STRATEGIES AND TACTICS IN ORGANIC SYNTHESIS, VOL. 5

Chapter 2

TOTAL SYNTHESIS OF THE AZINOMYCIN FAMILY OF ANTITUMOR AGENTS

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I. Introduction

The work detailed in this chapter provides our perspective on two natural products – azinomycins A (**1**) and B (**2**) – that possess remarkably intricate and unusual structures (Figure 1) and that exhibit potent and effective antitumor activity. Perhaps serving as defining examples of the oft-used term "deceptively simple" in describing a synthetic target (a fair comparison could be mitomycin C), these agents have presented a synthetic challenge that is notably greater than their size and functionality would suggest. This project began in the summer of 1988 with a naïve but prescient research proposal prepared as part of an academic job search, and has continued through the successful total synthesis of azinomycin A in 2001, and beyond.

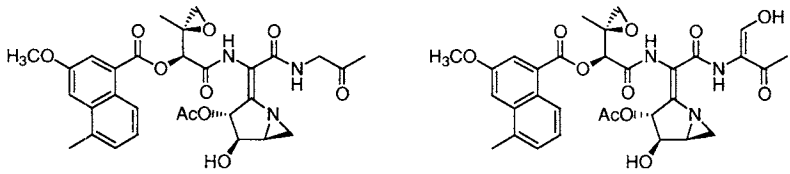


FIGURE 1
Structures of Azinomycin A and Azinomycin B

In 1954, there was a report on the isolation of a new antitumor agent from *Streptomyces sahachiroi*, named carzinophilin.[1] The compound exhibited effective and notable single-dose antitumor activity against several tumor cell lines implanted in mice and possessed significant antibiotic activity. These workers reported that carzinophilin was unstable and that the fermentation yields were poor and the structure of carzinophilin was not assigned. There were a number of reports detailing work on the mechanism of action of carzinophilin.[2]

Three decades later, Lown proposed the dimeric and C2-symmetric structure **3** (Figure 2) for carzinophilin that was based partly on degradation work.[3] The ^{1}H NMR spectra showed only 29 different protons whereas Onda and co-workers[4] had originally proposed a molecular formula $C_{50}H_{58}N_5O_{18}$.

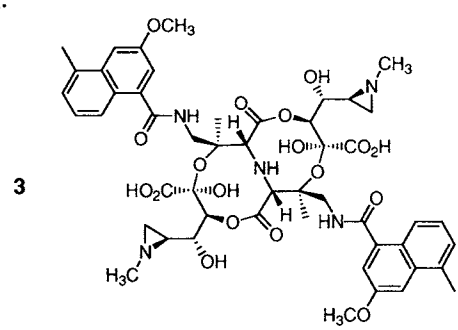


FIGURE 2
Dimeric Structure Proposed by Lown for Carzinophilin

The dimeric structure **3** of Lown was revised by Onda and co-workers[5] in 1983 to correspond to **4** (Figure 3), and carzinophilin was reported to possess a molecular formula $C_{31}H_{33}N_3O_{12}$. As it turned out, neither the Lown nor the Onda structures or molecular formulae were correct, but both were intriguing from both a mechanistic and synthetic viewpoint.

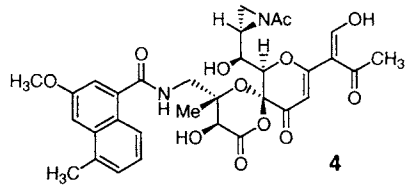


FIGURE 3
Structure of Carzinophilin Proposed by Onda

In 1986, two antitumor agents named azinomycin A (**1**) and azinomycin B (**2**) were reported.[6] These compounds were isolated from fermentation broths of *Streptomyces griseofuscus* S42227. Their unusual structures were determined by detailed NMR spectroscopic studies, and were shown to contain the structurally unprecedented 1-azabicyclo[3.1.0]hexane or aziridino[1,2-*a*]pyrrolidine ring system (Figure 4).[7] Given the presence of two potential electrophilic groups in the form of the spiroepoxide (C21) and aziridine (C10), we immediately realized that these antitumor agents could exert their biological activity through formation of DNA cross-links.

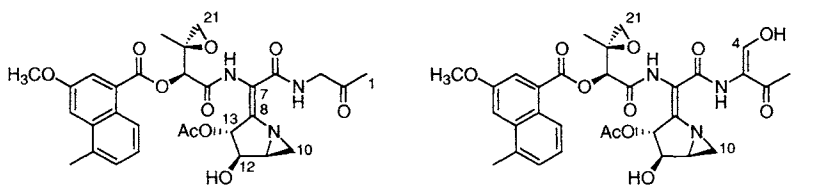


FIGURE 4
Structures of Azinomycin A and Azinomycin B

There is a comment in the azinomycin structure elucidation paper that the spectral data for azinomycins A and B and carzinophilin were similar, but this issue was not pursued further because at that time it was believed that the molecular formulae for azinomycin B and carzinophilin differed by one oxygen atom ($C_{31}H_{33}N_3O_{11}$ for azinomycin B versus $C_{31}H_{33}N_3O_{12}$ for carzinophilin). Armstrong and co-workers[8] later pointed out that the two compounds were identical, and carzinophilin/azinomycin B have since been used interchangeably in the literature.

The azabicyclic ring system of these agents was part of a dehydroamino acid fragment (Figure 4), wherein the aziridine nitrogen was conjugated with the C6 amide carbonyl group. This bridgehead nitrogen can-

not be planar because of the conformational restrictions imposed by the bicyclic ring system, but conjugation of the nitrogen lone pair would dictate sp^2 hybridization for such a vinylogous *N*-acyl aziridine. This suggested that this potential electrophile would have heightened reactivity towards ring opening due to the expected relief of ring strain, in addition to the energetically favorable rehybridization of the aziridine nitrogen upon cleavage of the C10-N9 bond. The selectively acylated 1,2-diol of the pyrrolidine ring system proved to be one of the more challenging aspects of the total synthesis.

The antitumor antibiotics azinomycins A and B were found to be active against Gram-positive, Gram-negative, and L5178Y cells in tissue cultures.[9] The azinomycins possess potent *in vitro* cytotoxicity, with IC_{50} values against L5178Y cells of 0.07 μg/mL and 0.11 μg/mL for azinomycins A (**1**) and B (**2**), respectively. *In vivo* antitumor activity was assessed using transplantable tumors in mice. Azinomycin B (**2**) showed marked activity against intraperitoneal-inoculated tumors such as P388 leukemia, B-16 melanoma and Ehrlich carcinoma. Administration of azinomycin B (32 μg/kg/d) resulted in 57% survivors at 45 d and 193% increase in lifespan (ILS) against P388 leukemia. As a comparison, mitomycin C exhibited 57% survivors and 204% ILS toward P388 leukemia, but at a higher dose (1 mg/kg/d). Azinomycin B (**2**) exhibited 63% survivors at 45 d and 161% ILS for Ehrlich carcinoma, but showed no beneficial effect on solid tumors such as Lewis lung carcinoma or Meth A fibrosarcoma. Azinomycin A (**1**) appeared less effective than azinomycin B (**2**) in these tests. The single-dose LD_{50} (i.p.) of azinomycin B was 190 μg/kg.

Acting in concert, the remarkably complex, novel structures of the azinomycins and the effective *in vitro* and potent *in vivo* antitumor activity have provided a target for total synthesis of significant importance. The fact that the agents are chemically unstable and consequently not suitable for clinical use provides additional impetus for synthetic studies.

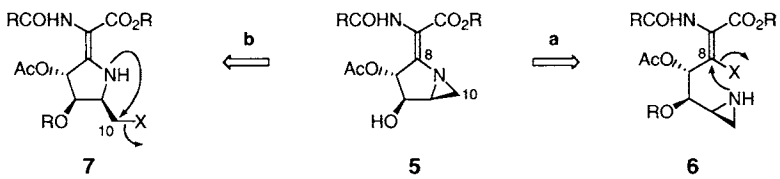
II. Retrosynthetic Analysis of the Azabicyclic Ring System

The aziridino[1,2-*a*]pyrrolidine ring system of the azinomycins presented the most challenging aspect of the natural product, and our initial efforts focused on the development of a synthetic route to this substructure. Critical issues that needed to be addressed included controlling the relative stereochemistry about the tetrasubstituted dehydroamino acid double bond and the development of a protecting group scheme allowing for introduction of the selectively acylated 1,2-diol system.

We surmised that the reactivity of the azabicyclic system would prevent carrying it very far along a sequence of synthetic transformations. Therefore, the key disconnection in planning our synthesis was the recognition that a greater chance of success could be had by introducing the azabicyclic ring system late in the synthetic pathway. Two disconnections were considered (Scheme 1):

- Introduction of the pyrrolidine ring by N–C8 bond formation through an addition-elimination reaction sequence between an aziridine nitrogen and an electrophilic β-haloacrylate (pathway **a**);

- Introduction of the aziridine ring by N–C10 bond formation through displacement of a C10 leaving group by the pyrrolidine nitrogen (pathway **b**).



SCHEME 1

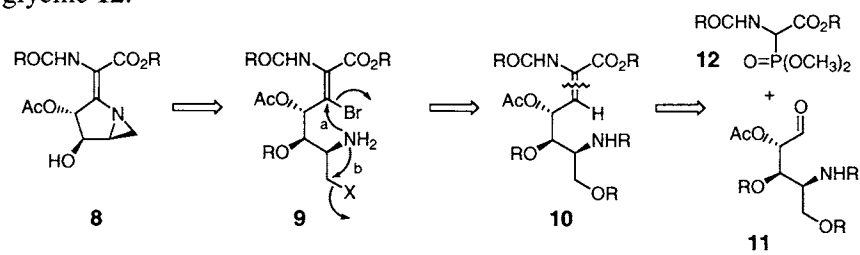
In the pathway **a** (Scheme 1), we anticipated that the aziridine nitrogen of **6**, while not as nucleophilic as a typical secondary amine, would be sufficiently reactive to effectively displace a vinylic leaving group such as a bromide in the intramolecular sense. This mode of reactivity, while lacking precedent as a cyclization reaction, was represented in the literature as an intermolecular reaction. The principle unproven issue with this strategy was the critical one of stereoselectivity in the vinylic displacement reaction, and the literature on this issue was murky.

In the second pathway **b** (Scheme 1), we anticipated that the sp^2 hybridized pyrrolidine nitrogen of **7** would be poorly nucleophilic due to resonance with the carboxylate, and hence would probably require deprotonation by strong base in order to induce cyclization. Furthermore, this disconnection still required that we address pyrrolidine ring introduction, and the original plan was to form the N–C8 bond of **7** in the same sense as in the first disconnection, that is, by an intramolecular addition-elimination reaction sequence with a β-haloacrylate, shown in **6**.

Two factors impacted the delay in development of a global plan on how to approach the total synthesis of the natural products. First, we

incorrectly assumed that the remainder of the agents did not appear to present any significant synthetic challenges. Second, this was to be the first project undertaken by the first graduate to join the incipient Coleman research group at South Carolina, and it seemed more prudent to focus such limited resources on this key aspect of the molecule.

The basic problem with respect to azabicyclic ring formation resided in the timing of processes **a** and **b** in structure **9** (Scheme 2), that is, whether the aziridine ring of **8** would be carried through the synthesis intact or would be formed as the final cyclization reaction, subsequent to process **a**. The vinyl bromide in **9** necessary to complete the addition-elimination reaction sequence would be installed at the unfunctionalized position of the dehydroamino acid olefin of **10** by electrophilic bromination. The dehydroamino acid would be formed by Wadsworth-Horner-Emmons olefination of the key suitably protected aldehyde **11** and the phosphonoglycine **12**.

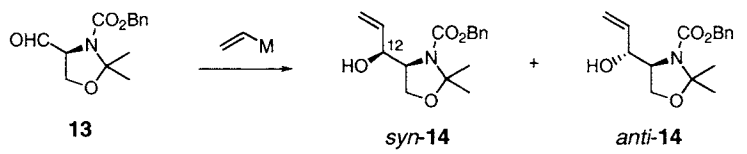


SCHEME 2

A number of questions were immediately apparent upon examining this proposed synthetic approach (Scheme 2). For example, will the olefin stereochemistry be maintained through the addition-elimination reaction sequence, that is, will the formation of the N–C8 bond (process a in **9**) be stereospecific? What factors will effect the stereoselectivity of this cyclization reaction? Can dehydroamino acids **10** be halogenated with control of stereoselectivity? Can we expect the dehydroamino acid olefin to act as a nucleophile in the bromination reaction of **10** and then expect it to mutate its reactivity to become electrophilic in the cyclization reaction process of **9**? In each case, there was either no literature precedent, or as is often the case, the literature was only slightly relevant. With so many ambiguities, it was with some amazement that a first-year graduate student, Andy Carpenter, eagerly chose this as his thesis project, and we proceeded (or more accurately stumbled) into this total synthesis in December of 1989. In retrospect, what has proven even more amazing is how the synthesis was accomplished so closely to the original 1988 plan.

III. Aldehyde Synthons – Stereoselective Addition of Vinyl Organometallic Reagents to α-Aminoaldehydes

Our initial approach towards a suitably protected five-carbon aldehyde was based on a process that adds a vinyl organometallic reagent to Garner's aldehyde **13** in a stereoselective manner to provide *syn*-**14** with the requisite azinomycin C12 stereochemistry (Scheme 3). At this time, there were a few reports in the literature on the addition of organometallic reagents to α-aminoaldehydes, and most reactions were either poorly stereoselective or afforded the undesired *anti* product as a result of so-called Felkin-Anh diastereoselection. In our hands (or more accurately, in Andy Carpenter's hands), addition of vinyllithium and vinylmagnesium bromide to **13** afforded products **14** with 1:5 and 1:3 ratios of syn/anti diastereoselection.



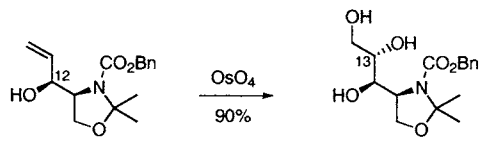
13 *syn*-**14** *anti*-**14**

SCHEME 3

Pre-complexation of the aldehyde with Lewis acids such as $TiCl_4$ or Et_2AlCl had no effect on this ratio. Moving away from THF to an ether/pentane solvent system and pre-forming what was presumably vinylzinc chloride from vinyllithium and $ZnCl_2$ completely switched the sense of diastereoselection and afforded a 6:1 ratio of *syn/anti*-**14**.[10] This was the first report of *syn*-selective addition of vinylic nucleophiles to α-aminoaldehydes, and which has become an expanding area of chemistry containing dozens of references. In the context of the azinomycin project, this was a minor victory with a seemingly trivial result, but the paper describing these results is the most highly cited from the Coleman group.

With this success in hand, it was disappointing to find that the olefin of *syn*-**14** could not be epoxidized successfully under any of a variety of conditions, including both of sets of conditions developed by Sharpless (vanadium and titanium-based reactions). Dihydroxylation of **14** with OsO_4 worked better and afforded **15** as a >95:5 ratio of diastereomers, unfortunately favoring the wrong absolute configuration at the emergent C13 stereogenic center (Scheme 4). After extensive and (ultimately successful) studies on inverting the C13 stereocenter, the whole reaction sequence became increasingly more unwieldy. Despite eventually arriv-

ing at functionalized pyrrolidine ring systems related to the azinomycin core, the entire strategy was abandoned as it became clear that the disadvantages of having to invert this key stereogenic center were insurmountable. This was especially true given the fact that a much better strategy had emerged in the interim.

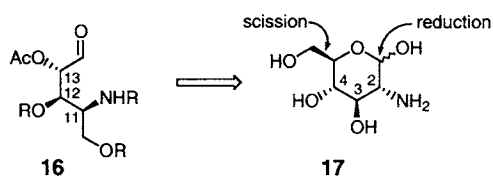


syn-**14** **15**

SCHEME 4

IV. D-Glucosamine Route to the Key Aldehyde

A new and improved strategy made efficient use of the chiral pool starting material D-glucosamine (Scheme 5). The absolute configuration of the C11, C12, and C13 stereogenic centers of the azinomycins, and hence of the aldehyde synthon **16**, were identical to the C4, C3, and C2 centers of D-glucosamine (**17**), respectively. Envisioning the emergence of the key aldehyde **16** from this chiral platform, we needed to arrange protecting groups appropriately around the periphery of pyran **17**, reduce C1 of the sugar to the alcohol oxidation state, and at some point cleave the C5–C6 bond so that C5 corresponds to the aldehydic carbon. One of the key issues, and a seemingly trivial one, was the selective protection of the C12/C13 diol. Given the vast literature on protecting group manipulations in the area of carbohydrate chemistry, this proved straightforward.



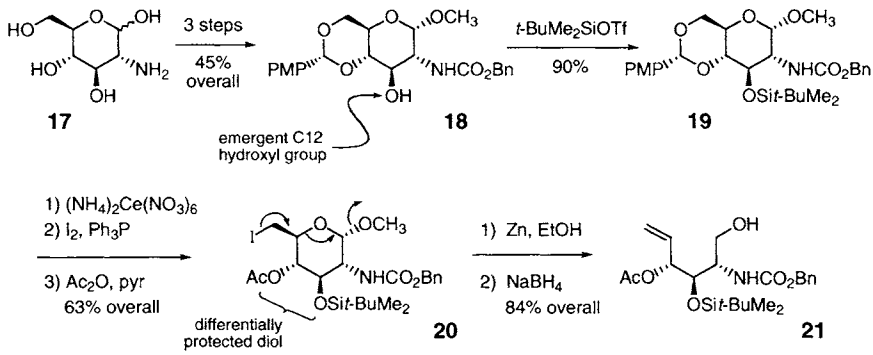
16 **17**

SCHEME 5

In April of 1991, a new graduate student, Yong Dong, was sent on an exploratory mission to see whether an efficient protecting group strategy and pyran ring scission method could be implemented.[11] This strategy was subject to several considerations, including the ability to smoothly unmask the unstable aldehyde at a later point in the synthesis and to produce a differentially protected *syn*-1,2-diol with the C12 hydroxyl group

protected in a form suitable for unmasking at the completion of the synthesis. This work culminated in the 1992 M.S. thesis of Yong Dong.

All of the functional groups of D-glucosamine (**17**) were easily differentiated making use of a methyl glycoside, benzyl carbamate, and *p*-methoxybenzylidene acetal, leaving a single unprotected hydroxyl group in the product **18** that would emerge in the azinomycins as the C12-hydroxyl (Scheme 6). This required us to choose the most appropriate protecting group at this position, from among the many possibilities.

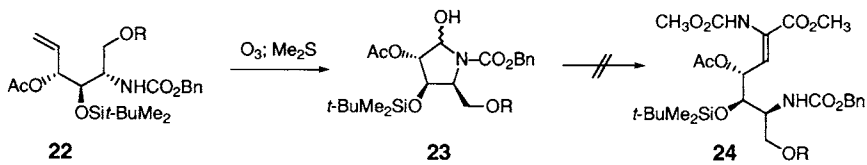


SCHEME 6

A benzyl ether could be installed with considerable difficulty due to competing cyclization of the intermediate alkoxide onto the proximal carbamate carbonyl group (Scheme 6), coupled with the sterically crowded environment around this position. The benzyl group survived subsequent transformations, but we could not find reaction conditions that would effect its removal at latter stages of the synthesis. Silyl protecting groups were much easier to install, and standard silylation with *t*-BuMe$_2$SiOTf afforded the ether **19** in 90% yield. The acetal of **19** was removed under oxidative conditions (73%), the primary C6-hydroxyl was selectively converted to the iodide (88%), and the remaining C4-hydroxy was acetylated (98%) to provide **20** bearing the important differentially protected 1,2-diol of the azinomycin azabicyclic system. The pyran ring was fragmented using a Vasella fragmentation[12] and without purification, the resulting C1-aldehyde was reduced to alcohol **21** (84% overall). More easily removable silyl groups such as the triethylsilyl ether did not survive the Vasella fragmentation conditions.

At this point Andy Carpenter and I had to make a decision regarding the timing of aziridine introduction, and commit to either pathway **a** or **b** shown in Scheme 1. In practice, the decision was made for us because of

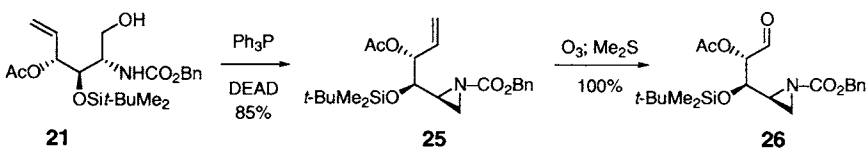
the following observation (Scheme 7). Ozonolysis of hydroxyl-protected forms of **22** afforded the pyrrolidine **23** as the sole product, and the hemiaminal of **23** was unreactive in subsequent Wadsworth-Horner-Emmons olefination reactions.



22 **23** **24**

SCHEME 7

Not wishing to burden the system with a second protecting group on nitrogen, we introduced the aziridine moiety by Mitsunobu reaction of aminoalcohol **21** to afford aziridine **25** (Scheme 8). Ozonolysis of the olefin of **25** proceeded smoothly to afford the key aldehyde **26**. Introduction of the aziridine at such an early stage of the synthesis was clearly a risk, but proved to be a significant advantage with respect to efficiency.



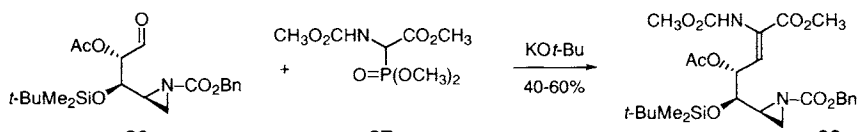
21 **25** **26**

SCHEME 8

V. Dehydroamino Acid Construction

Wadsworth-Horner-Emmons olefination of aldehyde **26** using the potassium salt of phosphonoglycine **27** was successful (Scheme 9), in only modest yields (40-60%). This was partly due to problems with elimination of acetate from **28** to form a 1-aza-1,3-butadiene system, and low yields in this reaction were to plague us continually throughout the remainder of our work. There was some thought given to reworking the protecting group scheme of aldehyde **26** so as to introduce the acetate subsequent to olefination, but this would necessitate at least two additional steps into the sequence, and would involve manipulations on dehydroamino acid systems related to **28**. It seemed far more efficient to optimize the olefination and deal with modest yields, particularly with an eye to the future when phosphonates of much greater complexity would be

olefination partners. As we learned, starting with this work by Andy Carpenter and proceeding through the total synthesis of the natural products, protecting group manipulations on late-stage intermediates were risky and oftentimes unsuccessful. Directness was always the most desirable strategy, and it was essential that the protecting group scheme for the differentiated diol be in place prior to olefination. This olefination reaction proved to be the key step in the total synthesis because it served to connect the relatively complex and chemically reactive aldehyde **26** with the remainder of the molecule. The reaction always proved to be reliable, although the yields were always less than optimal.

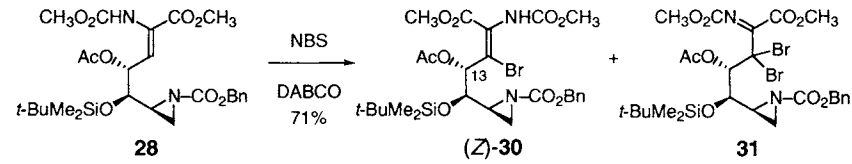


26 **27** **28**

SCHEME 9

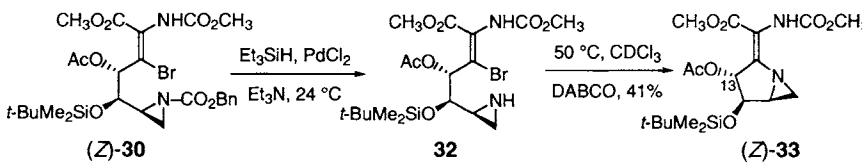
VI. Synthesis of Vinyl Bromides and Stereoselective Formation of the Aziridino-[1,2-*a*]pyrrolidine Substructure of the Azinomycins

Introduction of the vinylic bromide in preparation for azabicyclic ring formation occurred smoothly upon treatment of dehydroamino acid **28** with *N*-bromosuccinimide followed by amine base (Scheme 10). Two products were isolated from this reaction in a ratio of 4:1 and the similarities in the ^{1}H NMR spectra initially led us to assign them as olefin diastereomers. Assignment of olefin configuration of the major isomer **30** as the desired E-diastereomer seemed possible (or perhaps more likely, hopeful) based on literature precedent, but its configuration was far from confidently assigned based the observation of a very weak and negative nOe between the NH and C13-H in **30**. The minor product **31** showed no nOe at all and turned out to be the dibromoimine shown. We attempted to prove olefin configuration of **30** by chemical means, thinking that we could free the C13 hydroxyl group and induce cyclization onto the proximal carbamate if it were the E-isomer (compared to the proximal carboxylate in the Z-isomer), but all attempts to remove the acetyl group were unsuccessful. This further reinforced our previous decision to avoid protecting group manipulations on late-state intermediates, and without recourse, we moved ahead with the major product **30**, which we hoped – wrongly, as it turned out – to be the E-vinyl bromide.



SCHEME 10

Andy Carpenter and I had discovered a paper by Sakaitani and Ohfune[13] that we had subsequently highlighted in Chemtracts.[14] These workers had demonstrated the conversion of benzyl to other carbamates via the intermediacy of silyl carbamates. Under palladium catalyzed conditions, silanes such as triethylsilane or *tert*-butyldimethylsilane effect reductive transesterification, as reported earlier by Birkofer and co-workers.[15] In our hands, the benzyl carbamate of **30** was cleanly removed and the corresponding free aziridine was obtained upon aqueous work-up without observation of products resulting from reduction of the olefin or vinyl bromide (Scheme 11). In 2000, these mild reductive conditions were shown by Jikesh Shah, a masters student in my group at Ohio State, to be useful for selective removal of benzyl carbamates, benzyl esters and benzyl ethers in the presence of other easily reduceable groups.[16]

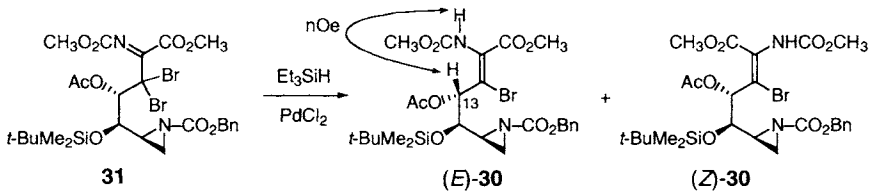


SCHEME 11

This surprisingly stable aziridine species (**32**) was induced to undergo cyclization upon warming in an NMR tube in the presence of DABCO (chosen conveniently because all 12 protons were chemical shift equivalent). A single stereoisomer of the azabicyclic system **33** was present, and could be isolated by chromatography (Scheme 11). There was no observable nOe between the NH and C13-H of **33**, which raised the anticipated but dreaded inference of having incorrect olefin stereochemistry.

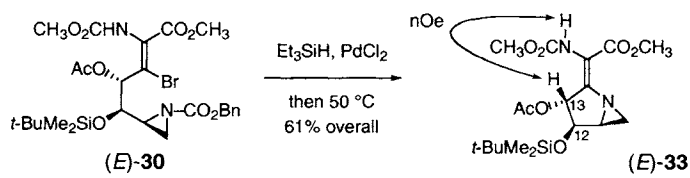
What seemed most desirable would be to have *both* olefin diastereomers of vinyl bromide **30** in order to make direct comparisons, and in fact, having both stereoisomers did prove essential for assigning the configuration about this tetrasubstituted double bond (Scheme 12). The *gem*-dibromide **31** underwent ready reduction to a mixture of E- and Z-vinyl bromides (*E*)-**30** and (*Z*)-**30** upon treatment with $Et_3SiH/PdCl_2$. The new

isomer now showed an unmistakably strong positive nOe between the NH and C13-H, confirming it as the E-isomer, and the previously obtained vinyl bromide as the undesired Z-isomer.



SCHEME 12

The E-vinyl bromide (*E*)-**30** underwent deprotection and the intermediate free aziridine cyclized to afford the E-stereoisomer of the azabicyclic system (*E*)-**33** (Scheme 13). There was a strong and reciprocal nOe between the NH and C13-H of (*E*)-**33**, confirming olefin configuration, and similarly confirming the stereochemistry of the previously obtained (*Z*)-**33** (Scheme 11). In both cases (*i.e.*, E- and Z-vinyl bromides), the cyclization provided the single stereoisomeric azabicyclic system (*E*)-**33**, and demonstrated that the cyclization was, in fact, stereospecific.



SCHEME 13

As a temporary solution to this stereochemical problem, we deliberately synthesized the *gem*-dibromide **31** (Scheme 12) by treatment of the dehydroamino acid **28** with excess NBS in the presence of DABCO. The separable, isomeric vinyl bromides (*E*)-**30** and (*Z*)-**30** could be produced in quantitative yield as a 1:1 mixture by $Na_2S_2O_4$ reduction of **31**. Our 1992 report on this achievement represented the first entry into the fully elaborated aziridino[1,2-*a*]pyrrolidine system of azinomycin A and B.[17]

Attempted removal of the *tert*-butyldimethylsilyl protecting group on the C12-hydroxyl of (*E*)-**30** was unsuccessful. At the stage of the vinyl bromide, basic reaction conditions resulted in acetate migration or 1,4-elimination, and acidic conditions effected aziridine ring opening, both at the stage of vinyl bromide **30** and bicyclic compound **33**. More easily removable silyl ethers such as triethylsilyl were insufficiently stable to

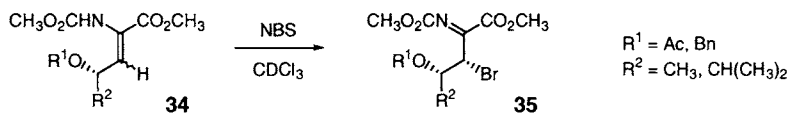
survive Vasella fragmentation. The issue of the C12-hydroxyl protecting group was to emerge as one of the most challenging aspects of the total synthesis, and would end up occupying an inordinate amount of time for solution. Our successful answer to this problem has proven unique with respect to other synthetic efforts in this area.

VII. Stereoselective Bromination of Dehydroamino Acids: Kinetic versus Thermodynamic Control

Literature results reported for the diastereoselectivity of the bromination of dehydroamino acids under conditions similar to those we used were inconsistent. There was no correlation of reaction conditions or substrate functionality with diastereoselectivity, and no uniform mechanistic interpretation of this reaction pathway was evident. It was from such an inconsistent body of literature that we undertook studies aimed at providing a stereoselective synthesis of the (*E*)-β-bromo-α,β-dehydroamino acids (*i.e.*, E-vinyl bromides) required for our azinomycin synthetic efforts (Scheme 14). We reported a mechanistic rationale on the stereochemical course of this reaction, and we clearly defined reaction conditions that allow for the stereodivergent preparation of both E- and Z-vinyl bromide products from either diastereomeric E- or Z-dehydroamino acid.

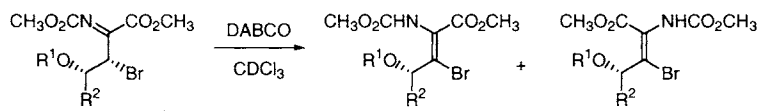
SCHEME 14

Treatment of a series of Z- or E-dehydroamino acids **34** with *N*-bromosuccinimide in $CDCl_3$ at 24 °C and following the reaction progress by ^1H NMR showed that a mixture of *syn*- and *anti*-α-bromoimines **35** were formed in ratios from 3:1 to 8:1 (Scheme 15). This ratio was found to be completely independent of the stereochemistry of the starting dehydroamino acid or protecting group on the allylic hydroxyl group.



SCHEME 15

Addition of the amine base DABCO (1,4-diazabicyclo[2.2.2]octane) – chosen solely because all 12 protons are equivalent – to the NMR tube containing the bromoimine **35** resulted in a rapid tautomerization from the imine to enamine structure (Scheme 16), affording **36** in E/Z ratios varying from 1:2 to 1:5. Again, there was no correlation between stereochemistry of the dehydroamino acid or syn/anti ratio of the intermediate α-bromoimines and the ultimate E/Z ratio of the vinyl bromide **36**, and in no instance did the desired E-vinyl bromide predominate. [In order to place this work in the proper temporal context, in 1992 the obscure Baylis-Hillman reaction had just come to the attention of synthetic organic chemists.]



SCHEME 16

These results suggested several possible mechanistic interpretations:

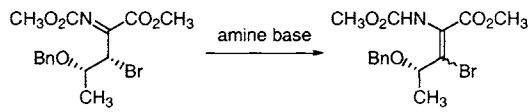
(1) The tautomerization of α-bromoimines *syn*-**35** and *anti*-**35** were only moderately stereoselective, with the *syn*-isomer leading predominately to the Z-vinyl bromide **36**, and the *anti*-isomer to the E-isomer of **36**. After separating the *syn*- and *anti*-bromoimines, this proved not to be true.

(2) Base-promoted epimerization of the intermediate α-bromoimines was occurring prior to a highly stereoselective (syn → Z, anti → E) tautomerization, thereby altering the correlation between syn/anti and E/Z ratios. This seemed possible, although DABCO is not very basic ($pK_{BH^+} = 8.8$).

(3) The E- and Z-vinyl bromide products **36** were equilibrating under the reaction conditions, and that there was no relationship between *syn*-selectivity and Z-selectivity.

The real breakthrough in this problem came after we followed the time course of the tautomerization using the stereoisomerically pure bromoimine *syn*-**35** (Scheme 17). After 15 min in the presence of DABCO, the vinyl bromides **36** were present in a 1:1.2 E/Z ratio, but the proportion of the Z-vinyl bromide slowly increased upon standing, until after 72 h the

ratio was less than 1:20. Repeating this experiment with Et_3N, we finally observed an initial ratio of products **36** that favored the E-vinyl bromide (E/Z 2.5:1), and which was invariant with time. The true proof of the mechanistic origin of the Z-vinyl bromide came when we added one equivalent of DABCO to the NMR tube containing the Et_3N experiment and observed the E/Z ratio change from 2.5:1 to 1:3.4 over the course of 16 h. This clearly demonstrated that the equilibration of (*E*)-**36** and (*Z*)-**36** was not base promoted but was due to the now well-known Baylis-Hillman conjugate addition-elimination process: all this work simply because of the simplicity of the ^1H NMR spectrum of DABCO!



35 **36**

SCHEME 17

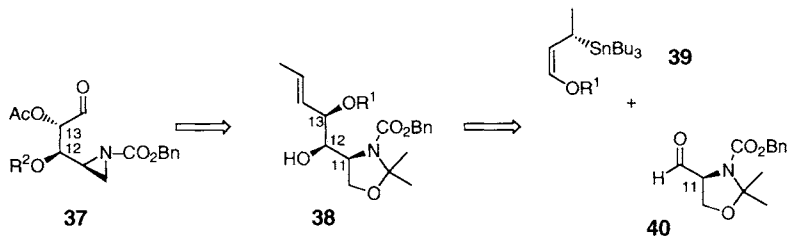
Additional work showed that sterically crowded amine bases such as 2,2,6,6-tetramethylpiperidine or alkoxide bases such as *t*-BuOK could effect a highly E-selective kinetic tautomerization process. Furthermore, stereoisomeric ratios of vinyl bromides **36** greater than 10:1 E/Z could be obtained in good chemical yields.

At this stage of the project, we had achieved several significant synthetic goals and we had reported the first synthesis of the fully differentiated azabicyclic substructure of the azinomycins. We had not yet dealt with protecting group issues for the C12-hydroxyl group, nor had we considered this chemistry in the larger context of a total synthesis. In addition, the synthetic route to the key aldehyde used for the olefination reaction was less than suitable for our purposes, and so we moved on to development of two different routes to this key intermediate.

VIII. The Allylstannane Route to the Key Aldehyde

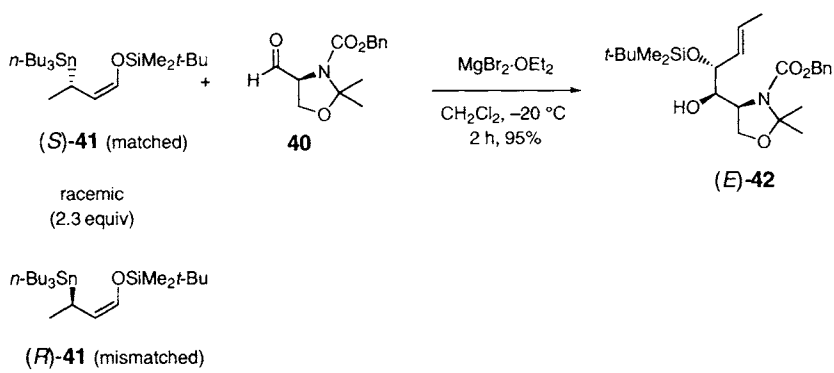
Our laboratories at South Carolina directly abutted Jim Marshall's, and my research group had developed satisfyingly collegial interactions with Jim's students and postdocs. The Marshall group had been publishing some of their initial work on γ-alkoxycrotylstannane chemistry.[18] Andy Carpenter was in the middle of his final year in graduate school (1994), and we had decided that the D-glucosamine route we had developed was unsuitable for a variety of reasons. Andy realized that Marshall's meth-

odology potentially could be applied to construction of the azinomycin core (Scheme 18). The C12/C13 selectively protected diol could be constructed by the anti S_E' chelation-controlled addition of the γ-alkoxycrotylstannane **39** to Garner's aldehyde (**40**), resulting in the C11/C12 *syn*-, C12/C13 *syn*-stereochemistry in the addition product **38**. More importantly, the C12 position would emerge unprotected, and so divergent introduction hydroxyl protecting groups could be achieved at this position, en route to the key aldehyde **37**.



SCHEME 18

In practice, the synthesis of enantiomerically pure crotylstannanes such as **39** is tedious and expensive, but we discovered that we could make use of the kinetic resolution that occurred when a racemic mixture of (*Z*)-γ-silyloxycrotylstannane **41** was reacted with Garner's aldehyde (**40**) in the presence of $MgBr_2$ (Scheme 19). Using 2.3 equivalents of the stannane **41** effected useful levels of kinetic resolution (>10:1 S/R) to provide the product (*E*)-**42** and obviated the need for tedious and expensive preparation of enantiomerically pure stannane (*S*)-**41**.

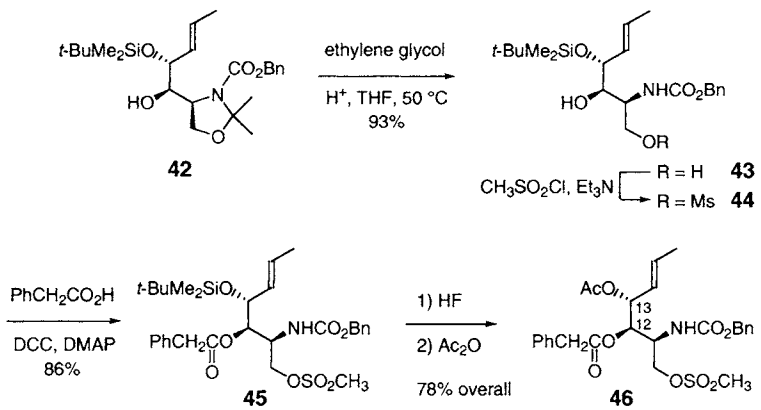


SCHEME 19

Using racemic crotylstannane **41** would seem to be highly atom uneconomic, since more than half of the stannane remained when the reaction was complete. In the conversion of **41** + **40** → **42** (Scheme 19), with regard to using the racemic **41** versus enantiomerically pure γ-alkoxycrotylstannane (*S*)-**41**, the overall atom economy based on total *n*-Bu_3SnH consumed is 6.3 mole of Sn per mole of **42** produced for racemic stannane, compared to 14.4 mole of Sn per mole of **42** for enantiomerically pure stannane. Surprisingly, the kinetic resolution is more than *twice* as efficient based on total tin consumed in the production of **42**, largely because of the low yields obtained in the more elaborate synthesis of the enantiomerically pure stannane. The expense of the reagents necessary for the synthesis of enantiomerically pure (*S*)-**41** increased the economic advantage of performing a kinetic resolution, considering that this was the first synthetic step in the total synthesis effort.

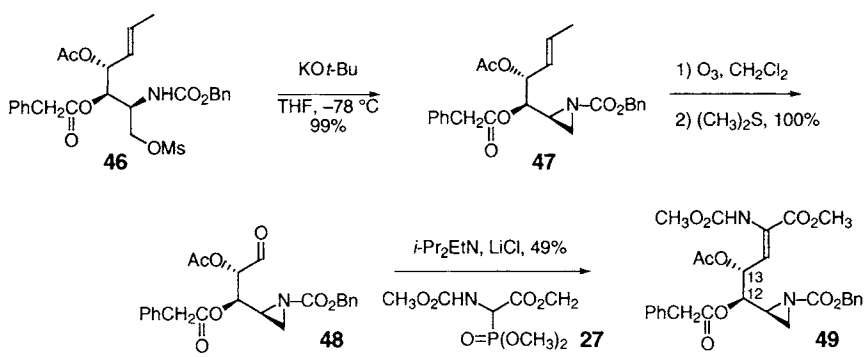
We selected a C12 ester group because of the flexibility with respect to reaction conditions for removal. In concurrent work detailed in the next section, we had also developed a route to the azinomycin substructure that alternatively relied on an ether group for this hydroxyl, and so these two routes were complementary. We were keenly aware of the considerable instability of systems with a free C12-hydroxyl group, and we decided that a protecting group for this alcohol must utilize especially mild deprotection conditions. After considerable experimentation with groups such as the self-immolative 4-azidobutyrate, a new student in my group at South Carolina, Tom Richardson, selected the enzymatically removable phenylacetate ester.

The sterically crowded hydroxyl group of **42** was unreactive towards even forcing acylation conditions, so we removed the N,O-acetonide prior to acylation (Scheme 20). Cleavage of the oxazolidine ring of **42** had a more practical advantage because these systems exist as a mixture of slowly interconverting rotamers that complicate NMR spectral analysis. Cleavage of the oxazolidine ring of **42** occurred upon treatment with ethylene glycol and acid, and the diol **43** was selectively acylated at the primary hydroxyl group with methanesulfonyl chloride to afford **44**. The secondary hydroxyl group of **44** was protected as the phenylacetate ester by treatment with the carboxylic acid and dicyclohexylcarbodiimide (DCC) to afford **45**. Protecting group interchange at the C13-hydroxyl group proceeded uneventfully to afford the acetate ester **46**.



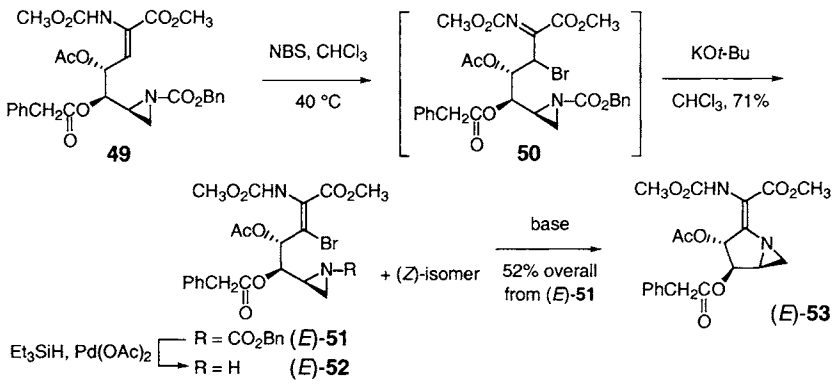
SCHEME 20

The remainder of the steps through the key Wadsworth-Horner-Emmons olefination occurred smoothly (Scheme 21). Base-promoted closure to the aziridine provided **47**, followed by ozonolysis of the double bond afforded the corresponding aldehyde **48**. Olefination with the glycine phosphonate **27**, this time using the Roush/Masamune conditions, allowed Tom Richardson to arrive at the fully elaborated, differentially protected dehydroamino acid **49** in a 1:1 ratio of olefin isomers, (irrelevant but for NMR assignments). The yield on the olefination was less than impressive (49%), as is characteristic for all systems we have worked with that possessed the α-acyloxyaldehyde functionality (rarely do yields top 70%). However, the desirability of having the C13 position of dehydroamino acid **49** acetylated prior to olefination outweighed any inconvenience due to lowered yields or exacting reaction conditions.



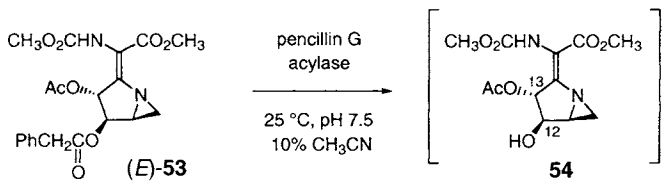
SCHEME 21

Bromination of **49** with *N*-bromosuccinimide at 40 °C proceeded smoothly to the corresponding α-bromoimine **50** (Scheme 22), but base-induced tautomerization proceeded non-stereoselectively to produce a separable 1:1 mixture of E- and Z-vinyl bromides **51**. This was a frustrating divergence from previous systems, where we were successful in effecting an E-selective tautomerization of the intermediate bromoimine. The aziridine *N*-benzyl carbamate of (*E*)-**51** could smoothly be removed under our now standard conditions ($PdCl_2$ or $Pd(OAc)_2$, Et_3SiH, Et_3N), and stereospecific cyclization of the free aziridine (*E*)-**52** was effected by warming the resulting free aziridine in $CDCl_3$ at 40 °C in the presence of amine base. Under these conditions, cyclization was accompanied by a competitive ring opening by the bromide ion liberated by elimination, but the presence of Dowex anion-exchange resin (carbonate ion form) served not only as a base, but also as a bromide ion scavenger; the azabicyclic product (*E*)-**53** was the sole product formed under these conditions.



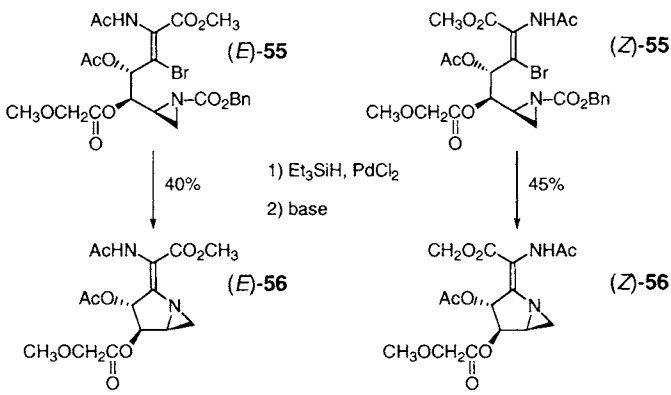
SCHEME 22

Deprotection of the phenylacetate ester of (*E*)-**53** was observed to occur with 5-10 mol% polymer-supported penicillin G acylase in a mixed solvent system of acetonitrile/aqueous buffer (Scheme 23). We knew that systems where the C12 hydroxyl group is unprotected were not sufficiently stable to permit isolation, and thus we attempted to characterize the product formed *in situ* using ^1H NMR (500 MHz, 9:1 D_2O/CD_3CN). The phenylacetate ester of (*E*)-**53** was removed with an approximate half-life of 2 h, and **54** could be observed as the minor of several products. Neither isolation nor further characterization of **54** could be achieved under these conditions.



SCHEME 23

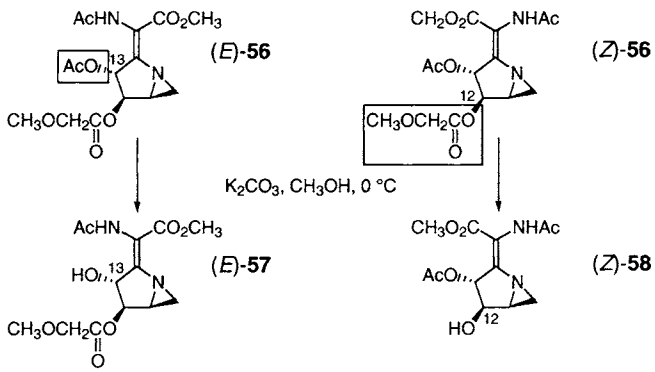
During these studies, Tom Richardson also had the opportunity to explore differentially reactive acyl protecting groups for the C12-hydroxyl group (Scheme 24). Methoxyacetate is significantly more labile than acetate, and we predicted that were we to construct systems such as (*E*)-**56** and (*Z*)-**56**, we could expect that under exceptionally mild hydrolytic conditions. In principle, there should be a significant difference in rate of hydrolysis between the acetate and methoxyacetate.



SCHEME 24

Principles are wonderful teaching tools, but are oftentimes unreliable in their application to complex systems (Scheme 25). When both the azabicyclic systems (*E*)-**56** and (*Z*)-**56** were subjected to mild hydrolysis conditions, we did in fact see effective differential reactivity of the two esters, but unfortunately, the origin of the difference lay in the stereochemistry of the dehydroamino acid double bond, and not in the ease of removal of the ester protecting groups. The principal product evident upon subjection of the correct isomer (*E*)-**56** to these reaction conditions was the C13-alcohol (*E*)-**57**, where the acetate had undergone a more rapid hydrolysis than the methoxyacetate. The converse was true with the undesired isomer (*Z*)-**56**, where we saw clean removal of the methoxy-

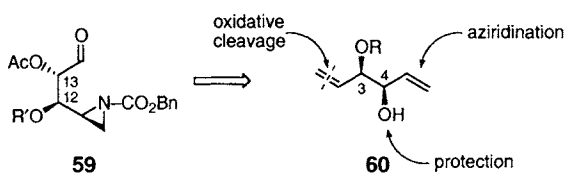
acetate to afford the C12-alcohol (*Z*)-**58** (which, by the way, was significantly more stable than the correct E-isomer). Both products were further hydrolyzed rapidly to the corresponding diol. This interesting difference, while probably deserving of further study in order to understand its origin, was the death knell for this synthetic route to the azinomycin azabicyclic ring system (although it also meant a Ph.D. for Tom in 2000).[19]



SCHEME 25

IX. The Pseudosymmetric Route to the Key Aldehyde

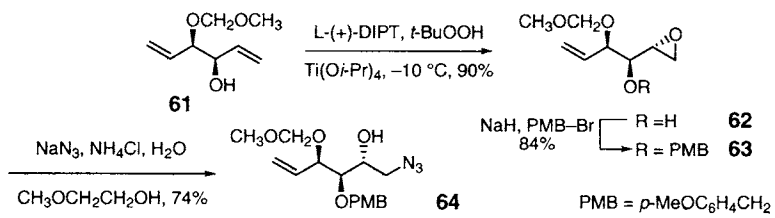
The third and ultimately successful route to the key aldehyde **59** and to the azabicyclic substructure of the azinomycins was based on the pseudosymmetry present in this target (Scheme 26). The basis of this plan was the recognition that an alkene could serve as a precursor to both the aldehyde and aziridine of **59**. Retrosynthetically this gives rise to pseudosymmetrical diene **60**, wherein differentiation of the *syn*-diol would permit introduction of the appropriate acylation pattern of the natural products and as a means for differentiation of the two olefins. 1,5-Hexadien-3,4-diol **60** is available in enantiomerically pure form using Brown's (γ-alkoxyallyl)diisopinocampheylborane reagent.[20]



SCHEME 26

Since Brown's methodology produces **60** with a methoxymethyl ether on the C3 hydroxyl group (R = CH_2OCH_3, **61**) while leaving 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 end differentiation of the two double bonds of **61**. The resulting 5,6-epoxide would then serve as a precursor to the aziridine of **59**. This meant that the C3 ether of **61** subsequently would be transformed to the C13 acetate ester of **59**, and that a suitable protecting group would be installed at the free C4 hydroxyl group of **61** that becomes C12 of **59**. We found that a *p*-methoxybenzyl ether was a partially effective solution for protection of the C12 hydroxyl group of the azinomycins, a seemingly trivial problem whose solution had escaped us to this point.

A new postdoc who had joined my group at South Carolina in 1995, Jian-she Kong, was able to develop an efficient sequence of transformations from protected diol **61**, prepared in 66% yield (>95% ee) following Brown, *et al.*, to the key olefin **69** (Scheme 28) that proceeded in greater than 35% yield for the eight-step conversion (Scheme 27). The two olefins of **61** were easily differentiated by Sharpless epoxidation by virtue of their allylically disposed hydroxyl groups (Scheme 27). Under standard conditions with the L-(+)-diisopropyl tartrate catalyst, epoxide **62** was obtained uneventfully in 90% yield and ≥ 98% enantiomeric excess.

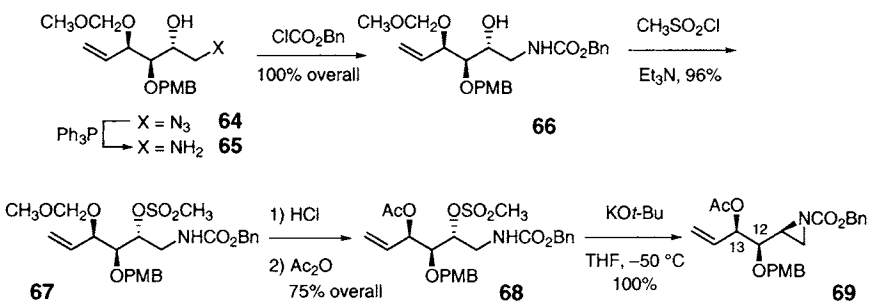


SCHEME 27

At this juncture – coincident with our move to Ohio State – we were faced once again choosing a C12-hydroxyl protecting group. We had gained considerable experience in this matter from Andy Carpenter's earlier studies, albeit without discovery of a viable protection scheme for the 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. After considering deprotection conditions that would be orthogonal with the reactivity patterns of various late synthetic intermediates and anticipating compatibility with pending transforma-

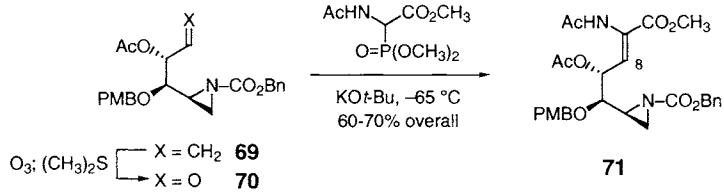
tions, we opted for the *p*-methoxybenzyl (PMB) ether, which can be removed under neutral, mildly oxidizing conditions. In addition, an ether protecting group was complementary to the concurrent crotylstannane based synthesis that used an ester for protection of this hydroxyl group (*vide supra*). Alkylation of the remaining alcohol of **62** with sodium hydride and *p*-methoxybenzyl bromide afforded **63** (84%) and occurred without rearrangement of the epoxide (Scheme 27). Addition of azide to the terminal carbon of epoxide **63** provided a 74% yield of primary azide **64**. On a larger scale, this reaction was difficult to force to completion and we would typically isolate unreacted epoxide starting material.

Transformation of the azide of **64** to the amine by reduction with triphenylphosphine in a toluene/water mixture followed by N-acylation of the resulting primary amine **65** with benzyl chloroformate and triethylamine afforded carbamate **66** in quantitative yield over the two-step procedure (Scheme 28). Manipulation of the hydroxyl protecting and activating groups began with acylation of the free secondary alcohol of **66** with methanesulfonyl chloride in the presence of triethylamine to afford mesylate **67** (96%). Subsequent cleavage of the methoxymethyl acetal with methanolic HCl (74%), and introduction of the azinomycin C13-acetate by standard acylation with acetic anhydride (99%) afforded **68**. Acid-catalyzed cleavage of the acetal of **67** was accompanied by a significant amount of *p*-methoxybenzyl ether cleavage, which could be minimized by monitoring the reaction as it progressed. Cyclization of **68** to the aziridine **69** occurred upon low temperature deprotonation of the carbamate of **68** with potassium *tert*-butoxide and effectively provided the pivotal intermediate **69** (100%) in an overall yield of >35% from **61**. This compound possesses all of the functionality and protecting groups for elaboration to the azinomycin core, including the essential C13-acetate ester and *p*-methoxybenzyl ether at the emergent C12 position.



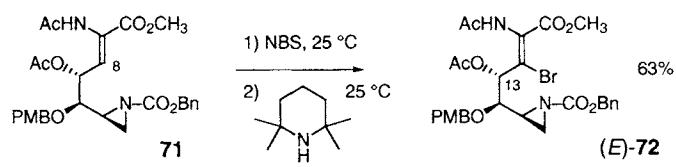
SCHEME 28

With the important aldehyde precursor **69** in hand and readily available in multigram quantities, Jian-she Kong proceeded along the established plan for elaboration to the azinomycin core substructure (Scheme 29). Installation of the dehydroamino acid system was preceded by oxidative cleavage of the terminal olefin of **69** using ozone with dimethylsulfide work-up to afford aldehyde **70** in high yield. This aldehyde was carried into the subsequent Wadsworth-Horner-Emmons olefination without purification using potassium *tert*-butoxide and the *N*-acetyl glycine phosphonate to afford olefin **71** as a >4:1 mixture of Z/E isomers in 60-70% yield.



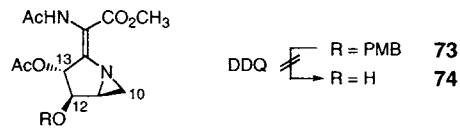
SCHEME 29

When dehydroamino acid **71** was treated with one equivalent 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*)-**72**, with >10:1 E/Z stereoselectivity as measured by ^{1}NMR analysis of the crude reaction mixture (Scheme 30). Confirmation of relative stereochemistry was made by the observation of a nuclear Overhauser enhancement between the NH and C13-H protons of (*E*)-**72**, and the lack of a similar enhancement with the minor Z-isomer. Furthermore, by this point we could make use of an extensive set of chemical shift correlations generated during our previous work. In this correlation, the allylic (C13) proton of E-vinyl bromides (*e.g.*, **72**) consistently resonated upfield (ca. 0.3 ppm) from the same proton in the Z-isomer, whereas the NH resonance in the E-vinyl bromides was consistently downfield (ca. 1.5-2.0 ppm) from the NH in the corresponding Z-isomer of **72**.



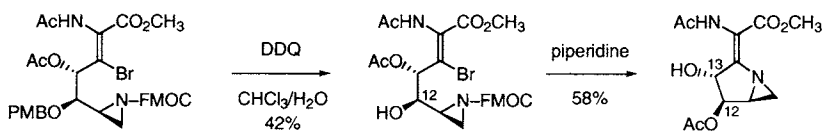
SCHEME 30

We were successfully able to convert (*E*)-**72** to the azabicyclic ring system, to afford **73** uneventfully (Scheme 31). However, under a wide variety of conditions using DDQ, we were unable to cleave the *p*-methoxybenzyl ether of **73** without destruction of the parent molecule. In no case was the alcohol **74** detected.



SCHEME 31

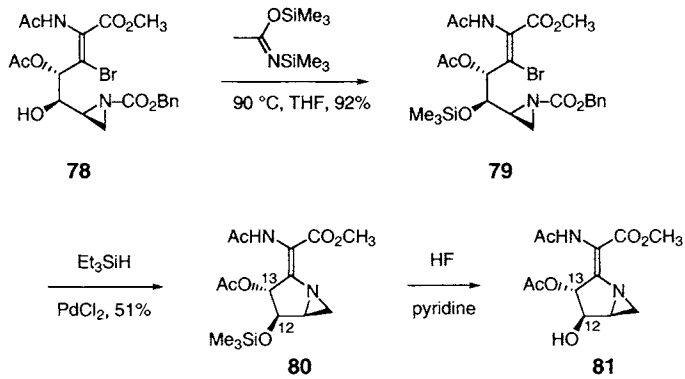
Backing up one step to the stage of the vinyl bromide **75** (Scheme 32), alternately bearing a 9-fluorenylmethoxycarbonyl (FMOC) protecting group on the aziridine because of previous problems in removing the corresponding benzyl carbamate, DDQ was effective at cleaving the *p*-methoxybenzyl ether to afford the alcohol **76**, although in low yields. Final piperidine cleavage of the FMOC protecting group effected subsequent cyclization to the azabicyclic system, which was unfortunately identified at the C12-acetate ester **77**, where the acetate group had migrated under the basic reaction conditions. This presumably occurred prior to cyclization, as it did not appear to be possible for the acetate to migrate in an intramolecular manner across the trans-diol of the azabicyclic system.



SCHEME 32

Backing up yet again, we could arrive at C12-alcohol **78**, now bearing a benzyl carbamate on the aziridine (Scheme 33). Lacking the ability to remove the *p*-methoxybenzyl ether at the stage of the azabicyclic system (*i.e.*, **73** in Scheme 31), or to carry the free alcohol through the cyclization reaction (*i.e.*, **76** → 77 in Scheme 32), we were left with few options. Answering the question of which protecting group would be most easily removed subsequent to formation of the unstable azabicyclic ring system, we arrived at the most basic of silyl ethers, the trimethylsilyl group. Silylation of the C12-hydroxyl group of **78** provided **79** in good yield. Now benzyl carbamate cleavage worked well to provide the free aziri-

dine, which underwent cyclization under the reaction conditions to afford the azabicyclic system **80**. Finally, HF·pyridine cleavage of the trimethylsilyl ether afforded **81**, the intact, fully elaborated aziridino[1,2-*a*]pyrrolidine ring system of the azinomycins.



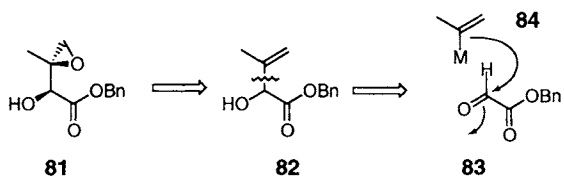
SCHEME 33

Compound **81** (Scheme 33) 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 and of azabicyclic systems with the C12-hydroxyl group protected. Repeated attempts to isolate **81** were unsuccessful, and even removing the reaction media *in vacuo* was sufficient to cause significant decomposition. Ultimately, we were able to obtain sufficient data from *in situ* ^{1}H NMR in THF-d_8 to provide characterization of **81**. The chemical shifts of the protons on the azabicyclic ring agreed well with those reported for the azinomycins. The synthesis of **81** proceeded in 15 steps, in very modest overall yield. The report of the synthesis of **81** in 1998 by Jian-she Kong was the first, and, to date, only route to the fully elaborated azinomycin ring system, including a description of a protecting group strategy (albeit a cumbersome one) for the selectively acylated C12/C13 diol of the natural products.[21] Full details of this route, along with the crotylstannane-based route developed by Tom Richardson were reported in a subsequent full paper.[22]

X. The Epoxyacid Fragment and the Perils of Optical Rotations

In the spring of 1995, coincident with the start of NIH funding for this effort, a new postdoc, Chris Sarko, joined the project to develop an efficient synthesis of the C17-C21 epoxyacid fragment.[23] Given that there were already a number of approaches to this substructure in the literature, we set as requirements of our approach brevity (number of synthetic operations), efficiency (overall mass throughput), and high enantioselectivity. Existing syntheses of the five-carbon epoxide substructure of the azinomycins were lengthy and/or inefficient with respect to chemical yield or stereoselectivity.

With substantial room for improvement, we reasoned that the trivial disconnection shown could be used to construct (±)-**82** (Scheme 34): the addition of a 2-propenyl organometallic reagent to benzyl glyoxylate followed by a Sharpless kinetic resolution should afford the target ester **81**. The only real issue was the chemical yield since we were limiting ourselves to no more than 50% chemical yield in the resolution step.

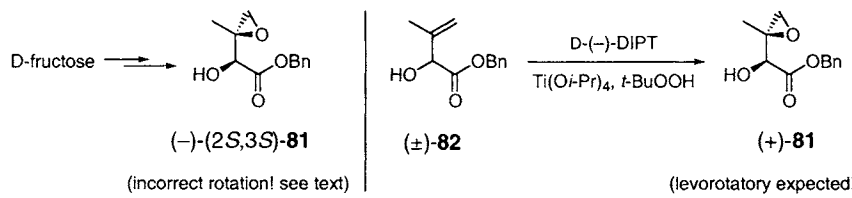


SCHEME 34

In practice, 2-lithiopropene (**84**, M = Li) could not be induced to add chemoselectively to the aldehyde of benzyl glyoxylate (**83**) even at –78 °C (Scheme 34). Addition of one equivalent of zinc chloride to the lithium reagent (**84**, M = ZnCl) modulated its reactivity so that clean conversion to the allylic alcohol (±)-**82** could be achieved in good yield (60%).

When we examined the kinetic resolution of racemic alcohol (±)-**82**, we found that this compound participated efficiently in the Sharpless asymmetric epoxidation as we expected. Without giving the matter a second thought, we had chosen D-(–)-diisopropyl tartrate (DIPT) as the chiral partner in this reaction based on the reliable predictability of the Sharpless epoxidation (Scheme 35). Shibuya and co-workers had prepared (2*S*,3*S*)-**81** by a lengthy but stereochemically unambiguous route from D-fructose, and so it was clear that D-(–)-DIPT would unquestionably provide the (2*S*,3*S*)-hydroxyepoxide **81** and recovered (2*R*)-hydroxyolefin **82**. Shibuya and co-workers had reported (2*S*,3*S*)-**81** to be levo-

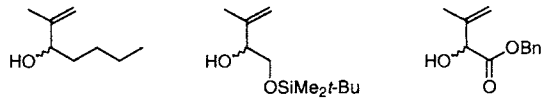
rotatory, but that the epoxide emerging from the Sharpless kinetic resolution of racemic alcohol (±)-**82** was dextrorotatory.[24] Chris naturally concluded that we had prepared the (2*R*,3*R*)-epoxide **81**, or the opposite enantiomer necessary for the azinomycin total synthesis. In apparent confirmation of this conclusion, our results agreed with those of Konda and co-workers,[25] who found that a *levorotatory* epoxide **81** was obtained with L-(+)-diethyl tartrate, which they assigned as (2*S*,3*S*)-**81** based on Shibuya's rotation. After reconfirming our result and carefully re-reading Sharpless' papers, we concluded that we had discovered an example of the Sharpless epoxidation of an allylic alcohol that occurred with opposite sense of asymmetric induction. The only examples of such reversals reported in the literature were with systems containing both allylic and homoallylic alcohols, where presumably the homoallylic hydroxyl group alters the coordination geometry about the titanium.



SCHEME 35

After a flurry of email exchanges with Barry Sharpless and M. G. Finn, both of whom were intrigued by our results, we set out to understand the origin of this apparent anomaly. A careful examination of the literature showed that there were no examples of Sharpless epoxidation of allylic alcohols bearing a carboxylate adjacent to the coordinating hydroxyl group. Thinking this must be the origin of the apparent reversal of stereoselection, my first new Ohio State student, Jason McKinley, set out in 1997 to prepare of series of compounds related to the key allylic alcohol **82** in order to demonstrate this anomaly. Our excitement was to be short-lived.

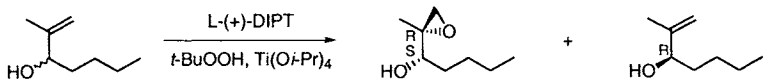
The thought process in this study was to design a series of 1,1-disubstituted olefins (Scheme 36) where the group distal to the double bond is varied from simple alkyl groups (**84**) to potentially coordinating groups (**82**). Our expectation was that there would be a switch in the sense of stereoselection with compound **82**.



84 **85** **82**

SCHEME 36

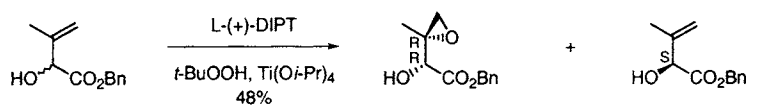
The kinetic resolution of racemic allylic alcohol (±)-**84** proceeded with the expected sense of enantioselectivity (Scheme 37) in accordance with earlier work of Sharpless. The enantiomeric excess of the resulting epoxide (2*R*,3*S*)-(−)-**86** was determined to be 98% by coupling with *R*-(+)-MTPA to afford the MTPA ester. In a similar fashion, kinetic resolution of allylic alcohol **85** using L-(+)-DIPT proceeded to afford the corresponding "normal" (2*R*,3*S*)-epoxyalcohol.



(±)-**84** (2*R*,3*S*)-(−)-**86** (3*R*)-**84**

SCHEME 37

When (±)-**82** was subjected to the identical reaction conditions (Scheme 38), the epoxyalcohol **87** that was isolated (93% ee) was levorotatory: $[\alpha]_D^{20}$ −11.10 (EtOH, *c* 1.90). According to Shibuya's results, this levorotatory epoxyalcohol should be (2*S*,3*S*)-**81**. However, Jason demonstrated that when alcohol **82** recovered from the Sharpless kinetic resolution was hydrogenated, *dextrorotatory* (2*S*)-(+)-2-hydroxyisovaleric acid was isolated, which compared in sign of rotation with authentic material prepared by Ourisson's procedure from (*S*)-valine. This was our first hint that something was amiss.



(±)-**82** (−)-**81** (3*S*)-**82**

SCHEME 38

Essentially coincident with this discovery, Professor Mike Shipman sent us a preprint of a paper describing a route to **81** that was based on a Sharpless asymmetric dihydroxylation protocol.[26] The specific rotation of

our epoxyalcohol (–)-**81**, at this point of undetermined absolute configuration, was opposite in sign and of the same magnitude as the rotation of (2*S*,3*S*)-(+)-**81** that was prepared by Shipman and co-workers. Given the fact that this group had been so scientifically rigorous as to confirm absolute stereochemistry of both the final product (2*S*,3*S*)-**81** and one of their intermediates by X-ray crystal structure analysis (using diastereomeric derivatives), this clearly meant that the absolute configuration of our levorotatory epoxyalcohol must be opposite to that present in azinomycin A and B. Thus, we unambiguously determined that we had synthesized (2*R*,3*R*)-(–)-**81**.

In Jason's studies,[27] he found that the rotation reported by Shibuya and co-workers was incorrect in both sign and magnitude,[28] a fact also discovered by Shipman. Not only had the Shibuya rotation led us astray (and created significant trouble for Shipman and co-workers), but Konda and co-workers assigned *levorotatory* epoxide (–)-**81** erroneously as (2*S*,3*S*) based on Shibuya's optical rotation. In the end, we found that the Sharpless kinetic resolution of (±)-**82** occurs with the *expected* sense of stereoinduction, where L-(+)-DIPT affords unnatural (2*R*,3*R*)-(–)-**81** contrary to our original report, and D-(–)-DIPT affords the desired (2*S*,3*S*)-(+)-**81**.

The detective work that went into these studies constituted the substantial 1998 M.S. thesis of Jason McKinley. We spent a great deal of effort to resolve absolute stereochemistry that had been based solely on one incorrectly measured optical rotation. Jason's studies served to confirm the death of, and firmly nail the lid on, the coffin of the decrepit technique of optical activity measurement. His work was the last time my research group used a polarimeter.

XI. Total Synthesis of Azinomycin A

By the five disconnections at ester, amide, olefin and C–N bonds **a**-**e** (Figure 5), we could arrive retrosynthetically at five simple fragments: naphthoic acid **87**; epoxyalcohol **81**; glycine phosphonate **88**; aziridine carboxaldehyde **89**; and 1-amino-2-propanol (**90**). An efficient and effectual synthetic route to naphthoic acid **87** had been published in 1992 by Shibuya as part of their earlier carzinophilin studies,[29] and we felt no need to spend time trying to improve on an already good route to this intermediate. Epoxyalcohol **81** was available in four steps using the Sharpless kinetic resolution described above. Known glycine phosphonate **88** was readily prepared starting from glyoxylic acid and benzyl urethane.

Aminopropanol **90** was available in both racemic and enantiomerically pure forms from Aldrich. The key intermediate, aldehyde **89**, was available from the corresponding olefin in a linear but efficient sequence of high yielding reactions. For the purposes of the total synthesis, we chose a triethylsilyl ether as the C12-hydroxyl protecting group.

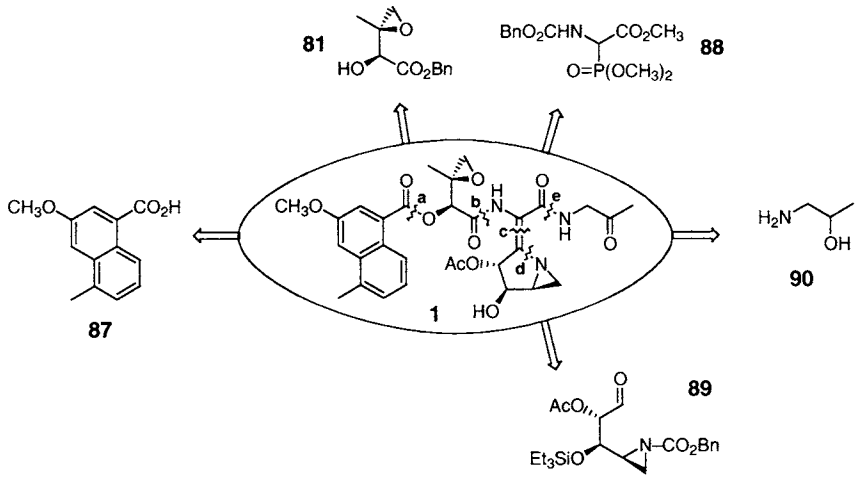


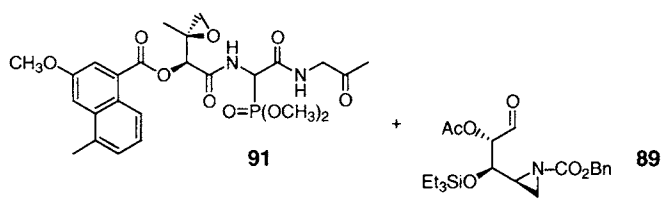
FIGURE 5
Modular and Convergent Retrosynthesis of Azinomycin A

During the earlier work of Andy Carpenter, we had used the *tert*-butyldimethylsilyl ether for this protection, but it could not be removed in model systems subsequent to azabicyclic ring formation. I had strongly urged Andy to examine the more labile triethylsilyl ether, but he found it would not completely survive the Vasella fragmentation en route from D-glucosamine. We abandoned further exploration of silyl ethers or alternative conditions for Vasella fragmentation in favor of carboxylic ester protecting groups and the crotylstannane-based route, eventually completed by Tom Richardson. This was clearly a case of finding the grass greener on the other side of the proverbial fence. I have found it both regrettable and ironic that my first choice for C12-hydroxyl protection, the triethylsilyl ether, originally examined and abandoned in 1992, was successfully used in the total synthesis completed nine years later.

When two new postdocs, Jing Li and Antonio Navarro, joined the group at Ohio State in late 1999, we proceeded to complete the total synthesis. Having arrived at suitable syntheses of key intermediates **81** and **89**, and developed a clear and stereocontrolled strategy for construction of

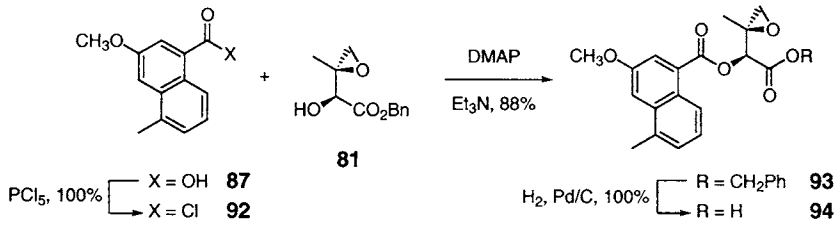
the aziridino[1,2-a]pyrrolidine ring system, we now had to plan how to put everything we had learned into practice (Figure 5). What order the steps **a**-**e** would be accomplished in the synthetic direction was not predetermined (*i.e.*, we had no clue how to bring these five pieces together to form the natural product). We had established clearly the importance of introducing the reactive azabicyclic system (bond **d**) as the penultimate step. We also knew that manipulations on systems bearing the dehydroamino acid double bond could be tricky, and so we felt that a late introduction of bond **c** would be wise. This left the question of whether the top framework should go into the Wadsworth-Horner-Emmons olefination step intact (*i.e.*, [**a** + **b** + **e**] + **c**), a strategy that would risk the survival of the labile epoxide, but which would reduce late stage transformations. The alternative equation was whether the left fragment should be incorporated after olefination (*i.e.*, [**e** + **c**] + **b** + **a** or [**e** + **c**] + [**a** + **b**]), which would require that one of the benzyl carbamate protecting groups on either **88** or **89** be changed (possibilities included the *tert*-butyl or 9-fluorenylmethoxy carbamate). The thought of trying to incorporate another dimension of orthogonality in protecting groups was nauseating at this point. Greene's book on protecting groups was dog-eared beyond repair.

Clearly the most direct route would be to synthesize the entire top piece **91** (Scheme 39), bearing a phosphonate suitable for subsequent olefination with the bottom piece **89**. Being a believer in Occam's Razor, this was the strategy upon which we embarked, as it would require no new transformations or protecting groups. Antonio Navarro put together the top piece **91**, and Jing Li began investigating olefinations of **89** and bromination reactions of the derived dehydroamino acid systems.



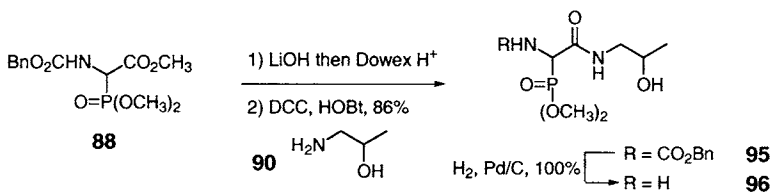
SCHEME 39

Initial ester bond between naphthoic acid **87** and epoxyalcohol **81** was most conveniently formed by way of the acid chloride **92** in the presence of *N*,*N*-dimethylaminopyridine to afford the left-half **93** (Scheme 40). Hydrogenolysis of the benzyl ester of **93** proceeded in quantitative yield under standard conditions to afford carboxylic acid **94.**



SCHEME 40

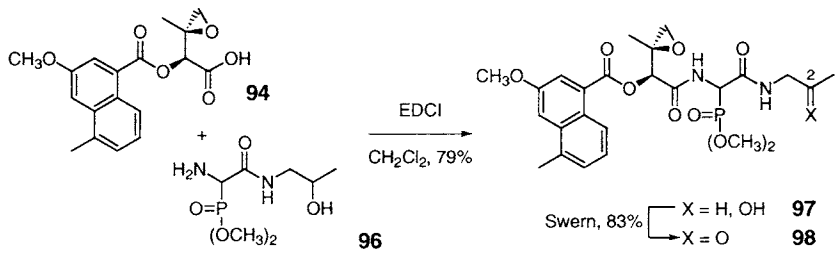
Saponification of the methyl carboxylate of glycine phosphonate **88** was not straightforward, as the methyl esters of the phosphonate moiety were subject to competing hydrolysis (Scheme 41). Carefully controlled stoichiometry of lithium hydroxide and non-aqueous work-up with Dowex cation exchange resin provided the corresponding carboxylic acid in quantitative yield. Direct coupling of the acid and 1-amino-2-propanol (**90**) with DCC provided the right-half phosphonate alcohol **95**. Hydrogenolysis of the benzyl carbamate of **95** afforded amine **96** quantitatively.



SCHEME 41

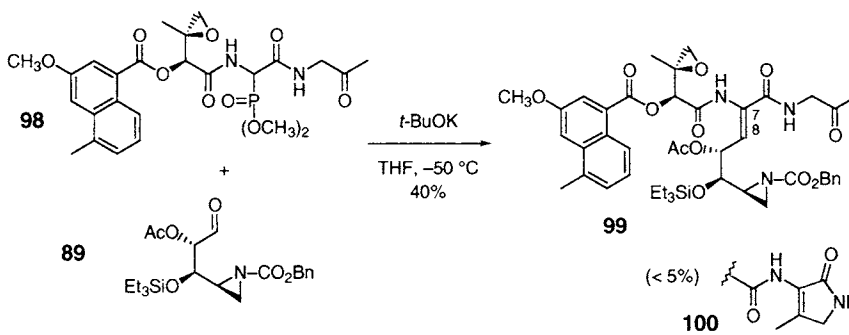
Coupling of the left (**94**) and right (**96**) fragments of the azinomycin top piece worked best using the carbodiimide EDCI, and effected amide bond formation in good yields to afford the fully elaborated phosphonate **97** (Scheme 42). Our initial conjecture was that the C2-hydroxyl group would need to be carried through the upcoming Wadsworth-Horner-Emmons reaction and then oxidized to the corresponding ketone in order to avoid a competing intramolecular olefination between the phosphonate anion and the C2-ketone to form a pyrrolinone ring. This would either require a hydroxyl protecting group (and two additional synthetic transformations on this late intermediate), or perhaps we could manage to perform the olefination with the unprotected alcohol. This latter option actually did work to afford the corresponding dehydroamino acid, but oxidation of the secondary alcohol to the ketone was problematic. Even though I had suggested to Jing Li that we protect the C2-hydroxyl group of **97**, it seems that Jing was also a strong believer in Occam's Razor. She went ahead with the more simple strategy of installation of the C2-ketone

(prior to olefination), and oxidation of **97** afforded **98** in good yield. Jing felt it was clearly better to know the answer to the question of intramolecularity versus bimolecularity in the Wadsworth-Horner-Emmons than to fool with protecting groups. It was a good decision on her part.



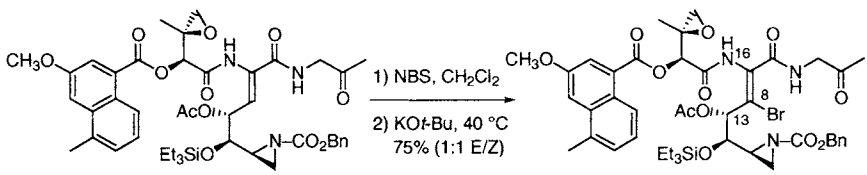
SCHEME 42

Treatment of phosphonate **98** with potassium *tert*-butoxide at low temperature followed by the addition of aldehyde **89** afforded the dehydroamino acid **99** (Scheme 43) in modest yield (not surprising given the normally low yields of this reaction and the complexity of the substrates). What was heartening, though, was that the intramolecular olefination product **100** was present in less than 5% yield, and oftentimes could not be detected in the crude reaction mixture. In retrospect, this is not entirely surprising. For the intramolecular olefination to occur, the amide bond of **98** must react from the less stable *cis*-amide conformation, and equilibration may be sufficiently slow at –50 °C to preclude competitive cyclization. In any event, this coupling reaction brought together the two key advanced intermediates **89** and **98** to form structure **99**, requiring only pyrrolidine formation to complete the total synthesis. In this case, the word "only" is used facetiously.



SCHEME 43

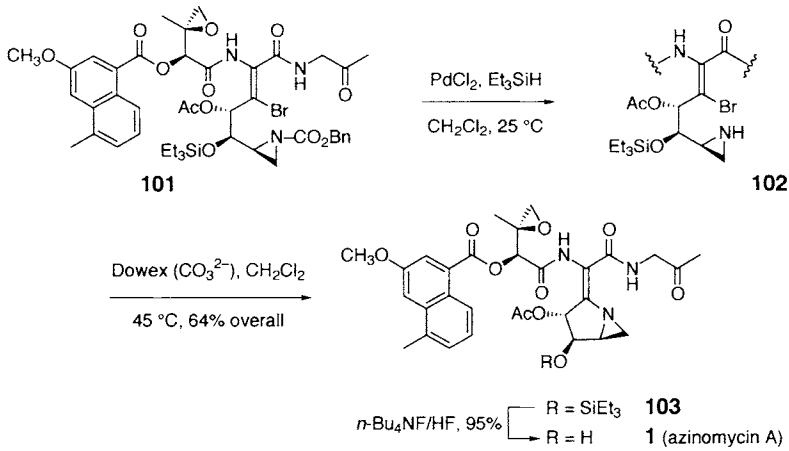
Bromination of dehydroamino acid **99** proved to be difficult, although after a somewhat extensive series of optimizations, Jing Li was able to effect introduction of the required C8-bromide (Scheme 44). The ratio of isomers was not favorable, typically slightly greater than 1:1 E/Z, but the isomeric vinyl bromides were separable. Once again, model studies did not prove valuable in predicting the outcome in the real system. This is a good example of what I once heard Sam Danishefsky refer to as the "Profumo Principle," so defined as an excessive reliance on models.[30] It may be that the high temperatures needed to induce tautomerization from the intermediate α-bromoimine to the vinyl bromide **101** may be largely responsible for the erosion of kinetic selection for the E-isomer. The stereochemistry about the tetrasubstituted olefin of **101** was demonstrated as in previous systems by the observation of a positive and reciprocal nOe between C13-H and N16-H of **101.**



99 **101**

SCHEME 44

Completion of the total synthesis of azinomycin A proceeded uneventfully (Scheme 45). Removal of the aziridine N-benzyl carbamate using the triethylsilane/palladium reduction conditions afforded the intermediate free aziridine **102**, which was carried into the cyclization reaction in the presence of Dowex anion exchange resin as a bromide ion scavenger, to provide O12-triethylsilyl azinomycin A (**103**) in good yield. The final fluoride-promoted deprotection of the triethylsilyl ether occurred in quantitative yield to afford the natural product (**1**). Jing Li completed the ^{1}H NMR of synthetic **1** at five o'clock on a Friday afternoon in early February of 2001, as I stood watching over her shoulder. Synthetic azinomycin A proved identical in all aspects with an authentic sample obtained by fermentation, so after the research group had consumed the appropriate number of beverages on Friday evening at a nearby watering hole, the paper went off by email to *Angewandte Chemie* at ten o'clock on the following Saturday morning.[31]



SCHEME 45

Acknowledgments

The work described in this review is the result of hard work, perseverance, patience, and determination by a highly talented group of co-workers, named specifically throughout the chapter. The early part of this work was funded by a Dreyfus Foundation Distinguished New Faculty Award (1989-94), an American Cancer Society Junior Faculty Research Award (1990-1993), the American Cyanamid Young Faculty Award (1993), and an Alfred P. Sloan Foundation Research Fellowship (1995). Work since 1995 has been funded by a grant from the National Cancer Institute (CA 65875).

References and Footnotes

1. Hata, T.; Koga, F.; Sano, Y.; Kanamori, K.; Matsumae, A.; Sugawara. R.; Hoshi, T.; Shima, T.; Ito, S.; Tomizawa, S. *J. Antibiotics, Ser. A* **1954**, 108.
2. Terawaki, A.; Greenberg, J. *Biochim. Biophys. Acta* **1966**, *119*, 59. Terawaki, A.; Greenberg, J. *Nature* **1966**, *209*, 481. Lown, J. W.; Majumdar, K. C. *Can. J. Biochem.* **1976**, *55*, 630.
3. Lown, J. W.; Hanstock, C. C. *J. Am. Chem. Soc.* **1982**, *104*, 3213.
4. Onda, M.; Konda, Y.; Noguchi, A.; Omura, S.; Hata, T. *Chem. Pharm. Bull.* **1971**, *19*, 2013.
5. Onda, M.; Konda, Y.; Hatano, A.; Hata, T.; Omura, S. *J. Am. Chem. Soc.* **1983**, *105*, 6311. Onda, M.; Konda, Y.; Hatano, A.; Hata, T.; Omura, S. *Chem. Pharm. Bull.* **1984**, *32*, 2995.

6. Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K,; Ishizeki, S.; Nakashima, T. *J. Antibiotics* **1986**, *39*, 1527.
7. Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554.
8. Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 3807.
9. Ishizeki, S.; Ohtsuka, M.; Irinoda, K.; Kukita, K.-I.; Nagaoka, K.; Nakashima, T. *J. Antibiotics* **1987**, *40*, 60.
10. Coleman, R. S.; Carpenter, A. J. *Tetrahedron Lett.* **1992**, *33*, 1697.
11. Coleman, R. S.; Dong, Y.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 3732
12. Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1984**, *67*, 1328.
13. Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870.
14. Coleman, R. S.; Carpenter, A. J. *Chemtracts – Organic Chemistry* **1991**, *4*, 213.
15. Birkofer, L.; Bierwirth, E.; Ritter, A. *Chem. Ber.* **1961**, *94*, 821.
16. Coleman, R. S.; Shah, J. A. *Synthesis* **1999**, 1399
17. Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 5813.
18. Marshall, J. A.; Seletsky, B.; Coan, P. S. *J. Org. Chem.* **1994**, *59*, 5139.
19. Coleman, R. S.; Richardson, T. E.; Carpenter, A. J. *J. Org. Chem.* **1998**, *63*, 5738.
20. Brown, H. C.; Jadhav, P. K.; Bhat, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 1535.
21. Coleman, R. S.; Kong, J.-s. *J. Am. Chem. Soc.* **1998**, *120*, 3528
22. Coleman, R. S.; Kong, J.-s.; Richardson, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 9088.
23. Coleman, R. S.; Sarko, C. R.; Gittinger, J. P. *Tetrahedron Lett.* **1997**, *38*, 5917.
24. Ando, K.; Yamada, T.; Shibuya, M. *Heterocycles* **1989**, *29*, 2209.
25. Konda, Y.; Machida, T.; Sasaki, T.; Takeda, K.; Takayanagi, H.; Harigaya, Y. *Chem. Pharm. Bull.* **1994**, *42*, 285.
26. The data in the Shipman paper (Bryant, H. J.; Dardonville, C. Y.; Hodgkinson, T. J.; Hursthouse, M. B.; Malik, K. M. A.; Shipman, M. *J. Chem. Soc., Perkin 1* **1998**, 1249) are unambiguous with respect to absolute configuration since both a key intermediate and the final product were characterized by X-ray crystallography as the camphorsulfonate or phenethylamine derivatives, respectively.
27. Coleman, R. S.; McKinley, J. D. *Tetrahedron Lett.* **1998**, *39*, 3433.
28. The specific rotation reported by Shibuya was $[a]_D^{20}$ –22.4 (EtOH, *c* 0.13). This means that the actual rotation measured on a polarimeter, assuming a standard 1 dm path length, was only –0.029°, which may be of insufficient magnitude to permit reliable measurement. Shipman's specific rotation of $[a]_D^{20}$ +11.5 (EtOH, *c* 1.9) for material of 95% ee was made at a much higher concentration giving an apparent measured rotation of +0.2185°, and is therefore more reliable. We feel this may be the origin of the discrepancy.
29. K. Shishido, T. Omodani, M. Shibuya, *J. Chem. Soc., Perkin 1* **1992**, 2053
30. For a less cynical view on the topic of "models that do not work", see Chapter 2 in: Sierra, M. A.; de la Torre, M. C. *Angew. Chem. Int. Ed.* **2000**, *39*, 1538.
31. Coleman, R. S.; Li, J.; Navarro, A. *Angew. Chem., Int. Ed.* **2001**, *43*, 1736.