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Synthesis and biological evaluation of pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) C8 cyclic amine conjugates

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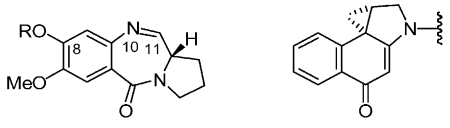
Abstract—We report examples of a series of novel pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) analogues **12**–**15** prepared from a common functionalized building block **11** that can be conveniently synthesized on a large scale and in optically pure form. Isoindoline analogue **15** is the most cytotoxic agent in this series, has the highest DNA-binding affinity, and shows significant activity in the in vivo hollow fibre assay.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs, e.g., **1a**–**c** in Fig. 1) are a group of antitumour agents which includes the natural products anthramycin and DC-81 (**1a**).[1] They exert their cytotoxic effect by covalently bonding to the exocyclic C2-NH_2 of guanine residues within the minor groove of DNA through their N10–C11 imine (or equivalent carbinolamine) functionality[1]. Synthetic PBD derivatives (e.g., **1b**–**c**) are of interest as potential anti-cancer drugs and also as gene targeting agents because they bind to guanine bases in duplex DNA in a sequence-selective manner with a preference for 5′-Pu-G-Pu motifs.[1–3] Recent research in this area has focused on improving and extending this sequence selectivity.[4] Various approaches have been explored, including the coupling of two PBD units through A-C8/A′-C8′[5–7] or C-C2/A′-C8′[8] linkages, or by conjugation of PBDs to secondary residues that possess complementary DNA sequence selectivity such as cyclopropylbenzindole[9,10] (e.g., CBI, **2** in Fig. 1) or distamycin/netropsin moieties.[11–13]

For studies involving coupling to an amine or alcohol, a building block such as **11** (Scheme 1) is required which has a carboxylate-terminated substituent at C8 and a protecting group at N10 that can be removed at the final synthetic stage to generate the DNA-interactive N10–C11 imine/carbinolamine moiety. Previously-reported building blocks have been prepared from intermediates of type **10** that contain Troc ester/Alloc,[9] methyl ester/Troc[11,12] and methyl ester/Fmoc[10] combinations at the C8/N10-positions, respectively. We report here a synthesis of building block **11** from intermediate **10** that utilizes benzyl and Boc protecting groups at C8/N10, respectively. With this combination of protecting groups, **11** can be produced efficiently on a large scale using simple procedures and without recourse to air-sensitive reagents. More importantly, stereochemistry is maintained at the C11a position of the PBD.



1a: R = H
1b: R = Me
1c: R = Bn

2

Figure 1. Generic structures of a pyrrolobenzodiazepine (PBD, **1a**–**c**) and cyclopropylbenzindole (CBI, **2**).

Keywords: DNA Binding; Pyrrolobenzodiazepines; PBDs; Sequence-selective; Gene targeting.
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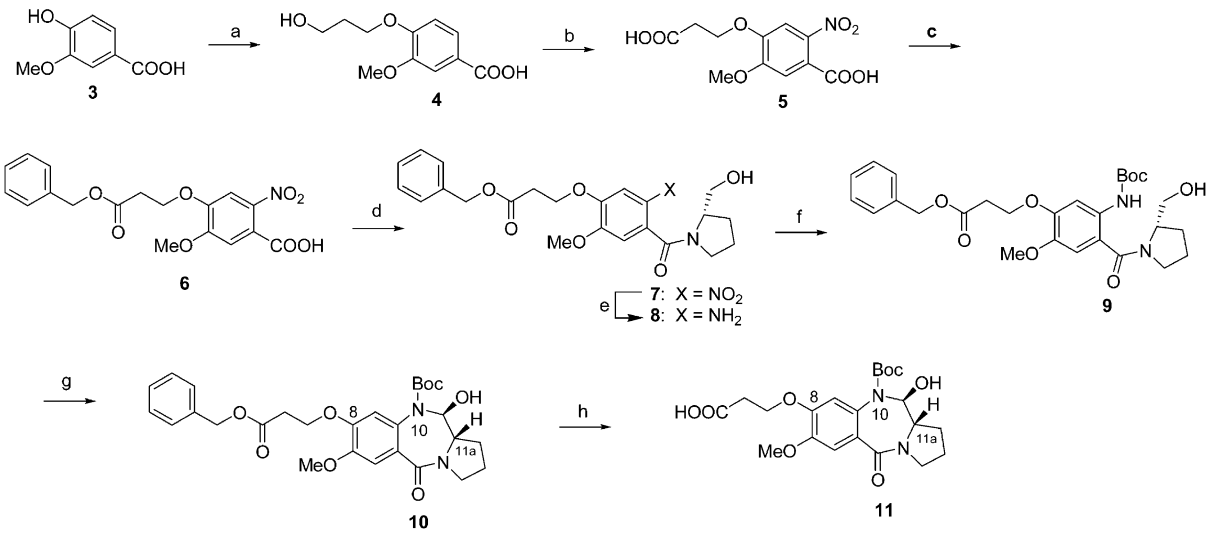
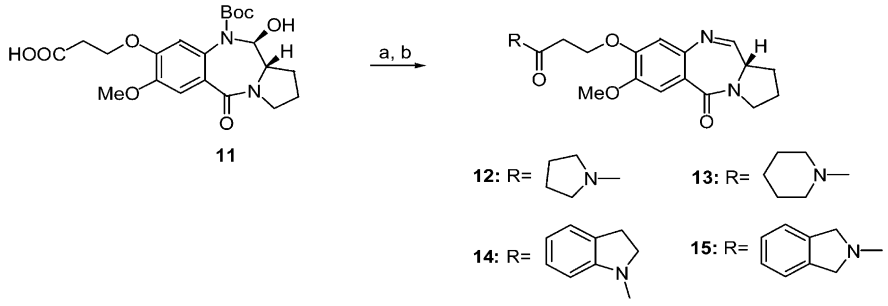


Scheme 1. (a) $Br(CH_2)_3OH$, $NaOH_{(aq)}$, reflux, 5 h, 71%; (b) 70% HNO_3, 94%; (c) $PhCH_2OH$, cat. *p*-TsOH, toluene, 66%; (d) (i) $(COCl)_2$/DMF-CH_2Cl_2, 18 h; (ii) (2*S*)-(+)-pyrrolidinemethanol, Et_3N, −50 °C to rt, 18 h, 98% or EDCI/DMAP/DMF, (2*S*)-(+)-pyrrolidinemethanol, 55%; (e) $SnCl_2{\cdot}2H_2O$/MeOH, reflux, 1 h, 94%; (f) $(Boc)_2O$, THF, reflux, 18 h, used without further purification; (g) pyridinium dichromate/CH_2Cl_2, 4 Å sieves, 1 h, 60% or DMSO, $(COCl)_2$, Et_3N, −40 °C, 66%; (h) 10% Pd/C/H_2, 16 psi/EtOH, 98%.



Scheme 2. (a) EDCI/DMAP/DMF, then pyrrolidine, piperidine, indoline or isoindoline; (b) 95% CF_3COOH, −10 °C.

We also report the use of this building block to synthesize four novel compounds **12–15** resulting from its conjugation to two cyclic amines (pyrrolidine and piperidine) and two benzo-fused heterocycles (indoline and isoindoline), all of which may be considered as substructures of the established DNA-binding CBI moiety (**2**, Fig. 1). These compounds have been screened in ovarian A2780 and CH1 cell lines and their cisplatin-resistant counterparts (A2780$^{cis\mathrm{R}}$ and CH1$^{cis\mathrm{R}}$, respectively), in SKOV-3 cells and also in the NCI 60-cell-line panel (data not shown). The isoindoline analogue **15** was sufficiently active to be progressed to the NCIs standard in vivo hollow fibre assay in which it achieved the significant total score of 40 (20 is the minimum total score to demonstrate antitumour activity according to NCI criteria).

The key core building block **11** was synthesized by the route shown in Scheme 1. Starting from commercially available vanillic acid (**3**), reaction with 3-bromopropanol gave the ether-alcohol **4**. Simultaneous nitration and oxidation with 90% nitric acid afforded the nitro di-acid **5** which, by chemoselective esterification with benzyl alcohol and *p*-toluenesulphonic acid, afforded the nitro monoester **6**. Careful addition of the acid chloride generated from **6** to a solution of (2*S*)-(+)-pyrrolidinemethanol and triethylamine in CH_2Cl_2 at −50 °C gave the coupled product **7** in excellent yield (98%). The coupling reaction was also effected but in lower yield (55%) using 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (EDCI) and 4-(dimethylamino)pyridine (DMAP).[14] Reduction of the coupled product **7** with tin(II) chloride in refluxing MeOH gave the amine **8** as a single product, which was reacted with di-*t*-butyl dicarbonate to give the Boc-protected amine **9**. As an alternative to the Swern oxidation of **9** to the benzyl ester-protected PBD (**10**) by the usual Fukuyama approach,[15] ring closure was achieved using pyridinium dichromate and 4 Å molecular sieves.[16] This allowed the oxidation to be carried out on a large scale (>60 g) with relative ease, although final yields tended to be poorer than for the Swern oxidation. After purification of **10** by flash chromatography, removal of the benzyl ester protecting group by palladium-catalyzed hydrogenolysis gave **11** in pure form.[17] Throughout this 8-step synthesis, each product was obtained in excellent yield and with only compound **10** requiring chromatographic purification.

Building block **11** was conjugated to each of the four amines using standard coupling methodology (EDCI/DMAP)[14] (Scheme 2). Pyrrolidine, piperidine and indoline were commercially available but isoindoline was prepared from phthalimide using a literature method.[18] Finally, the Boc protecting group was removed using

Table 1. In vitro cytotoxicity (in human ovarian cell lines) and induced thermal stabilization of DNA melting for the novel PBD–amine conjugates **12**–**15** and PBDs **1b**–**c**

Compd	Cytotoxicity (μM)[a]							Induced ΔT_m (°C)[c] after incubation at 37 °C for		
	SKOV-3	A2780	A2780^{cisR}	*RF*[b]	CH1	CH1^{cisR}	*RF*[b]	0 h	4 h	18 h
12	5.4	1.5	4.3	2.9	1.4	1.85	1.3	0.5	0.9	1.2
13	23.5	3.2	14.5	4.5	4.9	7.9	1.6	0.8	0.9	1.2
14	5.8	1.55	4.9	3.2	1.5	3.0	2	0.7	1.0	1.2
15	1.45	0.23	0.94	4	0.24	0.42	2	1.0	1.1	1.3
1b	1.70	0.064	0.155	2.4	0.082	0.11	1.3	0.2	0.4	0.6
1c	0.46	0.17	0.48	2.8	0.145	0.145	1.0	0.2	0.4	0.4

[a] Concentration of agent required to inhibit cell growth by 50% compared with PBD-free controls after incubation for 96 h at 37 °C.
[b] RF = resistance factor (IC_{50} cisplatin-resistant/parent) for A2780 or CH1 tumour cells.
[c] For a 5:1 molar ratio of duplex calf thymus (CT) DNA (100 μM in DNAp) and ligand (20 μM) in aqueous buffer (10 mM NaH_2PO_4/Na_2HPO_4 + 1 mM Na_2EDTA, pH 7.00 ± 0.01).[7,20,21] All values are ± 0.1 °C (typically ± < 0.06–0.08 °C) from at least 3 determinations.

95% trifluoroacetic acid to provide the target compounds **12**–**15**[19] in good yields. These molecules were evaluated for in vitro cytotoxicity in selected ovarian cell lines and in the NCI 60-cell-line panel (data not shown), and for DNA duplex binding affinity using a thermal denaturation assay with calf thymus (CT) DNA.[7,20,21] Results from these assays are shown in Table 1.

All new compounds were significantly cytotoxic in the ovarian cell lines, with IC_{50} values ranging from 0.23–23.5 μM (Table 1). Compound **15** had the consistently lowest IC_{50} values of < 1 μM, except in the SKOV-3 cell line (1.45 μM). Similarly, in the NCI screen, **15** had an average GI_{50} value of 2.14 μM across the 60-cell panel and was significantly more cytotoxic than compounds **12**–**14** (average GI_{50} values = 3.0, 72.4 and 19.2 μM, respectively). This biological activity is reflected in the thermal denaturation data (Table 1) which show that **15** develops 75% of its final helix-stabilizing effect to melting (i.e., $\Delta T_m = 1.3$ °C after 18 h) without prior DNA-drug incubation at 37 °C (i.e., $\Delta T_m = 1.0$ °C at $t = 0$). Compounds **12**–**14** effect a similar but slightly lower ΔT_m after 18 h incubation (i.e., 1.2 °C), although lower thermal stabilization is achieved at $t = 0$ (i.e., $\Delta T_m = 0.5$–0.8 °C). One interpretation of this result is that the isoindoline ring of **15** is connected to the PBD moiety such that the fused rings align to be coplanar with the A-ring of the PBD moiety. In this case the whole conjugate should fit into the minor groove of double-stranded DNA in a superior linear (and/or isohelical) fashion, thereby maximizing contact with the DNA bases and improving covalent adduct stability. Conversely, analogues **12** and **13** present fewer contacts to the minor groove due to their smaller pyrrolidine and piperidine rings. In the case of **14**, the linkage to the indoline unit should not allow a linear fit within the DNA minor groove. Interestingly, although **15** did not have an improved cytotoxicity profile compared to the known C8-OMe (**1b**) or C8–OBn (**1c**) DC-81 analogues, its relative induced ΔT_m values were significantly higher (by factors of ~5-fold and 3.4-fold at $t = 0$ and $t = 18$ h, respectively), supporting the notion that the isoindoline ring confers improved contacts with the minor groove floor and wall of the DNA host. Compound **15** also has significant activity in the NCI standard hollow fibre assay,[22,23] with intraperitoneal (ip), subcutaneous (sc) and total scores of 38, 2 and 40, respectively.

In summary, the novel PBD-building block **11** reported here can be synthesized in good yield on a large scale (> 60 g) and with maintenance of stereochemistry at the C11a position using simple procedures with minimum chromatography, and without recourse to air-sensitive reagents. The potential use of **11** for coupling to amine- or alcohol-functionalised substrates is illustrated by the synthesis of the four cyclic amine conjugates **12**–**15**. One of these (**15**) has significant in vitro cytotoxicity and DNA-binding affinity, and has promising antitumour activity as revealed by the hollow fibre assay data. The results of studies of **15** in human tumour xenograft experiments will be reported elsewhere.

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