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Chemical synthesis and mode of action of the azinomycins

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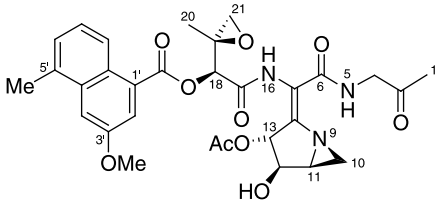
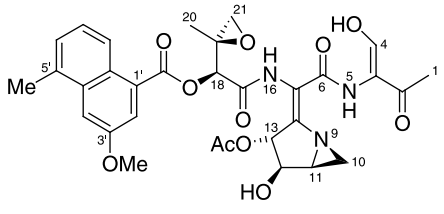
1. Introduction

Chemical agents capable of inducing DNA interstrand cross-links (ISCs) comprise an extremely important class of cancer chemotherapeutic agent.[1] Indeed, several important clinical drugs used for the treatment of this disease (e.g. cisplatin, chlorambucil and melphalan) are known to induce ISC formation. Azinomycins A and B are naturally occurring antibiotics which possess potent in vitro cytotoxic activity, significant in vivo antitumour activity and which appear to act by ISC formation (Fig. 1).[2–4] The azinomycins possess a truly remarkable structural motif in the form of the 1-azabicyclo[3.1.0]hex-2-ylidene dehydroamino acid fragment (i.e. C-6 to N-16). As far as we are aware, only one other natural product, ficellomycin, is known to contain a 1-azabicyclo[3.1.0]hexane ring system.[5] The background surrounding the azinomycins is complicated by the fact that azinomycin B is identical to carzinophilin,[6] a compound isolated over three decades earlier by Hata et al. from *Streptomyces sahachiroi*.[7] Whilst several attempts to deduce the molecular structure of carzinophilin have been published,[8–14] it transpires that these structural proposals are incorrect and that, in fact, carzinophilin and azinomycin B are the same compound.[6,15]

It has been widely accepted that the epoxide and aziridine



Azinomycin A, **1**



Azinomycin B, **2**

Figure 1.

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functionality are responsible for the ISC activity of the azinomycins, although definitive proof in support of this hypothesis is only just beginning to emerge.[16,17] Work in this area has been hampered by the limited availability of the natural products. It is known that cross-linking occurs between DNA bases, two residues apart on the complementary strands, with specificity for $^{5'}$GNC$^{3'}$ and $^{5'}$GNT$^{3'}$ sequences, with alkylation occurring via N-7 of both purine bases.[18] ISC formation by azinomycin B is pH dependent, with more rapid cross-link formation occurring at lower pH.[19] Since tumour cells are characterised by having a somewhat lower pH than normal cells, this may explain, in part, the selectivity of these agents.

The unusual structural features, present in the azinomycins, combined with their therapeutic potential has aroused much interest from the scientific community. The synthetic challenge presented by these natural products is very substantial, because, in addition to the density of functionality associated with their structures, they are quite unstable especially in acidic media.[2,7] The strained 1-azabicyclo-[3.1.0]hexane ring is also prone to opening at C-10 by nucleophiles.[20,21] Recently, it has been proposed that the C-12 hydroxyl group may contribute to the observed instability of the natural products.[22,23] Despite considerable efforts over the past 15 years, no total synthesis of these natural products has yet been accomplished.[67] Terashima has described the most advanced synthetic intermediates to date, this work culminating in the preparation of 4-*O*-methyl-13-desacetyl-12,13-di-*O*-benzylazinomycin.[21] In this report, we review all the chemistry and biology associated with this intriguing class of natural product, in the hope that it might stimulate further research in this field.

2. Isolation and structural elucidation

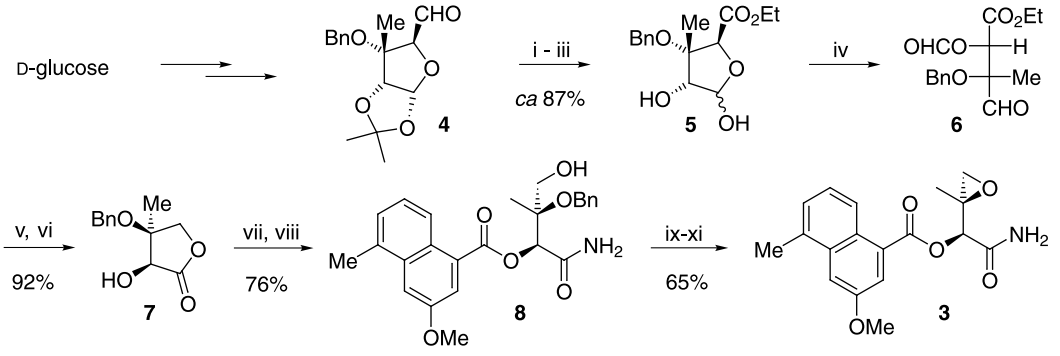
In 1986, azinomycins A and B, **1** and **2**, were isolated from the culture broths of *S. griseofuscus* S42227 by Nagaoka et al. (Fig. 1).[2] The producing strain was isolated from a soil sample obtained from Itakura, Japan. After fermentation, the natural products were isolated by a simple extraction and chromatographic procedure. The structures of the azinomycins were elucidated using a variety of physical and spectroscopic techniques in conjunction with chemical derivatisation studies.[3] NMR experiments based upon long-range selective decoupling techniques and NOE difference measurements were particularly useful in identifying the strained and chemically labile 1-azabicyclo-[3.1.0]hexane ring system. Other key functionalities of the azinomycins were identified as the *gem*-disubstituted epoxide, the disubstituted naphthoyl ester and the dehydroamino acid. Azinomycins A and B are very closely related, differing only in the nature of the substituent present at C-3. Whilst it appears to be widely accepted that the tetrasubstituted double bond possesses the (*E*)-configuration, this assignment was made solely on the basis of the surprisingly high chemical shift (δ 10.8) of the amide proton at N-5. An intramolecular hydrogen bond between this NH and the nitrogen of the aziridine ring was invoked by Yokoi et al. to account for this downfield shift, an interaction which requires the (*E*)-configuration of the double bond.[3] Some further evidence in support of this assignment has emerged.[24] The (18*S*,19*S*)-configuration of the 'left-hand' side of these natural products has been conclusively established by the synthesis of the epoxide domain from D-glucose (see Section 3.1).[25,26] The relative stereochemical relationships within the 1-azabicyclo[3.1.0]hexane ring system were determined by NOE difference measurements.[3] It would appear, however, that unambiguous proof regarding the *absolute* stereochemistry at C-11, C-12 and C-13 has not yet been published. This particular issue may only be satisfactorily resolved by the completion of the first total synthesis of these natural products.

3. Synthetic studies towards the azinomycins

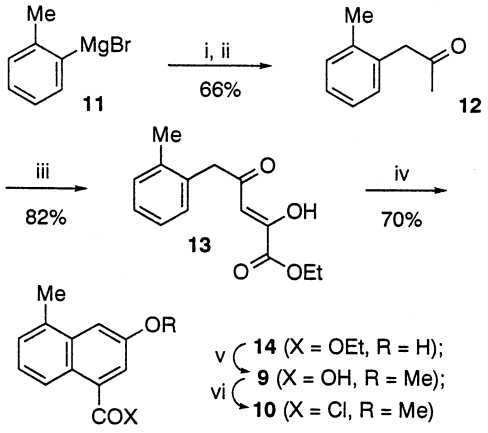
Many of the approaches to the azinomycins have strategically disconnected the molecule into two parts about the central amide bond and, it is therefore appropriate to review the synthetic routes to the epoxide and the 1-azabicyclo[3.1.0]hex-2-ylidene dehydroamino acid separately. The work, directed towards the complete carbon skeleton of the azinomycins, and to the preparation of analogues for biological and/or pharmacological investigations are surveyed in two further sections.

3.1. Approaches to the epoxide subunit

In 1987, the first approach to the epoxide subunit of the azinomycins was reported by Shibuya and Terauchi (Scheme 1).[25,26] Whilst somewhat lengthy (17 linear steps from D-glucose), this work was important because it



Scheme 1. *Reagents and conditions*: (i) Jones' reagent, Me_2CO; (ii) HCl (cat.), EtOH; (iii) 70% AcOH (aq.); (iv) $NaIO_4$ (2 equiv.), MeOH–H_2O (1:1); (v) $NaBH_4$, EtOH–THF (1:1); (vi) TsOH (cat.), benzene; (vii) 3-methoxy-5-methyl-1-naphthoyl chloride, Et^{i}Pr$_2$N, DMAP, CH_2Cl_2; (viii) ca 15% NH_3 in MeOH; (ix) H_2, 10% Pd-C, AcOH; (x) MsCl, Et^{i}Pr$_2$N, CH_2Cl_2; (xi) K_2CO_3, Me_2CO.



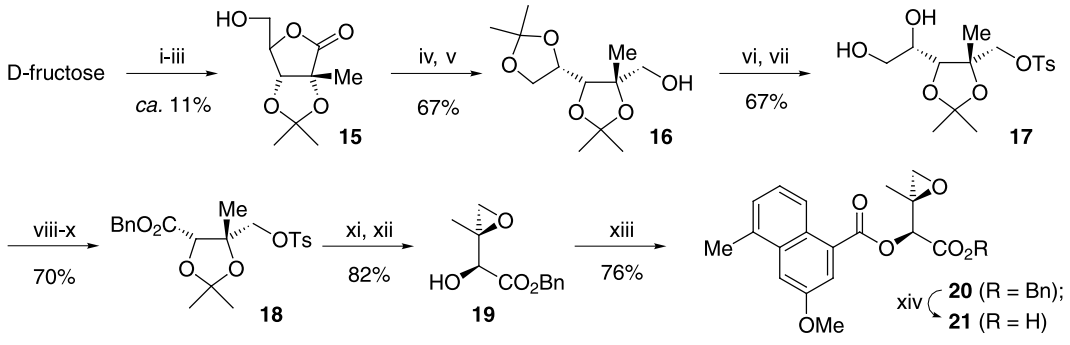
Scheme 2. *Reagents and conditions*: (i) propylene oxide, Et_2O then NH_4Cl (aq.); (ii) Jones' reagent, Me_2CO; (iii) $(CO_2Et)_2$, NaOEt; (iv) conc. H_2SO_4, $CHCl_3$; (v) $(MeO)_2SO_2$, NaOH (aq.); (vi) PCl_5, Et_2O.

established the (18*S*,19*S*)-stereochemistry of the natural products. This assignment was possible because the epoxy amide **3** was isolated from the culture broths of *S. griseofuscus* along with the azinomycins. By comparison of the optical rotations of the synthetic and natural epoxy amide **3** {$[\alpha]_D^{23}=+47.5$ (*c* 0.32, MeOH) (synthetic[26]); $[\alpha]_D^{25}=+48$ (*c* 0.33, MeOH) (natural[3])}, Shibuya was able to deduce the (*S*,*S*)-stereochemistry of this material, and hence the (18*S*,19*S*)-stereochemistry of the azinomycins. Their stereocontrolled synthesis of epoxy amide **3** began with the conversion of D-glucose in six steps into aldehyde **4** according to well-described methods (Scheme 1).[27] Oxidation of this aldehyde with Jones' reagent, esterification and subsequent hydrolysis of the acetonide ring with 70% acetic acid produced lactol **5** as a mixture of anomers, which was oxidatively cleaved with periodate to give the aldehyde **6**.[28] Treatment of **6** with sodium borohydride facilitated simultaneous cleavage of the formate group and reduction of the aldehyde, subsequent acid-catalysed cyclisation then yielding the γ-lactone **7**. Acylation of the free hydroxyl group within **7** with 3-methoxy-5-methyl-1-naphthoyl chloride followed by aminolysis with methanolic ammonia produced primary amide **8**. Finally, cleavage of the benzyl ether by hydrogenolysis, selective mesylation of the primary hydroxyl group, and subsequent potassium carbonate-induced ring-closure resulted in the formation of epoxy amide **3** in enantiomerically enriched form.[26]

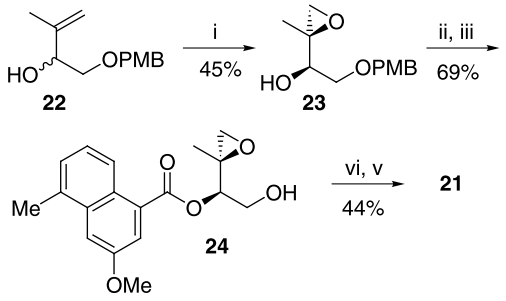
All the syntheses of the left-hand domain of the azinomycins utilise 3-methoxy-5-methyl-1-naphthoic acid **9** or the corresponding acid chloride **10** to introduce the aromatic chromophore. The method originally described by Shibuya for its synthesis has been adopted by all workers in this field,[28] although some minor modifications to the later stages have been reported.[29] The preparation of these naphthalene derivatives involves conversion of the Grignard reagent **11** into the ketone **12** by treatment with propylene oxide and oxidation of the resulting secondary alcohol with Jones' reagent (Scheme 2). After base-mediated condensation of **12** with diethyl oxalate, the resulting enol derivative **13** which is reported to be the sole product of the reaction, was cyclised using concentrated H_2SO_4 in $CHCl_3$ to give naphthol **14**. Subsequent ester hydrolysis and methylation produced carboxylic acid **9** in 92% yield from **14**, which can be further transformed into the corresponding acid chloride **10**.[28]

Shibuya has described a second route to the epoxide subunit of the azinomycins from D-fructose.[30] This carbohydrate was initially converted to γ-lactone **15**, then reduced to the corresponding triol using lithium aluminium hydride and selectively transformed into diacetonide **16** (Scheme 3). Tosylation of the remaining hydroxyl group and subsequent selective hydrolysis with aqueous acetic acid gave diol **17**. Oxidative cleavage of the diol with sodium periodate and further oxidation with Jones' reagent furnished the carboxylic acid, which was esterified to give benzyl ester **18**. Hydrolysis of the remaining acetal and base-induced ring-closure furnished epoxy alcohol **19**, which was condensed with 3-methoxy-5-methyl-1-naphthoic acid **9** to give diester **20**. Finally, selective cleavage of the benzyl ester by hydrogenation facilitated conversion to carboxylic acid **21**, which is suitable for coupling to the dehydroamino acid fragment of the azinomycins. It is pertinent to note that later studies have suggested that some of the optical rotation data reported in this paper are incorrect.[29,31–33] These findings are particularly significant because several groups have relied on this physical data to establish the absolute stereochemistry of their products (vide infra).

A number of groups have explored the use of the Sharpless asymmetric epoxidation (SAE) reaction to introduce the epoxide functionality and establish the relative and absolute stereochemistry of the 'left-hand' side of the azinomycins. The first such approach was reported by the group of



Scheme 3. *Reagents and conditions*: (i) $Ca(OH)_2$, H_2O, 8–10 weeks; (ii) $(CO_2H)_2{\cdot}2H_2O$; (iii) Me_2CO, H_2SO_4; (iv) $LiAlH_4$, Et_2O; (v) Me_2CO, TsOH; (vi) TsCl, DMAP, pyridine; (vii) 70% AcOH (aq.); (viii) $NaIO_4$, MeOH–H_2O (1:1); (ix) Jones' reagent, Me_2CO; (x) BnOH, DCC, DMAP, THF; (xi) 80% AcOH (aq.); (xii) K_2CO_3, Me_2CO; (xiii) **9**, DCC, DMAP; (xiv) H_2, 10% Pd-C, MeOH.

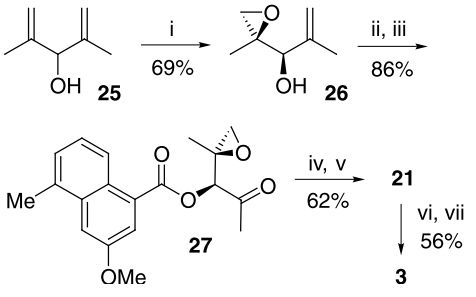


Scheme 4. *Reagents and conditions*: (i) D-(−)-DIPT, $Ti(O^iPr)_4$, ^tBuOOH, CH_2Cl_2; (ii) **9**, DCC, DMAP, CH_2Cl_2; (iii) DDQ, CH_2Cl_2–H_2O; (iv) Swern oxidation; (v) $NaClO_4$, NaH_2PO_4, THF–^tBuOH.

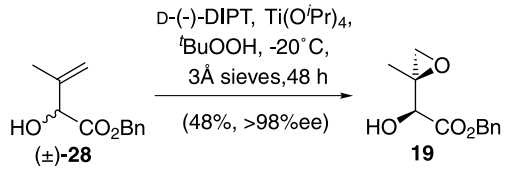
Armstrong who employed the alkenyl alcohol (±)-**22** as a substrate for the epoxidation (Scheme 4).[34] This compound was prepared in three steps from allyl alcohol in 24% overall yield. Subjection of (±)-**22** to SAE conditions using D-(−)-diisopropyl tartrate facilitated kinetic resolution of the substrate and resulted in the formation of the desired (*R*)-epoxy alcohol **23** in 88% ee and 45% chemical yield. DCC coupling of this alcohol with 3-methoxy-5-methyl-1-naphthoic acid **9** followed by oxidative deprotection of the *p*-methoxybenzyl ether yielded the alcohol **24**. Finally, stepwise oxidation of the resulting hydroxyl group afforded the carboxylic acid **21** in eight linear steps from allyl alcohol in 3% overall yield.

A more efficient synthesis relying upon the SAE reaction has been reported by Shishido et al. (Scheme 5).[31] The prochiral alcohol **25** was transformed into epoxy alcohol **26** in 69% yield according to the protocol of Schreiber.[35] Esterification using naphthoic acid **9** and oxidative cleavage of the alkene furnished methyl ketone **27**. Further conversion into carboxylic acid (2*S*,3*S*)-**21** was accomplished by formation of the enol carbonate and Lemieux–Johnson oxidation. This latter step was very sluggish and required over 10 days to produce an acceptable yield of **21**. The resulting acid was transformed into amide **3** by coupling with 4-methoxybenzylamine using PyBOP, then oxidative cleavage of the *p*-methoxybenzyl (PMB) group using DDQ. The epoxy amide (2*S*,3*S*)-**3** produced using this approach was identical with that isolated from *S. griseofuscus* S42227.

Two groups have reported kinetic resolution of racemic



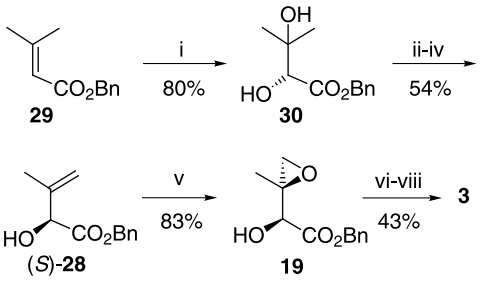
Scheme 5. *Reagents and conditions*: (i) D-(−)-DIPT, ^tBuOOH, $Ti(O^iPr)_4$, CH_2Cl_2; (ii) **9**, DCC, DMAP, CH_2Cl_2; (iii) $NaIO_4$, OsO_4, Et_2O–H_2O (1:1); (iv) LiHMDS, HMPA, $ClCO_2Me$, THF, −78°C; (v) $NaIO_4$, OsO_4, Et_2O–H_2O (1:1), 255 h; (vi) $PMBNH_2$, PyBOP, HOBt, Et_3N, DMF; (vii) DDQ, CH_2Cl_2–H_2O (18:1).



Scheme 6.

allylic alcohol **28** using the SAE method. The pioneering work was done by Konda,[36] with further refinements coming from the Coleman group.[33,37] Findings published by Shipman have revealed that the original conclusions made in these papers regarding the absolute sense of asymmetric induction in these reactions were incorrect.[29,32] Unfortunately, the Konda and Coleman groups were mislead by the erroneous optical rotation data for (2*S*,3*S*)-**19** reported by Shibuya (vide supra).[30] Shipman's revised interpretation of these reactions has been endorsed and confirmed independently by Coleman and McKinley,[33] and to prevent further confusion, we present the corrected data in this report. Kinetic resolution of the allylic alcohol (±)-**28** under SAE conditions using D-(−)-diisopropyl tartrate provides (2*S*,3*S*)-**19** in 48% isolated yield and 98% ee, along with recovered (*R*)-**28** (Scheme 6).[33,37] Lower levels of enantioselection were observed using the corresponding D-(−)-diethyl tartrate.[36] These kinetic resolutions proceed with the expected sense of stereoinduction and, furthermore, they are diastereospecific with none of the unwanted *anti*-epoxides being observed. Since several simple routes to (±)-**28** have been described,[36,37] this seems to be the most practical SAE approach to the epoxy alcohol motif of the azinomycins.

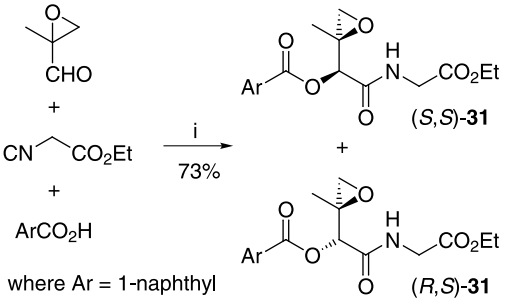
Shipman and coworkers have developed an alternative route to the left-hand portion of the azinomycins based upon the Sharpless asymmetric dihydroxylation (AD) reaction.[29,32] Using AD-mix-α, dihydroxylation of α,β-unsaturated ester **29** gave diol (*R*)-**30** in 80% yield and ≥95% ee (Scheme 7). This diol was converted into allylic alcohol (*S*)-**28** in three steps by selective mesylation, epoxide formation and subsequent acid-catalysed ring opening of the epoxide. A variety of oxidative methods were then examined for the stereocontrolled epoxidation of homochiral allylic alcohol (*S*)-**28**, the best levels of diastereoselectivity being achieved using an SAE reaction employing D-(−)-diethyl tartrate. Using the matched



Scheme 7. *Reagents and conditions*: (i) AD-mix-α, $NaHCO_3$, $MeSO_2NH_2$, ^tBuOH, H_2O; (ii) MsCl, CH_2Cl_2, Et_3N; (iii) Na_2CO_3, MeCN; (iv) CSA, toluene; (v) $Ti(O^iPr)_4$, D-(−)-DET, ^tBuOOH, CH_2Cl_2; (vi) **10**, Et_3N, DMAP, CH_2Cl_2; (vii) 10% Pd/C, H_2, MeOH; (viii) 35% NH_4OH, Et_3N, HOBt, PyBOP, DMF.

chirality of the substrate and epoxidising agent, epoxy alcohol (2*S*,3*S*)-**19** could be produced in 83% yield as a 96:4 mixture of separable diastereomers. Crystalline derivatives of both (2*S*,3*S*)-**19** and (*R*)-**30** were prepared and subsequent X-ray crystallographic studies unambiguously determined the relative and absolute stereochemistries of these materials. Epoxide **19** was converted into epoxy amide **3** via carboxylic acid **21** in a further three steps.

A very elegant approach to the epoxide fragment of the azinomycins has been devised by Moran and Armstrong using a Passerini three-component condensation (Scheme 8).[6] Simply stirring a mixture of 2-methylglycidal, ethyl isocyanoacetate and 1-naphthoic acid in ethyl acetate gave epoxy amide **31** in good yield as a 3.6:1 mixture of diastereomers. The major diastereomer was determined to possess the (*S*,*S*)-stereochemistry found in the natural products. This type of Passerini condensation was extended to other isocyanates incorporating a phosphonate or vinyl substituent at C-7 (azinomycin numbering). Armstrong has used this methodology to rapidly generate libraries of azinomycin analogues (see Section 3.4).[38,39]



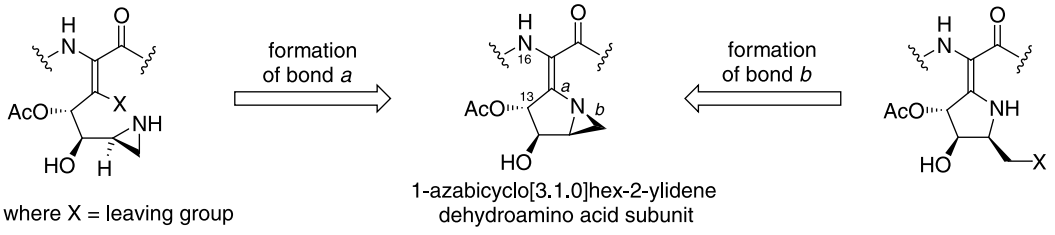
Scheme 8. *Reagents and conditions*: (i) EtOAc, 25°C, (*S*,*S*)-**30**/(*R*,*S*)-**30**, 3.6:1.

3.2. Synthesis of the 1-azabicyclo[3.1.0]hex-2-ylidene dehydroamino acid subunit

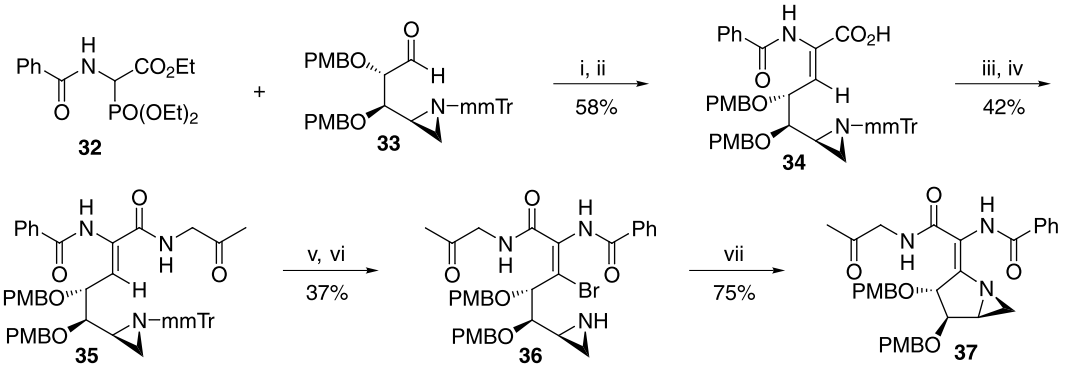
The preparation of the 1-azabicyclo[3.1.0]hexane ring system of the azinomycins is primarily very challenging because of its inherent ring strain, instability in acidic media,[2,7] and reactivity towards nucleophiles.[20,21] Control of the geometry around the tetrasubstituted alkene is a considerable challenge and, to make matters worse, determining the stereochemistry about this double bond is experimentally difficult. In some systems, NOE enhancements have been observed across the double bond between H-13 and N-16 on which to base an assignment.[23,40] In the natural products themselves,[3] and in other synthetic 1-azabicyclo-[3.1.0]hex-2-ylidene dehydroamino acids,[17,20,21] however, no useful NOE measurements have been observed and the stereochemical assignments have been tentatively made using other data. The synthetic challenge presented by this fragment of the natural products is further complicated by the fact that the C-12 and C-13 hydroxyl groups must be differentiated with only the C-13 position being acetylated. To date, only the most recent work of Coleman has satisfactorily addressed this issue.[23,41,42]

Two distinct strategies have emerged for the preparation of this ring system (Scheme 9). The research groups of Armstrong[24,40,43] and Coleman[23,41,42,44–46] have independently developed an intramolecular addition–elimination strategy to successfully prepare this ring system (i.e. formation of bond a). An alternative approach involving ring closure to form the aziridine ring (i.e. formation of bond b) has been devised by Terashima,[20,21,47–49] and further exploited by Shipman.[17,50]

The first approach to the 1-azabicyclo[3.1.0]hex-2-ylidene dehydroamino acid subunit was that described by Armstrong and Moran in 1992.[24,40] Whilst this early study ultimately produced a dehydroamino acid possessing the wrong alkene geometry, it did elegantly establish that the stereocontrolled formation of these systems via a conjugate addition–elimination strategy was viable. Furthermore, ^{1}H NMR comparisons with the data reported for the azinomycins helped in providing evidence for the (*E*)-alkene geometry within the natural products. The requisite cyclisation precursor was constructed from two components, namely, glycine phosphonate **32** and aldehyde **33** (prepared from D-arabinose). Whilst the synthesis of aldehyde **33** will not be discussed herein, it is pertinent to note that the use of the monomethoxytrityl (mmTr) protecting group proved to be essential for the successful synthesis of this aldehyde.[40] Condensation of phosphonate **32** with aldehyde **33** in the presence of LDA provided a mixture of the stereoisomeric dehydroamino esters {4:1; (*Z*)/(*E*)} (Scheme 10). Hydrolysis of this mixture then provided geometrically pure carboxylic acid (*Z*)-**34**, as the corresponding (*E*)-isomer was selectively destroyed under the reaction conditions. (*Z*)-**34** was coupled with 1-amino-2-propanol using DCC/HOBt, then oxidised under Swern conditions to provide (*Z*)-**35** containing the side chain of azinomycin A. Introduction of a leaving group at the β-position of (*Z*)-**35** was accomplished using bromine at −78°C followed immediately by treatment with DABCO which gave exclusively the isomerically pure vinyl bromide. Addition of Cl_3CCO_2H



Scheme 9.



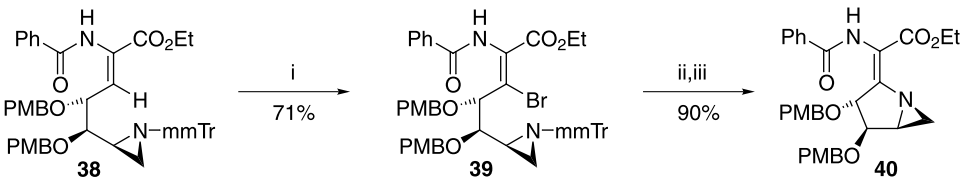
Scheme 10. *Reagents and conditions*: (i) LDA, THF, −78→0°C; (ii) LiOH, THF, H_2O, 50°C; (iii) $H_2NCH_2CH(OH)CH_3$, DCC, HOBt; (iv) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C→rt; (v) Br_2 then DABCO, CH_2Cl_2, −78→0°C; (vi) Cl_3CCO_2H, CH_2Cl_2 then Et_3N, rt; (vii) Et_3N, $CDCl_3$, 50°C, 8 h.

followed by quenching with Et_3N effected removal of the mmTr protecting group to give the free aziridine (*Z*)-**36**. Finally, subjection of (*Z*)-**36** to Et_3N in $CDCl_3$ at 50°C smoothly furnished 1-azabicyclo[3.1.0]hexane (*Z*)-**37** via conjugate addition–elimination. In other work, an aldehyde related to **33** in which the PMB protecting groups were replaced by triethylsilyl ethers was prepared from L-serine.[40] Whilst this aldehyde participated in Horner–Emmons olefinations with **32**, the authors indicate that this protecting group strategy is unworkable.[40,51] Indeed, in later work directed towards the entire azinomycin skeleton, a modified approach for the introduction of the protected C-12/C-13 hydroxyl groups was devised (see Section 3.3).[51]

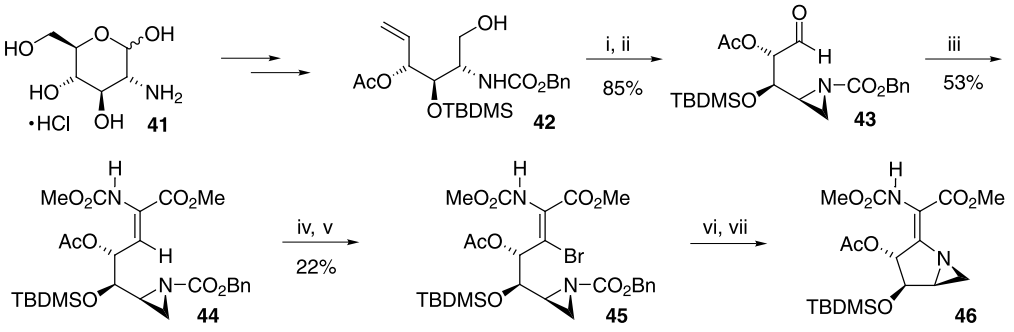
In further studies, Armstrong et al. devised a route to related 1-azabicyclo[3.1.0]hexanes containing the (*E*)-alkene geometry required for the natural products.[40,43] In a key discovery, they determined that the stereoselectivity of the bromination of ester (*Z*)-**38** was dependent on the precise reaction conditions. Thus, bromination of (*Z*)-**38** with NBS in CH_2Cl_2 provided exclusively (*E*)-**39** in 71% yield (Scheme 11), whereas treatment with bromine in the presence of 2,6-lutidine in CH_2Cl_2 at −78°C, followed by the addition of DABCO led to a mixture of (*E*)- and (*Z*)-isomers (1:1.5, respectively). Interestingly, treatment of (*E*)-**38** under the NBS conditions also produced exclusively (*E*)-**39** albeit in low yield (34%). The authors proposed that the bromination of both (*E*)- and (*Z*)-**38** proceeded via a common intermediate to account for these observations. Deprotection of the mmTr group from (*E*)-**39** and cyclisation proceeded with complete retention of stereochemistry to yield dehydroamino acid **40** with the requisite (*E*)-stereochemistry.

In 1992, Coleman reported the synthesis of a 1-azabicyclo-[3.1.0]hexane fragment containing differentially protected hydroxyl groups at C-12 and C-13 using a similar nucleophilic addition–elimination process.[22,44,45] D-Glucosamine hydrochloride **41** was converted in nine steps into differentially protected alkene **42**. A vasella fragmentation was used as a key step in this interconversion to introduce the olefin and open the pyranose ring (details not shown).[44,52] Mitsunobu cyclisation of **42** gave the corresponding aziridine which after ozonolysis of the terminal double bond yielded **43** (Scheme 12). Horner–Wadsworth–Emmons olefination of **43** produced dehydroamino acid **44** with >10:1 (*Z*)/(*E*) diastereoselectivity. The stereocontrolled introduction of the bromine atom into this system proved to be quite problematic, and detailed studies into halogenation reactions of this type were reported.[53] After extensive experimentation, it was determined that reaction of **44** with excess NBS in the presence of DABCO gave a *gem*-dibromoimine intermediate which upon treatment with sodium dithionite afforded the desired (*E*)-bromide **45**, along with an equal quantity of the undesired (*Z*)-isomer which could be separated by silica gel chromatography. In a recent article, it has been disclosed that potassium *t*-butoxide at −20°C can be used to perform this dehalogenation in a stereoselective fashion {(*E*)/(*Z*)≈3.5:1}, although no chemical yield is given using this method.[45] Finally, palladium-catalysed removal of the *N*-benzoxycarbonyl group from **45** provided the NH aziridine quantitatively, which, when warmed in the presence of DABCO, underwent a stereospecific intramolecular addition–elimination reaction to give dehydroamino acid (*E*)-**46**. Unfortunately, all attempts to remove the C-12 protecting group under either acidic or basic reaction conditions met with failure.

More recently, Coleman has described two further approaches to 1-azabicyclo[3.1.0]hexanes bearing differentially protected hydroxyl groups.[22,23,41,42] In these second-generation syntheses, C-12 deprotection to systems bearing a free hydroxyl group has been accomplished, although the compounds proved to be highly unstable and could only be characterised in situ. The first approach began



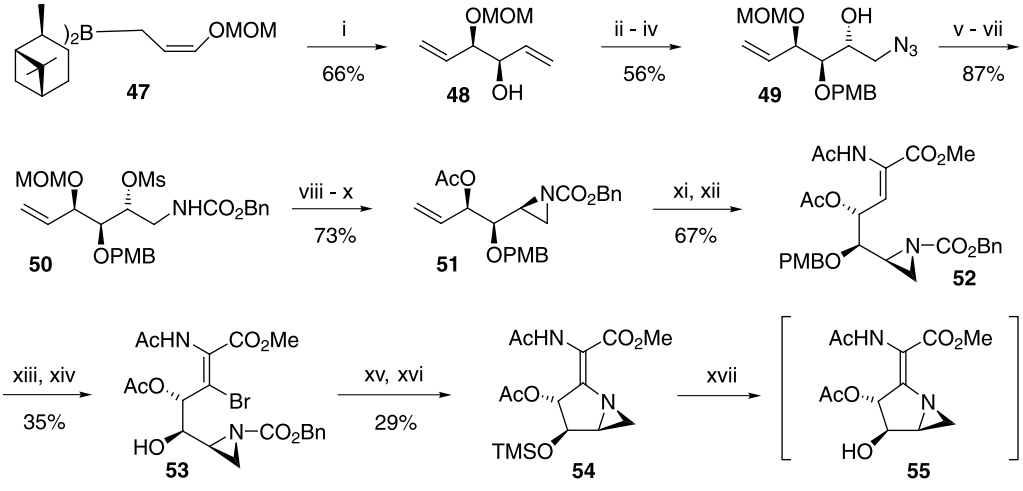
Scheme 11. *Reagents and conditions*: (i) NBS, $CHCl_3$; (ii) Cl_3CCO_2H, CD_3CN, rt; (iii) Et_3N, 50°C.



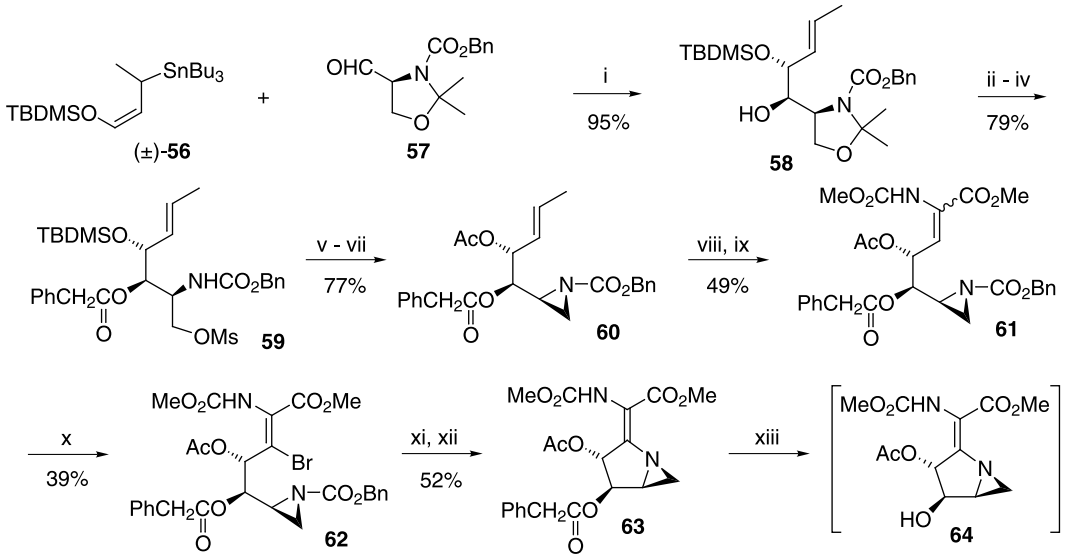
Scheme 12. *Reagents and conditions*: (i) Ph_3P, DEAD, THF, 23°C; (ii) O_3, CH_2Cl_2, −78°C then Me_2S; (iii) $MeO_2CNHCH[PO(OMe)_2]CO_2Me$, KO^{t}Bu, CH_2Cl_2, or THF; (iv) excess NBS, DABCO, CH_2Cl_2; (v) $Na_2S_2O_4$, THF–H_2O (1:1), 0°C, 15 min; (vi) Et_3SiH, $PdCl_2$, Et_3N; (vii) DABCO, $CDCl_3$, 50°C, 1 h.

with asymmetric allylation of acrolein using organoborane **47** to yield diene **48** (>95% ee) (Scheme 13).[23,41] SAE facilitated differentiation of the two double bonds, the hydroxyl group was protected and the epoxide ring then opened with sodium azide to give azide **49**. This compound was transformed using standard methods into aziridine **51**, containing all the required stereochemistry, via **50**. Oxidative cleavage of the double bond contained within **51**, followed by Wadsworth–Horner–Emmons olefination yielded dehydroamino acid **52** {>4:1, (*Z*)/(*E*)} in a satisfactory yield. At this juncture, several different strategies were explored to complete the synthesis, although only the most productive route is presented herein.[23,41] Bromination of (*Z*)-**52** gave the corresponding vinyl bromide{>5:1, (*E*)/(*Z*)}, which was transformed into alcohol **53** by oxidative deprotection of the PMB ether. Reprotection of this alcohol as its trimethylsilyl ether proved essential to the completion of the synthesis. Liberation of the aziridine NH, followed by treatment with piperidine, then facilitated cyclisation to 1-azabicyclo[3.1.0]hexane **54**. Deprotection of the C-12 ether from **54** with HF/pyridine afforded the target substructure **55**, which could not be isolated but could be characterised in situ by ^{1}H NMR and high resolution mass spectrometry.

The second approach explored the use of an enzyme-cleavable protecting group at C-12, namely a phenylacetyl ester.[23,42] Lewis acid-promoted addition of allylstannane **56** to aldehyde (*S*)-**57** (derived from L-serine) produced *syn*-alcohol **58** in near quantitative yield (Scheme 14). Whilst the reaction could be performed using homochiral (*S*)-**56**, it was more conveniently executed using an excess of racemic **56** (2.5 equiv.). Under these latter conditions, useful levels of kinetic resolution were observed {>10:1; (*S*)/(*R*)}, thus obviating the need to prepare (*S*)-**56** in enantiomerically pure form. Cleavage of the *N*,*O*-acetal from **58**, selective mesylation of the resulting primary hydroxyl group, and esterification of the remaining alcohol with phenylacetic acid then provided **59**. Replacement of the silyl ether at C-13 with an acetate (azinomycin numbering), and subsequent ring closure, gave aziridine **60**. Ozonolysis and olefination of **60** provided dehydroamino acid **61** in a rather modest yield as a mixture of geometric isomers. This and related olefinations proved difficult because of the presence of the proximal acetoxy group. Bromination of **61** gave (*E*)-**62**, which could be separated from the unwanted (*Z*)-isomer by column chromatography. Essentially, no stereocontrol could be accomplished in this bromination, a finding which contrasts with earlier work where good selectivity was achieved {typically ≥10:1; (*E*)/(*Z*)}. Removal of the Cbz group from (*E*)-**62** and cyclisation under basic conditions using Dowex resin provided 1-azabicyclo[3.1.0]-



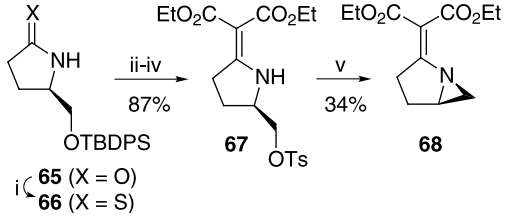
Scheme 13. *Reagents and conditions*: (i) acrolein, $BF_3{\cdot}Et_2O$; (ii) L-(+)-DIPT, ^{t}BuOOH, $Ti(O^iPr)_4$, −10°C; (iii) NaH, PMBBr; (iv) NaN_3, NH_4Cl, H_2O, $CH_3OCH_2CH_2OH$; (v) PPh_3, toluene, H_2O; (vi) $ClCO_2Bn$, Et_3N; (vii) MsCl, Et_3N; (viii) HCl, MeOH; (ix) Ac_2O, pyridine; (x) KO^{t}Bu, THF; (xi) O_3 then Me_2S; (xii) $AcHNCH[PO(OMe)_2]CO_2Me$, KO^{t}Bu, −65°C; (xiii) NBS, $CHCl_3$ then TMP; (xiv) DDQ, H_2O, $CHCl_3$; (xv) *N*,*O*-bis(trimethylsilyl)acetamide, 90°C, THF; (xvi) Et_3SiH, $PdCl_2$, Et_3N then piperidine, $CDCl_3$; (xvii) HF, pyridine.



Scheme 14. *Reagents and conditions*: (i) $MgBr_2{\cdot}Et_2O$, CH_2Cl_2; (ii) ethylene glycol, CSA, THF, 50°C; (iii) MsCl, Et_3N, CH_2Cl_2; (iv) $PhCH_2CO_2H$, DCC, DMAP; (v) HF, MeCN; (vi) Ac_2O, DMAP; (vii) KO^{t}Bu, THF, −78°C; (viii) O_3 then Me_2S; (ix) $MeO_2CHNCH[PO(OMe)_2]CO_2Me$, LiCl, ^{i}Pr$_2$EtN; (x) NBS, $CHCl_3$ then KO^{t}Bu; (xi) Et_3SiH, $Pd(OAc)_2$, Et_3N; (xii) Dowex 1X8-400, $CHCl_3$; (xiii) penicillin G acylase, pH 7.5, D_2O, D_3CCN.

hexane **63** in a stereospecific fashion. As well as serving as a base, the Dowex resin also acted as a bromide ion scavenger, suppressing ring opening of the aziridine by this anion. In an effort to produce **64** bearing a C-12 alcohol group, **63** was treated with 5–10 mol% polymer-supported penicillin G acylase in a mixed solvent system of acetonitrile/aqueous buffer. The phenylacetate could be removed with an approximate half-life of 2 h, but **64** could only be observed at best as the minor of several products in the reaction mixture as ascertained by 500 MHz ^{1}H NMR spectroscopy. As a result of all their efforts, the authors conclude that problems with deprotection of the C-12 hydroxyl group are not a result of the choice of inappropriate protecting groups, but are the result of the inherent instability of these systems.

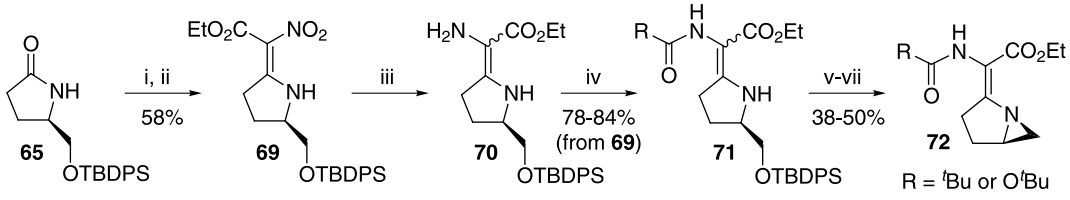
A considerable amount of work on the dehydroamino acid subunit of the azinomycins has been published by



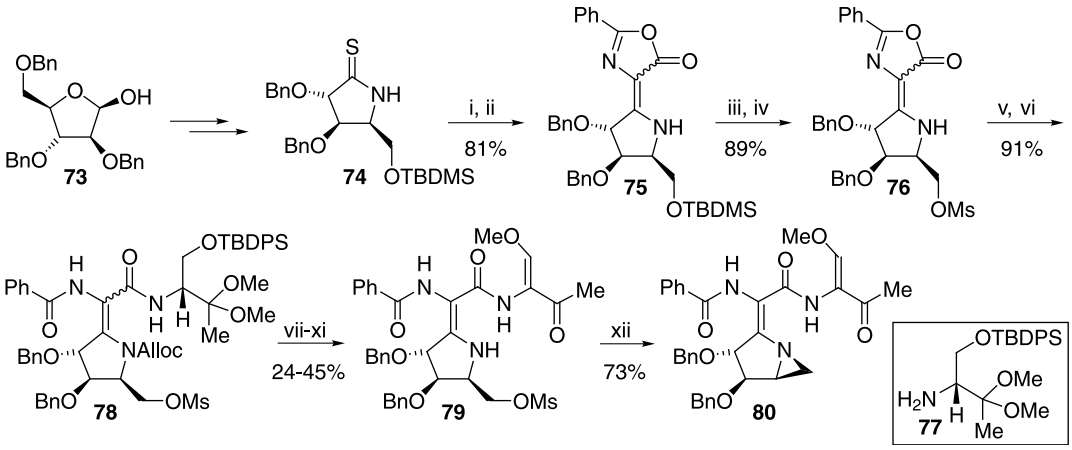
Scheme 15. *Reagents and conditions*: (i) Lawesson's reagent, toluene, reflux; (ii) $(EtO_2C)_2CHBr$, CH_2Cl_2 then $KHCO_3$ (aq.); (iii) TBAF, THF; (iv) TsCl, pyridine, CH_2Cl_2; (v) KH, THF, rt, 15 min.

Terashima and coworkers using bond disconnection b (Scheme 9).[20,21,47–49] Earlier model studies employed pyroglutamic acid as the starting material, which was transformed into lactam **65** using established methods.[47] The reaction sequence was perfomed using both enantiomers of pyroglutamic acid, although only the (*R*)-series is depicted (Scheme 15). The lactam functionality was converted to the corresponding thiolactam **66** using Lawesson's reagent in 97% yield, and **66** was subsequently reacted with diethyl bromomalonate via an Eschenmoser coupling to introduce the exocyclic double bond. Two further manipulations yielded tosylate **67**, which was suitably functionalised to evaluate the key aziridine ring closure reaction. Treatment of **67** with KH in THF afforded the 1-azabicyclo[3.1.0]hexane **68** which was isolated in 34% yield after Florisil® column chromatography. The authors noted that the cyclisation reaction was clean as determined by NMR but that degradation occurred during the purification. Intriguingly, the use of iodide rather than tosylate anion as the leaving group in the ring closure step resulted in the formation of substantial quantities of a dimeric compound.[47] Using a more densely-functionalised thiolactam derived from 2,3,5-tri-*O*-benzyl-β-D-arabinofuranose, a related 1-azabicyclo[3.1.0]hexane bearing benzyloxy groups at C-12 and C-13 with the correct stereochemistry has been produced using essentially the same chemistry.[48]

In an extension of his earlier work, Terashima explored



Scheme 16. *Reagents and conditions*: (i) $(MeO)_2SO_2$, benzene, 60°C; (ii) $O_2NCH_2CO_2Et$, 60°C; (iii) H_2 (5 atm), 10% Pd-C, toluene; (iv) Boc_2O, EtOAc or PivCl, $NaHCO_3$, EtOAc; (v) TBAF, THF; (vi) MsCl, Et_3N, CH_2Cl_2, −78°C; (vii) KH, THF.



Scheme 17. *Reagents and conditions*: (i) MeI, CH_2Cl_2; (ii) 2-phenyl-Δ^2-5-oxazolinone, toluene, 80°C; (iii) TBAF, THF; (iv) MsCl, Et_3N, CH_2Cl_2, −78°C; (v) $Alloc_2O$, DMAP, THF; (vi) **77**, toluene, then concentration at 50°C; (vii) HF–pyridine, pyridine; (viii) PDC, 4 Å MS, CH_2Cl_2; (ix) TsOH, THF–H_2O; (x) CH_2N_2, THF–Et_2O; (xi) $Pd(Ph_3P)_4$, Ph_3P, AcOH, THF; (xii) TBAF, 4 Å MS, THF.

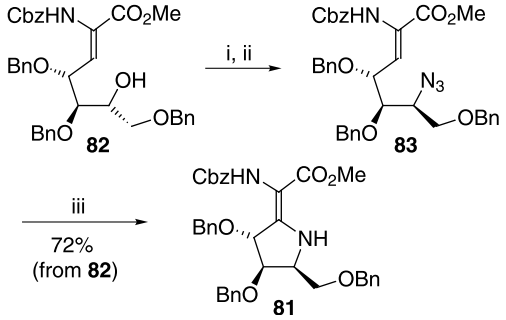
methods for the introduction of the dehydroamino acid group to produce substrates more closely related to the azinomycins (Scheme 16).[20] Lactam **65** was *O*-methylated with dimethyl sulfate to yield the corresponding imidate. Condensation with ethyl nitroacetate then provided nitromethylidene **69**, which was obtained as a stereoisomeric mixture, the ratio varying in different solvents [1:1 (C_6D_6), 6:4 ($CDCl_3$) and 1:0 (CD_3OD)]. X-ray diffraction, however, showed that **69** existed entirely as the (*Z*)-isomer in the crystalline state. Reduction of the nitro group by catalytic hydrogenation provided the unstable enamide **70**, which was immediately acylated with either Boc_2O or PivCl to give the protected enamine **71** (R=^{t}Bu or ^{t}BuO) as 7:1 [(*E*)/(*Z*)] mixtures of stereoisomers. Removal of the TBDPS group, activation of the hydroxyl group as the methanesulfonyl ester and ring closure with KH yielded the target 1-azabicyclo[3.1.0]hex-2-ylidene **72** (R=^{t}Bu or ^{t}BuO). The authors commented that **72** was very unstable to silica gel or Florisil® chromatography. The double bond geometry of **72** was tentatively assigned as possessing the (*E*)-configuration on the basis of ^{1}H NMR chemical shifts.

In further elegant work, Terashima used this approach to make a 1-azabicyclo[3.1.0]hex-2-ylidene subunit incorporating essentially all the features found in the 'right-hand' domain of azinomycin B (Scheme 17).[49] Thiolactam **74**, prepared from 2,3,5-tri-*O*-benzyl-β-D-arabinofuranose **73**,[48] was *S*-methylated with methyl iodide and then coupled with 2-phenyl-Δ^2-5-oxazolinone to give **75** as an inseparable mixture of isomers. Next, **75** was converted to the corresponding mesylate **76** prior to introduction of the azinomycin B side chain. Interestingly, this mesylate was initially produced as a 6:4 mixture of (*E*)- and (*Z*)-isomers after chromatography but underwent stereoisomerisation to give pure (*Z*)-**76** after recrystallisation from ethyl acetate/hexane (>90% recovery). In order to ring open the 4-methylene-Δ^2-oxazolin-5-one ring system, acylation of the pyrrolidine nitrogen atom was required. Thus, **76** was reacted with $Alloc_2O$ then 2-aminobutane **77**, obtained in five steps from *N*-benzoxycarbonyl L-threonine, to yield amides (*E*)-**78** (68%) and (*Z*)-**78** (23%) which were separated by column chromatography. Both (*E*)-**78** and (*Z*)-**78** were separately converted into (*E*)-**79** in a further five steps. Significantly, in the final step of this sequence in which the *N*-alloc group was removed by palladium catalysis, the (*Z*)-isomer cleanly isomerised to the more stable (*E*)-isomer. Finally, (*E*)-**79** was converted to 1-azabicyclo[3.1.0]hexane **80** as a single stereoisomer using TBAF as the base in combination with 4 Å molecular sieves. Whilst the stereochemistry about the methyl vinyl ether double bond was readily established using NOE measurements, the assignment about the C-7/C-8 double bond (azinomycin numbering) could only be made by comparison with the ^{1}H NMR spectum of 4-*O*-methyl azinomycin B.[3]

Whilst Konda et al. have not reported the construction of 1-azabicyclo[3.1.0]hexane ring systems, they have described a novel route to dehydroamino acid **81**, which could in principle be transformed into bicyclic systems.[54] The key steps in this synthesis involved conversion of **82** (prepared from tri-*O*-benzyl-D-arabinose) into azide **83** with stereochemical inversion followed by thermolysis to yield dehydroamino acid **81** (Scheme 18). The authors propose that the pyrrolidine-forming reaction proceeds through an initial 1,3-dipolar cycloaddition between the azide moiety and the alkene, which is followed by loss of molecular nitrogen from the intermediate triazoline.

3.3. Synthetic endeavours towards the natural products

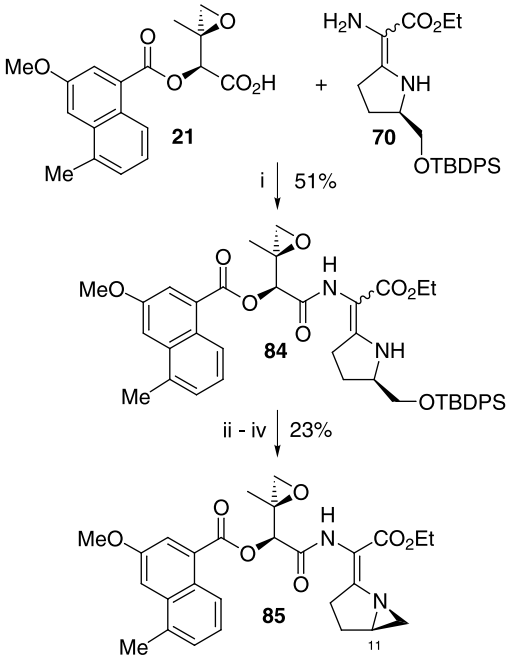
In this section, we review work dealing with the assembly of



Scheme 18. *Reagents and conditions*: (i) Tf_2O, 2,6-lutidine, −40°C; (ii) NaN_3, $BnEt_3NBr$, DMF; (iii) 60°C, THF.

molecules incorporating all the key functionality of the natural products (i.e. epoxide, aziridine and naphthoyl ester). The most complex structures have been reported by Terashima, who has described the synthesis of 4-*O*-methyl-13-desacetyl-12,13-di-*O*-benzylazinomycin.[21,67] As will be seen later, problems with the removal of protecting groups thwarted the completion of the total synthesis of the natural product itself. In 1996, Armstrong indicated that his group had prepared 13-desacetyl-12,13-di-*O*-*p*-methoxybenzyl-azinomycin A, although the details of this work have not yet appeared in the literature.[51]

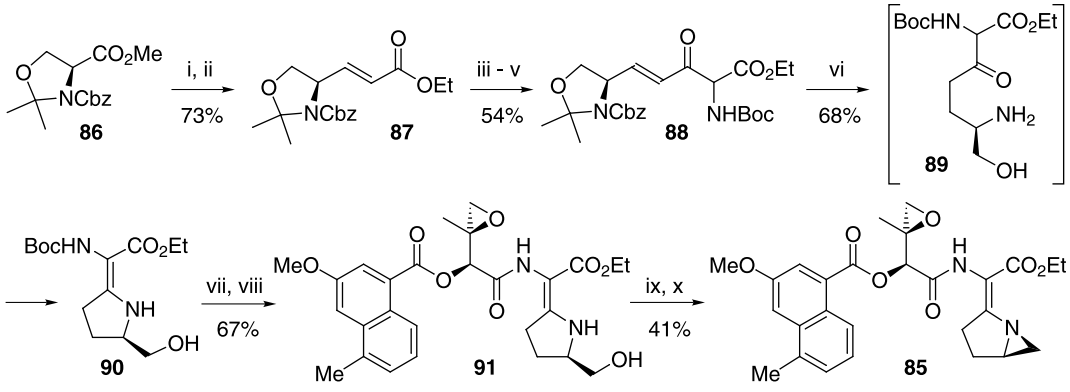
Terashima was the first to describe the synthesis of a compound containing both 'halves' of the azinomycin skeleton.[20] Enamine **70**, the synthesis of which has been described earlier (Scheme 16), was coupled with carboxylic acid **21** (prepared using the methods of Shibuya[28] and Hirama[55] with slight modifications) to give **84** as a tautomeric mixture (Scheme 19). Deprotection, mesylation and base-induced cyclisation then afforded epoxy aziridine **85** as a single stereoisomer. In this example, cyclisation was effected more cleanly using KHMDS rather than potassium hydride. The stereochemistry about the double bond was tentatively assigned as the (*E*)-configuration using ^{1}H NMR chemical shifts. Starting from the opposite enantiomer of pyroglutamic acid, a diastereomer of **85** possessing (11*S*)-stereochemistry was also prepared.



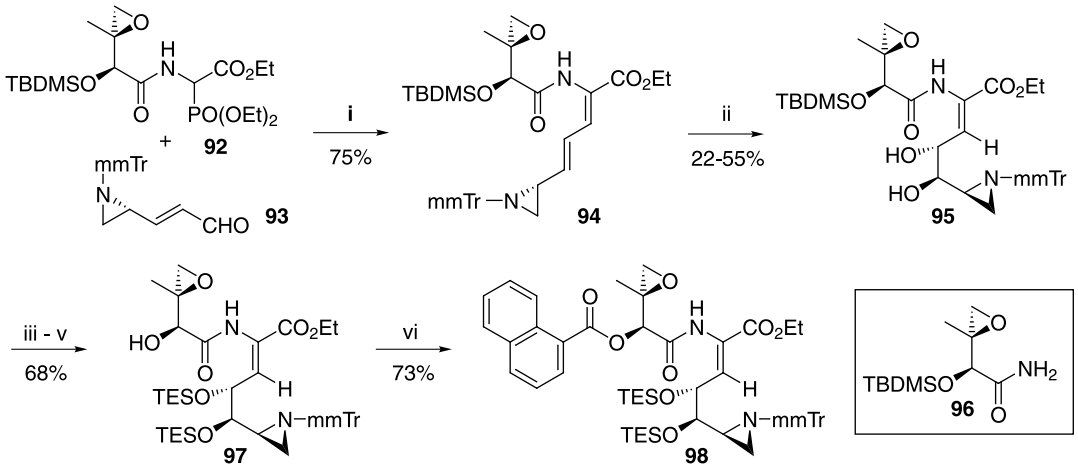
Scheme 19. *Reagents and conditions*: (i) DCC, HOBt, THF; (ii) TBAF, THF; (iii) MsCl, Et_3N, −78°C; (iv) KHMDS, THF.

An alternate synthesis of epoxy aziridine **85** has been reported by Shipman (Scheme 20).[17] Homochiral ester **86**, readily obtained from L-serine in three steps, was transformed into α,β-unsaturated ester **87** by DIBAL reduction and subsequent olefination using (carboethoxymethylene)-triphenylphosphorane. Further homologation of **87** to α-amino-β-ketoester **88** was accomplished by conversion to the acid chloride and coupling with $EtO_2CCH(CO_2H)$-(NHBoc) in the presence of magnesium ethoxide. The pivotal step in the synthesis was then executed by hydrogenation of **88** in the presence of aqueous hydrochloric acid to give dehydroamino acid **90** in 68% yield. The authors propose that the reaction proceeds via γ-amino ketone **89** which spontaneously cyclises and tautomerises to the thermodynamically more stable **90**. Dehydroamino acid **90** was produced as a variable mixture of geometric isomers in favour of the desired (*E*)-isomer [(*E*)/(*Z*); 10:1 to 6:1 (in $CDCl_3$)] and in reproducibly high enantiomeric excess [(*E*)-isomer=90% ee; (*Z*)-isomer=89% ee]. *N*-Boc deprotection and coupling of the resultant amino alcohol with carboxylic acid **21** yielded **91** as a mixture of geometric isomers [9:1 (in $CDCl_3$)]. Finally, ring closure to 1-azabicyclo[3.1.0]hexane **85** was accomplished according to the methodology devised by Terashima via the corresponding mesylate using TBAF as the base for the aziridine ring-forming step.[20] The stereochemical assignment about the tetrasubstituted double bond could only be tentatively assigned as *trans* by comparison with earlier synthetic intermediates.

Whilst Armstrong's synthesis of 13-desacetyl-12,13-di-*O*-*p*-methoxybenzylazinomycin A has not appeared in the literature, he has published the synthesis of some advanced synthetic intermediates which could potentially be transformed into the natural products using the bromination/ring closure methods developed in his group (Section 3.2).[51] The formation of the C-7/C-8 alkene bond is the

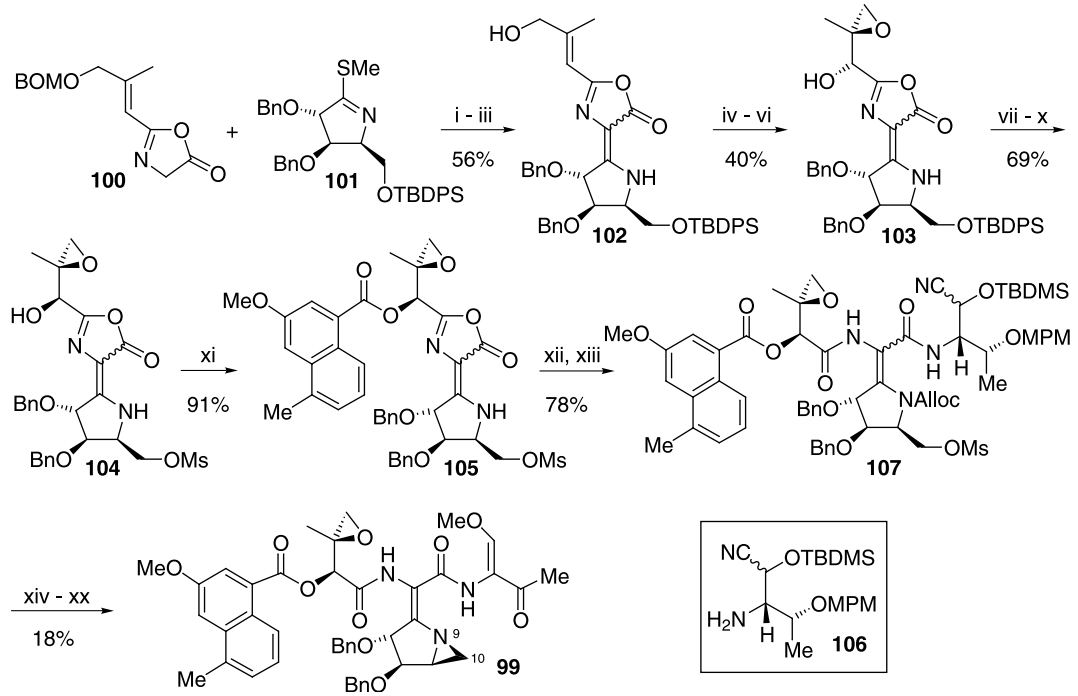


Scheme 20. *Reagents and conditions*: (i) DIBAL, toluene, −78°C; (ii) $Ph_3P{=}CHCO_2Et$, toluene; (iii) NaOH, THF, H_2O; (iv) $(COCl)_2$, DMF (cat.), CH_2Cl_2; (v) $EtO_2CCH(CO_2H)(NHBoc)$, $Mg(OEt)_2$, CCl_4; (vi) H_2, Pd/C, EtOH, HCl; (vii) TFA, CH_2Cl_2; (viii) **21**, PyBOP, HOBt, Et_3N, DMF; (ix) MsCl, Et_3N, −78°C, CH_2Cl_2; (x) TBAF, 4 Å molecular sieves, THF, 15 min.



Scheme 21. *Reagents and conditions*: (i) LDA, THF; (ii) $K_2OsO_2(OH)_4$, DHQD-*p*-chlorobenzoate ligand, $K_3Fe(CN)_6$, K_2CO_3, $^tBuOH–H_2O$ (1:1); (iii) TBAF, THF; (iv) TESCl, Et_3N, DMAP, CH_2Cl_2; (v) TBAF, THF; (vi) 1-naphthoic anhydride, DMAP, Et_3N.

central element of this approach. The sequence begins with the formation of the alkene bond by Horner–Emmons condensation of phosphonate **92** with aldehyde **93** (derived from L-serine)[40] to give diene **94** (Scheme 21). Initially, this condensation yielded a 4:1 ratio of [(*Z*),(*E*)]/[(*E*),(*E*)]-isomers, although the [(*E*),(*E*)]-diene decomposed via aziridine ring opening upon silica gel chromatography, leaving pure [(*Z*),(*E*)]-**94**. Sharpless AD of the distal double bond of **94** was then investigated and it was found, in general, that the best results were obtained when the reaction was run on a small scale (<0.3 mmol) to about 50% conversion. The reported yield of diol **95** is therefore based upon recovered starting material. In this dihydroxylation reaction, a minor diol isomer (typically 1:3 to 1:7 ratio) was also formed. In addition, epoxy amide **96** was produced in approximately the same yield as **95** but it becomes predominant at higher percentage conversions (>50%). The authors concluded that only modest regioselectivity was observed in the dihydroxylation reaction in favour of the distal double bond. The formation of **96** was rationalised as resulting from the dihydroxylation of the proximal double bond, followed by bond fission. Other phosphonates incorporating different ester (or amide) groups were evaluated in the Horner–Emmons condensation/dihydroxylation sequence although less favourable results were obtained. Diol **95** was subjected to TBAF deprotection to afford the corresponding triol, which was converted into alcohol **97** by trisilylation with TESCl followed by selective deprotection. Esterification of **97** with 1-naphthoic anhydride, thus provided naphthoate **98**. Further progression of these



Scheme 22. *Reagents and conditions*: (i) toluene, 60°C; (ii) DDQ; (iii) $NaBH_4$; (iv) $(DHQ)_2PHAL$, OsO_4 then H_2S; (v) MsCl, γ-collidine; (vi) DBU; (vii) Dess–Martin reagent; (viii) TBAF; (ix) MsCl, γ-collidine; (x) $NaBH_4$, $CeCl_3·7H_2O$, 80% de; (xi) **9**, WSCI.HCl, DMAP; (xii) $Alloc_2O$, cat. DMAP; (xiii) **106**; (xiv) DDQ; (xv) Dess–Martin reagent; (xvi) $Pd(PPh_3)_4$, AcOH; (xvii) TBAF, AcOH; (xviii) aq. $NaHCO_3$; (xix) CH_2N_2; (xx) TBAF, 4 Å MS.

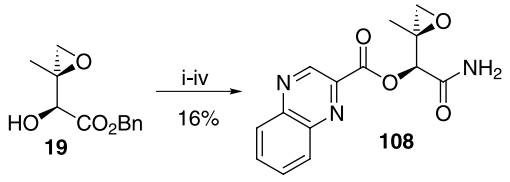
intermediates towards the azinomycins has not yet been disclosed.

Terashima's extensive efforts in this area recently culminated in the synthesis of 4-*O*-methyl-13-desacetyl-12,13-di-*O*-benzylazinomycin **99**.[21] Whilst the 'right-hand' domain was assembled largely according to his earlier studies, the chiral epoxide domain was installed in a somewhat different fashion using a Sharpless AD reaction. Excess 2-oxazolin-5-one **100**, synthesised in five steps from methyl hydroxymethyl-2-butenoate, was combined with thioimidate **101**[48,49] to give the condensation product in 58% yield as an inseparable mixture of (*E*)- and (*Z*)-isomers (Scheme 22). Deprotection of the BOM group in two steps provided the allylic alcohol **102**. Modified Sharpless dihydroxylation of **102** using stoichiometric amounts of OsO_4 and $(DHQ)_2PHAL$, followed by decomposition of the resulting osmate ester with H_2S, afforded the corresponding triol in excellent diastereomeric excess (>95% de). Selective mesylation of the primary hydroxyl group and DBU-induced ring closure provided epoxide **103**. Inversion of the C-18 stereochemistry was accomplished by an oxidation–reduction sequence. Towards this end, **103** was first oxidised with the Dess–Martin reagent to the corresponding epoxyketone. The TBDPS ether was removed and the resulting hydroxyl group converted to its methanesulfonate ester prior to stereocontrolled reduction of the C-18 carbonyl group under Luche conditions {(*S*)/(*R*)=9:1}. Esterification of **104** with acid **9** then proceeded uneventfully to give **105** in 91% yield. Introduction of the C-1 to N-5 side chain of azinomycin B was achieved by reaction of **105** (after prior *N*-alloc activation) with amine **106** (prepared from methyl L-threoninate in six steps). This reaction furnished predominantly the desired (*E*)-amide **107** in 78% yield, along with a small amount of the (*Z*)-isomer (2%). Seven further manipulations were required to complete the synthesis of 4-*O*-methyl-13-desacetyl-12,13-di-*O*-benzylazinomycin **99**. These involved removal of the MPM group, oxidation of the resulting C-2 hydroxyl group, cleavage of the *N*-alloc group, liberation and *O*-methylation of the formyl group from the cyanohydrin and, finally, closure of the aziridine ring using TBAF. The synthesis of **99** using this approach stands as a strong testament to the viability of the ring closure strategy devised by Terashima. The synthesis of the natural product itself, however, could not be achieved from **99** because attempts to effect reductive debenzylation at C-12 and C-13 resulted in cleavage of the aziridine ring via rupture of the N-9/C-10 bond.

3.4. Chemical synthesis of analogues

A variety of novel chemical structures related to those of the azinomycins have been synthesised to help elucidate the mechanism of action of these antitumour agents, and to identify novel agents with enhanced chemical stability and/or better biological profiles. The chemistry underpinning these studies is presented in this section. Most work has centred on the epoxide domain of the azinomycins as it is readily amenable, displays good chemical stability and is a potent cytotoxic agent in its own right (see Section 4.3).

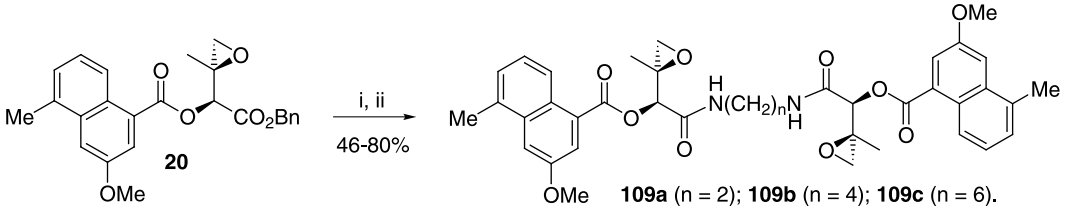
Shipman and coworkers have made a variety of simple alterations to the 'left-hand' side to explore the influence of such changes on cytotoxicity towards human tumour cell lines.[56] These derivatives were prepared from homochiral **19**, made using their Sharpless AD strategy.[29,32] A variety of changes to the nature of the aromatic chromophore, the absolute configuration of the compounds (by switching to AD-mix-β), and the amide substitution pattern were accomplished. The general strategy is illustrated by the preparation of epoxy amide **108** bearing a quinoxaline group (Scheme 23).



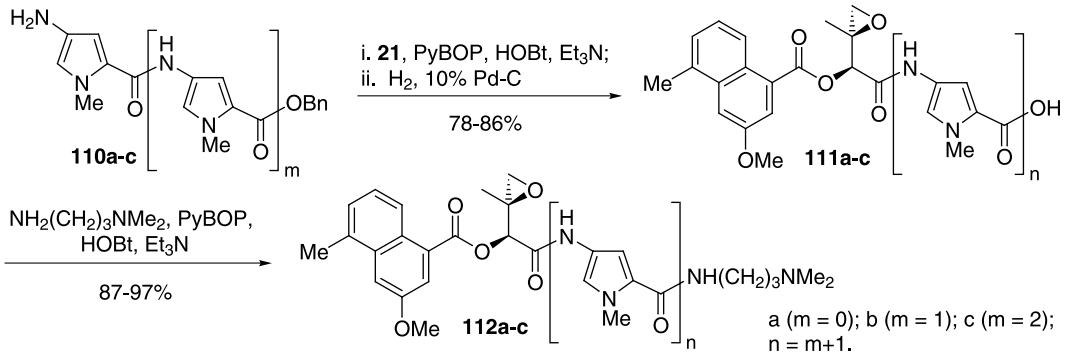
Scheme 23. *Reagents and conditions*: (i) 2-quinoxaloyl chloride, DMAP, Et_3N, CH_2Cl_2; (ii) H_2, 10% Pd-C, EtOAc; (iii) $PMBNH_2$, Et_3N, HOBt, PyBOP, DMF; (iv) CAN, $MeCN/H_2O$.

This work has been extended to the synthesis of symmetrical bisepoxides **109a–c** which have been demonstrated to act as powerful DNA interstrand cross-linking agents.[57] These compounds were readily made in moderate to good yields by reacting carboxylic acid **21** (formed by catalytic hydrogenation of benzyl ester **20**) with 0.5 M equiv. of a diamine (ethylenediamine, 1,4-diaminobutane or 1,6-diaminohexane) under PyBOP/HOBt coupling conditions (Scheme 24).

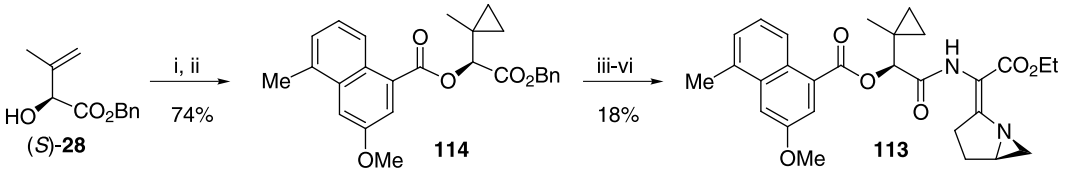
Shishido et al. have designed and synthesised several enantiomerically enriched azinomycin–lexitropsin hybrid molecules which incorporate the epoxide domain of the azinomycins (Scheme 25).[58] These compounds were prepared using the SAE approach devised by some members of this group.[31] Condensation of aminopyrrole **110a–c** with **21** yielded pyrrolecarboxylic acid **111a–c** after catalytic hydrogenation to remove the benzyl ester. Further analogues containing the unnatural (18*R*,19*R*)-stereochemistry



Scheme 24. *Reagents and conditions*: (i) 10% Pd/C, H_2, MeOH, 1 h; (ii) ethylenediamine (**109a**, 0.5 equiv.), 1,4-diaminobutane (**109b**, 0.5 equiv.) or 1,6-diaminohexane (**109c** 0.5 equiv.), PyBOP, Et_3N, HOBt, DMF.



Scheme 25.

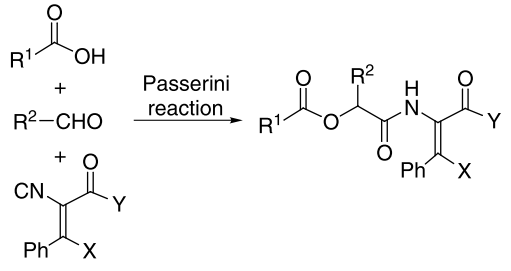


Scheme 26. *Reagents and conditions*: (i) Et_2Zn, CH_2I_2, CH_2Cl_2; (ii) **10**, DMAP, Et_3N, CH_2Cl_2; (iii) H_2, Pd/C, MeOH; (iv) **90** (pre-treated with TFA), PyBOP, HOBt, Et_3N, DMF; (v) MsCl, Et_3N, CH_2Cl_2; (vi) TBAF, 4 Å molecular sieves, THF.

were made by using L-(+)-DIPT in the SAE reaction. Introduction of the dimethylaminopropyl appendage found in distamin A into **111a–c** provided **112a–c** in excellent yields.

In order to study the role of the epoxide functionality in DNA interstrand cross-link formation, Shipman has reported the synthesis of cyclopropane analogue **113** (Scheme 26).[17] Cyclopropanation of enantiomerically pure allylic alcohol (*S*)-**28**,[56] and subsequent esterification of the hydroxyl group with acid chloride **10**, yielded ester **114** in good yield. This material was converted into aziridine **113** in a further four steps along similar lines to the chemistry used for the preparation of **85** depicted in Scheme 20.

Much less work has been published regarding the synthesis of analogues in which the unstable 1-azabicyclo[3.1.0]hexane ring system has been modified. Armstrong has successfully prepared a range of dehydroamino acids using a multiple-component condensation (MCC) reaction in which the bicyclic system has been substantially simplified.[38,39] The chemistry is based upon his earlier work on the Passerini reaction (Scheme 8).[6] Initially, a small library of analogues was synthesised using a polymer-supported strategy. A photocleavable polymer linker was, however, required so that the resultant azinomycin analogues could be removed from the resin under mild conditions without recourse to acid or base treatment.[38] Analogues more closely related to the natural products were made by applying this MCC technology in the solution phase (Scheme 27).[38,39] Indeed, a variety of compounds incorporating some or all of the key functionality of the natural products (epoxide, aziridine and naphthoyl ester) were made using this technology. The chemical structures of a number of the derivatives made using this chemistry can be found in Section 4.3 (Table 1, entries 10–16). Cytotoxicity data obtained for these compounds have helped in the development of structure–activity relationships (SARs).



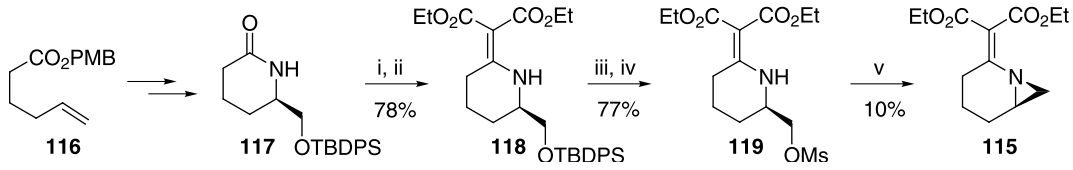
Scheme 27.

Shipman and coworkers have synthesised ring-expanded 1-azabicyclo[4.1.0]heptane **115** and its enantiomer, reasoning that the introduction of an additional methylene carbon into the five-membered ring might lead to a reduction in ring strain, thereby producing more chemically robust compounds (Scheme 28).[50] Homochiral δ-lactam (*R*)-**117** was made in high enantiomeric excess (≥95% ee) starting from alkenyl ester **116**.[59] By adaptation of the methodology reported by Terashima,[47] this lactam was converted into 1-azabicyclo[4.1.0]heptane **115** in a further five chemical steps by way of alkene **118** and mesylate **119**. While the crude ^1H NMR spectrum indicated that the final ring-closure reaction proceeded cleanly, purification of **115** proved to be rather difficult and it could be obtained in only 10% yield after careful chromatography on Florisil®.

4. Biological mode of action of the azinomycins

4.1. Antitumour activity

The azinomycins display in vitro cytotoxic activity against the L5178Y tumour cell line. The concentrations for 50% inhibition of cell growth (IC_{50}) for azinomycins A and B were found to be 0.07 and 0.11 μg/ml, respectively.[2] These



Scheme 28. *Reagents and conditions*: (i) Lawesson's reagent, toluene; (ii) $(EtO_2C)_2CHBr$, CH_2Cl_2, then aq. K_2CO_3; (iii) TBAF, THF; (iv) MsCl, CH_2Cl_2; (v) KH, THF, reflux, 90 min.

natural products are also active against Gram-positive and Gram-negative bacteria, but inactive against yeast and fungi. Ishizeki et al. undertook in vivo testing in mice to determine the antitumour activities of the azinomycins against the intraperitoneally inoculated tumours P388 leukaemia, P815 mastocytoma, B-16 melanoma and Ehrlich carcinoma, in addition to the solid tumours Lewis lung carcinoma and Meth A fibrosarcoma.[4] Intraperitoneal adminstration of azinomycin B (32 μg/kg/day) produced 57% survivors at 45 days and a 193% increase in lifespan (ILS), compared to 57% survivors at 45 days and an ILS of 204% for mitomycin C but at a higher dose (1 mg/kg/day). For Ehrlich carcinoma, azinomycin B gave 161% ILS and 63% survivors at 45 days, but solid tumours such as Lewis lung carcinoma and Meth A fibrosarcoma were not susceptible, even after repeated adminstration of the compound. Azinomycin A proved to be somewhat less effective than azinomycin B in the tumour systems evaluated. As part of this investigation, the acute toxicity of azinomycin B by a single intraperitoneal injection was determined (LD_{50} 190 μg/kg). It should be noted that Hata et al. had demonstrated in much earlier studies that carzinophilin (azinomycin B) has marked inhibitory activity against transplanted tumours (Yoshida sarcoma, Ehrlich carcinoma and ascitic hepatoma) and prolongs the survival time of the treated rats or mice.[7] One clinical study in humans has been disclosed in which it was determined that azinomycin B can be remarkably effective in the treatment of malignant neoplasm of the connective tissue.[60] Improvements were observed in patients suffering from various other types of inoperable tumours.

4.2. Mode of action of the natural products

The first indications of the mode of action of the azinomycins were made by Terawaki and Greenberg in 1966,[61,62] who found that azinomycin B markedly inhibited DNA synthesis but not RNA or protein synthesis in *E. coli* B_0 (a strain sensitive to azinomycin B) at doses which inhibit colony formation.[61] In addition, it was determined that azinomycin B induces ISCs in the DNA of *E. coli* B_0 and of *Bacillus subtilis* and that this cross-linking effect disappeared either in cells or in vitro during post-treatment incubation.

Lown and Majumdar used ethidium fluorescence assays to undertake extensive investigations into the interaction of azinomycin B with DNA.[19] They established that this compound rapidly produces covalent links between the complementary strands of a variety of DNAs without prior activation. Upon treatment with progressively increasing concentrations of azinomycin B, extensive alkylation was also observed as detected by reduced fluorescence. Both the cross-linking and alkylation of DNA were determined to be pH dependent, with more rapid cross-link formation occurring at lower pH. It was concluded that, since tumour cells are characterised as having a somewhat lower pH than normal cells (due to the production of lactic acid), this may account, in part, for the selectivity of this agent. In accordance with earlier findings,[61,62] incubation of DNA (previously cross-linked with azinomycin B) for prolonged periods resulted in a reduction in the number of observed cross-links, indicating that the process is reversible. Using a radiolabelled synthetic polynucleotide, it was further determined that the alkylation and ISC processes occur without loss of the purine or pyrimidine bases. Using DNAs of differing GC content, it was established that azinomycin B shows a preference for guanine residues. Evidence was put forward suggesting that the alkylation process does not involve N-7 of guanine, although more recent data contradicts this observation.[18]

Using ^{32}P-labelled synthetic oligonucleotides, Armstrong et al. determined that azinomycin B causes interstrand cross-links between purine residues which are two bases removed from one another on the complementary DNA strands.[18] Specificity for $^{5'}GNC^{3'}$ and $^{5'}GNT^{3'}$ sequences was established by PAGE analysis, after treatment of the cross-linked DNA with piperidine to reveal the labile cleavage sites and, separately, after reaction with the chemical nuclease reagent (1,10-phenanthroline)copper. By incorporating 7-deazaguanosine or deoxyinosine into the DNA duplexes, it was additionally determined that both purine residues react through the N-7 position, a finding which contradicts the earlier work of Lown et al.[19] Further experiments revealed that azinomycin B alkylates all G residues in either of the single strands of DNA, but fails to react with any A residues. Given the differences in nucleophilicity of N-7 in G and A, it was concluded that, for cross-links involving these purine bases (i.e. the $^{5'}GNT^{3'}$ sequence), the first alkylation occurs at G which is followed by a template-directed alkylation at A. Finally, the formation of cross-links at purine residues located in the 5′ direction led the authors to the preliminary conclusion that the natural product reacts in the major DNA groove.

Saito and coworkers have investigated the reactions of both azinomycin B and its 4-*O*-methyl derivative with the self-complementary oligodeoxynucleotide $[d(TAGCTA)_2]$ work which provided the first direct evidence of the involvement of the electrophilic epoxide and aziridine moieties in the DNA cross-linking event.[16] Using HPLC analysis in conjunction with mass spectrometry, they determined that both azinomycin B and 4-*O*-methyl azinomycin B reacted with the DNA duplex to provide a monoadduct and an interstrand cross-linked adduct. Thermolysis (90°C, 5 min) of

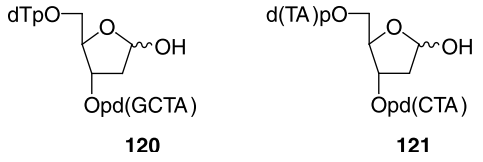
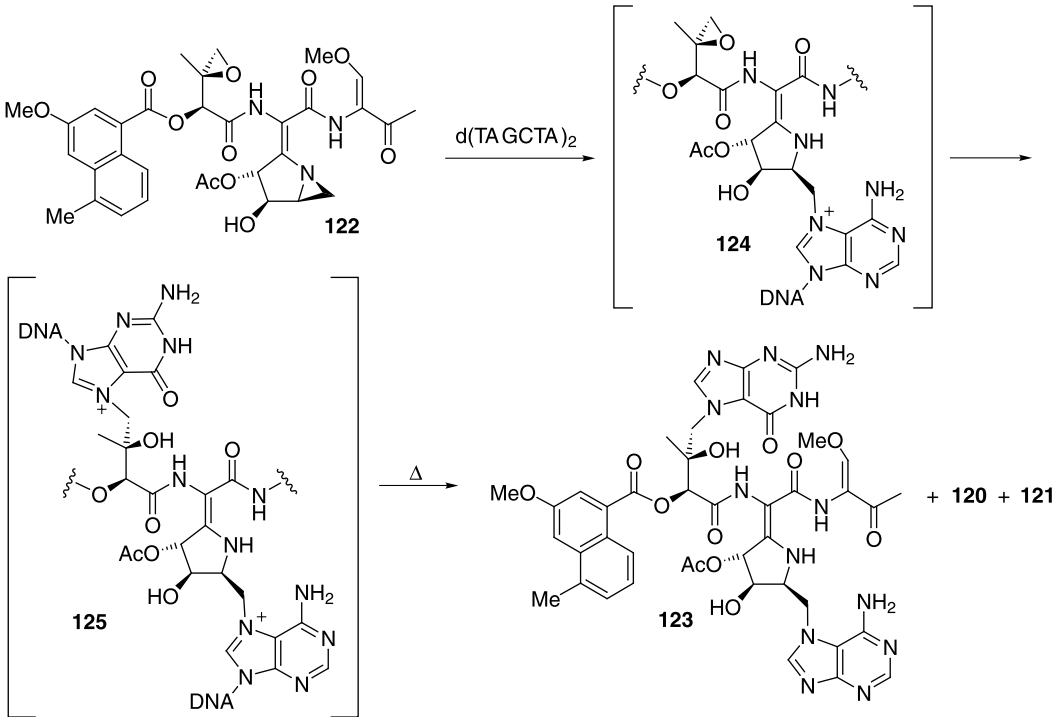


Figure 2.

they were able to determine that epoxy aziridine **85** produces DNA cross-links (100% cross-link formation at 100 μM after 1.5 h exposure) whereas **113** and **91**, devoid of the epoxide and the aziridine, respectively, show little activity above background levels. The presence of both functional groups is therefore essential for ISC activity. In a Taq DNA polymerase stop assay, all three analogues



Scheme 29.

the monoadduct yielded oligodeoxynucleotide **120**, whilst similar treatment of the cross-linked adduct provided both **120** and **121**, suggesting that initial alkylation at A then leads to an A to G cross-link (Fig. 2).

More detailed characterisation of the reaction products was achieved using 4-*O*-methyl azinomycin B **122**.[16] In this example, an additional compound was isolated which, after chemical degradation studies, was assigned as **123**, in which guanine was attached to C-21 and the adenine to C-10 (Scheme 29). Time-dependent HPLC analysis of the reaction indicated that the cross-linking proceeds via a two-step mechanism in which adenine N-7 first reacts with the aziridine moiety to give **124**, and then guanine N-7 cross-links by a second reaction at the epoxide centre producing **125**. No monoadduct between the epoxide and the oligonucleotide could be detected by HPLC. Clearly, the conclusions reached by Armstrong[18] and Saito[16] are not completely consistent with regard to which purine residue (A or G) is involved in the first alkylation event.

Using the fully synthetic compounds **85**, **91** and **113**, Shipman and coworkers have been able to provide additional evidence in support of the role of the epoxide and the aziridine in the cross-linking process (Fig. 3).[17] By means of an agarose gel DNA cross-linking assay, induced Taq stops preferentially at G residues, indicating alkylation at these bases.

Chemical studies on azinomycin-like structures support the idea that the initial alkylation involves the aziridine C-10 position. Reaction of **85** with 2 equiv. of thiophenol, for example, leads to the production of monoadduct **126** and bisadduct **127** (Scheme 30).[20] After 4 h, the monoadduct **126** was found to be the major product (62%), along with

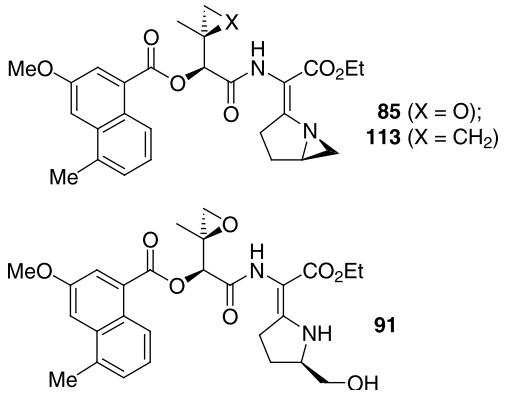
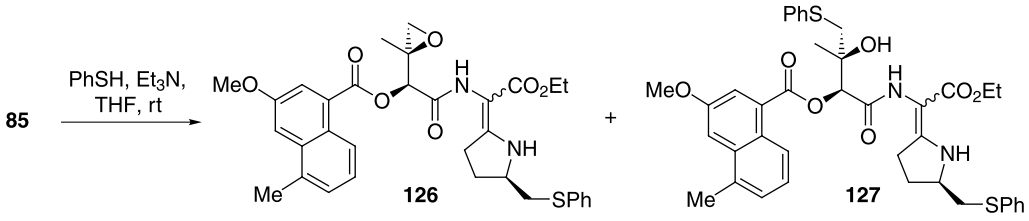


Figure 3.



Scheme 30.

a smaller amount of **127** (27%) but, after 10 h, only **127** was detected. In accordance with these findings, Lown and Majumdar noted that the extent of DNA cross-link formation markedly decreased when a freshly-prepared solution of azinomycin B was kept at 22°C for 20 h prior to use,[19] the extent of alkylation remaining constant, suggesting that one of the reactive sites in azinomycin B is much more labile than the other site. Attempts to react azinomycin B with 2′-deoxyguanosine have not been successful.[63] Decomposition of the drug occurs more rapidly than addition of G, and spectroscopic analysis suggests that a hydrolysed product is formed as a result of attack of water at C-10 of the aziridine ring. Interestingly, an azinomycin B-guanine monoadduct could be obtained when the drug was treated with calf thymus DNA and thermally degraded.[63]

Alcaro and Coleman have developed a modified AMBER* force field and used it in conjunction with DNA cross-linking-based filtering to identify low energy conformations of the azinomycins using molecular modelling.[64] In further work, they have reported computational methods to study the formation of the covalent interstrand DNA cross-links involving azinomycin B.[65] Four different monoalkylated DNA–azinomycin adducts were subjected to simulations using Monte Carlo-based methods, to determine if the populated conformations were geometrically competent to proceed to a cross-linked adduct. They concluded that only the monoadduct produced by reaction between adenosine (N-7) and C-10 of azinomycin B was capable of fulfilling these criteria. They noted that minimal structural reorganisation of the agent or the B-DNA duplex was necessary for cross-link formation to occur. This study provides evidence consistent with Saito's proposal that DNA cross-linking by azinomycin B occurs through an initial alkylation of the adenosine (N-7) by the aziridine C-10 carbon, followed by a second alkylation of the guanine (N-7) by the epoxide carbon at C-21.[16]

Whilst the basic mechanism of DNA ISC formation by the azinomycins is largely established, little is known about whether the other functionalities contained within these natural products are involved in binding to the DNA helix or play a role in the alkylation events. The intrinsic fluorescence of the naphthalene chromophore of azinomycin B (excitation at 346 nm, emission at 427 nm) has been reported to show a progressive enhancement up to 200% when aliquots of calf thymus DNA are added, indicating that this residue intercalates between the DNA bases.[12] The addition of an intercalating agent (ethidium bromide) to DNA prior to treatment with azinomycin B inhibited the cross-linking process.[19] It remains unclear, however, whether intercalation is a prerequisite for ISC formation.

4.3. Structure–activity relationships

A wide range of azinomycin-like structures have been screened for cytotoxicity against a variety of human tumour cell lines, including L5178Y (lymphoma), P388 (murine leukaemia), A2780 (ovarian), CH1 (ovarian), HT29 (colon), K562 (leukaemia), SKOV-3 (ovarian) and HCT116 (colon), as well as the drug resistant lines, CH1cisR and A2780cisR (both with acquired resistance to cisplatin), HCT116/VM46 (expressing the multidrug resistance phenotype) and HCT116/VP35 (resistant to verapamyl and topoisomerase II-type drugs). These data are collated and presented in Table 1. Whilst the use of differing tumour cell lines means that caution needs to be exercised in analysing the data, a number of SARs do seem apparent.

Consistent with the mechanism of action, compounds containing the epoxide and the 1-azabicyclo[3.1.0]hexane ring system display substantial cytotoxic activity (Table 1, entries 1–5). Chemical inactivation of either the epoxide (Table 1, entries 4 and 9),[17] or both the epoxide and the aziridine (Table 1, entries 4 and 8),[20] result in a marked reduction in potency. Activity is observed in compounds containing a simple monocyclic aziridine rather than the more complex bicyclic system found in the natural products (Table 1, entries 10 and 11).[38] Structures based solely upon the bicyclic aziridine subunit appear to possess little or no cytotoxic activity (Table 1, entries 30–36).[20,47,48,50]

The simple epoxy amide **3**, isolated along with the natural products from *S. griseofuscus*, was originally reported to display no antibacterial or antitumour activity.[4] Terashima subsequently noted, however, that this molecule is highly cytotoxic (Table 1, entry 17).[20] The findings that more complex derivatives devoid of the 1-azabicyclo[3.1.0]-hexane ring system display prominent activity (Table 1, entries 6 and 7) can be rationalised in light of this observation. Detailed SAR studies have been undertaken on compounds related to epoxy amide **3**.[56] Zang and Gates have obtained compelling evidence that the naphthalene portion of epoxy amide **3** intercalates within the DNA duplex,[66] and this is borne out in the trends in cytotoxicity seen across a range of related derivatives (Table 1, entries 17–22).[56] The influence of changing the absolute configuration of the molecule, replacing the epoxide, and altering the amide side chain have also been examined (Table 1, entries 23–26). It has been determined that epoxy amide **3** alkylates but does not induce ISCs in duplex DNA.[57] In a recent development, potent DNA ISC agents based solely upon the epoxide domain have been reported (Table 1, entries 27–29).[57] Significantly, these dimeric molecules are much more chemically and thermally stable than the

Table 1.

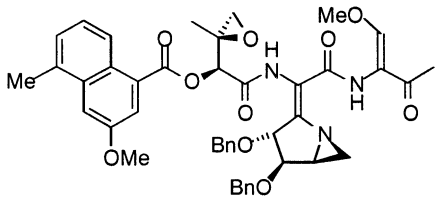
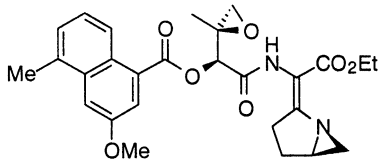
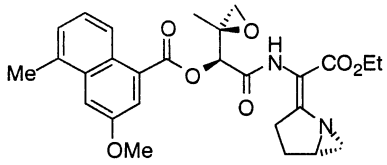
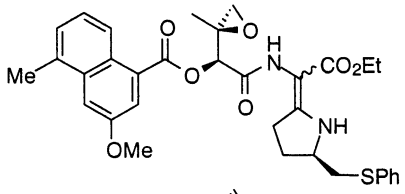
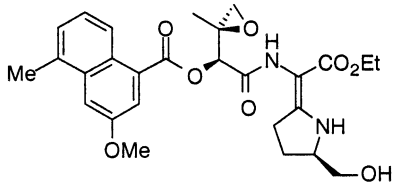
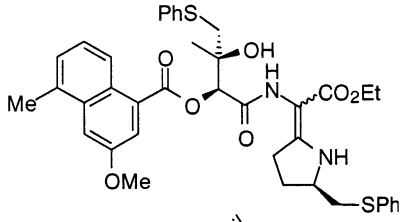
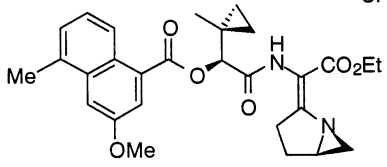
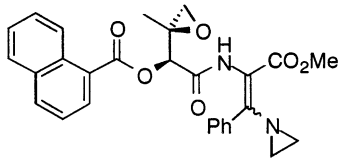
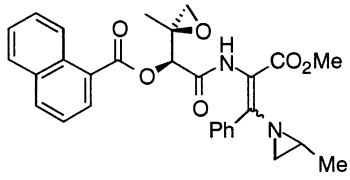
Entry	Chemical structure	Cytotoxicity, IC_{50} (cell line)	Reference
1	Azinomycin A	0.12 μM (L5178Y)[a]	2
2	Azinomycin B (carzinophilin)	0.18 μM (L5178Y)[a] 0.838 μM (HCT116)	2 38
3		0.50 μM (P388)[a]	21
4		0.0047 μM (P388)[a] 0.076 μM (A2780); 0.28 μM (A2780cisR); 0.078 μM (CH1); 0.087 μM (CH1cisR); 2.1 μM (SKOV-3); 0.52 μM (HT29); 0.143 μM (K562)	20 17
5		0.0073 μM (P388)[a]	20
6		0.058 μM (P388)[a]	20
7		0.06 μM (A2780); 0.14 μM (A2780cisR); 0.058 μM (CH1); 0.035 μM (CH1cisR); 0.56 μM (SKOV-3); 0.55 μM (HT29); 0.52 μM (K562)	17
8		2.7 μM (P388)[a]	20
9		5.2 μM (A2780); 10 μM (A2780cisR); 8.6 μM (CH1); 5.8 μM (CH1cisR); 29 μM (SKOV-3); 10 μM (HT29); 1.9 μM (K562)	17
10		(*E*)-isomer 4.39 μM (HCT116); 5.56 μM (HCT116/VM46); 5.27 μM (HCT116/VP35) (*Z*)-isomer: 12.4 μM (HCT116); 13.2 μM (HCT116/VM46); 11.0 μM (HCT116/VP35)	38 38
11		(*E*)-isomer: 5.4 μM (HCT116); 5.4 μM (HCT116/VM46); 1.6 μM (HCT116/VP35) (*Z*)-isomer: 6.76 μM (HCT116); 7.7 μM (HCT116/VM46); 6.4 μM (HCT116/VP35)	38 38

Table 1 (*continued*)

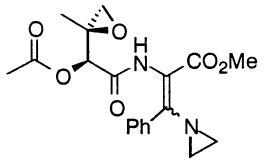
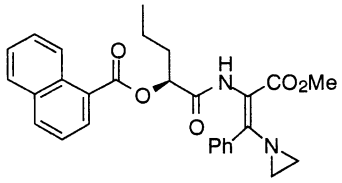
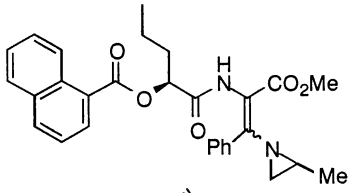
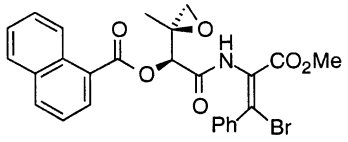
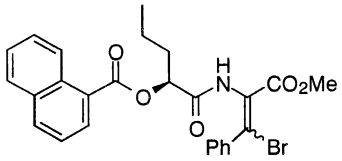
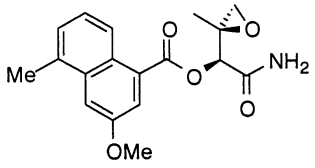
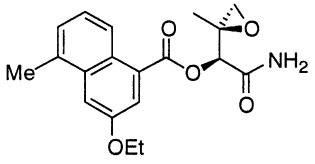
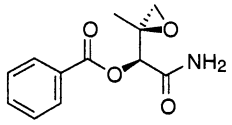
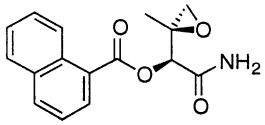
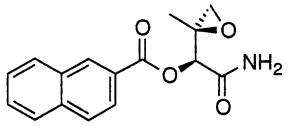
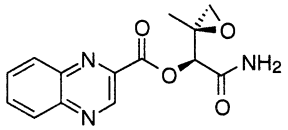
Entry	Chemical structure	Cytotoxicity, IC_{50} (cell line)	Reference
12		(*E*)-isomer: >30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM? (HCT116/VP35) (*Z*)-isomer: >30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM (HCT116/VP35)	38 38
13		(*E*)-isomer: >30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM (HCT116/VP35) (*Z*)-isomer: 28.6 μM (HCT116); 38.4 μM (HCT116/VM46); 27.3 μM (HCT116/VP35)	38 38
14		(*E*)-isomer: >30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM (HCT116/VP35) (*Z*)-isomer: 25.3 μM (HCT116); 27.2 μM (HCT116/VM46); 25.5 μM (HCT116/VP35)	38 38
15		>30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM (HCT116/VP35)	38
16		>30 μM (HCT116); >30 μM (HCT116/VM46); >30 μM (HCT116/VP35)	38
17		'no anti-bacterial or anti-tumour activities' 0.011 μM (P388)[a] <0.05 μM (A2780); 0.076 μM (A2780cisR); <0.05 μM (CH1); 1.25 μM (SKOV-3); 0.33 μM (HT29)	4 20 56
18		1.8 μM (A2780); 3.5 μM (A2780cisR); 1.4 μM (CH1); 12.0 μM (SKOV-3); 4.3 μM (HT29)	56
19		20.5 μM (A2780); >25 μM (A2780cisR); >25 μM (CH1); >25 μM (SKOV-3); >25 μM (HT29)	56
20		0.44 μM (A2780); 1.1 μM (A2780cisR); 0.55 μM (CH1); 5.1 μM (SKOV-3); 2.15 μM (HT29)	56
21		0.39 μM (A2780); 1.35 μM (A2780cisR); 0.17 μM (CH1); 13.0 μM (SKOV-3); 2.6 μM (HT29)	56
22		>25 μM (A2780); >25 μM (A2780cisR); >25 μM (CH1); >25 μM (SKOV-3); >25 μM (HT29)	56

Table 1 (*continued*)

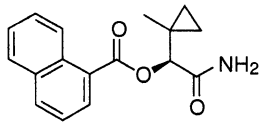
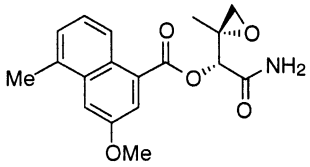
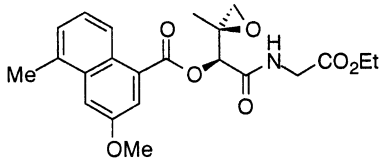
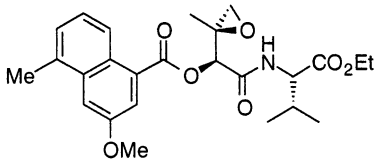
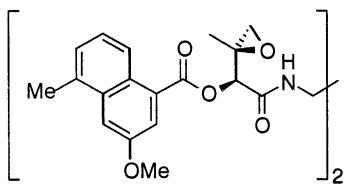
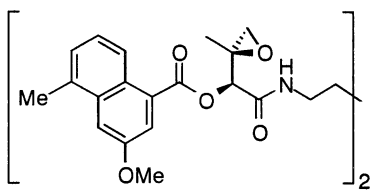
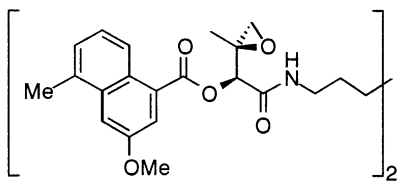
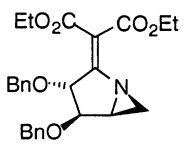
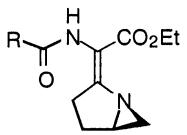
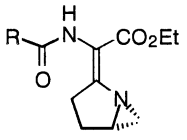
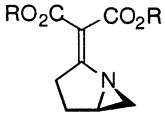
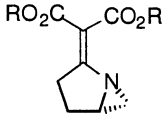
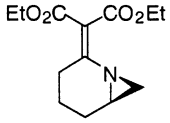
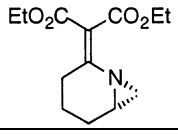
Entry	Chemical structure	Cytotoxicity, IC_{50} (cell line)	Reference
23		>25 μM (A2780); >25 μM (A2780cisR); >25 μM (CH1); >25 μM (SKOV-3); >25 μM (HT29)	56
24		0.058 μM (A2780); 0.155 μM (A2780cisR); 0.062 μM (CH1); 2.3 μM (SKOV-3); 0.61 μM (HT29)	56
25		0.34 μM (A2780); 0.83 μM (A2780cisR); 0.26 μM (CH1); 7.3 μM (SKOV-3); 2.25 μM (HT29)	56
26		0..35 μM (A2780); 0.56 μM (A2780cisR); 0.315 μM (CH1); 2.95 μM (SKOV-3); 2.25 μM (HT29)	56
27		<0.05 μM (A2780); <0.05 μM (CH1); <0.05 μM (CH1cisR); 1.4 μM (SKOV-3); 0.42 μM (HT29); 0.067 μM (K562)	57
28		0.065 μM (A2780); <0.05 μM (CH1); <0.05 μM (CH1cisR); 0.82 μM (SKOV-3); 0.55 μM (HT29); 0.027 μM (K562)	57
29		0.11 μM (A2780); 0.08 μM (CH1); 0.08 μM (CH1cisR); 1.2 μM (SKOV-3); 0.50 μM (HT29); 0.062 μM (K562)	57
30		6.4 μM (P388)[a]	48
31		R=^{t}BuO: >35 μM (P388)[a] R=^{t}Bu: 35 μM (P388)[a]	20 20
32		R=^{t}BuO: 34 μM (P388)[a] R=^{t}Bu: 33 μM (P388)[a]	20 20

Table 1 (*continued*)

Entry	Chemical structure	Cytotoxicity, IC_{50} (cell line)	Reference
33		R=Et: 13 μM (P388)[a]	47
34		R=Bn: 21 μM (P388)[a] R=Et: 3.1 μM (P388)[a]	47
35		R=Bn: 32 μM (P388)[a] >25 μM (A2780); >25 μM (A2780cisR); >25 μM (CH1); >25 μM (SKOV-3); >25 μM (HT29)	50
36		>25 μM (A2780); >25 μM (A2780cisR); >25 μM (CH1); >25 μM (SKOV-3); >25 μM (HT29)	50

[a] Cytotoxicity value originally reported in μg/ml.

parent azinomycins. Finally, some azinomycin–lexitropsin hybrid molecules incorporating the epoxide domain of the azinomycins have been shown to display potent DNA-cleaving activity, although the mechanism of action of these compounds has not yet been established (Scheme 25).[58]

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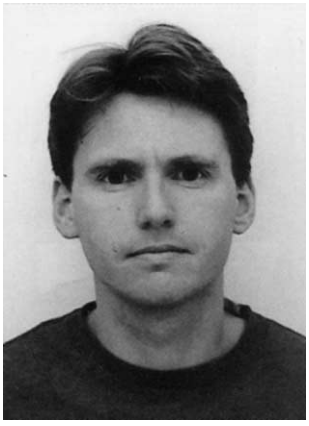
67. During the very late stages of the production of this issue, the authors became aware that Coleman et al. had completed the first total synthesis of azinomycin A, see: Coleman, R.S.; Li, J.; Navarro, A. *Angew. Chem. Int. Ed.* **2001**, *40* (9), in press.

Biographical Sketch



Mike Shipman was born in Exeter on 16 February 1967, where he grew up. He graduated in chemistry from Imperial College, London with first class honours in 1987 and remained there to work with Prof. W. B. Motherwell on novel transition metal-catalysed cycloaddition reactions, obtaining his PhD in 1990. After 2 years as an SERC/NATO postdoctoral fellow with Prof. A. I. Meyers at Colorado State University, Fort Collins, USA, he returned to the UK to take up a lectureship in organic chemistry at Loughborough University. In 1996, he moved to his present position as senior lecturer in organic chemistry at the University of Exeter. Dr Shipman has received the Meldola Medal of the Royal Society of Chemistry (1996) and an EPSRC Advanced Research Fellowship (1999–2004). His research interests include heterocyclic chemistry, the reactivity of strained ring systems, carbohydrate chemistry, natural product synthesis and anticancer chemistry.

Timothy Hodgkinson was born in Chesterfield in May 1968. He graduated with an honours degree in chemistry from the University of Teesside in 1991. After obtaining an MSc degree under the guidance of Prof. McKillop at the University of East Anglia, he undertook his PhD studies with Mike Shipman at the University of Exeter on work related to the synthesis of the azinomycins. After the completion of his doctorate, Dr Hodgkinson undertook postdoctoral work at the Universities of Nottingham and Sheffield (with Drs J. S. Clark and J. C. Anderson, respectively).