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The synthesis and biological activity of *C*2-fluorinated pyrrolo[2,1-*c*][1,4]benzodiazepines

Ian A. O'Neil,[a,*] Stephen Thompson,[a] S. Barret Kalindjian[b] and Terence C. Jenkins[c]

[a]*Department of Chemistry, University of Liverpool, Crown St, Liverpool L69 7ZD, UK*
[b]*James Black Foundation, 68 Half Moon Lane, Dulwich, London SE24 9JE, UK*
[c]*Yorkshire Cancer Research Laboratory of Drug Design, University of Bradford, West Yorkshire BD7 1DP, UK*

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Abstract—The novel *C*2-fluorinated pyrrolobenzodiazepines (**1**, **2** and **3**) have been prepared from commercially available *trans*-hydroxyproline in good overall yield and were screened for in vitro cytotoxicity against a number of cancer cell lines. The 2*R*-fluoro isomer **2** exhibits an activity of 76 nM against the CH1 cell line.

Within the scientific community, there is a considerable amount of interest in the design and synthesis of small molecules that can actively recognise and bind to specific base-tract sequences of DNA. The pyrrolo[1,4]-benzodiazepine (PBD) ring system is found in a number of antitumour agents belonging to the anthramycin family of antibiotics. Members of this family include anthramycin, porothramycin B and prothracarcin (Fig. 1). These agents exert their biological activity by covalently binding to the C2-NH_2 of the guanine base in PuGPu sequences in the minor groove of DNA.[1]

In order to probe their mode of action and biological activity, several synthetic analogues have been prepared with the vast majority bearing modifications to the aromatic A-ring,[1,2] with fewer reports of B-[3] and C-ring modifications.[4] Dugave et al.[5] have reported the synthesis of Boc- and Fmoc-protected 4-fluoro- and 4,4′-difluoroprolines from the commercially available *trans*-4-hydroxyproline. Given our interest in defining structural activity relationships for substituted PBDs, we envisaged these compounds would be extremely useful in the synthesis of the *C*2-fluoro-substituted PBDs **1**, **2** and **3** using the Staudinger/aza-Wittig methodology that we[6] and others[7] have previously reported. We now wish to report the successful synthesis and biological activity of the novel *C*2-fluoro-analogues **1**, **2** and **3** using this methodology.

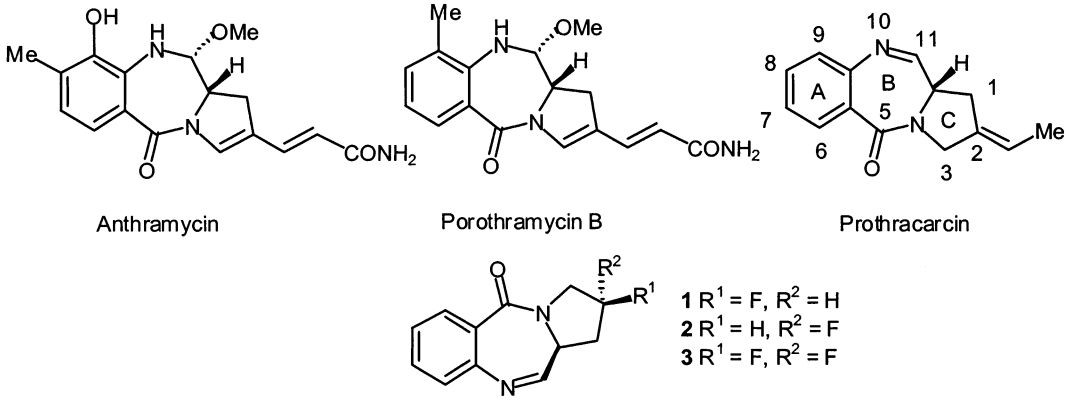

Figure 1.

* Corresponding author. Tel./fax: 0044 (0)151 794 3485/3588; e-mail: ion@liv.ac.uk

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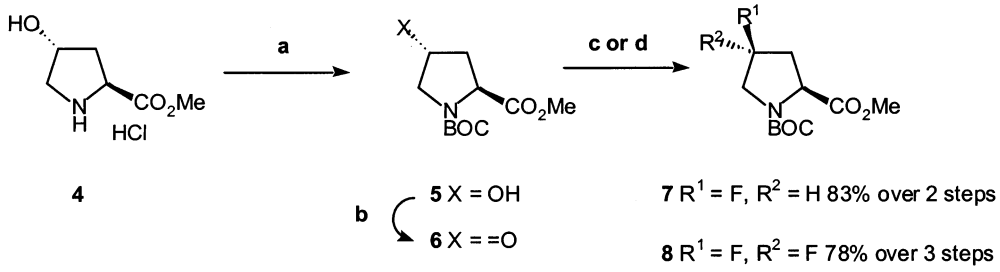
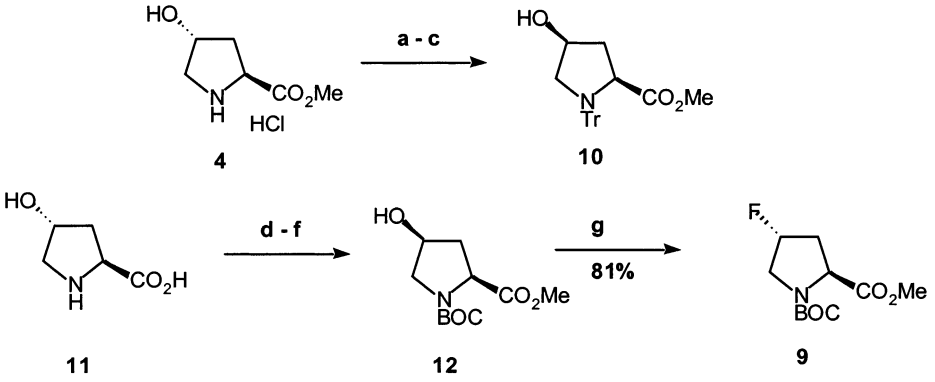
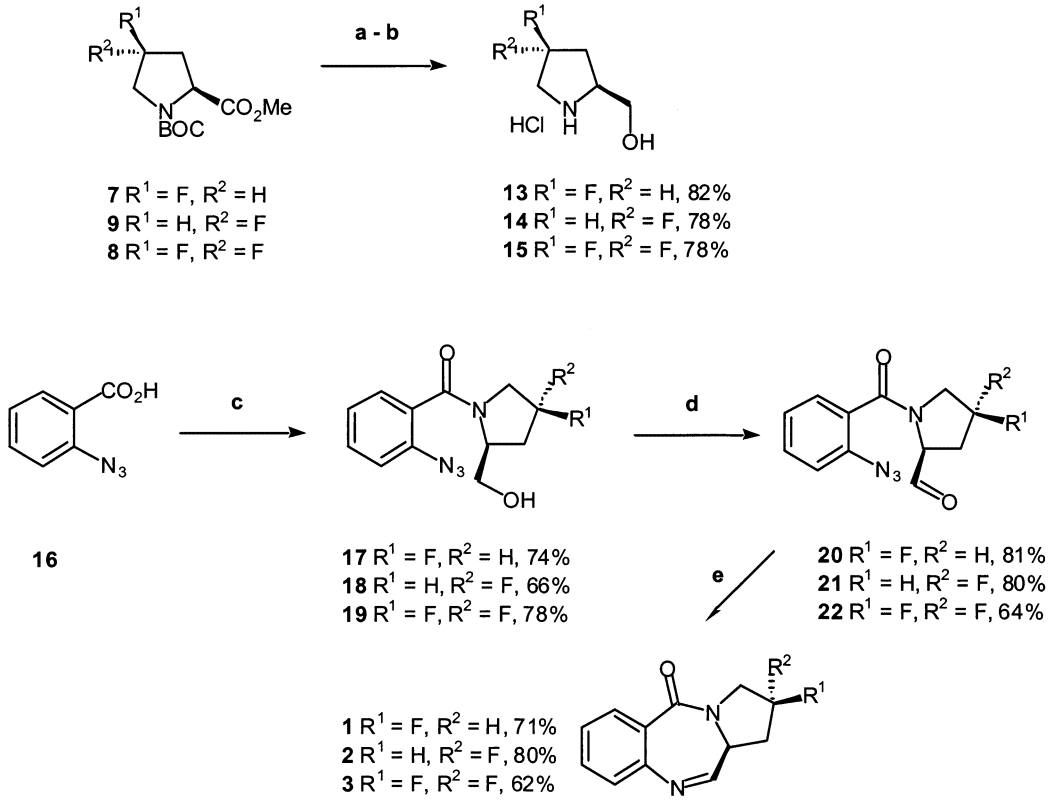


Scheme 1. *Reagents*: (a) BOC_2O, Et_3N, DCM, 0°C to rt, 2 h, 95%; (b) $(COCl)_2$, DMSO, Et_3N, DCM, −78°C, 93%; (c) **5**–**7**, DAST (2.5 equiv.), DCM, −78°C to rt, o/n, 85%; (d) **6**–**8**, DAST (5 equiv.), DCM, −78°C to rt, o/n, 90%.

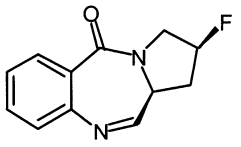
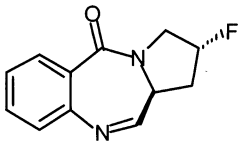
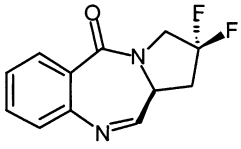
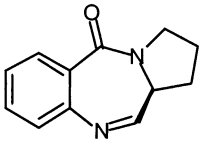
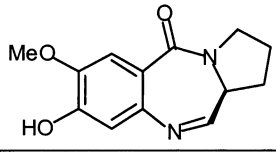


Scheme 2. *Reagents*: (a) TrCl, Et_3N, DCM, 0°C to rt, 2 h; (b) DEAD, DPPE, $PhCO_2H$, toluene, rt, 2 h; (c) 5% KOH/MeOH, 2 h; (d) Boc_2O, 10% aq. NaOH, THF:H_2O (2:1), 0°C, o/n, 95%; (e) DEAD, PPh_3, THF, 0°C to rt, o/n, 65%; (f) Amberlyst-15®, MeOH, rt, o/n, 80%; (g) DAST, DCM, −78°C to rt, o/n, 81%.



Scheme 3. *Reagents*: (a) DIBAL-H, THF, −78°C to rt, o/n; (b) 4 M HCl in 1,4-dioxane, o/n; (c) (i) $(COCl)_2$, DCM cat. DMF; (ii) **13**, **14** or **15**, Et_3N, DCM, −78°C to rt, o/n; (d) Dess–Martin periodinane, DCM, rt, o/n; (e) DPPE, THF, 2 h.

Table 1. In vitro cytotoxicity for fluorine-substituted C-ring modified PBDs

		$IC_{50}(\mu M)$[a, b]		
Entry	**PBD**	**A2780**	**CH1**	**L1210**
1	 **1**	1.1	0.22	1.9
2	 **2**	0.43	0.076	0.64
3	 **3**	1.8	0.56	3.2
4	 **23**	-	42	>100
5	 **24**	-	0.10	0.38

a: Determined after 72 h (L1210 mouse leukaemia) or 96 h (A2780 or CH1 human ovarian carcinoma cell lines) continuous exposure to the PBD agent.

b: Concentration of drug (+/-10-15%) required to effect 50% cell killing.

Table 2. Effect upon thermal denaturation of calf thymus DNA by C-ring modified PBDs

		ΔT_m/°C after incubation[a]			
Entry	PBD	0 h	4 h	18 h	48 h
1	**1**	0.41+/−0.06	0.63+/−0.07	0.85+/−0.07	0.98+/−0.11[b]
2	**2**	0.57+/−0.07	0.82+/−0.08	1.03+/−0.10	1.21+/−0.12[c]
3	**3**	0.37+/−0.07	0.54+/−0.06	0.78+/−0.09	0.86+/−0.10
4	**23**	0.35+/−0.09	0.51+/−0.09	0.74+/−0.10	0.88+/−0.13
5	**24**	0.31+/−0.10	0.49+/−0.13	0.65+/−0.14	0.72+/−0.11

[a] Determined with either a Varian-Carey 100 or Varian-Carey 1E spectrophotometer fitted with a Peltier temperature controller, following incubation at 37.0+/−0.1°C within an external water bath for the times shown. Values represent the mean+/−s.e.m. from at least three determinations.

[b] Value of 0.99+/−0.12°C after 72 h incubation.

[c] Value of 1.25+/−0.11°C after 72 h incubation.

Key to the strategy was the synthesis of BOC-protected *cis* and *trans* 4-fluoro- **7** and **9** and 4,4′-difluoroproline methyl esters **8**. The syntheses of **7** and **8** were achieved in 83 and 78% yield, respectively, from *trans*-4-hydroxyproline methyl ester hydrochloride **4**, using similar methodology to Dugave[5] (Scheme 1).

Attempts to synthesise *N*-BOC-*trans*-4-fluoroproline methyl ester **9** employing the conditions reported by Dugave proved to be inconsistent in our hands. Purification of the crude *N*-trityl-*cis*-4-hydroxyproline **10** by column chromatography gave low yields of the desired product (Scheme 2). These results were attributed to the acidity of the silica gel employed during purification, resulting in the removal of the trityl protecting group. Use of base-treated silica gel and basic alumina also failed to give any of the desired product, therefore an alternative strategy was sought. BOC-protection of *trans*-4-hydroxyproline **11** followed by an intramolecular Mitsunobu reaction and ring-opening of the resultant lactone with Amberlyst-15® in methanol afforded the desired *N*-BOC-*cis*-4-hydroxyproline methyl ester **12** in 50% yield over the three steps. Treatment of **12** with 2.5 equivalents of DAST afforded the desired *trans*-4-fluoro analogue **9** in 81% yield.

Reduction of esters **7**, **9** and **8** with DIBAL-H followed by removal of the BOC-protecting group by treatment with 4 M HCl in 1,4-dioxane afforded the *cis*-4-fluoro-**13**, *trans*-4-fluoro-**14** and 4,4′-difluoroprolinol **15** hydrochlorides in 82, 78 and 78% yields, respectively (Scheme 3). Coupling of **13**, **14** and **15** with 2-azidobenzoic acid (**16**) via its acid chloride furnished azido-alcohols **17**, **18** and **19** in 74, 66 and 81% yields, respectively. Oxidation of alcohols **17**, **18** and **19** with Dess–Martin periodinane afforded the corresponding aldehydes **20**, **21** and **22** in 81, 80 and 64% yields, respectively. Aldehydes **20**, **21** and **22** underwent a Staudinger/aza-Wittig cyclisation upon treatment with DPPE in THF, to give the desired fluoro-substituted PBDs **1**, **2** and **3** in 71, 80 and 62% yields, respectively.

The C-ring fluorinated PBDs **1**, **2** and **3** were screened for in vitro cytotoxicity (IC_{50}) against the A2780[8] and CH1 human ovarian carcinoma cell lines as well as the L1210 mouse leukaemia cell line and for DNA-binding affinity by means of the stabilisation given to the duplex form calf thymus DNA towards thermal denaturation.[9] The data in Tables 1 and 2 indicate that the rank order of DNA reactivity and stabilising efficiency for the C-ring fluorinated PBDs is:-

$$2R\text{-F } \mathbf{2} > 2S\text{-F } \mathbf{1} > 2\text{-}F_2 \mathbf{3}$$

These results suggest that the 2*R*-F isomer offers the better presentation of the fluorine group whilst the PBD is bound, leading to a 550-fold increase in activity against the CH1 cell line when compared to the unsubstituted PBD **23** (Table 1, entry 2 versus entry 4). Initial molecular modelling studies indicate that the 2*R* orientation is favoured as it enables superior alignment of the PBD within the minor groove without wall clash. Nevertheless, both the 2*S*-F **1** and 2-F_2 **3** PBDs are 190- and 75-fold, respectively, more active than the unsubstituted PBD **23** against the CH1 cell line (Table 1, entries 1 and 3 versus entry 4), indicating the presence of considerably favourable stereoelectronic effects. It is of note that the 2*R*-F isomer **2** is more active against the CH1 cell line than the naturally occuring PBD DC-81 **24**.

As can be seen in Table 2, the fluorinated PBDs **1**, **2** and **3** significantly increase the thermal stability of the calf thymus DNA duplex. Longer DNA-PBD contact times lead to increased thermal stabilisation, a feature commonly associated with PBDs. In addition, in all cases the differential effects upon the low-*T* and high-*T* portions of the melting curves suggest that the guanine tracts (the higher melting temperature events) are selectively modified. Again, it is noteworthy that **1**, **2** and **3** all exhibit greater DNA binding affinity than the naturally occuring PBD DC-81 **24**.

In summary, the replacement of a hydrogen with a fluorine atom in the *C*2-position of the PBD ring system leads to a significant increase in cytotoxicity.

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