

The Chemistry and Biology of the Azinomycins

Introduction:

Compounds endowed with the ability to cross-link DNA have, on many occasions, demonstrated significant medicinal properties.¹ Azinomycin A **1** and azinomycin B **2** are capable of DNA intercalation, interstrand cross-linking (ISC)^{2,3} and demonstrate sub-micromolar *in-vitro* and *in-vivo* cytotoxic activity.⁴ Complicated isolation procedures have inhibited detailed evaluation and a lack of availability, combined with their unique structures, make them attractive targets for total synthesis. The construction of **1** was recently achieved⁵ and this talk is a review of this highly functionalized, biologically active and synthetically challenging natural product.

Isolation/Characterization:

Carzinophilin **2** was first reported in 1954 during a random screening of soil anaerobes for antitumor activity and was isolated from the fermentation broth of *streptomyces sahachiroi*.⁴ Compound **2** demonstrated significant anti-tumor and antibiotic properties in model systems, providing up to a four fold increase in life expectancy in rodents injected intra peritoneally with Ehrlich carcinoma and Yoshida carcinoma cells. This isolated small molecule had up to a ten fold range between the minimum effective and maximum tolerated doses making it a therapeutic candidate. However, the isolated materials proved too unstable for crystallographic analysis

Figure 1:

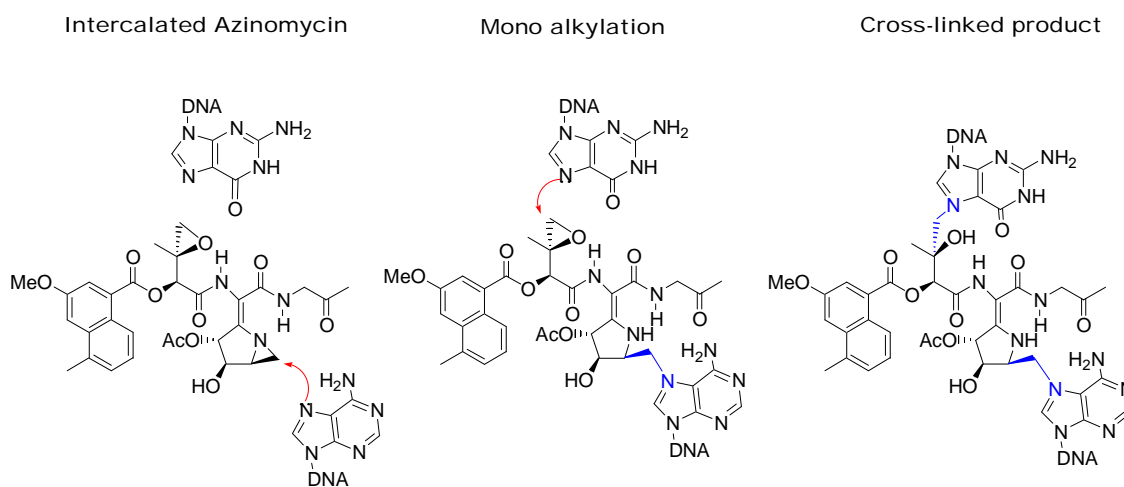


leaving absolute structure determination to, partial syntheses, NMR studies and mass spectroscopy.^{6,7} Azinomycin A **1** and azinomycin B **2** were independently isolated in 1986 from

streptomyces grizeofuscus and were found to possess anti-tumor activity analogous to carzinophilin.⁸ Later, analysis by high resolution spectroscopic methods and comparison of synthetic materials proved azinomycin B to be identical to carzinophilin.⁹

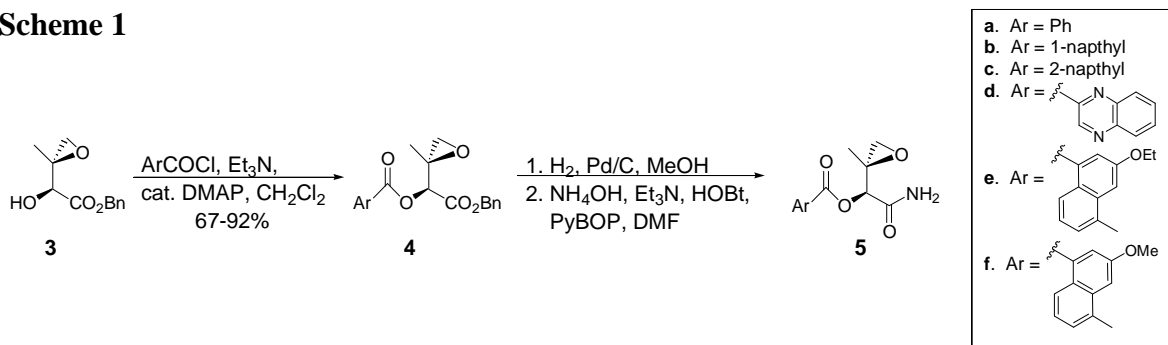
Structure function relationship:

Figure 2



Azinomycin electrostatically associates with the major groove of 5'GNT3' and 5'GNC3' double stranded oligonucleotide sequences and forms an ISC through the N-7 position of the purine base as shown in Figure 2.¹⁰ The uncharged trisubstituted naphthoate moiety of **1** efficiently intercalates into DNA;¹¹ intercalation promotes attack of the aziridine by the N7 purine base of bases leading to mono alkylated duplex.¹² The contribution of intercalation, based on alterations of IC₅₀ values, was eloquently elucidated by the synthesis of **5 a-f** (Scheme 1)

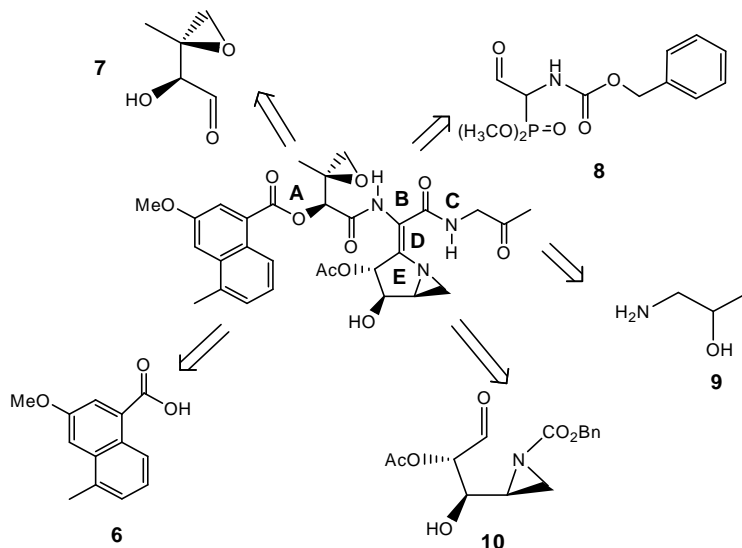
Scheme 1



and by the results of subsequent cytotoxicity assays based on these aryl substitutions.¹³ When nestled in the major groove, proximity and orientation of the epoxide lead to aminolysis of the ring by the upstream guanine base to give the mono alkylated product.^{14,15}

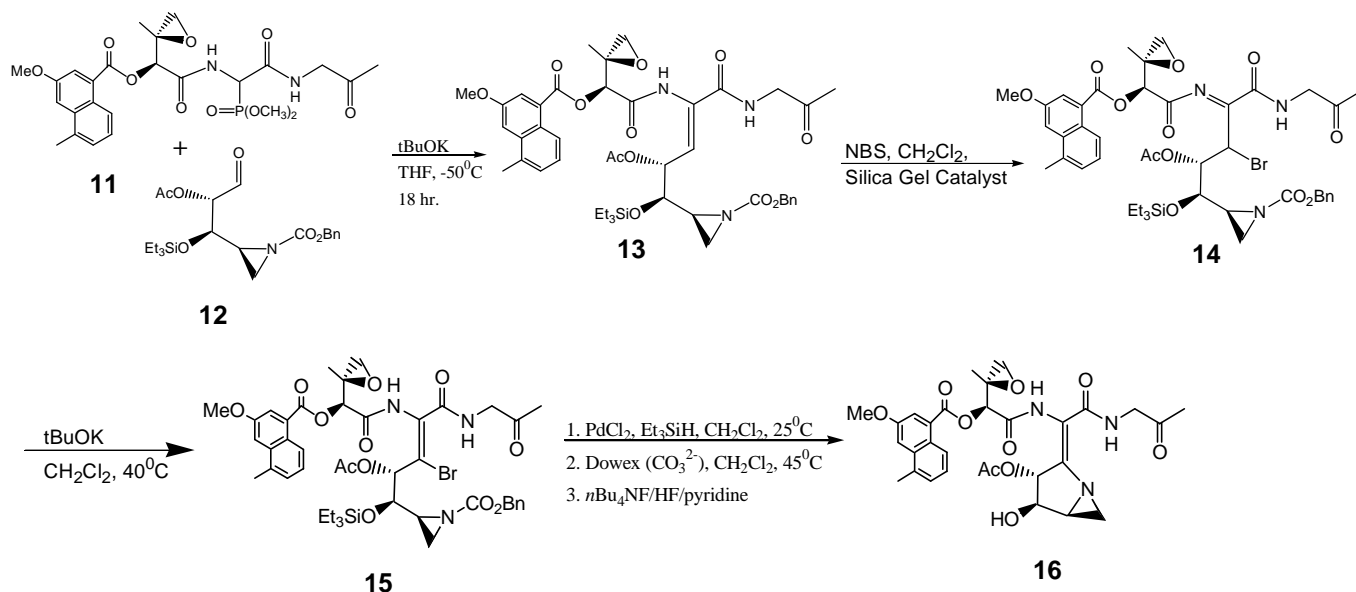
Asymmetric Total Synthesis:

Scheme 2



The total synthesis of azinomycin A by the Coleman group was envisioned as a five subunit convergent synthesis from naphthoic acid **6**,¹⁶ the epoxy aldehyde **7**,^{17,18} glycine phosphonate **8**, commercially available 1-amino-2-propanol **9** and aziridine carbaldehyde **10**.^{19,20} The successful synthesis of this molecule relied on work from a diverse group investigators over a span of 30 years. The most notable contributions are, the naphthoylepoxide system elucidated by Shibuya, and the 1-azabicyclo[3.1.0]hexane portion developed by Terashima. Additionally, pioneering work accomplished by the Armstrong group established the importance of Horner-Wadsworth-Emmons (HWE) coupling to install the aziridine containing **10**.²¹ The order of bond formation as shown above starts with the ester linkage **A** between naphthoic acid **6** and epoxy aldehyde **7**. Separately, the amide bond **C** is formed between the phosphonate **8** and the amino alcohol **9**. The products from the two condensation reactions were coupled via amide linkage **B** to form the completed upper half skeletal system of Azinomycin A. The D-glucosamine derived lower half aziridine carbaldehyde **10** resulted from a reaction series involving a Mitsunobu to form the CBZ protected aziridine in 20% over eleven steps.

Scheme 3



Formation of the complete backbone of Azinomycin was accomplished from these synthons *via* a challenging HWE olefination involving **11** and **12**. The choice of aziridine carbaldehyde O-protection on **13** affected the ratio of isomers formed during the coupling step between the phosphonate and the aldehyde, therefore, limiting the efficacy of protection. The major product of this reaction was the undesired (*Z*) olefin while the minor (*E*) product was formed in 40% yield with a surprisingly low, <5%, yield of formation of phosphonate dimer. Silica gel catalyzed bromination of **13** with NBS was accomplished in 75% yield to afford α bromoimine **14**. NBS mediated bromination with a afforded the α -bromoimine **14** at 75% yield. The tBuOK mediated tautomerization of **14** gave a mixture of separable (*Z*) and desired (*E*) **15** at an unspecified yield.^{22,23} Silane enhanced palladium deprotection of the carbamate yielded the free aziridine without concomitant ketone or vinyl bromide reduction. Cyclization was achieved over Dowex anion exchange resin to give the silyl ether of **16**. Deprotection of this alcohol with a mixture of TBAF/HF/Pyridine gave azinomycin A in 95%. The product was characterized by TLC and ¹H NMR, regrettably instability of **1** prevented crystallization.

The inherent reactivity of the electrophilic epoxide and azabicyclic domains, while contributing to their activity, have historically prevented the use of azinomycins as practical

chemotherapeutics. However, studies of this compound have elucidated the link between structure and activity of each of the three main subdomains. These independent substructures when appended to novel synthetic targets may endow these compounds with predetermined function. Further advances in creating these types of structure/function relationships may facilitate future development of rationally designed therapeutics.

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