophile (process a); the order of these two steps was not obvious at the inception of the synthesis. Olefin **5** would arise from aldehyde **6** by a stereoselective Wadsworth-Horner-Emmons (WHE) olefination[14] using the appropriate glycine phosphonate **7**.



Impending questions surrounding the stereoselectivity of the transformation of olefin **5** to vinyl bromide **4**, and more importantly, the question of whether the pyrrolidine cyclization (**4** → **3**) would occur stereospecifically and with useful levels of stereoselection were as yet unanswered, and the literature offered little guidance in this area. Clearly, the viability of our planned synthesis was conditional on successful resolution of these issues. Introduction of the C8-vinyl bromide necessary for pyrrolidine ring formation makes use of the nucleophilic enamine character of dehydroamino acid systems, whereas cyclization requires that the same system act as an electrophilic acrylate. A notable feature of our synthetic plan employs the potentially mutable reactivity of these systems in consecutive synthetic operations.

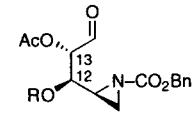


N-acyl enamine
(nucleophile)



β-bromoacrylate
(electrophile)

In practice, we found that it was optimal to introduce the aziridine ring early in the synthetic scheme, prior to Wadsworth-Horner-Emmons olefination, and we have developed routes to the appropriate aldehydes **8a** and **8b** starting from D-glucosamine with either a benzyl (Bn) or *tert*-butyldimethylsilyl (TBS) protecting group at the C12 alcohol (R); this work has been described elsewhere.[15] Herein, we describe full details of our work on the elaboration of the aziridino[1,2-*a*]pyrrolidine ring system of the azinomycins, and particularly, our results on the stereoselective introduction of the C7-C8 E-olefin of the target molecules.



R = CH_2Ph **8a**
R = $SiMe_2t$-Bu **8b**

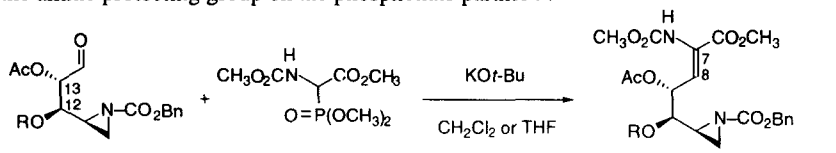
RESULTS

Wadsworth-Horner-Emmons Olefinations

Early in our synthetic studies, we had found that the Wadsworth-Horner-Emmons olefination reaction of aldehydes was a viable and convergent method for the construction of the important C7-C8 dehydroamino acid double bond. Olefination of aldehydes **8a** and **8b** proceeded as planned with phosphonate **9** to afford the corresponding dehydroamino acids **10a** and **10b**, but in only modest yields. High Z-selectivity was observed (6-10:1 Z/E) with potassium as the counter-ion. This reaction worked in significantly higher yields with substrates containing simple alkoxy groups α to the aldehyde (MeO, BnO), but placement of an electron withdrawing acyl group at the C13 position significantly hampered the reaction process. We became increasingly aware as the project proceeded that manipulation of protecting groups after introduction of the dehydroamino acid was fraught with problems, and this reinforced the desirability of having the C13 position acetylated prior to olefination. Thus, the inconvenience of lower yields or exacting reaction conditions caused by the presence of the C13-acetoxy group was transcended by the necessity of bringing this appendage into the olefination intact. In addition, since we were to rely on this same process using phosphonates bearing the intact top half of the azinomycins in the course of the total synthesis, it seemed like a good investment to optimize this key bond construction process.

In most cases, the major problem was simply low yields, but under more forcing reaction conditions, we occasionally observed 1,4-elimination of HOAc from the product **10** to form a 1-aza-1,3-butadiene system. Particularly important in achieving higher yields was the careful control of reaction temperature with KO*t*-Bu (< –20 °C). [More recently, we have succeeded in promoting olefination of similar aldehyde substrates[16] with other bases (tetramethylguanidine[17] or *i*-Pr_2NEt/LiBr[18]), but not in a predictable manner with respect to vari-

ous aldehyde substrates.] In addition, we found little variation in the success of the olefination reaction as a function of the amine protecting group on the phosphonate partner **9**.



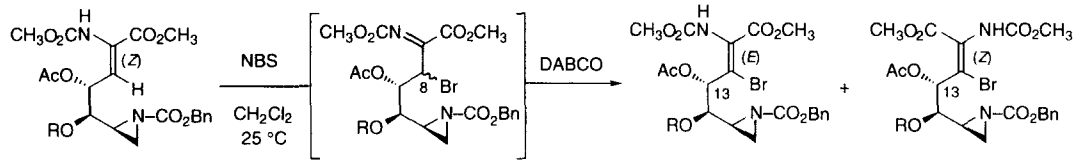
8a R = CH_2Ph
8b R = $SiMe_2t$-Bu

9

(*Z*)-**10a** (48%) R = CH_2Ph
(*Z*)-**10b** (53%) R = $SiMe_2t$-Bu

Brominations[19]

Introduction of a vinyl bromide[20] necessary for pyrrolidine ring construction via the proposed addition-elimination reaction sequence turned out to be a particularly difficult transformation to achieve with the desired stereoselection. Bromination occurred upon treatment of olefin (*Z*)-**10a** with 1.2 equivalents of *N*-bromosuccinimide (NBS) in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO, 1.2 equiv) in CH_2Cl_2 and afforded directly a 4:1 mixture of vinyl bromides, tentatively (or hopefully) assigned as desired (*E*)-**12a** (major) and undesired (*Z*)-**12a** (minor), respectively. Olefin (*Z*)-**10b** afforded similar levels of stereoselection.



(*Z*)-**10a** R = CH_2Ph
(*Z*)-**10b** R = $SiMe_2t$-Bu

11a R = CH_2Ph
11b R = $SiMe_2t$-Bu

(*E*)-**12a** R = CH_2Ph (*Z*)-**12a**
(*E*)-**12b** R = $SiMe_2t$-Bu (*Z*)-**12b**

In the absence of base, treatment of (*Z*)-**10a** or (*Z*)-**10b** with 1.0 equivalent of NBS generated an intermediate α-bromo imine **11a** or **11b** which could be isolated by chromatography. These α-bromoimines were recognizable by their characteristic downfield resonance in the ^1H NMR spectrum (300 MHz, $CDCl_3$) at δ 5.33 (d, J = 9.6 Hz) and δ 5.64 (d, J = 5.9 Hz), respectively, corresponding to C8-H. Subsequent base-induced tautomerization promoted by DABCO of purified α-bromoimines **11a** and **11b** resulted in the formation of a single diastereomer presumed to be vinyl bromides (*E*)-**12a** and (*E*)-**12b**, respectively, in quantitative yield.

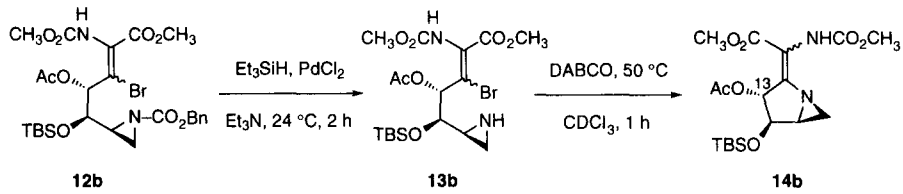
Tentative support for E-olefin configuration of the major vinyl bromide **12b** came from the observation of a *weak* negative nuclear Overhauser enhancement of the NH in the NOE difference spectrum of **12b** when C13-H was irradiated (500 MHz, $CDCl_3$). No enhancement was seen for the minor (presumably Z) isomer of vinyl bromide **12b**. The absence of a stronger, more reliable NOE in the major isomer was thought to result from the inherent conformational flexibility of the acyclic system. Attempts to prove olefin configuration by chemical means were without success.[21] At this stage, our "assignment" of stereochemistry of product vinyl bromides was precarious.

We felt, perhaps, that it would be easier to assign olefin configuration at the stage of the cyclized product, which would possess a more rigid system better suited to spectroscopic analysis. There was significant literature precedent[22] for addition-elimination reactions of acrylate systems that occur with retention of olefin configuration, and thus, if a more reliable NOE signal could be obtained from a product aziridino[1,2-*a*]pyrrolidine, we felt that this stereochemical assignment could be extrapolated back to the starting vinyl bromide with a higher level of confidence.

Cyclizations

Up to this point the benzyloxycarbonyl group had proven effective as an amino-protecting group, but initial attempts to remove it at the stage of vinyl bromide **12** using standard conditions were futile. Hydrogenolysis using various palladium catalysts [Pd–C, $Pd(OH)_2$, Pd black, $Pd–Al_2O_3$] were found to be incompatible with the vinyl bromide (and/or enamine) moiety of **12**. An initial solution to this problem was to remove the benzyl carbamate at the stage of olefin **10** (H_2, Pd black), and replace it with the base-labile 9-fluorenylmethoxycarbonyl (FMOC) group. While this was successful in eventually permitting deprotection of the aziridine in the presence of the vinyl bromide [$NaN(SiMe_3)_2$, THF, –78 °C], the yields for the protecting group interchange were never better than 62% overall for the additional two-step sequence, and this was judged unacceptable.

We turned to a procedure by Sakaitani and Ohfune[23] describing the conversion of the benzyl carbamate protecting group into a trialkylsilyl carbamate ($NH–CO_2SiR_3$). The silyl carbamates could be isolated or hydrolyzed to the corresponding carbamic acids, which decarboxylate spontaneously to generate the corresponding amines. In our case isolation of the silyl carbamate was unnecessary, and so we used to the earlier conditions of Birkhofer[24] ($PdCl_2$, Et_3SiH, Et_3N, 25 °C) for deprotection of the benzyloxycarbonyl group. Application of the Birkhofer protocol quantitatively converted the $N–CO_2Bn$ aziridine of **12b** to the corresponding free aziridine **13b** upon workup, without disturbing the integrity of the vinyl bromide. Warming **13b** at 50 °C in the presence of 1.0 equiv of 1,4-diazabicyclo[2.2.2]octane (DABCO) in $CDCl_3$ effected the desired addition-elimination reaction to afford a *single* isomer of the target aziridino[1,2-*a*]pyrrolidine **14b** in good yield. Presumably, this meant that the reaction occurred stereospecifically, but no firm assignment of stereochemistry of the tetrasubstituted double bond had yet been made.

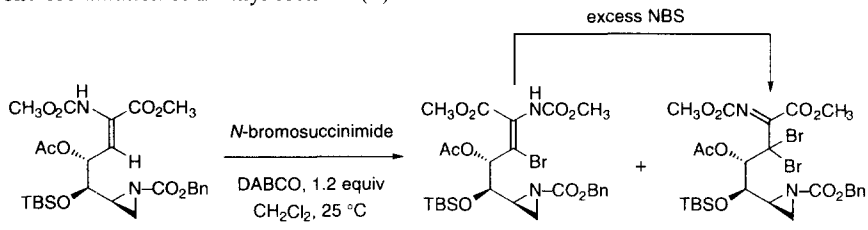


12b **13b** **14b**

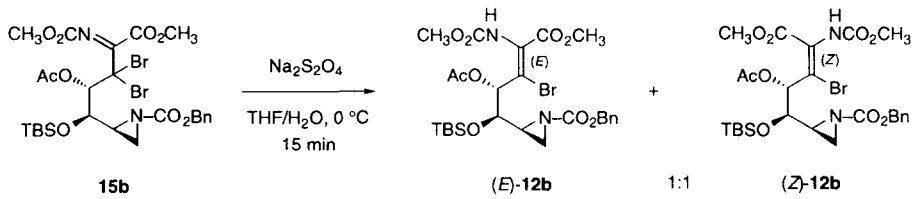
Distressingly, *no NOE enhancement* was seen between the C13-H and the NH of **14b**, raising serious doubts as to the proposed E-double bond stereochemistry. Ultimately, these results along with other published

results[12] forced us to conclude that aziridino[1,2-*a*]pyrrolidine **14b** must unfortunately possess the undesired Z-olefin stereochemistry. In addition, this suggested that our initial assignment of E-stereochemistry to the major product **12b** from the bromination was incorrect, and that the *major* vinyl bromide probably possessed the Z-configuration, assuming that the cyclization of **13b** → **14b** occurred with retention of olefin stereochemistry. Thus, at this stage of the synthesis, it appeared that the inherent stereochemical preferences of these systems were in opposition to our plans.

We expected that the isomeric minor vinyl bromide (presumably the E-isomer) would cyclize to afford the isomeric (*E*)-aziridino[1,2-*a*]pyrrolidine, but under similar deprotection conditions the *minor* isomer of vinyl bromide **12b** failed to afford the corresponding aziridino[1,2-*a*]pyrrolidine. These inexplicable results were resolved when mass spectral analysis of *minor* vinyl bromide isomer of **12b** revealed the presence of *two* bromine atoms by isotope cluster abundance calculations. Thus, the compound we had assigned as the *minor* vinyl bromide isomer of **12b** was not the vinyl bromide but instead was the *gem*-dibromo imine **15b**, resulting from a second bromination of Z-vinyl bromide (*Z*)-**12b**.

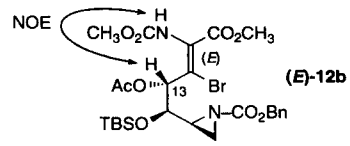


Consistent with this result, dibromide **15b** could not undergo imine-enamine tautomerization and failed to exhibit the expected nuclear Overhauser enhancements since no NH was present. We found that dibromide **15b** could be produced in high yield by treatment of (*Z*)-**10b** with excess *N*-bromosuccinimide in the presence of DABCO. Reduction of the *gem*-dibromo imino functionality of **15b** occurred efficiently with sodium dithionite ($Na_2S_2O_4$) in a nonstereoselective manner to afford a separable 1:1 mixture of vinyl bromides (*E*)-**12b** and (*Z*)-**12b**. Now for the first time we were in possession of confirmed samples of *both* stereoisomers of the vinyl bromide **12b**, and hence in a much better position to assign olefin configuration with confidence.



Nuclear Overhauser enhancement experiments with the newly obtained E-vinyl bromide (*E*)-**12b** confirmed the stereochemistry as assigned by the observation of a *strong positive* reciprocal enhancement

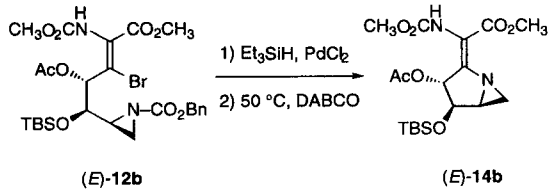
between the C13-H and NH protons. With a confirmed sample of the E-vinyl bromide **12b** now accessible, although as a separable 1:1 mixture with its Z-diastereomer, we were in a position to complete the construction of the azinomycin core substructure. Clearly, this was an instance where a non-stereoselective reaction proved to be advantageous, as possession of both isomeric E- and Z-stereoisomers of **12b** made assignment of stereochemistry much easier.



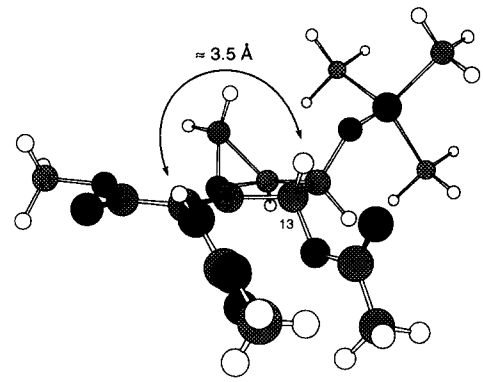
nuclear Overhauser enhancement confirms E-olefin geometry

Synthesis of the Azinomycin Aziridino[1,2-a]pyrrolidine Ring System

As the pure E-stereoisomer, (*E*)-**12b** was treated with Et_3SiH in the presence of $PdCl_2$ and Et_3N (25 °C, 30 min), which effected quantitative removal of the *N*-benzoxycarbonyl protecting group to afford the free aziridine. The isolated and purified aziridine was induced to undergo the desired intramolecular Michael addition-elimination reaction sequence upon warming in the presence of base (DABCO, $CDCl_3$, 50 °C) and thereby afford the targeted aziridino[1,2-*a*]pyrrolidine (*E*)-**14b**. Assignment of E-olefin geometry to **14b** was based primarily on correlation of spectral data with related compounds, and was confirmed by the observation of a strong positive nuclear Overhauser enhancement of the NH in the NOE difference spectra of **14b** when C13-H was irradiated (500 MHz, $CDCl_3$). The calculated distance in the energy-minimized structure (MMX forcefield) was about 3.5 Å, well within the expected value for observation of an NOE effect.

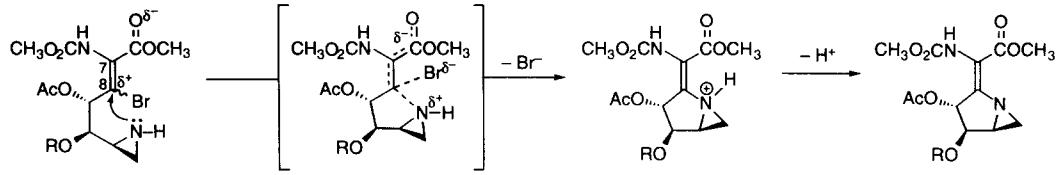


(*E*)-**12b** (*E*)-**14b**



The key cyclization sequence starting from E- and Z-vinyl bromides **13** to the product E- and Z-aziridino[1,2-*a*]pyrrolidine systems **15** characteristic of the azinomycins proved ideal for the synthesis of this complex system. The actual cyclization reaction was found to be stereospecific, and furthermore was observed to occur with complete stereoselectivity within the limits of detection by ^1H NMR. While we have depicted this reaction process in our retrosynthetic analyses as a Michael addition-retro Michael sequence, it seems unlikely that this is the case in view of the stereochemical outcomes of the cyclization reactions of the E- and Z-vinyl bromides.[22,25] We view this process to be much closer to a direct displacement reaction,[26] where the structure **16** is either a short-lived intermediate or a transition state between starting material **13** and product **17** (or **15**). In any case, it is clear that the C7-C8 olefin never exists in a state such that the barrier to rotation

about this bond is sufficiently low to permit isomerization, as we have never detected any stereochemical "leakage" during the cyclization reactions.



13 16 17 15

In addition to the NOE results used in confirmation of stereochemistry, we observed a highly reliable correlation of proton chemical shift values within this series of compounds (Table 1). For dehydroamino acids **10**, vinylic C8-H and allylic C13-H protons of the Z-isomers resonated upfield relative to the corresponding E-isomers. A similar correlation was observed for the C13-H protons of the E-isomers of vinyl bromides **12** and aziridino[1,2-*a*]pyrrolidines **14**, which resonated upfield relative to the corresponding Z-isomers. The opposite correlation was observed for the NH protons of **12** and **14**. These correlations have held throughout a large number of related compounds.

Table 1
^{1}H NMR Chemical Shift Values for E- and Z-Isomers (δ)

Compound[a]	C8-H	C13-H	NH
Z-**10a**	6.1	5.3	–
E-**10a**	6.8	6.3	–
E-**12b**	–	5.5	8.5
Z-**12b**	–	5.8	6.8
E-**14b**	–	5.2	7.5
Z-**14b**	–	5.8	6.3

[a]Note the change of CIP priorities: *Z*-**10a** corresponds to *E*-**12a**

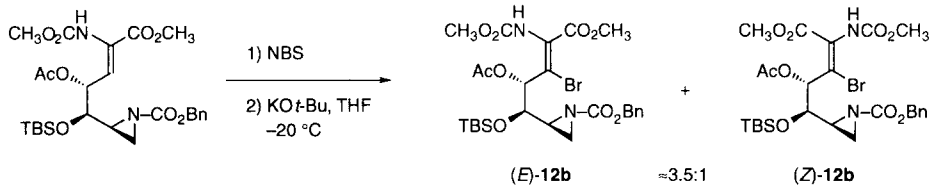
Deprotection of C12-Hydroxyl

Synthesis of the desired aziridino[1,2-*a*]pyrrolidine characteristic of the azinomycins requires the removal of the C12-silyl protecting group. Unfortunately, all attempts to remove the *tert*-butyldimethylsilyl protecting group at the vinyl bromide stage met with failure. Under basic conditions, acetate migration and 1,4-elimination were observed. Under acidic conditions, 1,4-elimination occurred along with acid-promoted aziridine ring opening. In fact, at no synthetic intermediate could the seemingly inert silyl group be removed. Although the synthesis of an aziridino[1,2-*a*]pyrrolidine derivative had been achieved, an alternative to the *tert*-butyldimethylsilyl ether would need to be developed for C12 hydroxyl group protection. This has been the subject of further investigations, which will be reported separately.

Stereoselective Brominations

The problem of stereocontrol in the bromination of dehydroamino acids was eventually solved in a detailed mechanistic study.[27] In these model systems, we found that we could control the stereoselectivity of the bromination reaction based on the observation that the desired E-vinyl bromides were produced as the kinetic products of the tautomerization of the intermediate α-bromoimines, and that these kinetic E-vinyl bromides were isomerized to the thermodynamic Z-isomers by the nucleophilic base DABCO. Useful levels of E-stereoselectivity were observed when hindered bases were used in the tautomerization of the intermediate α-bromoimines.

In the system at hand, bromination of (*E*)-**10b** afforded the corresponding intermediate α-bromoimine **11b** as before. Now base-promoted tautomerization with potassium *tert*-butoxide at low temperature afforded a mixture of the E- and Z-vinyl bromides **12b** favoring the desired E-isomer by a useful 3-4:1 ratio. Amine bases such as 2,2,6,6-tetramethylpiperidine or $KSi(NMe_3)_3$ failed to successfully promote the tautomerization of the intermediate α-bromoimine, instead effecting reduction to afford a stereoisomeric mixture of starting olefins. This result was particularly satisfying, as stereocontrol in the bromination reaction of **10b** to **12b** was the pivotal element in a stereoselective synthesis of the azinomycin core substructure.



Conclusion

An effective, stereocontrolled synthesis of the fully elaborated aziridino[1,2-*a*]pyrrolidine core substructure (***E***)-**14** of the azinomycins was achieved. Key transformations included a Wadsworth-Horner-Emmons olefination reaction between aldehyde **8** and phosphonate **9** for formation of the C7-C8 olefin, a stereoselective bromination to introduce the corresponding C8-bromide, and a final stereospecific intramolecular addition-elimination reaction sequence for formation of the central pyrrolidine ring system.

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on Brüker AM 300 or AM 500 instruments. Mass spectra were recorded on a VG 7070S high resolution instrument. Infrared spectra were recorded on a Varian 1600 FTIR. All extraction and chromatography solvents were distilled before use. Reaction solvents were distilled under N_2 immediately prior to use from the following drying agents: THF (Na, benzophenone), CH_2Cl_2 (CaH_2). Flash chromatography was performed using E. Merck silica gel 60 (240-400 mesh) following the procedure of Still, Kahn, and Mitra (*J. Org. Chem.* **1978**, *43*, 2923). Thin layer chromatography was performed using precoated plates purchased from E. Merck (silica gel 60 PF_{254}, 0.25 mm). *N*-Bromosuccinimide was

recrystallized from H_2O and stored in the dark; DABCO was dried by azeotropic removal of water with benzene and stored under Ar; triethylamine was distilled from CaH_2; triethylsilane was distilled from 4 Å molecular sieves. For NOE studies, deuterochloroform ($CDCl_3$) was distilled from P_2O_5 and stored under Ar, otherwise it was passed through a small plug of neutral Al_2O_3 immediately prior to use.

(2*Z*,4*R*,5*R*) Methyl 4-Acetoxy-5-[(2*S*)-*N*-(benzoxycarbonyl)aziridin-2-yl]-5-[(*tert*-butyldimethylsilyl)oxy]-2-[*N*-(methoxycarbonyl)amino]-2-pentenoate [(*Z*)-10b]. Phosphonate **7** (1.95 g, 7.7 mmol, 1.5 equiv) was added to a slurry of potassium *tert*-butoxide (0.80 g, 7.1 mmol, 1.4 equiv) in CH_2Cl_2 (25 mL) at –78 °C under N_2 and the reaction was allowed to warm to 0 °C and was stirred for 15 min before recooling to –78 °C. The resulting solution was added *via* cannula over a 30 min period to a solution of aldehyde **6** (2.15 g, 5.1 mmol) in CH_2Cl_2 (25 mL) at –78 °C. The reaction mixture was stirred for 1 h at –78 °C and was allowed to warm to 24 °C. After 1 h at this temperature the reaction was quenched by the addition of NH_4Cl (10 mL). EtOAc (50 mL) was added and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and saturated aqueous NaCl (2 × 50 mL), and were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (3.4 × 20 cm silica, 20-30% EtOAc/hexanes) to afford olefin **(*Z*)-10b** (1.24 g, 44%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5 H, ArH), 6.08 (d, J = 9.8 Hz, 1 H, C8-H), 5.38 (dd, J = 9.8, 5.7 Hz, 1 H, C13-H), 5.09 (s, 2 H, OCH_2Ph), 3.77 (s, 3 H, CO_2CH_3), 3.68 (s, 3 H, CO_2CH_3), 3.58 (apparent t, J = 6.5 Hz, 1 H, C12-H), 2.57 (apparent td, J = 6.5, 3.7 Hz, 1 H, C11-H), 2.33 (d, J = 6.4 Hz, 1 H, C10-H), 2.15 (d, J = 3.7 Hz, 1 H, C10-H), 2.04 (s, 3 H, CH_3CO), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.08 and 0.07 (2 s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 164.5, 162.9, 154.5, 135.6, 131.1, 128.5, 128.4, 123.4, 74.0, 72.2, 68.4, 52.8, 52.7, 39.2, 29.3, 25.7, 21.0, 18.1, –4.5, –4.8; IR (neat) ν_{max} 3324, 2954, 1730, 1225, 838, 779, 698 cm^{-1}; EIMS, *m/z* (relative intensity) 550 (M^+, 1), 389 (22), 357 (30), 293 (35), 246 (22), 201 (36), 91 (base); HRMS, *m/z* calcd for $C_{26}H_{38}N_2O_9Si$: 550.2347; found: 550.2339.

(4*R*,5*R*) Methyl 4-Acetoxy-5-[(*tert*-butyldimethylsilyl)oxy]-5-[(2*S*)-*N*-(benzoxycarbonyl)aziridin-2-yl]-3-bromo-2-[*N*-(methoxycarbonyl)imino]pentanoate (11b). A solution of olefin **(*Z*)-10b** (105 mg, 0.20 mmol) in CH_2Cl_2 (5 mL) under N_2 was treated with *N*-bromosuccinimide (39 mg, 0.22 mmol, 1.1 equiv) and the reaction mixture was allowed to stir at 24 °C for 45 min during which time a light yellow color appeared. The reaction mixture was concentrated *in vacuo* to provide a yellow oil which was directly purified by flash chromatography (0.9 × 15 cm silica, 20-30% EtOAc/hexanes) to afford bromoimine **11b** (100 mg, 83%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (br m, 5 H, ArH), 5.64 (d, J = 5.9 Hz, 1 H, C8-H), 5.46 (dd, J = 5.9, 5.2 Hz, 1 H, C13-H), 5.11 (AB q, J = 12.1 Hz, Δν = 14.7 Hz, 2 H, OCH_2Ph), 3.84 (m, 4 H, overlapping C12-H and CO_2CH_3), 3.77 (s, 3 H, CO_2CH_3), 2.74 (m, 1 H, C11-H), 2.34 (d, J = 6.6 Hz, 1 H, C10-H), 2.28 (d, J = 3.7 Hz, C10-H), 2.09 (s, 3 H, CH_3CO), 0.84 (s, 9 H, $SiC(CH_3)_3$), 0.08 and –0.01 (2 s, 6 H, $Si(CH_3)_2$).

(4*R*,5*R*) Methyl 4-Acetoxy-5-[(*tert*-butyldimethylsilyl)oxy]-5-[(2*S*)-[*N*-(benzoxycarbonyl)]aziridin-2-yl]-3,3-dibromo-2-[*N*-(methoxycarbonyl)imino]pentanoate (15b). A solution of olefin **(*Z*)-10b** (66 mg, 0.12

mmol) in $CHCl_3$ (1 mL) under N_2 was treated with *N*-bromosuccinimide (107 mg, 0.60 mmol, 5.0 equiv) and 1,4-diazabicyclo[2.2.2]octane (DABCO, 34 mg, 0.30 mmol, 2.5 equiv) and the reaction mixture was allowed to stir at 24 °C for 8 h, during which time a dark red precipitate formed in a yellow solution. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to provide a yellow oil, which was purified directly by flash chromatography (0.9 × 15 cm silica, 20-30% EtOAc/hexanes) to afford the corresponding dibromide **15b** (45 mg, 53%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5 H, ArH), 6.00 (d, J = 5.9 Hz, 1 H, C13-H), 5.09 (AB q, J = 12.1 Hz, Δν = 14.3 Hz, 2 H, OCH_2Ph), 3.90 and 3.82 (2 s, 6 H, 2 CO_2CH_3), 3.68 (dd J = 8.5, 5.9 Hz, 1 H, C12-H), 2.76 (ddd, J = 8.5, 6.3, 3.7 Hz, 1 H, C11-H), 2.40 (d, J = 6.3 Hz, 1 H, C10-H), 2.28 (d, J = 3.7 Hz, 1 H, C10-H), 2.17 (s, 3 H, CH_3CO), 0.83 (s, 9 H, $SiC(CH_3)_3$), 0.09 and 0.08 (2 s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 163.1, 160.1, 159.3, 159.2, 152.6, 136.0, 128.9, 128.7, 76.7, 75.5, 68.7, 63.6, 54.6, 54.3, 41.0, 32.2, 26.2, 21.4, 18.3, –3.6, –4.0; IR (neat) ν_{max} 3390, 1738, 1292, 840, 780 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 706/708/710 (M^+, rel. 1/2/1), 631 (10), 491 (25), 438 (92), 215 (base).

(2*E*,4*R*,5*R*) Methyl 4-Acetoxy-5-[(2*S*)-*N*-(benzoxycarbonyl)aziridin-2-yl]-3-bromo-5-[(*tert*-butyldimethylsilyl)oxy]-2-[*N*-(methoxycarbonyl)amino]-2-pentenoate [(*E*)-12b]. A solution of dibromide **15b** (100 mg, 0.14 mmol) in 1:1 THF/H_2O was cooled to 0 °C and treated with $Na_2S_2O_4$ (49 mg, 0.28 mmol, 2.0 equiv) for 15 min. The reaction mixture was extracted with EtOAc (3 × 5 mL), and the combined organic extracts were washed with water (10 mL) and saturated aqueous NaCl (2 × 10 mL), and were dried (Na_2SO_4) and concentrated *in vacuo* to afford a mixture of isomeric vinyl bromides **(*Z*)-12b** and **(*E*)-12b**. Purification of the residue by flash chromatography (0.9 × 20 cm silica, 25-30% EtOAc/hexanes) afforded pure **(*E*)-12b** (37 mg, 42%) as a colorless oil. The E-isomer was characterized: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (br s, 1 H, NH), 7.39-7.27 (m, 5 H, ArH), 5.51 (d, J = 8.1 Hz, 1 H, C13-H), 5.10 (AB q, J = 12.1 Hz, Δν = 21.0 Hz, 2 H, OCH_2Ph), 4.07 (br m, 1 H, C12-H), 3.82 (s, 3 H, CO_2CH_3), 3.66 (s, 3 H, CO_2CH_3), 2.57 (br m, 1 H, C11-H), 2.37 (d, J = 3.7 Hz, 1 H, C10-H_{exo}), 2.33 (d, J = 6.3 Hz, 1 H, C10-H_{endo}), 2.11 (s, 3 H, CH_3CO), 0.85 (s, 9 H, $SiC(CH_3)_3$), 0.09 and 0.08 (2 s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 169.4, 164.2, 163.2, 154.0, 136.1, 134.1, 128.9, 128.9, 128.7, 74.4, 72.0, 68.8, 53.1, 38.8, 29.3, 26.0, 21.3, 18.5, –4.0, –4.2; IR (neat) ν_{max} 3314, 1728, 1225, 837, 777 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 629 (M^+ + H , 10), 571 (13), 491 (10), 91 (base); HRMS, *m/z* calcd for $C_{26}H_{38}N_2O_9SiBr$ + H: 629.1530; found: 629.1526. The Z-isomer was characterized: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 5 H, ArH), 6.56 (br s, 1 H, NH), 5.77 (d, J = 8.8 Hz, 1 H, C13-H), 5.08 (s, 2 H, OCH_2Ph), 3.86 (s, 3 H, CO_2CH_3), 3.72 (s, 3 H, CO_2CH_3), 3.57 (apparent t, J = 8.8 Hz, 1 H, C12-H), 2.82 (ddd, J = 8.5, 6.3, 3.7 Hz, 1 H, C11-H), 2.35 (d, J = 6.3 Hz, 1 H, C10-H), 2.14 (d, J = 3.7 Hz, 1 H, C10-H), 2.03 (s, 3 H, CH_3CO), 0.82 (s, 9 H, $SiC(CH_3)_3$), 0.07 and 0.04 (2 s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 163.2, 162.6, 153.6, 136.0, 132.6, 128.9, 128.8, 128.7, 75.2, 73.8, 68.7, 53.7, 40.0, 30.7, 26.0, 21.3, 18.4, –3.9, –4.4; IR (neat) ν_{max} 3390, 1738, 1292, 840, 780 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 629 (M^+ + H , 11), 571 (13), 491 (80), 416 (36), 359 (45), 169 (50), 91 (base); HRMS, *m/z* calcd for $C_{26}H_{38}N_2O_9SiBr$ + H: 629.1530; found: 629.1527.

Aziridino[1,2-*a*]pyrrolidine (*Z*)-14. A mixture of **(*Z*)-12b** (35 mg, 56 μmol), Et_3SiH (0.2 mL, 1.3 mmol), and Et_3N (15 μL, 11 μmol, 2.0 equiv) was treated with $PdCl_2$ (10 mg, 56 μmol, 1.0 equiv) at 25 °C under N_2. Gas evolution was observed as the clear reaction mixture slowly turned black while stirring at 25 °C for 2 h. Direct purification of the reaction mixture by flash chromatography (0.9 × 10 cm silica, 50-75% EtOAc/hexanes) afforded aziridine **(*Z*)-13** as a colorless oil. A solution of the free aziridine in $CDCl_3$ (0.4 mL) was treated with 1,4-diazabicyclo[2.2.2]octane (DABCO, 6 mg, 56 μmol, 1.0 equiv) and the reaction mixture was warmed at 50 °C for 1 h. The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography (0.9 × 15 cm silica, 50-75% EtOAc/hexanes) to afford **(*Z*)-14** (11 mg, 41%) as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 6.34 (br s, 1 H, NH), 5.81 (br s, 1 H, C13-H), 4.50 (dd, J = 4.8, 1.1 Hz, 1 H, C12-H), 3.74 and 3.72 (2 s, 6 H, 2 CO_2CH_3), 3.07 (apparent td, J = 5.3, 4.8 Hz, 1 H, C11-H), 2.46 (dd, J = 5.3, 1.6 Hz, 1 H, C10-H_{exo}), 2.28 (d, J = 3.7 Hz, 1 H, C10-H_{endo}), 2.06 (s, 3 H, CH_3CO), 0.86 (s, 9 H, $SiC(CH_3)_3$), 0.10 and 0.06 (2 s, 6 H, $Si(CH_3)_2$); FABMS, *m/z* (relative intensity) 415 (M^+ + H, 8), 355 (18), 191 (42), 165 (base); HRMS (FAB), *m/z* calcd for $C_{18}H_{30}N_2O_7Si$ + H: 415.1901; found: 415.1896.

Aziridino[1,2-*a*]pyrrolidine (*E*)-14. ^1H NMR (500 MHz, $CDCl_3$) δ 7.50 (br s, 1 H, NH), 5.25 (br s, 1 H, C13-H), 4.51 (br d, J = 4.4 Hz, 1 H, C12-H), 3.81 (s, 3 H, CO_2CH_3), 3.68 (s, 3 H, CO_2CH_3), 3.16 (apparent q, J = 4.5 Hz, 1 H, C11-H), 2.62 (br m, 1 H, C10-H_{exo}), 2.56 (d, J = 3.7 Hz, 1 H, C10-H_{endo}), 2.10 (s, 3 H, CH_3CO), 0.85 (s, 9 H, $SiC(CH_3)_3$), 0.06 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 164.2, 154.7, 152.3, 120.3, 84.7, 76.8, 53.2, 52.7, 39.0, 26.0, 21.2, 18.4, –4.6, –4.7; IR (neat) ν_{max} 3324, 1734, 1251, 838, 779 cm^{-1}; HRMS, *m/z* calcd for $C_{18}H_{30}N_2O_7Si$: 414.1822; found: 414.1819.

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19. Note the change in CIP priorities as the olefins **10** are transformed to vinyl bromides **12** (Z-olefin corresponds to E-vinyl bromide).
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21. In theory, removal of the allylic C13 acetate would afford the corresponding alcohol, which would be poised to cyclize to either an oxazinone or a lactone system depending on the olefin configuration. Analysis of the infrared spectra of cyclized products could be used to definitively assign configuration. In practice, subjection of **12b** to hydrolytic conditions (NH_3/CH_3OH or $NaOCH_3/CH_3OH$) promoted 1,4-elimination of HOAc to afford an intermediate 1-aza-1,3-butadiene, which reacted with solvent.
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