Design and Efficient Synthesis of Novel DNA Interstrand Cross-Linking Agents: C2-Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimers

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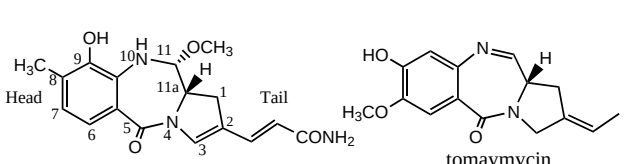
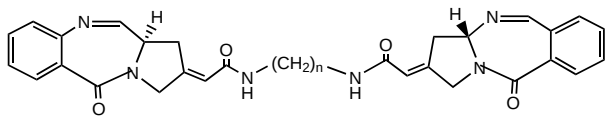
Abstract: The design and facile synthesis of C2-linked pyrrolo[2,1-*c*][1,4]benzodiazepines **(1a-c)** are described. The compounds are prepared with varying degrees of linker length in order to probe the structural requirements for optimal DNA interstrand cross-linking. The products formed are exclusively of the E-configuration and this is the first report on the synthesis of these kind of dimers.

Key words: minor groove binders, pyrrolo[2,1-c][1,4]benzodiazepine, lexitropsins, cross-linking agent, bisalkylators

There has been increasing interest in the synthesis of DNA-sequence selective agents for the last decade. It has been observed that an increasing range of DNA sequences may be recognized by small molecules which have DNA binding properties including pyrrolo[2,1-c][1,4]benzodiazepines (PBDs), and lexitropsins. PBDs, are a group of naturally occuring antibiotics, examples of which include anthramycin, tomaymycin, sibiromycin and the neothramycins A and B (Fig. 1).[1] A key feature of these molecules with respect to their mechanism of action is the N10-C11 carbinolamine (or imine equivalent). Nucleophilic attack by the 2-NH_2 of a guanine base at the C11 position of PBD forms a covalent adduct in the minor groove of DNA.[1] Furthermore, the PBDs bind to DNA sequence-selectively[2] and have potential not only as antitumor agents but as gene regulators and probes of DNA structure.[3]





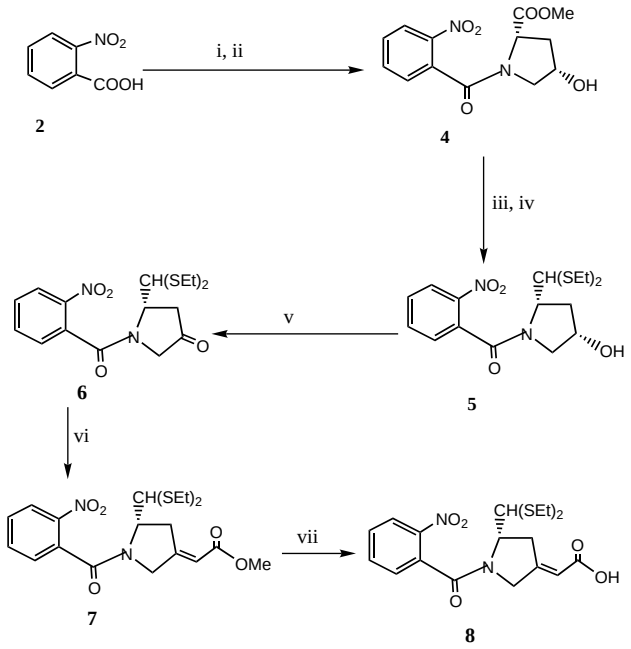
1 a-c (n = 3, 4, 5)

Figure 1

In an earlier study, Wang et al.[5] showed that two tomaymycin molecules can be covalently bound to a 12 mer duplex DNA, where the drug molecules are on opposite strands six base pairs apart. Later, Mountzouris et al.[6] demonstrated that DSB-120 forms a guanine-guanine interstrand cross-link. They showed that the tomaymycin tail is close to the floor of the minor groove, while the five membered ring of DSB-120 is more shallowly immersed, perhaps due to strain from cross-linking with a very short linker unit. These results prompted us to design the synthesis of tail to tail dimers of the PBD molecule, in order to probe and optimize the selectivity and DNA binding efficiency. Considerable literature is available on various PBD conjugates and head to head dimers but this is the first report on synthesis of tail to tail PBD dimers. As part of our continuing efforts on the studies of synthetic DNA minor groove binding agents bearing more than one type of reactive center, we recently synthesized a novel type of lexitropsin-pyrrolobenzodiazepine hybrids,[7] to study their sequence selectivity and binding efficiency. Now we herein report the efficient synthesis of bifunctional DNA alkylating C2-linked PBD dimers in order to probe the DNA cross-linking efficiency and structural requirements for the optimum interstrand cross-linking as well as cytotoxicity.

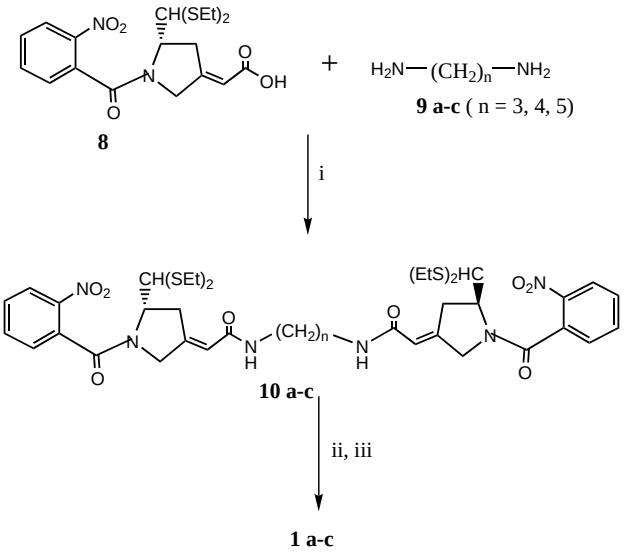
Because of the unreactive nature of the hydroxy group at the C2 position in the PBD moiety, it is very difficult to functionalize at that position in contrast to the case of DSB-120.[8] That is possibly why no attempt has been found in the literature to prepare C2 linked dimers by an ether linkage. We designed instead the synthesis of C2-linked dimers to proceed via peptide bond formation. This versatile approach makes the synthesis straightforward and convenient. Compounds, **(1a-c)** were made according to the routes described in schemes 1 and 2. The precursor PBD-acid was synthesized according to scheme 1. The coupling of 2-nitrobenzoic acid (**2**) via its acid chloride with the methyl-(2S)-trans-4-hydroxycarboxylate ester hydrochloride (**3**) gave methyl-(2S)-N-(2-nitrobenzoyl)-4-hydroxypyrrolidine-2-carboxylate (**4**) in 75-80% yield. Reduction of nitroester (**4**) with DIBAL-H to produce the corresponding aldehyde, followed by protection of the aldehydic group with ethanethiol to give rise to the corresponding diethylthioacetal compound (**5**). Several unsuccessful attempts were made to oxidize the C-2 hydroxy group with various oxidizing agents including pyridinium chlorochromate and Jones reagent. In all these attempts, the yield of the isolated product was less than 10%. The Swern oxidation with DMSO, oxalylchloride, and Et_3N afforded no better results. Finally, satisfactory results were obtained with the reagent DMSO/Ac_2O. With this reagent, the oxidation of C2-hydroxy group was achieved under mild conditions and afforded an approxi-

mately 60% yield of the corresponding oxo compound (**6**). The principal side product isolated in this oxidation reaction was the acetylated compound which, upon hydrolysis, led to the recovery of the starting hydroxy compound (**5**) quantitatively. The Wittig reaction of the keto compound (**6**) with methyl(triphenylphosphoranylidine)acetate afforded the methyl ester in 80% yield. In this reaction, the (E) ester[9] (**7**) was obtained exclusively (vide infra) which upon hydrolysis with ethanolic NaOH produced the corresponding acid compound (**8**) in 90% yield.



Scheme 1: (i) $SOCl_2$,C_6H_6,r.t,2-3h; (ii) trans-4-Hydroxy-L-prolin methyl ester hydrochloride (**2**),Et_3N,CH_2Cl_2,0 °C; (iii) DIBAL-H, CH_2Cl_2,r.t, 20-24h. ; (iv) EtSH,$(CH_3)_3SiCl$,CH_2Cl_2,r.t.,20-24h; (v) DMSO,Ac_2,r.t., 18h; (vi)$(Ph)_3P$=CHCOOMe,C_6H_6, reflux, 12h;(vii) Ethanolic NaOH,Δ,12h

The formation of the dimeric PBDs is shown in scheme 2. Two moles of the acid (**8**) were coupled with one mole of one of the diamines (**9a-c**) (n = 3, 4, 5 respecively), with EDCI and HOBT to afford the corresponding nitrodithiolane dimers (**10a-c**)[$,] with different lengths of linker chain. The yields in the coupling steps were good and approximately 60-65% compared with the coupling using DCC and HOBT (yield is 20-25%). Hydrogenation of **10a-c** with H_2/Pd-C afforded 100% conversion of nitro compounds to the corresponding amino compounds which were then subjected to deprotective cyclization with $HgCl_2$/HgO. The latter conditions produced dimeric PBD imines (**1a-c**)[#] in 30-35% isolated yields. These dimeric compounds, linked via peptide bonds, were comparatively more polar than the corresponding monomers such as DC-81. Purification by column chromatography with 10% MeOH in CH_2Cl_2 resulted in an equilibrium mixture of imine and carbinolamine methyl ether.



Scheme 2: (i) EDCI, HOBT, DMF, r.t.; (ii) Pd/C, H_2, 50 psi, MeOH, 2h (iii) $HgCl_2$, HgO, 25% aq CH_3CN

Assigning the configuration of the Wittig product: The Wittig reaction of the compound (**6**) resulted in the formation of exclusively one product (**7**).[9] A proton decoupling experiment was performed on the corresponding acid (**8**) NMR spectrum. Double irradiation on the δ 5.9 ethylenic proton signal affected only one proton signal corresponding to that at the C4 position and there is no effect on any other proton, which confirms the E-configuration of the Wittig product (Fig. 2).[10]

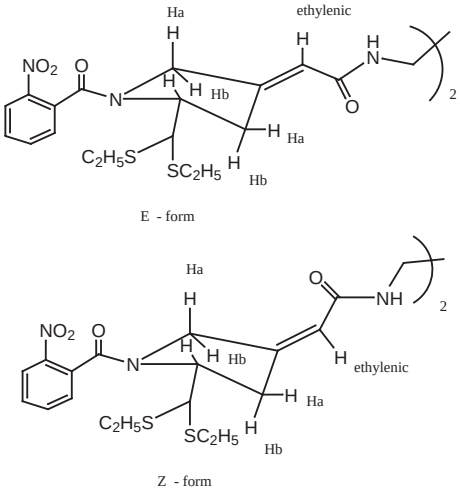


Figure 2

In conclusion, in this communication, we describe the design and synthesis of novel C2-linked PBD dimers. The products are formed exclusively with the E-configuration. The compounds were prepared with varying degrees of linker length in order to probe the DNA sequence selectiv-

ity and structural requirements for optimal DNA cross-linking. The biological studies of these compounds are in progress and the results will be published in due course.

Acknowledgement

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References and Notes

$ *General procedure* **10a**: To a solution of **8** (200 mg, 0.4872 mmol, 1 eq) in dry DMF, EDCI (93 mg, 0.4872 mmol), HOBT (66 mg, 0.4872 mmol) and 1,3-diaminopropane (18 mg, 0.2436 mmol, 0.5 eq) (**9a**) were added under nitrogen atmosphere and stirred for 6 h. When TLC indicated the absence of starting material, DMF was removed under reduced pressure. The dark residue was dissoloved in ethyl acetate and washed with satd. $NaHCO_3$ solution (2 x 10 ml) and then with water (1 x 10 ml). The organic layer was dried under anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting solid was purified by silica gel flash column chromatography using ethyl acetate : Hexane (1:2) as eluent. *spectral data for* ***10a*** : mp. 65-67 °C; ^{1}H NMR ($CDCl_3$, 360 MHz): δ 1.24−1.38 (m, 6H), 1.50−1.60 (m, 2H), 2.65−2,82 (m, 4H), 3.05-3.10 (m, 3H), 3.82-3.88 (d, J = 6.0 Hz, 1H), 4.02-4.08 (dd, J_1 = 4.0 Hz, J_2 = 0.5 Hz, 1H), 4.50 (d, J = 1.2 Hz, 1H), 5.42 (bs, 1H), 5.95 (s, 1H), 6.42 (t, 1H), 7.42-7.45 (m, 1H), 7.50-7.60 (m, 1H), 7.65-7.80 (m, 1H), 8.15-8.20 (m, 1H); MS m/z 859.4 (M^{+1}), 846, 611, 510, 412, 331, 237, 135.

General Procedure **1a**: To a solution of **10a** (170 mg, 1976 mol) in methanol was added 10 % Pd/C (100 mg) and the compound was hydrogenated in a Parr shaker at 50 psi pressure for 2 hrs. TLC indicated the complete consumption of starting material, and the reaction mixture was filtered on a small celite bed to remove Pd/C and then the filtrate was concentrated under reduced pressure to remove methanol completely. The amine was a pale yellow compound to which 10 ml of 25% acetonitrile in water together with $HgCl_2$ (118 mg) and HgO (117 mg). The reaction mixture was stirred for 12 h. TLC indicated the completion of the reaction, (the Rf values for the products, 1a-c are between 0.2 to 0.3 in dichloromethane: methanol: aq ammonia, 9.0:1.0:0.2), so the reaction mixture was filtered and directly charged on to a small silica column. The $HgCl_2$ was eluted first with ethyl acetate and then the solvent system was changed to 5% methanol in dichloromethane. The product was obtained after evoparation of the solvent as pale yellow compound. *spectral data for* ***1a*** : mp. 73 °C (dec.); ^{1}H NMR (CD_3OD, 360 MHz): δ 1.62-1.70 (m, 2H), 3.05-3.12 (m, 4H), 3.40-3.48 (m, 4H), 4.20-4.72 (m, 6H + OCH_3 of methyl ether form), 5.70-5.80 (m, 2H), 6.70-6.78 (m, 2H), 7.20-7.32 (m, 2H), 7.45-7.68 (m, 4H), 7.80 (d, 2H, J = 4.4 Hz, imine proton), 7.90-8.10 (m, 2H); MS m/z 551.5 (M^{+1}, imine), 308.8, 237.0, 134.9, 119.1; ES^+ Calcd. 551.2406, found 551.2399.

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