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Synthesis and DNA-binding ability of *C2R*-fluoro substituted DC-81 and its dimers

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Abstract—*C2R*-Fluoro substituted DC-81 and its dimers have been synthesized that exhibit significant DNA-binding ability, particularly the five carbon alkane spacer compound (**6c**) showed the helix melting temperature (ΔT_m) of 18.8 °C after incubation of 36 h at 37 °C.

There has been growing interest in anticancer agents, such as pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), that can recognize and bond to specific sequence of DNA. They are potential gene regulators with possible therapeutic applications in the treatment of genetic disorders, including some cancers, as selective anti-infective agents, and as probes and tools for use in molecular biology.[1] PBDs are a group of naturally occurring antitumour antibiotics, members of which include anthramycin, tomaymycin, sibiromycin, chicamycin, and DC-81.[2] The cytotoxicity and antitumour activity of these agents are attributed to their property of sequence-selective covalent binding to the N2 of guanine in the minor groove of duplex DNA[3] via an acid labile aminal bond to the electrophilic imine at the N10–C11 position. The N10–C11 carbinolamine form may exist in the equivalent imine or carbinolamine methyl ether form depending on the precise structure of the compound and the method of isolation.[4] Thurston and co-workers[5] have synthesized C8-linked PBD dimers (DSB-120), which form an irreversible interstrand cross-link between two guanine bases within the minor groove via their exocyclic N2 atoms and actively recognizing a central 5′-GATC sequence.[6] Molecular modeling studies suggested that C8-linked PBD dimers have greater isohelicity with the minor groove of DNA[7] compared with the C7-linked dimers. Further, C2-methylene DC-81 dimer and imine amide mixed PBD dimers have been synthesized and they exhibited interesting biological profile[8] (Fig. 1).

The uses of organofluorine compounds have been found rapidly increasing in the areas of agrochemicals, pharmaceuticals, and fluoropolymers. A number of antiviral, antitumour, and antifungal agents have been developed in which fluorine substitution has been a key to their biological activity.[9,10] Many organofluorine derivatives have been used as probes for studying biochemical process. More importantly, this demonstration of the extraordinary potential of fluorine substitution to alter and enhance the pharmacological properties of organic molecules has become the basis of a powerful strategy for lead development in the pharmaceutical industry. There are also a few recent reports in the literature wherein fluorinated analogues have improved the biological activity profile of some pharmacologically important compounds.[11–17]

A number of naturally occurring PBDs namely anthramycin, tomaymycin, sibiromycin, and neothramycin have different types of substitution in the C-ring. It is interesting to note that these C-ring modified PBDs appear to provide both greater differential thermal stabilization of DNA duplex and significantly enhance kinetic reactivity during covalent adduct formation. Similarly, the C2-substituted naturally occurring PBDs exhibit more cytotoxicity compared to their unsubstituted PBDs. More recently a series of *C2*-fluorinated PBDs[18] have been synthesized and screened for in vitro cytotoxicity (IC_{50}) against a number of cancer cell lines. In recent years a large number of hybrid molecules

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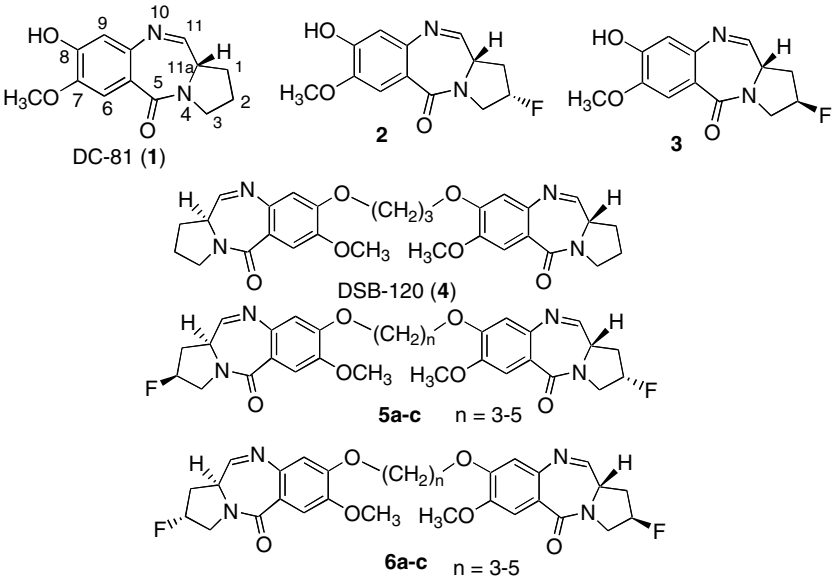


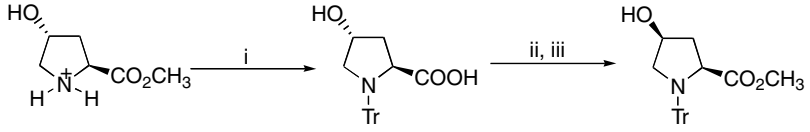
Figure 1.

containing the PBD ring system have been synthesized leading to novel sequence selective DNA cleaving and cross-linking agents. Therefore, based on these observations and our own findings in the synthesis and biological evaluation of PBDs with fluorine substitution,[19] it has been considered of interest to design and synthesize *C2R*-fluoro substituted DC-81 and its dimers to evaluate their DNA-binding potential.

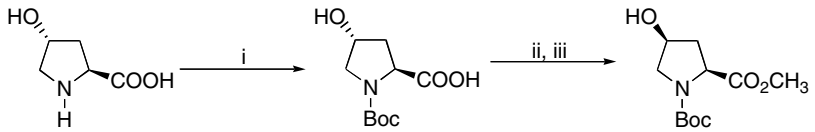
The synthesis of *cis*-4-hydroxyproline methyl ester has been carried out by employing the *trans*-4-hydroxyproline methyl ester by the methods reported in the literature.[20] Purification of the crude *N*-trityl-*cis*-4-hydroxyproline methyl ester by column chromatography has given low yields of the desired product. Therefore, an alternative strategy has been sought by opting the Boc-protection of *trans*-4-hydroxyproline followed by intramolecular Mitsunobu reaction and ring opening of the resultant lactone with Amberlyst-15® in methanol to afford the desired *N*-Boc-*cis*-4-hydroxyproline methyl ester (Schemes 1 and 2).

Synthesis of *C2R*-fluoro-DC-81 and its dimers is of considerable interest as molecular modeling studies indicated that these PBDs may have a better DNA-binding potential than the *C2S*-fluoro PBDs. These *C2R*-fluoro-DC-81 and its dimers have been synthesized by using the literature synthetic approach.[19a] Methyl-(2*S*,4*R*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-fluoropyrrolidine-2-carboxylate (**8**) has been synthesized by employing methyl-(2*S*,4*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-hydroxy pyrrolidine-2-carboxylate (**7**) and DAST in dichloromethane. (2*S*,4*R*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-fluoropyrrolidine-2-carboxaldehyde diethyl thioacetal (**10**) has been prepared by literature method,[21] which upon debenzylation with $BF_3{\cdot}OEt_2$ affords the compound **11**. Further, this upon reduction followed by deprotection of aminothioacetal precursor (**12**) affords the target *C2R*-fluoro substituted PBD **3** (Scheme 3).

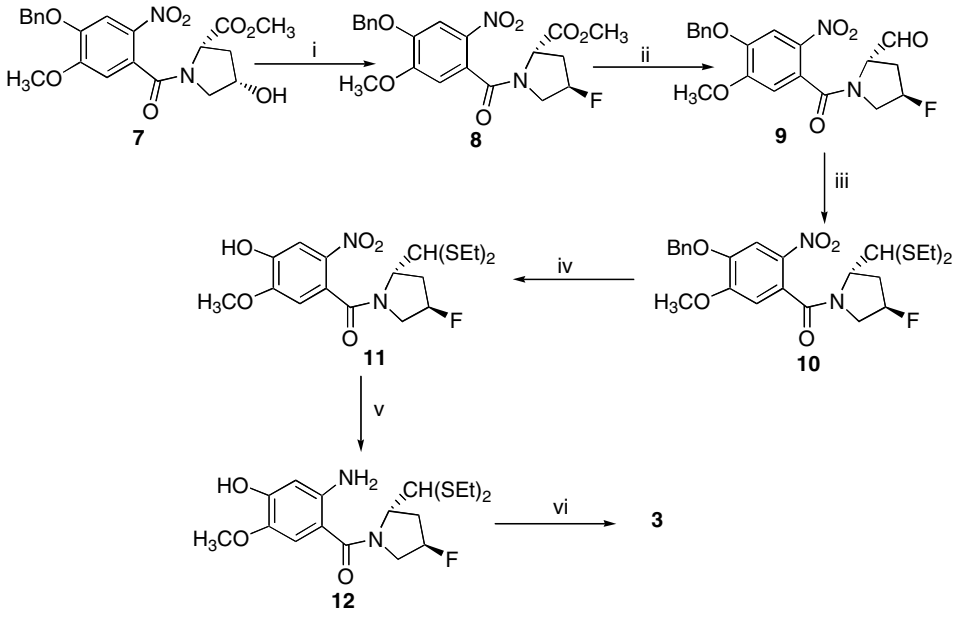
The synthesis of *C2R*-fluoro dimers has been carried out by the etherification of (2*S*,4*S*)-*N*-(4-hydroxy-5-meth-



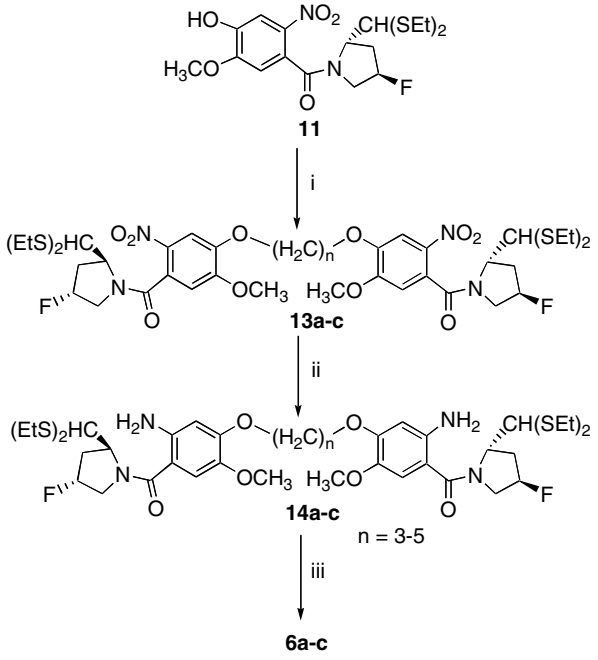
Scheme 1. Reagents and conditions: (i) TrCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (ii) DEAD, PPh_3, $PhCO_2H$, toluene rt, 2 h; (iii) 5% KOH/CH_3OH, 2 h.



Scheme 2. Reagents and conditions: (i) Boc_2O, 10% aq NaOH, THF/H_2O (2:1), 0 °C, o/n, 95%; (ii) DEAD, PPh_3, THF, 0 °C to rt, o/n, 65%; (iii) Amberlyst-15®, CH_3OH, rt, o/n, 80%.



Scheme 3. Reagents and conditions: (i) DAST, CH_2Cl_2, −78 °C, 12 h, rt, 87%; (ii) DIBAL-H, CH_2Cl_2, −78 °C, 45 min, 80%; (iii) EtSH-TMSCl, $CHCl_3$, 24 h, rt, 85%; (iv) EtSH-BF_3OEt_2, CH_2Cl_2, 12 h, rt, 80%; (v) $SnCl_2{\cdot}2\,H_2O$, CH_3OH, reflux, 2 h, 82%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 74%.



Scheme 4. Reagents and conditions: (i) dibromoalkanes, K_2CO_3, acetone, 36–48 h, reflux, 85–95%; (ii) $SnCl_2{\cdot}2H_2O$, CH_3OH, reflux, 2 h, 68–75%; (iii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 63–75%.

oxy-2-nitrobenzoyl)-4-fluoropyrrolidine-2-carboxaldehyde diethyl thioacetal (**11**) with dibromoalkanes to provide **13a**–**c**. Further, these upon reduction followed by deprotection of aminothioacetal precursors **14a**–**c** afford the desired *C2R*-fluorinated PBD dimers **6a**–**c** in good yields (Scheme 4).[22]

The DNA-binding ability of these new *C2R*-fluorinated DC-81 and its dimers has been investigated by thermal

Table 1. Thermal denaturation data for C2-fluorinated PBDs with CT-DNA

Compounds	[PBD]:[DNA] molar ratio[b]	ΔT_m[a] (°C) after incubation at 37 °C for		
		0 h	18 h	36 h
2[c]	1:5	0.3	1.0	2.2
5a[c]	1:5	3.1	4.9	6.2
5b[c]	1:5	4.6	12.3	13.8
5c[c]	1:5	14.2	16.0	17.4
3	1:5	0.7	2.1	2.3
6a	1:5	3.1	5.2	6.2
6b	1:5	4.8	12.4	14.0
6c	1:5	15.6	16.9	18.8
DSB-120 (**4**)	1:5	10.2	15.1	15.4
DC-81 (**1**)	1:5	0.3	0.7	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.8 °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 PBD concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

[c] Literature values.

denaturation studies using calf thymus (CT) DNA.[23] Melting studies show that these compounds stabilize the thermal helix coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. In case of *C2S*-fluorinated DC-81 (**2**) the helix melting temperature has marginally increased after 18 h of incubation in comparison to naturally occurring DC-81 (**1**). Similarly, *C2R*-fluorinated DC-81 (**3**) has exhibited slightly higher DNA melting temperature compared to the *S*-isomer (**2**). On the other hand in the case of *C2R*-fluorinated dimers it is interesting to observe that the

C2R-fluorinated dimer **6a** shows lower DNA melting temperatures compared to DC-81 dimer (**4**), while as the linker length increases from three to five the helix melting temperature of CT-DNA increases to 16.9 °C after incubation of 18 h for compound **6c** as shown in Table 1. Further, like many other hybrids of PBD the linker length plays an important role in these compounds as well. However, some of these newly synthesized fluorinated PBD helix-melting temperatures are higher than the DC-81 and its dimer (DSB-120).

The newly synthesized *C2R*-fluorinated DC-81 and its dimers have shown significant DNA-binding ability. Amongst these **6c** showed high helix melting temperature 16.9 °C after 18 h incubation. *C2R*-fluorinated DC-81 (**3**) has exhibited slightly higher DNA melting temperatures compared to the *C*2-*S* isomer (**2**). These results suggest that *C2R*-fluoro isomer offers the better orientation of the fluorine group in the *C*2-position for an efficient binding of the PBD ring system to the DNA.

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22. Spectral data for compound **6c**: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.00–2.22 (m, 4H), 2.30–2.80 (m, 6H), 3.58–3.85 (m, 6H), 3.92 (s, 6H), 4.00–4.25 (m, 4H), 5.35 (dt, 2H, J = 53.2, 5.4 Hz), 6.80 (s, 2H), 7.50 (s, 2H), 7.70 (d, 2H, J = 4.3 Hz); FAB MS: 597 (M^++1); Anal. Calcd for $C_{31}H_{34}F_2N_4O_6$: C, 62.57; H, 5.75; N, 9.41; Found: C, 62.49; H, 5.66; N, 9.34.
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