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# Synthesis of the First Example of a C2-C3/C2′-C3′-*endo* Unsaturated Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimer

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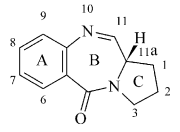
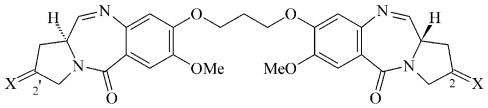
**Abstract**—We report the first example of a C2-C3/C2′-C3′-*endo* unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) dimer **16** synthesised through a new and efficient route, thus establishing that C2-C3-*endo* unsaturation enhances both cytotoxicity and DNA-binding affinity in A-Ring-linked PBD dimers but to a lesser extent than C2/C2′-*exo*-unsaturation. This new route has allowed the preparation of multi-gram quantities of the related clinical candidate **1** and should lead to more structurally diverse PBD dimer analogues. 

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a class of antitumour antibiotics known to interact covalently with the N2 of guanine residues within the minor groove of DNA via an electrophilic N10-C11 imine moiety.[1] Synthetic derivatives possessing the PBD ring system (Fig. 1) are generating interest[1,2,3] as potential anticancer and gene targeting agents due to their sequence specificity when binding to duplex DNA. As part of an ongoing program of research on these agents, we are focusing on extending base pair coverage and sequence specificity in order to enhance in vivo antitumour activity. One approach has involved joining two PBD units together through a C8/C8′ diether linkage to produce PBD dimers such as DSB-120 (**2**)[4,6] and SJG-136 (**1**).[5] These compounds exhibit greater biological activity than their parent monomers due to their ability to irreversibly interstrand cross-link DNA.[6,7] NMR and molecular modelling studies have demonstrated that PBD dimers of this type span six DNA base pairs and recognise embedded GATC sequences.[8,9] Significantly, in recent experiments performed by the National Cancer Institute (NCI, USA), the C2/C2′ *exo*-methylidene analogue **1** has displayed substantial in vivo antitumour activity in hollow fibre and human xenograft studies.[10] As a result, dimer **1** has been selected for clinical evaluation.

Recently, we reported the synthesis of C2-*exo*/*endo* unsaturated PBD monomers using an efficient and versatile route.[11,12] We now report the application of this linear methodology to the synthesis of the novel C2-C3/C2′-C3′-*endo* unsaturated PBD dimer **16**. In addition, this new route can be used for the synthesis of multi-gram quantities of PBD dimers, and this is illustrated by the production of gram quantities of **1**.




PBD Ring system and numbering




**1** X = $CH_2$
**2** X = H,H

**Figure 1.**

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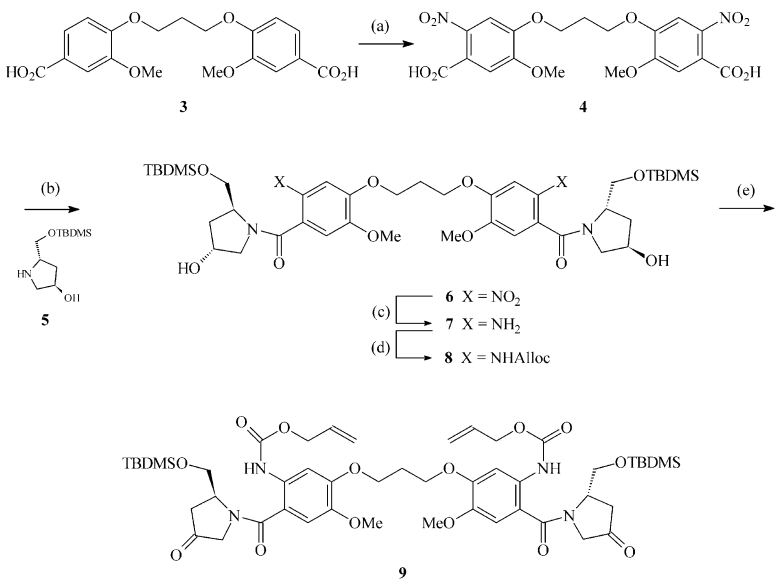


**Scheme 1.** (a) 70% $HNO_3$, 10 °C, 12 h, 70%; (b) $(COCl)_2$, DMF, THF, 16 h, then **5**, TEA, $H_2O$, 0 °C, 16 h, 62%; (c) Raney Ni, $H_2NNH_2$, MeOH, Δ, 1 h, 93%; (d) Alloc-Cl, pyridine, $CH_2Cl_2$, 0 °C, 2.5 h, 84%; (e) $(COCl)_2$, DMSO, TEA, $CH_2Cl_2$, −60 °C, 59%.

The known bis-acid **3**[4] was nitrated in high yield using 70% nitric acid to furnish the bis-nitro compound **4** with the required regiochemistry (Scheme 1). This method for introduction of the nitro groups is advantageous compared to the original $SnCl_4/f$ $HNO_3$ procedure of Thurston[4] because it avoids the preliminary esterification step and is more amenable to large-scale synthesis. The pyrrolidine **5**[11] was coupled to the acid chloride of **4** to provide the bis-amide **6** in moderate yield. The nitro groups were subsequently reduced in excellent yield with Raney nickel in refluxing MeOH, and the resulting bis-aniline **7** treated with allyl chloroformate in the presence of pyridine to give the bis-Alloc protected compound **8**. Swern oxidation of **8** provided the *pro*-C2/C2′-ketone **9**. Significantly, multigram quantities of this versatile key intermediate can be produced using this route.

The bis-ketone **9** was then subjected to the Horner–Emmons reaction which proceeded smoothly to give exclusively the bis-*endo*-unsaturated ester **10** in 79% yield (Scheme 2).[13,14] By contrast, the Wittig reaction on **9** gave the desired bis-*exo*-olefin **11** in moderate 51% yield. Near-quantitative cleavage of the TBDMS-ethers was achieved upon acid hydrolysis of **10** to give the bis-alcohol **12**. Similarly, bis-alcohol **13** was obtained in high yield by treating **11** with HF/pyridine.

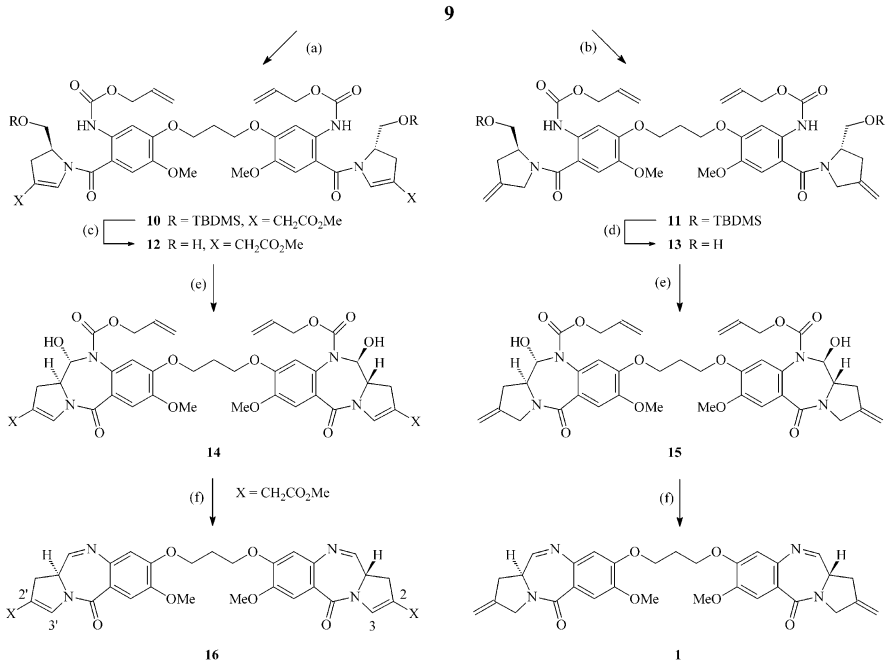
The penultimate step in the synthesis was formation of the B-ring via oxidation of the bis-alcohol to the corresponding bis-aldehyde with resultant spontaneous cyclisation.[15] The previously reported first-generation synthesis of **1** utilised TPAP to effect this transformation but in low yield.[5,7] The route reported here to precursor **13** produced significantly more material thus allowing a thorough investigation of Swern oxidation conditions (i.e., different dilutions and stoichiometries) for this substrate. As a result, we were able to optimise the yield of **15** without undesired concurrent formation of the tetralactam.[5,16] Similarly, bis-Alloc protected carbinolamine **14** was obtained in 78% yield following Swern oxidation of **12**. Treatment of the Alloc protected PBD precursors **14** and **15** with $Pd(PPh_3)_4$ in the presence of pyrrolidine furnished the novel C2-C3/C2′-C3′-*endo* unsaturated PBD dimer **16**[17] and **1** in good yield.

PBD dimer **16** was evaluated for in vitro cytotoxicity[18] and DNA binding affinity using a thermal denaturation assay,[19] and compared to the known PBD dimers **1** and **2**. The data presented in Table 1 indicate that **16** is more potent than the fully C-ring saturated dimer **2** in each of the cell lines examined, but significantly less potent (i.e., by two or three orders of magnitude) than the C2-*exo* unsaturated analogue **1**. This trend is also reflected in the thermal denaturation data consistent with the known mechanism of action of this family of agents.

**Table 1.** In vitro cytotoxicity and thermal denaturation data for **1, 2** and the novel PBD dimer **16**

| Compd | $IC_{50}$ (μM)[a] | | | $\Delta T_m$ (°C)[d,e] |
|---|---|---|---|---|
| | A2780[b,e] | A2780 *cis*R[b,e] | *RF*[c,e] | |
| **2** | 0.0072 (0.064) | 0.21 (0.155) | 29.2 (2.4) | 15.0 (0.57) |
| **16** | 0.0054 (0.36) | 0.058 (0.46) | 10.7 (1.3) | 21.7 (5.94) |
| **1** | 0.0000225 (0.15) | 0.000024 (0.36) | 1.0 (2.4) | 33.6 (2.38) |

[a]Dose of PBD required to inhibit cell growth by 50% compared with PBD-free controls as measured by the sulforhodamine B (SRB) growth delay assay. The cells were incubated with the compounds for 96 h at 37 °C.
[b]Human ovarian carcinoma cell lines: A2780 and cisplatin resistant counterpart A2780*cis*R.
[c]*RF* is the resistance factor ($IC_{50}$ resistant/parent).
[d]Increase in calf thymus DNA (CT-DNA) melting temperature after 18 h incubation with the PBD dimer. For CT-DNA at pH 7.00 ± 0.01, $\Delta T_m = 67.83 \pm 0.06$ °C (mean value from 50 separate determinations). All $\Delta T_m$ values ± 0.1–0.3 °C. For a 1:5 molar ratio of [ligand]/[DNA], ligand concentration = 20 μM and calf thymus DNA concentration = 100 μM in aqueous sodium phosphate buffer (10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01).
[e]Data for corresponding C8-methoxy substituted monomers in parentheses.[11,12]




**Scheme 2.** (a) $(EtO)_2P(O)CH_2CO_2Me$, NaH, THF, 0 °C, 16 h, 79%; (b) $Ph_3PCH_3Br$, KO$^t$Bu, THF, 0 °C, 2.5 h, 51%; (c) AcOH/THF/$H_2O$ (3:1:1), 16 h, 94%; (d) HF–pyridine complex, THF, 0 °C, 1.5 h, 99%; (e) $(COCl)_2$, DMSO, TEA, $CH_2Cl_2$, −45 °C, 78% for **14**, 77% for **15**; (f) $Pd(PPh_3)_4$, $PPh_3$, pyrrolidine, $CH_2Cl_2$, 1.5 h, 66% for **16**, 77% for **1**.

Interestingly, no similar trends are obvious for the structurally analogous series of 7,8-dimethoxy-PBD monomers (results given in parentheses in Table 1; structures not shown), presumably reflecting the different mechanism of action of the two series of compounds (i.e., monoalkylation for PBD monomers versus cross-linking for PBD dimers).

In summary, we have developed a versatile and efficient synthetic route to C-ring unsaturated PBD dimers of types **1** and **16**. This has enabled the preparation of gram quantities of the clinical candidate **1** and should allow the synthesis of more structurally diverse PBD dimer analogues. Furthermore, it has been established that C2-*exo*-unsaturation enhances both cytotoxicity and DNA-binding affinity to a greater degree than C2-C3-*endo*-unsaturation in A-ring-linked PBD dimers.

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16. Experimental procedure for Scheme 2 step (e): A solution of DMSO (0.55 mL, 7.75 mmol) in dry $CH_2Cl_2$ (10 mL) was added drop-wise over a 15 min period to a stirred solution of oxalyl chloride (0.32 mL, 3.67 mmol) in $CH_2Cl_2$ (10 mL) at −45 °C under a nitrogen atmosphere. The reaction mixture was allowed to stir for 35 min at −45 °C followed by addition of the bis-alcohol **13** (1.01 g, 1.32 mmol) in $CH_2Cl_2$ (10 mL) at the same temperature over 15 min. After a further 45 min a solution of triethylamine (1.50 mL, 10.76 mmol) in $CH_2Cl_2$ (10 mL) was added over a period of 15 min. The reaction mixture

was allowed to stir at −45 °C for 30 min before being allowed to warm to room temperature over 45 min. The reaction mixture was then diluted with water and the phases allowed to separate. The organic phase was washed with 1 M HCl (3×50 mL), brine (50 mL) and dried over $MgSO_4$. Evaporation of solvent in vacuo yielded a crude product which was purified by flash column chromatography (1.5% MeOH, 98.5% $CHCl_3$) to afford **15** as a white solid (0.785 g, 77%).

17. Selected data for **16**: $[\alpha]_D^{20} = +500°$ (*c* 0.043, $CHCl_3$); $^1H$ NMR (270 MHz, $CDCl_3$) (imine form): δ 7.83 (d, 2H, $J = 4.4$ Hz), 7.47 (s, 2H), 7.06–6.92 (m, 2H), 6.84 (s, 2H), 4.30–4.26 (m, 6H), 3.91 (s, 6H), 3.73 (s, 6H), 3.32–3.15 (m, 8H), 2.42–2.17 (m, 2H); $^{13}C$ NMR (67.8 MHz, $CDCl_3$) (imine form): δ 170.7, 162.6, 161.4, 151.0, 148.0, 140.2, 126.5, 111.8, 110.9, 65.4, 56.2, 53.8, 52.2, 37.4, 33.6, 28.7; FAB MS *m*/*z* (relative intensity): 673 ($[M+H]^{+\cdot}$, 2), 185 (55), 93 (100); IR (neat): 3583, 3394, 2997, 2950, 1736, 1717, 1628, 1596, 1511, 1483, 1451, 1431, 1382, 1273, 1245, 1197, 1152, 1068, 995, 963, 914, 842, 753 $cm^{-1}$.

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