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Effect of C2-*exo* Unsaturation on the Cytotoxicity and DNA-Binding Reactivity of Pyrrolo[2,1-*c*][1,4]benzodiazepines

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Abstract—A series of novel C2-*exo* unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) has been synthesised via a versatile *pro*-C2 ketone precursor. C2-*exo*-unsaturation enhances both DNA-binding reactivity and in vitro cytotoxic potency.

The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) antitumour antibiotics have generated interest as potential anticancer and gene-targeting agents.[1] All biologically active PBDs (e.g. DC-81 **1** and tomaymycin **2**) have an electrophilic imine or carbinolamine moiety at the N10-C11 position and (*S*)-stereochemistry at C11a which enables a snug fit in the minor groove of DNA followed by covalent bonding to guanine at N2. Bonding occurs in a sequence specific fashion with a preference for PuGPu motifs. Typical examples of PBD natural products such as DC-81 and tomaymycin possess the same aromatic substitution pattern, although tomaymycin possesses C2-*exo* unsaturation. As tomaymycin is significantly more potent than DC-81,[2–4] a series of analogues of tomaymycin has been synthesised to confirm the significance of C2 unsaturation.

Access to C2-*exo* unsaturated compounds was obtained via the versatile *pro*-C2 ketones, **15a**,**b** (Scheme 1). These provided the opportunity to prepare a series of diverse C-ring-unsaturated PBDs such as the four novel *exo* unsaturated structures reported here, as well as a series of C2-*endo* unsaturated relatives.[5]

The ketone intermediates **15a**,**b** were prepared from commercially available L-*trans*-4-hydroxyproline (**5**), which was *N*-protected as the benzyl carbamate **6** and then converted to the methyl ester **7** in quantitative yield. Reduction to the diol **8** using $LiBH_4$, and subsequent selective TBDMS protection furnished the silyl ether **9** in 70% yield. Cleavage of the *N*-Cbz group by catalytic hydrogenation afforded amine **10**, which was coupled to the 4-methoxy[7] (**11a**) or 4-benzyloxy[6] (**11b**) benzoyl chloride derivatives of **11** to yield amides **12a**,**b** in high yield. Reduction of the nitro groups with Raney nickel followed by Alloc protection of the resulting anilines **13a**,**b** gave the allyl carbamates **14a**,**b** in high yield. Swern oxidation of the C2-hydroxyl groups afforded the crucial C-ring ketones **15a**,**b** in excellent yield.

The *exo*-methylene compounds **16a**,**b** were prepared by Wittig olefination of **15a**,**b** at 0 °C, whereas reflux conditions were required for introduction of the *E*/*Z* ethylidene moiety in **16c**,**d** (Scheme 2). Treatment of the silyl ethers **16a–d** with TBAF in THF generated the primary alcohols **17a–d** in good yields. B-ring cyclisation was achieved by subjecting alcohols **17a–c** to Swern conditions[8] to give the N10-Alloc-protected carbinolamines **18a–c** in moderate yield. Oxidation with Dess–Martin periodinane effected cyclisation of **17d** to the PBD precursor **18d** in 83% yield. Finally, the Alloc protecting groups were removed upon treatment with catalytic

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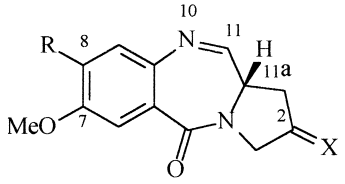
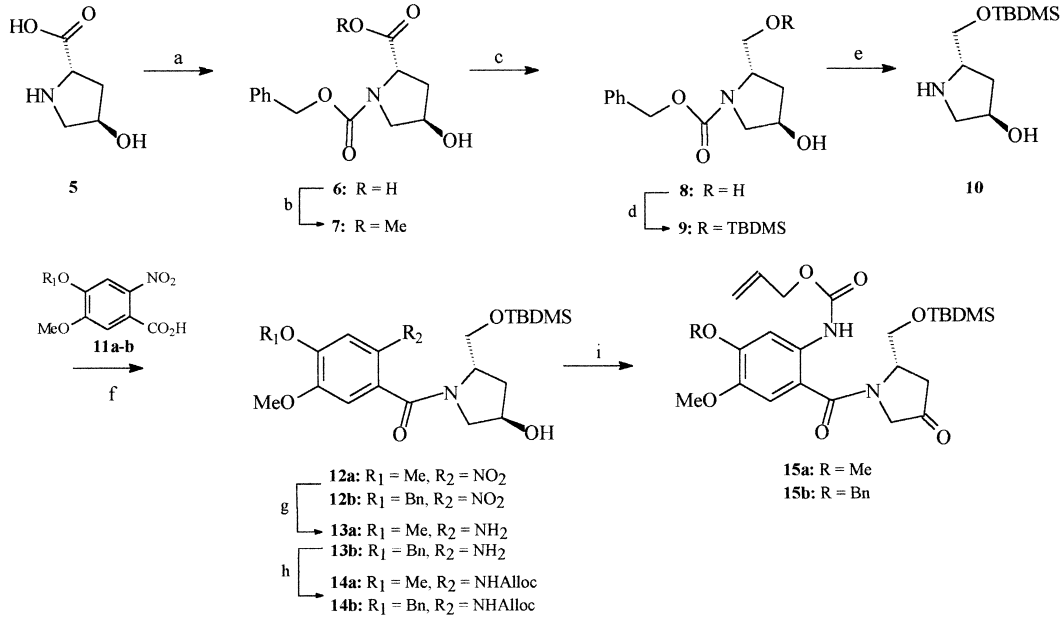


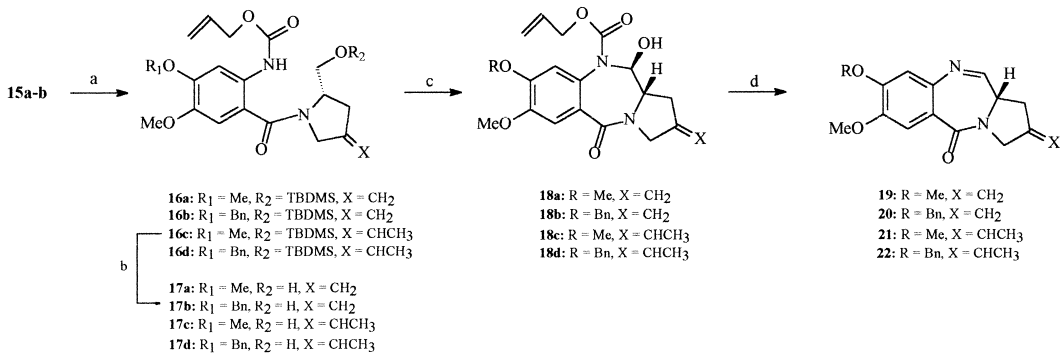
Figure 1.

Pd(0) in the presence of pyrrolidine as an allyl scavenger to give the target PBDs **19**–**22**[12] in quantitative yield. The C2-ethylidine compounds **21** and **22** were obtained as mixtures of *E*- and *Z*-isomers (e.g., 9:1 *Z*:*E* ratio[9] for **21**) which were not separated. This behaviour contrasts with the natural product tomaymycin and its Cδ-*O*-methyl-ated derivative, which exist exclusively in the *E*-form.[10]

The new PBD compounds were assessed for cytotoxic potency in three human ovarian carcinoma cell lines and two derived cisplatin-resistant counterparts (Table 1) using a published procedure.[11] Their DNA-binding reactivities were examined from the time course and extent of effects on double-stranded calf thymus DNA using a thermal denaturation (T_m) assay designed to probe drug-induced helical stabilisation.[3,4] Compared to the C2-unsubstituted C8-methyl DC-81 (**3**), the C2-methylene (**19**) and C2-ethylidene (**21**; examined as a mixture of geometric isomers) compounds significantly enhanced the stabilisation of DNA (ΔT_m values: ×6.0, ×4.5 and ×4.1 for **19**, and ×3.5, ×3.4 and ×3.3 for **21** at 0, 4 and 18 h, respectively). Although cytotoxicity did not change significantly in the A2780 and CH1 lines, potency was increased by approximately 4- and 19-fold for **19** and **21** in the SKOV-3 line. Similarly, compared to **4** (C8-benzyl-DC-81), **20** and **22** (as a mixture of isomers) significantly enhanced the stabilisation of DNA (ΔT_m values: ×5.0, ×3.0 and ×3.3 for **20**, and ×4.6, ×4.1 and ×5.8 for **22** at 0, 4 and 18 h, respectively). However, cytotoxic potency increased in both the A2780 (i.e., ×5.8



Scheme 1. (a) CBzCl, $NaHCO_3$, Et_2O, quant.; (b) MeOH, H_2SO_4, Δ, quant.; (c) $LiBH_4$, THF, 0 °C, 97%; (d) TBDMSCl, TEA, DBU, CH_2Cl_2, 70%; (e) 10% Pd–C, H_2, EtOH, quant.; (f) $(COCl)_2$, DMF, CH_2Cl_2, then **10**, TEA, CH_2Cl_2, 0 °C, 75% (**12a**), 61% (**12b**); (g) Raney Ni, H_2NNH_2, MeOH, Δ, 95% (**13a**), 92% (**13b**); (h) AllocCl, pyridine, CH_2Cl_2, 0 °C, 87% (**14a**), 65% (**14b**); (i) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −60 °C, 98% (**15a**), 95% (**15b**).



Scheme 2. (a) Ph_3PMeBr, KO^{t}Bu, THF, 0 °C, 86% (**16a**), 68% (**16b**); Ph_3PEtBr, KO^{t}Bu, THF, 0 °C → Δ, 64% (**16c**), 35% (**16d**); (b) TBAF, THF, 0 °C: 89% (**17a**), 88% (**17b**), 77% (**17c**), 72% (**17d**); (c) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −45 °C, 41% (**18a**), 66% (**18b**), 51% (**18c**); Dess–Martin periodinane, CH_2Cl_2, 0 °C, 83% (**18d**); (d) $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, quant. (**19**–**22**).

Table 1. In vitro cytotoxicity (human ovarian) and thermal denaturation data (calf thymus DNA) for the novel C2-*exo* unsaturated PBDs **19–22** and parent compounds **3** and **4**

	Cytotoxicity (μM)[a]							Induced ΔT_m (°C)[b,d] after incubation at 37 °C for:		
	SKOV-3	A2780	A2780$^{cis\mathrm{R}}$	*RF*[c]	CH1[d]	CH1$^{cis\mathrm{R}}$	*RF*[c]	0 h	4 h	18 h
19	0.39	0.15	0.36	2.4	0.066	0.084	1.3	1.46	1.95	2.38
20	0.35	0.029	0.20	6.9	0.017	0.082	4.8	1.01	1.22	1.33
21[e]	0.135	0.071	0.14	1.9	0.035	0.056	1.6	0.84	1.47	1.86
22[e]	0.081	0.034	0.066	1.9	0.031	0.031	1.0	0.92	1.65	2.34
3	1.70	0.064	0.155	2.4	0.082	0.11	1.3	0.24	0.43	0.57
4	0.46	0.17	0.48	2.8	0.145	0.145	1.0	0.20	0.40	0.40

[a]Dose required to inhibit cell growth by 50% compared with PBD-free controls after incubation for 96 h at 37 °C.
[b]For a 5:1 molar ratio of duplex-form CT-DNA (100 μM) and ligand (20 μM) in aqueous buffer (10 mM NaH_2PO_4/Na_2HPO_4 + 1 mM Na_2EDTA, pH 7.00±0.01). All values are ± < 0.08–0.13 °C.
[c]*RF* = resistance factor (IC_{50} cisplatin-resistant/parent).
[d]For comparison: IC_{50} value for tomaymycin (**2**) in CH1 is 0.00013 μM, and ΔT_m values are 0.97, 2.38 and 2.56 °C after incubation at 37 °C for 0, 4 and 18 h, respectively.
[e]Evaluated as a mixture of *E*/*Z* geometric isomers.

for **20**; ×3.4 for **22**) and SKOV-3 lines (×5.0 for **22**). Furthermore, **22** was 7.2-fold more cytotoxic in the cisplatin-resistant A2780$^{cis\mathrm{R}}$ line than **4**. It should be noted that tomaymycin, which differs in structure from **21** only in having a C8-OH rather than a C8-OCH_3 substituent, raises T_m significantly over the three time points and is 270-fold more cytotoxic in CH1. This establishes the importance of a free hydroxyl group at the C8-position in maximising both DNA stabilisation and cytotoxicity.

In conclusion, we have developed a versatile synthetic route to C2-*exo* unsaturated PBD analogues which is illustrated by the syntheses of **19–22**. The versatile ketone intermediates **15a**,**b** can be efficiently synthesised on a large scale (i.e., >20 g), and should be applicable to the synthesis of a wide range of C2-*exo* and C2-*endo*[5] derivatives. Cytotoxicity and thermal denaturation data for **19–22** confirm that C2-unsaturation enhances both cytotoxic potency and DNA-binding affinity.

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12. Data for **19** $[\alpha]_D^{22} = +583.14°$ ($c = 1.42$, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.69 (d, 1H, $J = 4.39$ Hz), 7.51 (s, 1H), 6.82 (s, 1H), 5.21–5.17 (m, 2H), 4.44–4.23 (s, 2H), 3.96–3.81 (m, 7H), 3.17–3.08 (m, 1H), 2.95 (d, 1H, $J = 14.29$ Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 164.7, 162.6, 151.5, 147.6, 141.6, 140.8, 119.8, 111.2, 109.4, 109.4, 56.2, 56.1, 53.8, 51.4, 35.5; MS (EI), *m*/*z* (relative intensity) 272 ($[M]^{\cdot+}$, 100), 257 (19), 243 (7), 230 (6), 212 (3), 191 (16), 164 (19), 136 (22), 93 (6), 82 (7), 80 (3), 53 (3); IR (neat) 3312 (br), 3083, 2936, 2843, 1624, 1603, 1505, 1434, 1380, 1264, 1217, 1180, 1130, 1096, 1069, 1007, 895, 837, 666, 594, 542 cm^{-1}; exact mass calcd for $C_{15}H_{16}N_2O_3$ *m*/*z* 272.1161, obsd *m*/*z* 272.1154.