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Effect of C2/C3-*endo* 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/C3-*endo* unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) has been synthesised via cleavage of the N10-Alloc protecting group from appropriate precursors. Biophysical and biological evaluations show that the presence of C2/C3-*endo* unsaturation in the PBD C-ring enhances both DNA-binding reactivity and in vitro cytotoxic potency. © 2000 Elsevier Science Ltd. All rights reserved.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a class of antitumour antibiotics known to interact covalently in the minor groove of DNA in a sequence-selective manner.[1] Covalent bonding occurs between the C11 position of the PBD and the exocyclic N2 group of guanine, giving rise to a preference for purine–guanine–purine sequences.[1] Of the two best known natural products, anthramycin (**1**) and tomaymycin (**2**) (Fig. 1), the former binds to DNA more efficiently[2], a characteristic thought to be associated with the C2/C3-*endo* unsaturation of anthramycin.

Although structure–activity relationship (SAR) information regarding both A-ring substitution[3] and C-ring C2-*exo* unsaturation[2,4] is available, little is known about the effect of varying C2-substituents in C-ring C2/C3-*endo* unsaturated PBDs. Here we report the synthesis and biophysical and biological evaluation of five novel C2/C3-*endo* unsaturated PBDs with unique C2-substituents not found in PBD natural products isolated to date.

The C2/C3 unsaturation under investigation was introduced by Horner–Emmons olefination of ketones **4a**,**b** (Scheme 1).[4] In the presence of excess ylid (2.2 equiv), spontaneous migration of the initially formed *exo* double bond into the ring occurred to give the C2/C3-*endo* unsaturated compounds **5a**–**c** in high yields. A similar bond migration process in related molecules was observed by both Kaneko and Leimgruber.[5,6] TBAF was found to be incompatible with amides **5a**–**c**, so the TBDMS protecting groups were instead removed by treatment with AcOH/THF/H_2O to provide alcohols **6a**–**c** in high yields.[7] B-ring closure was accomplished by oxidation under Swern conditions[8] to afford **7a**–**c** in good yield. Further elaboration of the C-ring involved $LiBH_4$ reduction of ester **5b** to provide the homoallylic alcohol **8** (55% yield), followed by acetylation under standard conditions to give the acetate **9** in 58% yield (Scheme 2).

Selective cleavage of the TBDMS ether proceeded under mild conditions to furnish the primary alcohol **10** in 81% yield. Spontaneous oxidation/cyclisation under Swern conditions gave the Alloc-protected carbinolamine **11** (82%) which was hydrolysed in aqueous K_2CO_3 to afford the PBD precursor **12** in 78% yield. The *N*-protected carbinolamines (**7a–c**, **11**, **12**) were converted to the novel PBD derivatives **13**–**17**[9,10] in good yields using the method of Deziel[11] (Scheme 3).

The data presented in Table 1 show that the five C2/C3-*endo* unsaturated PBDs **13–17** exhibit sub-micromolar IC_{50} values in a panel of human ovarian tumour cell

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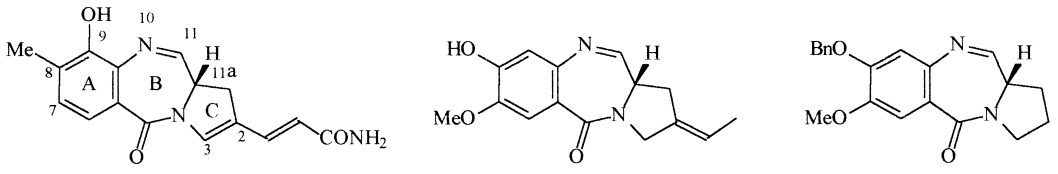


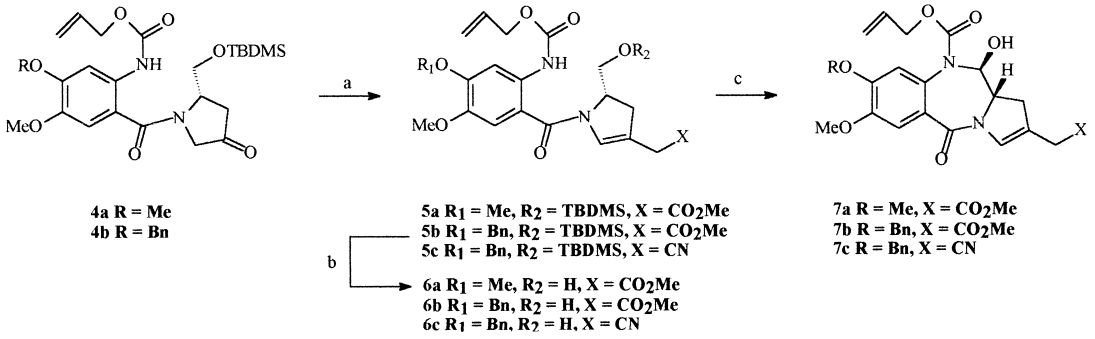
1 **2** **3**

Figure 1.

Table 1. In vitro cytotoxicity (human ovarian) and thermal denaturation data (calf thymus DNA) for the novel C2-*endo* unsaturated PBDs **13-17** and parent compounds **1** and **3**

Compound no.	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
13	0.45	0.36	0.46	1.3	0.115	0.15	1.3	3.41	4.63	5.94[d]
14	0.12	0.0145	0.12	8.3	0.016	0.04	2.5	3.08	3.96	4.70[d]
15	0.52	0.155	0.43	2.8	0.105	0.27	2.6	1.84	2.31	3.48[d]
16	0.46	0.10	0.27	2.7	0.105	0.16	1.5	1.57	2.11	2.53
17	0.105	0.07	0.105	1.5	0.09	0.037	0.4	3.98	4.61	5.60
1	0.16	0.155	0.160	1.03	0.062	0.05	0.81	9.40	11.20	13.0
3	0.46	0.17	0.48	2.8	0.145	0.145	1.0	0.20	0.40	0.40

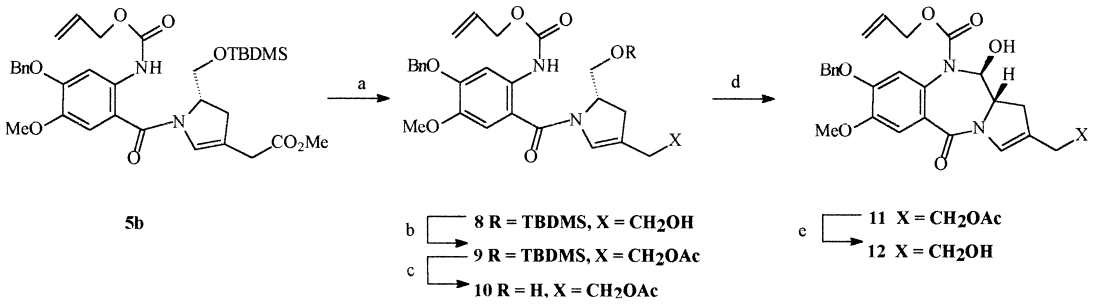
[a]Dose of PBD required to inhibit cell growth by 50% compared with PBD-free controls after incubation for 96 h at 37 °C.
[b]*RF* = resistance factor (IC_{50} cisplatin-resistant/parent).
[c]For a 5:1 molar ratio of duplex CT-DNA (100 μM) and ligand (20 μM) in aqueous buffer (10 mM NaH_2PO_4/Na_2HPO_4 + 1 mM Na_2EDTA, pH 7.00±0.01). All values are ± < 0.09–0.13 °C.
[d]ΔT_m values of 6.65, 4.97 and 4.31 °C (all ± < 0.19 °C) were determined for PBDs **13**, **14** and **15**, respectively, after incubation for 36 h.



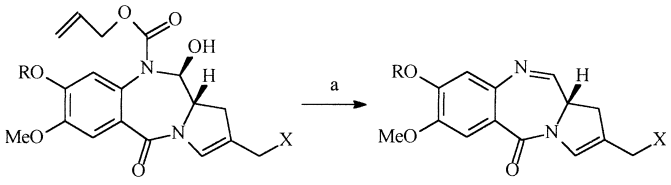
Scheme 1. (a) $(C_2H_5O)_2P(O)CH_2CO_2CH_3$, NaH, THF, 0°C, 73% for **5a**, 80% for **5b**; $(C_2H_5O)_2P(O)CH_2CN$, NaH, THF, 0°C, 63% for **5c**; (b) AcOH:THF:H_2O (3:1:1), 98% for **6a**, 90% for **6b**, quant for **6c**; (c) $(COCl)_2$, DMSO, TEA, CH_2Cl_2,−45°C, 54% for **7a**, 66% for **7b**, 68% for **7c**.

lines.[12] In this particular assay, the homoallylic alcohol PBD **17** and the methyl ester **14** are the most potent compounds. Unusually, **17** had a resistance factor of 0.4 in the CH1/CH1^{cisR} pair, indicating that this compound has more activity in the CH1^{cisR} cell line than the parent line suggesting that it may have potential in the treatment of cisplatin-resistant disease. The compounds were also evaluated for their ability to raise the melting temperature (T_m) of calf thymus (CT) DNA.[2] The data illustrate that the C2/C3-*endo* unsaturated PBDs have a greater ability to stabilise CT-DNA than compounds with fully saturated C-rings or C2-*exo*-unsaturation.[3,4] There is a broad correlation between DNA stabilisation and cytotoxicity, with **14** and **17** having two of the highest ΔT_m values. However, **13** gave the highest ΔT_m of all the synthetic compounds which may reflect the presence of the smaller C8-methoxy substituent. Interestingly, the novel PBDs are of similar cytotoxic potency to the natural product anthramycin (**1**), although the latter elevates the DNA melting temperature by a far greater extent. These results suggest that the relationship between T_m and cytotoxicity may not be as straightforward as previously thought.[1–3]

In summary, the ketone intermediates **4a**,**b** are ideal substrates for the introduction of C2/C3-*endo* unsaturation into the C-ring of PBDs. Compared to C8-*O*-benzyl DC-81 which has a saturated and unsubstituted C-ring (**3**), the enhanced DNA binding affinity and submicromolar cytotoxicity observed for **13–17** illustrates the significant effect that C-ring unsaturation has on both biological activity and biophysical properties.



Scheme 2. (a) $LiBH_4$, THF, 0°C, 55%; (b) $(Ac)_2O$, pyridine, 25°C, 58%; (c) AcOH:THF:H_2O (3:1:1), 81%; (d) $(COCl)_2$, DMSO, TEA, CH_2Cl_2,−45°C, 82%; (e) K_2CO_3, MeOH, CH_2Cl_2, 78%.



7a R = Me, X = CO_2Me
7b R = Bn, X = CO_2Me
7c R = Bn, X = CN
11 R = Bn, X = CH_2OAc
12 R = Bn, X = CH_2OH

13 R = Me, X = CO_2Me
14 R = Bn, X = CO_2Me
15 R = Bn, X = CN
16 R = Bn, X = CH_2OAc
17 R = Bn, X = CH_2OH

Scheme 3. (a) $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, quant (**13**–**17**).

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