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Stereospecific Synthesis of a Novel Azetido[2,1-*c*][1,4]-benzodiazepine (ABD) Ring System with DNA Recognition Potential

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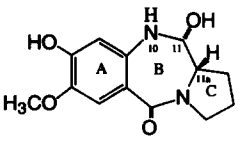
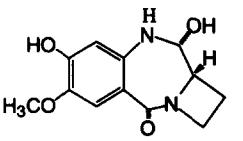
Abstract: The first stereospecific synthesis and reactivity of a novel azetido[2,1-*c*][1,4]benzodiazepine (ABD) ring system with DNA recognition potential is described. © 1997 Elsevier Science Ltd.

In the area of molecular recognition there is growing interest in studying and modifying the recognition patterns of DNA-binding ligands primarly due to their involvement in carcinogenesis and their use as antitumour agents and probes of DNA structure[1]. The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) family of DNA-binding antitumour antibiotics[2] are a group of biosynthetically derived compounds produced by *Streptomyces species,* collectively known as the anthramycins, and represented by anthramycin, tomaymycin, chicamycin, neothramycin and DC-81 (**1**). These compounds exert their biological activity through sequence-selective covalent binding to the N2 of guanine in the minor groove of DNA, via an electrophilic imine or carbinolamine functionality at N10-C11. Fluorescence, NMR, molecular modelling and DNA foot printing-type studies[2,3] have established that the PBDs recognize a three base-pair motif with a preference for purine-guanine-purine triplets. More recent studies[4] have started to relate the DNA binding affinity and sequence selectivity of the PBDs to their biological activity.



1 PBD



2 ABD

A rational approach to the development of clinically useful gene targeted drugs in this series has been suggested, and a few groups including our own, have embarked upon the production of rationally designed anologues. The results of a number of SAR investigations and also the synthetic aspects of the PBDs have recently been reviewed[5].

In the effort towards the synthesis of modified PBD ring systems, the synthesis of PBD dimers joined through their aromatic A-rings via a flexible linker has recently been reported[6]. These compounds produce DNA-interstrand cross-link and have potent cytotoxicity. Our efforts have been directed towards the synthesis of modified PBD ring systems with the C-ring replaced by an azetidine ring, to produce the

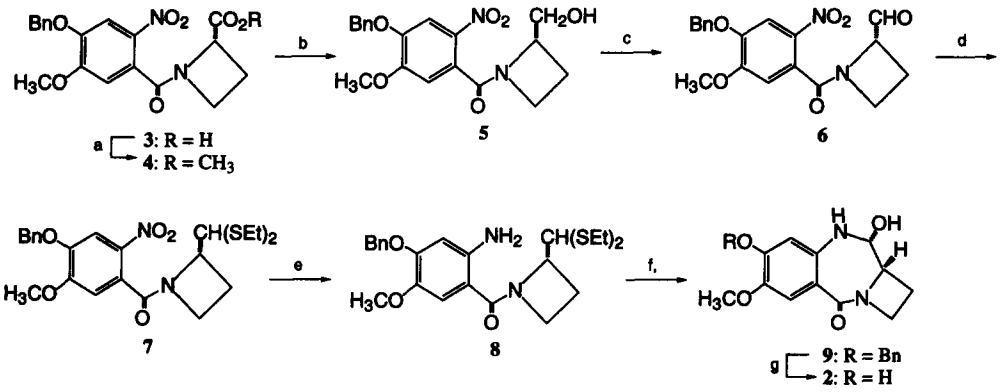
previously unknown azetido[2,1-*c*][1,4]benzodiazepine ring system (ABD) (**2**), with potential DNA-binding affinity and sequence selectivity. The ABD ring system **2** obtained through **8** by *in situ* cyclization of the aldehyde prepared by oxidation of the labile primary alcohol in **10** occurred without detectable racemization, and **2** is the first of its kind to be synthesised.

The major problem encountered in the synthesis of PBDs is the installation of the sensitive imine or carbinolamine functionality at N10-C11, which is usually incorporated at the final synthetic step. In the present study, a further complication is the presence of the inherently strained 4-membered ring system. It is well known that all biologically active PBDs possess the (S)-configuration at the chiral C11a position which provides the molecule with a right-handed twist necessary for a snug fit within the minor groove of DNA. Racemization at C11a could adversely affect the DNA-binding potential of a PBD.

Inspection of the target molecule (**2**) reveals a number of synthetic challenges that include a) incorporation of the highly strained azetidine ring with appropriate functionality, b) preservation of stereochemical integrity at C11a, and c) formation of a carbinolamine or imine moiety in a mild and non-racemizing environment. The synthetic approach described below meets all of these criteria and, in addition, is adaptable for a convergent synthesis.

The synthetic route to the desired target (Scheme 1) includes the crucial electrophilic N10-C11 cyclization (**8**-->**9**) to give the imine moiety of the B-ring. The approach towards the key intermediate **3**

Scheme-1



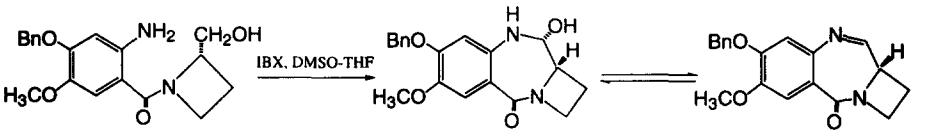
Reagents: (a) $SOCl_2$, CH_3OH (b) $LiBH_4$, THF, 0°C, r.t. (c) IBX, DMSO, r.t, 0.5 h (d) EtSH, TMSCl, r.t, 12 h (e) $SnCl_2.2H_2O$, CH_3OH, reflux, 40 min (f) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O (4:1), 2.5 h (g) 10% Pd-C, cyclohexadiene, EtOH, 45 min.

involved initial preparation of the 4-benzyloxy-5-methoxy-2-nitrobenzoic acid[7] representative of A-ring fragment. Subsequently, the derived acid chloride (oxalyl chloride/THF) was coupled to (S)-azetidine-2-carboxylic acid[8] in the presence of Et_3N to give the corresponding amide **3** which, without purification, was converted to its methyl ester **4** using $SOCl_2$/MeOH. Based on past experience[7b], **4** was treated with DIBAL-H at -78°C in order to transform it to the corresponding aldehyde. However, this led to a complex mixture of products, necessitating chromatographic separation. In order to circumvent this problem, the ester **4** was reduced with lithium borohydride[10] to afford the alcohol **5** in 78% yield. Conversion of **5** into the

corresponding nitro aldehyde **6** was problematic. A variety of oxidising protocols, such as PCC, Swern, and TPAP etc. were unsatisfactory in our hands. However, oxidation proceeded smoothly in 85% yield upon treatment of **5** with O-iodoxybenzoic acid (IBX)[9] (DMSO, 0.5 h) at room temperature. The product was relatively unstable although no racemization was detected at the critical pro-C11a position.

The aldehyde (**6**) was therefore immediately converted into the diethyl thioacetal derivative **7** with $EtSH/TMSCl/CHCl_3$ which was subsequently reduced ($SnCl_2.2H_2O$ in CH_3OH) to the amine **8** in 76% yield. Finally, deprotection of the thioacetal group of **8** was performed with $HgCl_2/CaCO_3$ resulting in concomitant ring closure to give the carbinolamine **9** in 80% yield[12]. A significant feature of this compound was the down-field shift of the H11a proton which was found at δ 4.32 compared to δ 3.92 observed for the DC-81 analogue this was attributed to the diminutive size of the azetidine ring system. Debenzylation was effected by treatment with 10% Pd-C in 1,4-cyclohexadiene[7a], to afford **2** in 89% yield.

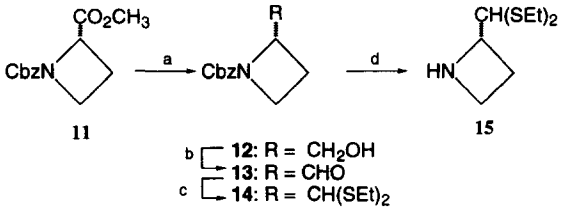
Since the IBX reagent was found to be far superior to any other oxidising agents in the above synthetic route, we decided to try this reagent in the direct preparation of the ABD ring system from the amino



10 **9** **9a**

alcohol **10** obtained from **5** by reduction with $SnCl_2.2H_2O$. Thus, oxidation of **10** with 1.1 eq. of IBX, gave the carbinolamine **9** directly in reasonably good yield (72%). To our knowledge, this represents the first example of an oxidative ring closure of this type[11]. Ring closure was relatively slow (4.5 h) at room temperature, and the product tends to stay preferably in the carbinolamine (**9**) rather than the imine (**9a**) form.

In an alternative approach, the intermediate (2S)-azetidine-2-carboxaldehyde diethyl thioacetal **15** was prepared from **11** in large quantities, *via* intermediates **12, 13** and **14**. Reaction of **15** with 4-benzyloxy-5-



Reagents: (a) $LiBH_4$, THF, 0°C, 4 h, 75% (b) IBX, DMSO, r.t, 0.5 h (c) EtSH, TMSCl, CH_2Cl_2, 12 h, 84% (d) TMS-I, CH_2Cl_2, r.t, 0.5 h, 65%

methoxy-2-nitrobenzoyl chloride gave the amide **7** in 86% yield. Compound **15** thus provides the opportunity for condensation with a variety of A ring fragments in a convergent approach.

In conclusion, this IBX approach to the ABD ring system should allow the synthesis of a number of ABD/PBD analogues for evaluation as potential DNA-binding ligands and cytotoxic agents. DNA binding properties and *in vitro* cytotoxicity of **2** will be reported elsewhere.

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12. All new compounds gave satisfactory spectroscopic and analytical data. **9** : ^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.55-2.73 (m, 1 H), 2.76-2.95 (m, 1 H), 3.42-3.51 (bs, 2 H, exchange with D_2O), 3.95 (s, 3 H), 4.05 (d, 1 H, J = 6.4 Hz), 4.12-4.26 (m, 2 H), 4.32 (t, 1 H, J = 8.0 Hz), 5.23 (s, 2 H), 7.12 (s, 1 H), 7.29-7.52 (m, 5 H), 7.65 (s, 1 H). MS (imine), m/z (relative intensity) : 322 (M^+, 20%), 277(15), 231(27), 203(9), 91(100), 43(48). HRMS : Calc. for 322.131743 ($C_{19}H_{18}N_2O_3$), found : 322.131718: $[\alpha]^{23}_D$ = +33 (c = 0.3, $CHCl_3$). **5** : ^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.01-2.18 (m, 1 H), 2.24-2.46 (m, 1 H), 3.30 (bs, 1 H, exchange with D_2O), 3.65-3.91 (m, 3 H), 3.95 (s, 3 H), 4.23-4.33(m, 1 H), 4.73-4.88 (m, 1 H), 5.20 (s, 2 H), 6.83 (s, 1 H), 7.32-7.51 (m, 5 H), 7.75 (s, 1 H). $[\alpha]^{23}_D$ = -11.5 (c = 0.5, $CHCl_3$). **14** : ^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.21-1.39 (m, 6 H), 1.78-1.95 (m, 1 H), 2.38-2.69 (m, 4 H), 2.71-2.92 (m, 2 H), 3.41-3.76 (m, 2 H), 3.85 (d, 1 H, J = 3.5 Hz), 5.22 (s, 2 H), 7.33 (s, 5 H). $[\alpha]^{23}_D$ = +36 (c = 0.9, $CHCl_3$). MS (CI), m/z (relative intensity) : 325 (M^+, 10%), 264(100), 220(30), 174(15), 130(48), 117(74), 91(66), 43(98).

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