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Design and Synthesis of a Novel Epoxide-Containing Pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) via a New Cyclization Procedure

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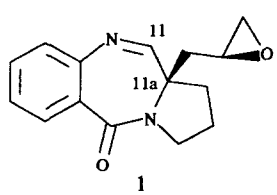
Abstract: The synthesis of a potential DNA-crosslinking pyrrolo[2,1-*c*]-[1,4]benzodiazepine (PBD) substituted at the C8-position with a 2,3-epoxypropaneoxy moiety using a new cyclization procedure is described.

There is growing interest in DNA-binding ligands such as the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) as the basis for potential gene targeted drugs[1]. The PBD family of antitumour antibiotics are a group of biosynthetically derived compounds produced by *Streptomyces* species; well known members include tomaymycin, sibiromycin, DC-81 and the neothramycins A and B[1]. Their antitumour activity is due to covalent binding in the minor groove of DNA through nucleophilic attack of the N2 of a guanine base on the electrophilic C11-position of the PBD. The adducts span three base-pairs with a preference for 5'-PuGPu sequences[1]. All biologically active PBDs possess the (S)-configuration at the chiral C11a position which provides the molecule with a right-handed twist when viewed from the C-ring towards the A-ring, thus providing the appropriate 3-dimensional shape for a snug fit within the minor groove of DNA.

In order to increase cytotoxicity through the production of DNA cross-links, PBD-dimers have been recently synthesised, consisting of two PBD units joined through their A-rings[2]. Similarly, with the aim of producing a PBD monomer with DNA-crosslinking activity, Confalone and co-workers have synthesised[3] a PBD analogue with an epoxide group substituted at the C11a position (**1**), although no DNA-binding data was reported. However, subsequent computer modelling studies in this laboratory suggested that C8 would be the preferred position for attachment of a second alkylating group, as attachment at the C11a position could sterically hinder fit within the minor groove as well as interfere with nucleophilic attack of the guanine N2 on the C11 position. For these reasons, an epoxide-containing PBD (**2**) was designed in which a 2,3-epoxypropaneoxy group is attached to the C8-position of the PBD.



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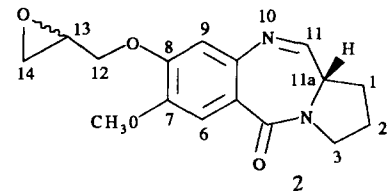
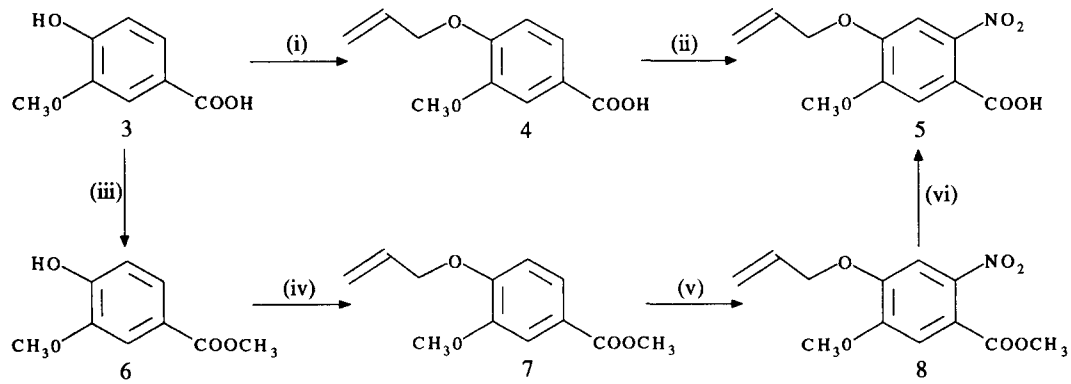


2

The main difficulty associated with PBD synthesis is the formation of the unstable N10-C11 carbinolamine-imine functionality to afford PBDs in practical yields[4]. For the target molecule **2**, this problem is further complicated by the requirement to include an equally labile epoxide group[5]. Preliminary investigations showed that the epoxide could not be generated in the presence of the free amine whilst production of the amine by reduction in the presence of the epoxide lead to ring opening. These problems were overcome by the design of a synthetic route based upon a strategy for PBD synthesis initially reported by Hurley and co-workers[6], but later modified by Fukuyama and co-workers[7], involving cleavage of N10-trifluoroacetyl- or N10-allyl carbamate-protected PBD-carbinolamines, respectively. However, for the synthesis of **2**, an amine protecting group was required that would be stable to epoxidation conditions but easily removed in the presence of the labile epoxide group.

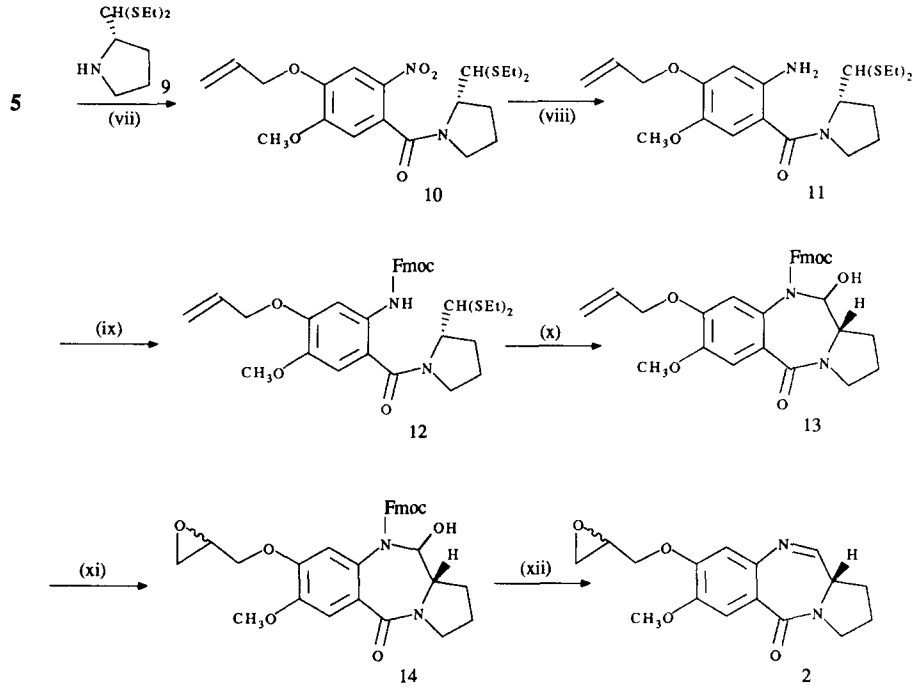
It has been reported that the 9-fluorenylmethyloxycarbonyl (Fmoc) group can be used to protect amines in high yields (88%-98%)[8], followed by cleavage with tetrabutylammonium fluoride (TBAF) at room temperature[9]. Other reports suggest that the epoxide group is relatively stable to fluoride ion at this temperature[5]. For these reasons, the Fmoc group was utilized for the synthesis of the epoxide-PBD (**2**).

The propeneoxy-substituted A-ring (**5**) was first synthesised by two different routes using commercially available vanillic acid (**3**) as starting material. The first route involved conversion of **3** to its propeneoxy substituted analogue (**4**) with NaH/allyl bromide in THF, followed by nitration with $SnCl_4/HNO_3$ to give the desired A-ring (**5**). In the second route, **3** was esterified to its methyl ester (**6**) with H_2SO_4/CH_3OH, and then substituted with the propeneoxy group using NaH/allyl bromide in DMF to give **7**. The final two steps involved nitration with either $SnCl_4/HNO_3$ or 70% HNO_3 to afford **8**, followed by cleavage of the methyl ester using NaOH/THF. Although the second route required two additional steps, it resulted in a higher overall yield of **5** (73% compared to 55%) and a shorter overall reaction time.



Reagents: (i) THF/NaH/rt/1 h - THF/Allyl Bromide/0°C/45 min - Δ/5 days. (ii) $CH_2Cl_2/SnCl_4/HNO_3$/-25°C/10 min. (iii) $CH_3OH/H_2SO_4/\Delta$/24 h. (iv) DMF/NaH/rt/2 h - Allyl Bromide/rt/24 h. (v) Method A: CH_2Cl_2/ $SnCl_4/HNO_3$/-25°C/30 min. Method B: HNO_3/0°C/1 h - rt/48 h. (vi) THF/NaOH(aq)/ rt/18 h.

(2S)-pyrrolidine-2-carboxaldehyde diethyl thioacetal (**9**), prepared *via* literature methods[4,10], was coupled to the propeneoxy-substituted A-ring (**5**) using DCC to afford the nitro thioacetal (**10**). The nitro group was reduced to the amine with $SnCl_2.2H_2O$ in methanol to afford **11**, followed by protection of the amine with Fmoc-Cl in dioxane to give **12** in high yield (88%). Cleavage of the diethyl thioacetal group with $HgCl_2/CaCO_3$ resulted in immediate ring closure to give the Fmoc-protected carbinolamine (**13**) in 95% yield. The final two steps of the synthesis involved epoxidation of the propeneoxy group with *m*-chloroperbenzoic acid to afford a diastereomeric mixture of the epoxide (**14**, 55% yield), followed by removal of the Fmoc protecting group with TBAF in DMF to form the N10-C11 imine while leaving the epoxide group intact. The resulting epoxide-PBD (**2**) was obtained in good yield (79%) and was characterised by NMR and MS[11].



Reagents: (vii) CH_2Cl_2/DCC/rt/4 h - **9**/rt/16 h. (viii) $CH_3OH/SnCl_2.2H_2O/\Delta$/3 h. (ix) Dioxane/$Na_2CO_3$(aq)/ Fmoc-Cl/0°C/4 h - rt/16 h. (x) $CH_3CN/H_2O/HgCl_2/CaCO_3$/rt/48 h. (xi) CH_2Cl_2/*m*-CPBA/rt/72 h. (xii) DMF/TBAF/rt/15 min.

The advantages of using Fmoc as a N10-protecting group for PBD synthesis, which include rapid cleavage under mild conditions, are apparent from this work. This strategy may be generally applicable to the synthesis of other PBD analogues, particularly those containing acid or base-sensitive functional groups.

Preliminary studies indicate that **2** has significant DNA-binding properties and *in vitro* cytotoxicity, and this will be the subject of a future publication.

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11. **2**: ^{1}H-NMR (270MHz, $CDCl_3$): δ 2.02-2.11 (m, 2H, 2×H-1), 2.29-2.37 (m, 2H, 2×H-2), 2.75-2.80 (m, 1H, H-14), 2.90-2.94 (m, 1H, H-14), 3.40-3.43 (m, 1H, H-13), 3.53-3.87 (m, 3H, 2×H-3, H-11a), 3.95 (s, 3H, OCH_3), 3.99-4.23 & 4.25-4.38 (m, 2H, 2×H-12), 6.85 (s, 1H, H-9), 7.53 (s, 1H, H-6), 7.67 (d, 1H, J = 4.4 Hz, H-11). ^{13}C-NMR ($CDCl_3$): δ 24.1 & 24.2 (C-1), 29.6 & 29.7 (C-2), 44.9 (C-14), 46.7 (C-3), 49.8 & 49.9 (C-13), 53.7 (C-11a), 56.1 & 56.2 (CH_3O), 69.6 & 70.0 (C-12), 111.0 & 111.1 (C-9), 111.8 (C-6), 121.0 (C-7), 140.5 (C-8), 147.8 (C-9a), 150.2 (C-5a), 162.6 (C-11), 164.5 (C-5); MS (EI) m/z (relative intensity): 302 (M^+, 100), 273 (5), 259 (4), 245 (16), 217 (3), 70 (9), 57 (8). HRMS: Calc. for $C_{16}H_{18}N_2O_4$ (302.1266), found 302.1209.

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