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Synthesis and DNA-binding ability of pyrrolo[2,1-*c*][1,4]benzodiazepine-azepane conjugates☆

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Abstract—A series of pyrrolobenzodiazepine–azepane conjugates linked through different alkane spacers have been prepared and their DNA thermal denaturation studies have been carried out. One of the compound (**4b**), elevates the DNA helix melting temperature of the CT-DNA by 2.0 °C after incubation for 36 h at 37 °C.

In recent years, there has been considerable interest in DNA-binding molecules, particularly due to their involvement in carcinogenesis and their use as antitumour agents and probes of DNA structure. Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), a group of potent naturally occurring antibiotics isolated from *Streptomyces* species, are of considerable interest due to their potential as antitumour agents, gene regulators and DNA probes.[2] The PBDs interact within the minor groove of DNA by forming a covalent bond between their electrophilic C11-position and the exocyclic C2-NH_2 moiety of a guanine residue.[3] Bonding occurs in a sequence-specific fashion with a preference for PuGPu motifs. Typical examples of PBD natural products[4] are DC-81 (**1**) and sibiromycin (**2**). A survey of established DNA-binding antibiotics reveals that a significant number of them have minor groove-binding elements composed of oligosaccharides.[5] Hydroxyazepanes have several properties that make them potentially useful as minor groove-binding ligands (MGBLs).[6] The flexibility of the seven-membered ring (compared with that of a five- or six-membered ring) would allow the hydroxyl groups to adopt a variety of positions increasing the probability of their forming hydrogen bonds with the N-3 of the purine, the urea carbonyl of the pyrimidine bases (H-bond acceptors), or the 2-amino of guanine (H-bond donor) which point into the minor groove (Fig. 1).

The primary advantage of the level of hydroxylation in hydroxyazepanes is their high water solubility, allowing them to circumvent the problem of poor bioavailability seen with many other MGBLs and the chirality allows improved sequence selectivity. Recently, polyhydroxylated bis-azepanes have been reported as new motifs for DNA-minor groove-binding agents.[6] Further, there are many instances where the PBD-based molecules have problems of solubility and bioavailability.[7] Therefore, design of conjugates like PBD-azepanes could not only address this issue but moreover these compounds will have both the covalent and non-covalent moieties. Inspired by these observations and on the basis of these presumptions and our earlier efforts on the design and development of structurally modified PBDs,[8] we herein report the synthesis of PBD-azepanes linked through different alkane spacers to evaluate their DNA-binding potential.

The desired final target molecules **4a–d** have been obtained from the key intermediates **13a–d** and **14**. The intermediates **13a–d** have been obtained from the compounds **7** and **10**. The bis-epoxide (**7**) has been obtained from D-mannitol (**5**) as the starting material and the other compound 4-acetoxyphenylamine (**10**) has been obtained from 4-nitrophenol (**8**) as the starting material. The precursor **14** has been obtained by literature methods.[9] One of the key intermediates bis-epoxide (**7**) has been obtained by converting D-mannitol (**5**) to its triacetonide using acetone and catalytic amount of

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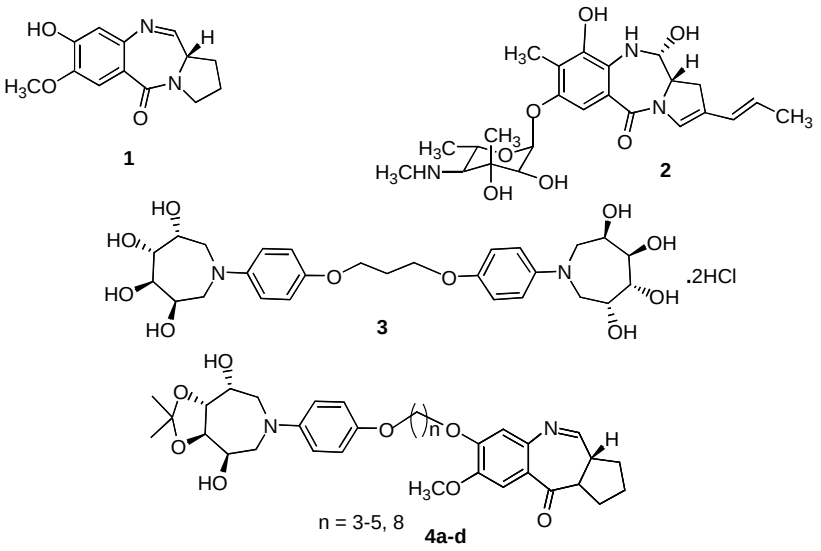
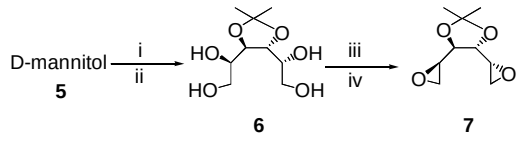


Figure 1. DC-81 (**1**), sibiromycin (**2**), bis-azepane (**3**), azepane-PBDs (**4a–d**).

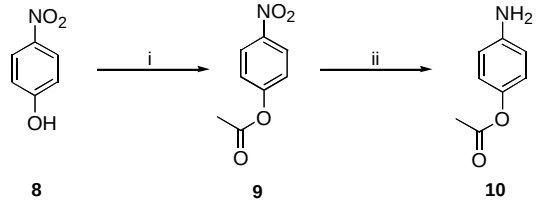
H_2SO_4, followed by removal of terminal acetonides using aq acetic acid, providing the monoacetonide (**6**). The monoacetonide (**6**) on tosylation using pyridine and tosyl chloride in dichloromethane at 0 °C for 4 h provides the 1,6-di-*O*-tosyl-3,4-*O*-isopropylidene-D-mannitol which on treatment with anhydrous K_2CO_3 in methanol for 2.5 h at 25 °C affords the bis-epoxide (**7**) (Scheme 1).[10]

Another intermediate, 4-acetoxyphenylamine (**10**), has been obtained from 4-nitrophenol (**8**) by simultaneous acetylation (**9**) employing acetyl chloride, triethylamine and DMAP in dichloromethane and nitro group reduction employing H_2/Pd-C in methanol (Scheme 2).

Refluxing the compound **12** and dibromoalkanes in acetone employing K_2CO_3 as the base gave the compounds **13a–d**. The compound **12** has been obtained by deacetylation of the compound **11** using 2M NaOH and methanol, which inturn have been obtained from



Scheme 1. Reagents and conditions: (i) acetone, H_2SO_4; (ii) 70% AcOH; (iii) TsCl, pyridine, CH_2Cl_2; (iv) K_2CO_3, MeOH.



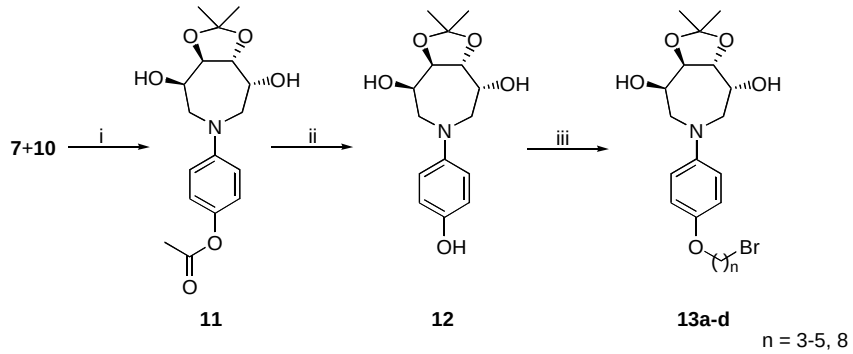
Scheme 2. Reagents and conditions: (i) acetyl chloride, NEt_3, DMAP, CH_2Cl_2; (ii) H_2/Pd-C, MeOH.

the intermediate compounds **7** and **10** in water at 95 °C (Scheme 3).[11]

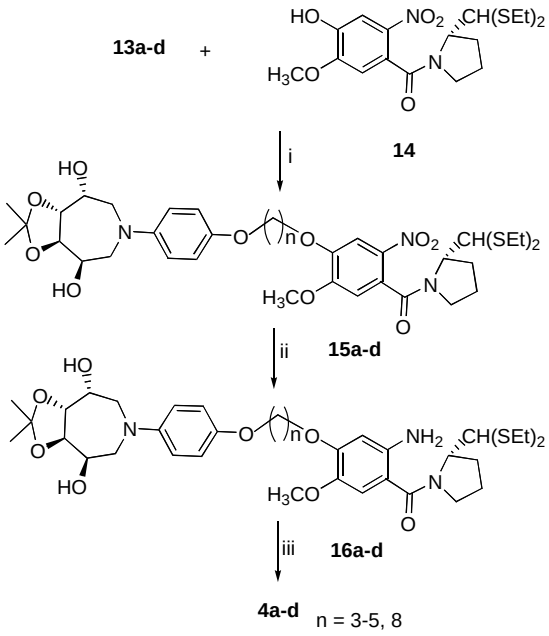
The compounds **15a–d** have been obtained by coupling of compounds **13a–d** and **14**. These upon reduction of the nitro group employing H_2/Pd-C in ethanol followed by deprotection of thioacetal group afford the target compounds **4a–d** (Scheme 4).[12]

The DNA-binding affinity of the novel azepane-linked PBDs (**4a–d**) was investigated by thermal denaturation studies using calf thymus (CT) DNA.[13,14] The studies for these compounds (**4a–d**) were carried out by DNA/ligand molar ratios of 5:1. The increase in the helix melting temperature (ΔT_m) for each compound was examined after 0, 18 and 36 h incubation at 37 °C. Data for compound **1** are included in Table 1 for comparison. The compound **4b** elevates the helix melting temperature of the CT-DNA by 2.0 °C after incubation at 37 °C for 36 h. The naturally occurring DC-81 (**1**) gives a ΔT_m of 0.7 °C under identical experimental conditions. This result illustrates the effect of introducing the azepane moiety through different alkane spacers at C8-position of the DC-81 (Table 1).

It is observed from the thermal denaturation study data that when azepane is linked to PBD through a three-carbon spacer (**4a**) there is no DNA-binding. However, in case of compound **4b** when the azepane sub-unit is linked to the PBD through a four-carbon alkane spacer it exhibits maximum DNA-binding which eventually increases to 2.0 °C upon incubation for 36 h at 37 °C. Probably the azepane sub-unit attains a proper alignment for the non-covalent interactions that could take place when the alkane spacer comprises of four carbon units. In case of compounds with five and eight alkane spacers, the ΔT_m value is marginally enhanced to 1.1 °C after incubation for 36 h at 37 °C compared to DC-81 (**1**), which exhibits 0.7 °C under identical experimental conditions (Table 1). Therefore, similar to other hybrids of PBD the linker length plays an important role



Scheme 3. Reagents and conditions: (i) H_2O, 95 °C; (ii) 2M NaOH, MeOH (iii) dibromoalkanes, K_2CO_3, reflux.



Scheme 4. Reagents and conditions: (i) K_2CO_3, acetone, reflux, 48 h; (ii) H_2/Pd-C, EtOH, 24 h; (iii) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O (4:1), 8 h.

Table 1. Thermal denaturation data for PBD–azepane conjugates with calf thymus (CT) DNA

Compound	[PBD]:[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for		
		0 h	18 h	36 h
4a	1:5	0.0	0.0	0.0
4b	1:5	1.1	1.0	2.0
4c	1:5	−0.8	1.0	1.1
4d	1:5	0.2	1.1	1.1
DC-81 (**1**)	1:5	0.3	0.7	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C.

[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

in these compounds as well. However, the bis-azepane (**3**) exhibits the helix melting temperature of 0.7 °C at pH 5.0 after incubation for 12 h.[6]

In conclusion, synthesis and DNA thermal denaturation studies of pyrrolobenzodiazepine–azepane conjugates linked through different alkane spacers are described. The biological screening of these compounds is under investigation. Further research will involve the preparation of azepanes with more number of hydroxyl groups, or with the hydroxyls modified as their methyl ethers, and are linked to the PBD moiety which may exhibit better minor groove-binding abilities. These compounds may form stable complexes with the double-stranded DNA and are expected to possess improved bioavailability.

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