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Synthesis of fluorinated analogues of SJG-136 and their DNA-binding potential

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Abstract—A series of C2-*exo*-fluorounsaturated pyrrolobenzodiazepines (PBDs) have been synthesized. These C2-*exo*-fluorounsaturated PBD dimers have exhibited remarkable DNA-binding affinity.

There is a growing interest in agents, such as pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), that can recognize and bond to specific sequences of DNA. They are potential gene regulators with possible therapeutic applications in the treatment of genetic disorders, including some cancers, as selective antiinfective agents, and as probes and tools for use in molecular biology.[1] PBDs are a group of naturally occurring antitumor antibiotics, members of which include anthramycin, tomaymycin, neothramycins A and B, sibiromycin, mazethramycin, chicamycin, prothracarcin, DC-81, and dextochrysin.[2] The PBDs interact within the minor groove of DNA by forming covalent bond between their elecrophilic C11-position and the exocyclic C2–NH_2 moiety of a guanine residue.[3] Bonding occurs in sequence specific fashion with a preference for PuGPu motifs. Typical examples of PBD natural products such as DC-81 (**1**) and tomaymycin (**2**) have the same substitutions in the aromatic A-ring, although **2** has an additional C2-*exo* unsaturation. In order to understand the structure–activity relationship studies many synthetic analogues have been prepared mainly with modifications and substitutions in the aromatic A-ring and not much attention has been given to the *C*-ring modifications.

A novel sequence selective PBD dimer (SJG-136, **5**) has been developed that comprises of C2-*exo*-methylene-substituted DC-81 subunits tethered through their C8 positions via an inert propanedioxy linker that has exhibited efficient minor groove interstrand cross-linking property.[4,5] This compound shows significant in vivo anticancer activity and has been selected for clinical trials.[6] It is known in the literature that in case of PBD dimers, they span approximately six base pairs of DNA and in which the sequence selectivity also increases (e.g., purine–GATC–pyrimidine)[7] in comparison to the PBD monomers that have sequence selectivity for three base pairs. Therefore, design and synthesis of symmetrical dimers of PBD has been considered interesting from the point of view of DNA interaction.

In the literature, a number of antiviral and antitumor agents have been developed in which fluorine substitution has played a key role in their biological activity. For example, the discovery of 2′-difluoro modified gemcitabine exhibited promising biological activity such as antitumor and antiviral activity, which is being used clinically for the treatment of various solid tumors.[8,9] Therefore, the importance of fluorine substitution in pharmaceutical development is evident in a large number of fluorinated compounds that have been approved by the Food and Drug Administration (FDA) as drugs.[10,11] In view of this, it has been considered of interest to design molecules with fluorine substitutions in the pyrrolo[2,1-*c*][1,4]benzodiazepine ring system.

Keywords: C2-*exo*-fluorounsaturated pyrrolobenzodiazepine; DNA-binding ability.

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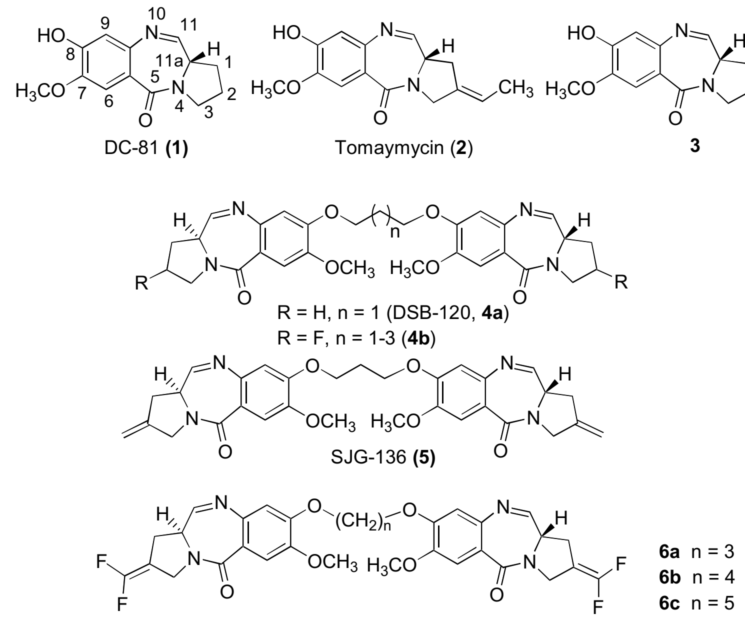
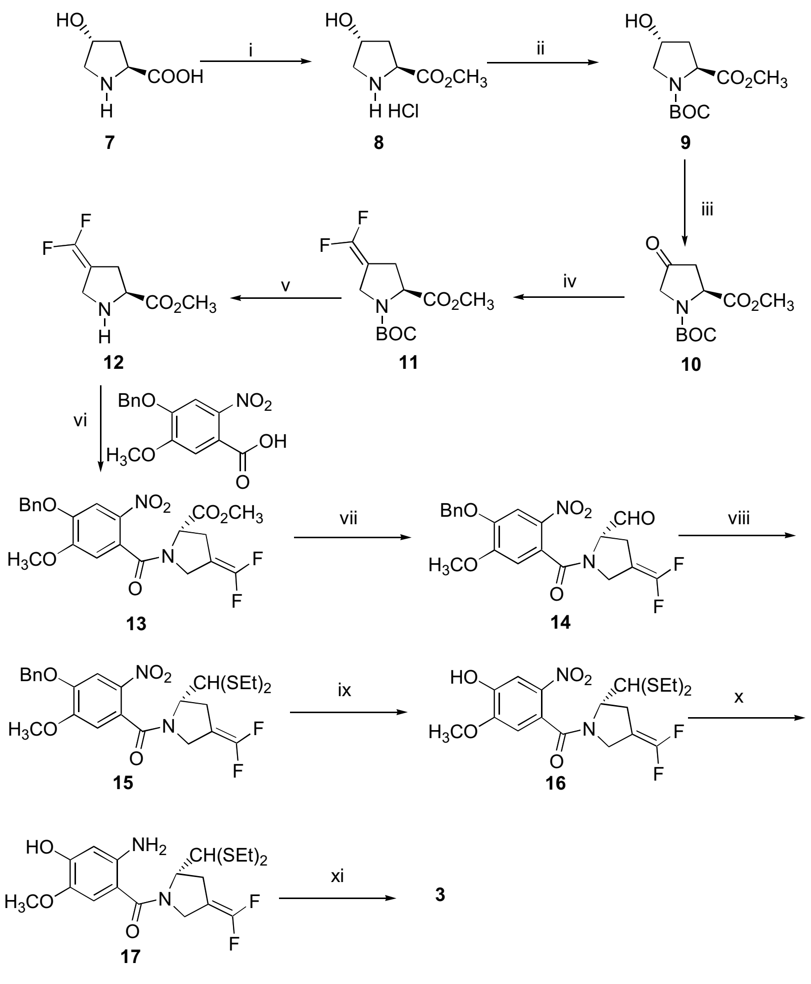


Figure 1.



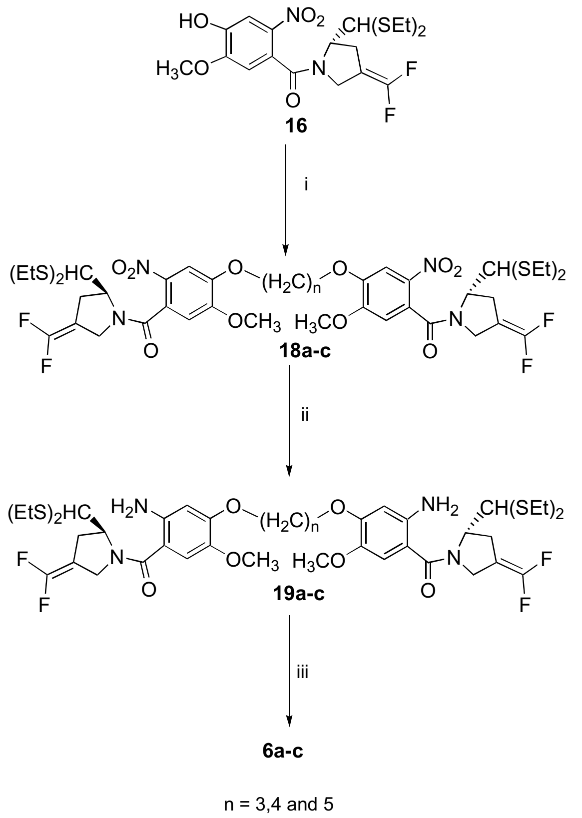
Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH; (ii) Boc_2O, 2N NaOH, THF–H_2O (2:1), rt; (iii) trichloroisocyanuric acid, Tempo, CH_2Cl_2; (iv) CF_2Br_2, HMPT, Zn, THF; (v) TFA, CH_2Cl_2; (vi) $(COCl)_2$, DMF, THF, Et_3N, THF; (vii) DIBAL-H, CH_2Cl_2, −78 °C, 45 min, 80–85%; (viii) EtSH–TMSCl, $CHCl_3$, 24 h, rt, 90%; (ix) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 80%; (x) $SnCl_2{\cdot}2H_2O$, CH_3OH, reflux, 2 h, 80–85%; (xi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 68–86%.

Therefore, on the basis of this presumption and our earlier efforts on the design and development of structurally modified PBDs,[12] we herein report the synthesis of C2-*exo*-difluoromethylene analogue of compound **1** and fluorinated analogues of SJG-136 (**6**) to explore their DNA-binding affinity in comparison with their non-fluorinated counterparts. Moreover, we have recently reported the synthesis of C2-fluorinated PBD analogues (**4b**) and their DNA-binding potential[13] (Fig. 1).

The synthesis of C2-*exo*-difluoromethylene DC-81 (**3**) has been carried out by employing commercially available L-*trans*-4-hydroxyproline (**7**), which is *N*-protected by Boc via its methyl ester to give compound **9** in quantitative yield. The oxidation of 2-hydroxy group of compound **9** with trichloroisocyanuric acid and Tempo gives the key precursor with C2-ketone (**10**) in excellent yield.[14] This has been converted to its C2-*exo*-fluoromethylene compound **11** employing dibromodifluoromethane in the presence of HMPT, Zn.[15] This upon deprotection of Boc provides the required precursor methyl (2*S*)-4-difluoromethylidenepyrrolidine-2-carboxylate (**12**). The other precursor 4-benzyloxy-5-methoxy-2-nitrobenzoic acid has been prepared by earlier reported methods[16] and is coupled to compound **12** via its acid chloride to give methyl (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-difluoromethylidenepyrrolidine-2-carboxylate (**13**). Reduction by DIBAL-H gives the corresponding aldehyde (**14**), which upon EtSH protection followed by deprotection of benzyl group by using EtSH–BF_3OEt_2 and subsequent reduction with tin chloride provides the key intermediate amino thioacetal (**17**). This upon deprotective cyclization affords the target C2-*exo*-difluoromethylene DC-81 (**3**) as shown in Scheme 1.[17]

The synthesis of C2/C2′-*exo*-difluoromethylene PBD dimers has been carried out by the etherification of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-difluoromethylidenepyrrolidine-2-carboxaldehyde diethyl thioacetal (**16**) with dibromoalkanes to provide **18a**–**c**. These upon reduction with $SnCl_2·2H_2O$ gave the corresponding aminodiethyl thioacetals (**19a**–**c**). Deprotection of these amino thioacetals by usual literature methods employing $HgCl_2$–$CaCO_3$ provided the required products **6a**–**c** in 60–65% yields. However, amino thioacetal functionality of **19a**–**c** has also been deprotected by employing bismuth triflate to afford the desired C2/C2′-*exo*-difluoromethylene PBD dimers **6a**–**c** in 65–70% yields (Scheme 2).[17] The use of catalytic amount of bismuth triflate in biphasic system for such deprotections has been earlier investigated in our laboratory.[18] It is interesting to note that this procedure not only provides better yields with relatively easier work up procedure but more importantly the products are free from trace impurities of mercuric salts.

The DNA-binding ability for these C2-*exo*-difluoromethylene PBDs and their dimers has been examined by thermal denaturation studies using calf thymus (CT) DNA. These studies were carried out at PBD/DNA molar ratio 1:5. In case of C2-*exo*-difluoromethylene DC-81 (**3**) helix melting temperature marginally increased after 18 h of incubation in comparison to naturally occurring DC-81. It is observed from the data one of the fluoro substituted PBD dimer **6a** stabilizes the double-stranded CT-DNA in an efficient manner. This compound with a three carbon alkane spacer elevates



Scheme 2. Reagents and conditions: (i) Dibromoalkanes, K_2CO_3, acetone, 36–48 h, reflux, 89–91%; (ii) $SnCl_2·2H_2O$, CH_3OH, reflux, 2 h, 70–72%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h (or) $Bi(OTf)_3·xH_2O$, CH_2Cl_2–H_2O, 4 h, rt.

Table 1. Thermal denaturation data for C2/C2′-*exo*-fluoromethylene PBDs with calf thymus (CT) DNA

Compounds	[PBD]:[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C		
		0 h	18 h	36 h
3	1:5	1.0	1.7	2.2
6a	1:5	20.8	24.6	24.9
6b	1:5	2.1	5.2	6.5
6c	1:5	16.2	19.2	21.3
4a	1:5	10.2	15.1	15.7
5[c]	1:5	25.7	33.6	
1	1:5	0.3	0.7	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, $T_m = 69.6\,°C \pm 0.01$ (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].

[c] Literature values.[5]

the helix melting temperature of the CT-DNA by a remarkable 24.6 °C after incubation at 37 °C for 18 h. In the same experiment the DC-81 dimer (DSB-120) gives a ΔT_m of 15.1 °C. On the other hand, ΔT_m of compound **6c** having five carbon alkane spacer is 19.2 °C after incubation for 18 h at 37 °C. It is interesting to observe that, both the PBD dimers **6a** and **6c** the ΔT_m values are higher when the length of alkyl chain spacers is three and five but they are not as high as SJG-136 as illustrated in Table 1. However, compound **6b** with a four carbon alkane spacer shows comparatively lower ΔT_m value (5.2 °C) after 18 h of incubation. Probably because of unfavorable fit of this molecule within the minor groove of host DNA duplex. Therefore, it appears that the linker length in these dimers is probably modulating the DNA reactivity potential.

In summary, new C2-*exo*-difluoromethylene DC-81 and its dimers have been synthesized that exhibit remarkable DNA-binding ability. The detailed mechanistic and molecular modeling studies for these fluorinated PBDs are in progress.

Acknowledgements

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