Synthesis of a novel C2/C2′-*exo* unsaturated pyrrolobenzodiazepine cross-linking agent with remarkable DNA binding affinity and cytotoxicity

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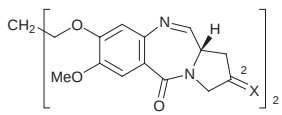
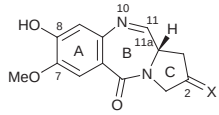
A C2/C2′-*exo* unsaturated pyrrolobenzodiazepine dimer 1 has been synthesised which is cytotoxic at the picomolar level and has remarkable covalent DNA binding affinity, raising the melting temperature of duplex-form calf thymus DNA by 34 °C after 18 h incubation.

There is presently interest in low molecular weight ligands that can interact with nucleic acids in a sequence-selective manner. Such agents have potential use in the validation of DNA sequences as potential therapeutic targets, in the therapy of genetic-based diseases (*e.g.* cancer[1,2]), and in the development of diagnostic agents. The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of antitumour antibiotics derived from various *Streptomyces* species that exert their biological activity by interacting with DNA in a sequence-selective fashion, forming a covalent bond between their electrophilic C11-position and the exocyclic C2-NH_2 group of a guanine base in the minor groove of DNA.[3] Recently, it has been demonstrated that PBDs can inhibit both endonuclease activity[4] and *in vitro* transcription[5] in a highly sequence-selective manner.

Although the parent PBDs span approximately three base pairs with a preference for purine–guanine–purine (*e.g.* AGA) sequences, a series of C-ring-unsubstituted C8-diyldioxy ether-linked PBD dimers have been synthesised (*e.g.* DSB-120 **4**) that



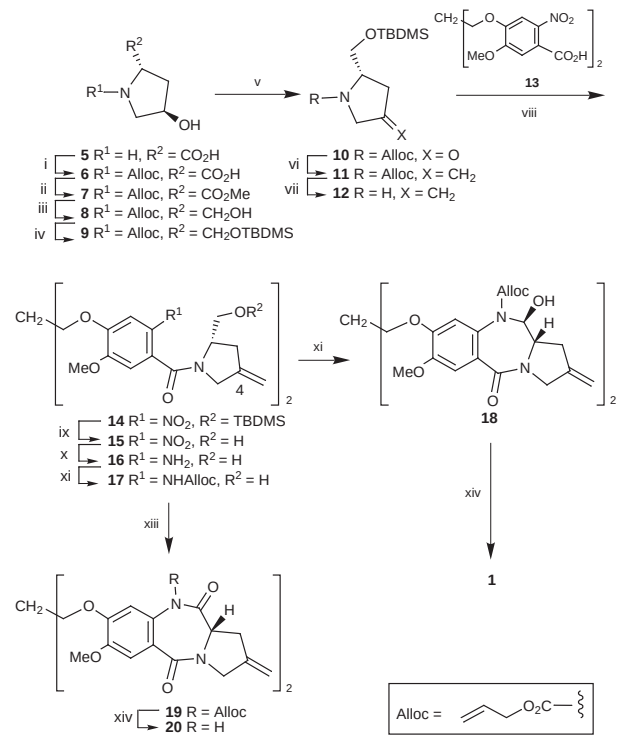
1 X = CH_2
4 X = H,H



2 X = H, H
3 X = CHMe

span approximately six base pairs of DNA and have enhanced sequence selectivity (*e.g.* purine-GATC-pyrimidine for DSB-120).[6,7] The sub-micromolar cytotoxicity of DSB-120 has been attributed to its ability to irreversibly cross-link DNA *via* guanine residues on opposite strands.[8] In an attempt to further extend base-pair span and recognition behaviour, we have investigated the inclusion of C2/C2′ substituents that should follow the contour of the host minor groove. Here, we report a novel synthesis of SJG-136 **1**, a C2/C2′*exo*-methylene analogue of DSB-120. This molecule has exquisite cytotoxicity in the picomolar region (*i.e.* IC_{50} = 0.000024 μM) in the cisplatin-resistant A2780cis human ovarian carcinoma cell line, some 9000-fold more potent than DSB-120 (IC_{50} = 0.21 μM). Furthermore, SJG-136 raises the melting temperature of calf thymus (CT) DNA by a record value of 33.6 °C after 18 h incubation at a [PBD] : [DNA] ratio of 1 : 5.

Synthesis of the target molecule was initially approached using the thioacetal method of Thurston and co-workers.[9,10] However, this had to be abandoned due to the unwanted addition of EtSH across the C4-*exo*-methylene of intermediates of type **11** during attempted thioacetal formation. Instead, synthesis of **1** was achieved by employing the B-ring cyclisation strategy first reported by Fukuyama and co-workers[11] (Scheme 1). Commercially available *trans*-4-hydroxy-L-proline **5** was initially *N*-protected as carbamate **6** in 87% yield.[12] Following esterification in disappointing yield (43%) using catalytic H_2SO_4 in refluxing MeOH, the resulting ester **7** was reduced with $LiBH_4$ to give diol **8** in quantitative yield. Selective silylation of the primary alcohol (**8** → **9**) was achieved using DBU as a silyl transfer agent. Disilylated product and unreacted diol were removed by column chromatography to provide the TBDMS ether **9** in 52% yield. Oxidation to the ketone **10** was achieved using either the Swern reaction or tetrapropylammonium perruthenate (TPAP) in the presence of NMO and 4 Å molecular sieves, both methods producing **10** in almost quantitative yield. The key C4 (*pro*-C2/C2′) unsaturation was introduced by performing a Wittig reaction on **10** to afford the olefin **11** in 87% yield. Initial attempts to deprotect **11** using



Scheme 1 *Reagents and conditions*: i, Alloc-Cl, aq. NaOH, THF, 0 °C, 87%; ii, MeOH, H_2SO_4, Δ, 43%; iii, $LiBH_4$, THF, 0 °C, 99%; iv, TBDMS-Cl, Et_3N, DBU, CH_2Cl_2, 52%; v, TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, MeCN, 92% or $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −70 °C, 95%; vi, Ph_3PCH_3Br, $KOBu^t$, THF, 0 °C, 87%; vii, Bu_3SnH, $Pd(PPh_3)_2Cl_2$, H_2O, CH_2Cl_2, 77%; viii, $(COCl)_2$, DMF, THF, then **12**, Et_3N, H_2O, 0 °C, 74%; ix, TBAF, THF, 0 °C, 94%; x, $SnCl_2.2H_2O$, MeOH, Δ, 61%; xi, Alloc-Cl, pyridine, CH_2Cl_2, 0 °C, 50%; xii, TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, MeCN, 32%; xiii, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −45 °C, 51%; xiv, $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, MeCN, 0 °C, 77% for **1** and 43% for **20**.

$PPh_3/Pd(PPh_3)_4$ in the presence of a suitable allyl scavenger (*e.g.* pyrrolidine, dimedone, 2-ethylhexanoic acid)[13,14] were unsuccessful. Eventually, the Alloc group was cleaved by palladium-catalysed hydrostannolysis[15] with Bu_3SnH to provide the amine **12** in 77% yield.

The known PBD dimer core **13**[6,7] was converted to the corresponding acid chloride, and coupled to **12** to furnish the bis(nitro amide) **14** in 74% yield. The TBDMS protecting groups were removed rapidly and selectively under mild conditions using TBAF in THF to produce the bis(nitro alcohol) **15** in 94% yield. Reduction of the nitro groups while retaining the C4/C4′ unsaturation intact was achieved in 61% yield by employing $SnCl_2 \cdot 2H_2O$ in refluxing MeOH. The resulting *bis*-aniline **16** was Alloc-protected at the *pro*-N10/N10′ positions (**17**), before subjecting it to Swern conditions in order to bring about oxidative cyclisation to give the bis-N10-protected product **18**. Unfortunately, **18** was prone to over-oxidation and only the tetralactam **19** was obtained under these conditions. However, oxidation with TPAP, NMO and 4 Å molecular sieves afforded the required **18** in 32% yield with no contaminating tetralactam. Deprotection of **18** with $Pd(PPh_3)_4$, PPh_3 and pyrrolidine[13] afforded the novel PBD dimer **1** in 77% yield. Treatment of **19** under identical conditions afforded **20**, the first example of a PBD dimer tetralactam, in 43% yield.

The C2/C2′-methylene groups of **1** were clearly visible in the ^{1}H NMR (broad singlets at δ 5.17 and 5.20) and ^{13}C NMR (δ 109.4) spectra.† Similarly, the diagnostic N10-C11/N10′-C11′ imine signals could be observed at δ 7.68 (d, J 4.4 Hz) and δ 162.6, respectively. FAB MS gave parent ions at 665 and 773, corresponding to single and double thioglycerol addition adducts, respectively. In addition, the observed $[\alpha]_D^{21}$ value of +357.7 (*c* 0.07, $CHCl_3$) compared favourably with that for DSB-120[7] ($[\alpha]_D^{23}$ +330 (*c* = 0.6, $CHCl_3$)], confirming that the C11a/C11a′ stereochemistry crucial for DNA interaction had been maintained throughout the synthesis.

The data presented in Table 1 show that SJG-136 **1** is the most potent DNA-stabilising agent known to date according to this particular assay.[16] For a 1 : 5 molar ratio of [PBD] : [DNA], the PBD dimer elevates the helix melting temperature of CT DNA by an unprecedented 33.6 °C after incubation for 18 h at 37 °C. Under identical conditions, the C-ring-unsubstituted dimer DSB-120 **4** provides a ΔT_m of 15.1 °C, demonstrating the extraordinary effect of introducing C2/C2′-unsaturation. In common with other PBD dimers, **1** exerts most of its effect upon the GC-rich or high temperature regions of the DNA melting curves. In a similar fashion to DSB-120, it provides some 60–80% of its stabilising effect without prior incubation, suggesting a kinetic effect in the reactivity profile. However, the comparative ΔT_m curves show that, on a concentration basis alone, SJG-136 is ≥10-fold more effective than DSB-120. Even at a [PBD] : [DNA] molar ratio of 1 : 100, SJG-136 effects significantly better DNA binding affinity than the monomer tomaymycin **3** at a 1 : 5 molar ratio (see Table 1).

Table 1 Thermal denaturation with calf thymus DNA[a] at a [PBD] : [DNA] molar ratio of 1 : 5[b] and *in vitro*[c] cytotoxicity data in the A2780 and A2780cisR cell lines for SJG-136 **1** and DSB-120 **4**

	Induced ΔT_m/°C[a,b,d] after incubation at 37 °C for			IC_{50}/μM[c]		
Compound	0 h	4 h	18 h	A2780	A2780cisR	RF[e]
SJG-136 **1**	25.7	31.9	33.6	0.0000225	0.000024	1.1
DSB-120 **4**	10.2	13.1	15.1	0.0072	0.21	29.2
Cisplatin	—	—	—	0.265	8.4	32

[a] For CT-DNA at pH 7.00 ± 0.01, T_m = 67.83 ± 0.06 °C (mean value from 30 separate determinations). All ΔT_m values ± 0.1–0.2 °C. [b] For a 1 : 5 molar ratio of [ligand] : [DNA], where CT DNA concentration = 100 μM in aqueous buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01]. [c] Dose of PBD required to inhibit cell growth by 50% compared with PBD-free controls. The cells were incubated with the compounds for 96 h at 37 °C. [d] For comparative purposes: ΔT_m of tomaymycin **3** = 0.97, 2.38 and 2.56 °C at 0, 4 and 18 h, respectively. [e] RF is the resistance factor (IC_{50} resistant/parent).

Representative cytotoxicity data for SJG-136 in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant subline A2780cisR are shown in Table 1, together with data for DSB-120 and cisplatin for comparison. Relative to the parental line, the A2780cisR subline is known to have elevated GSH levels, an increased level of repair of DNA–cisplatin adducts, and a decreased ability to uptake cisplatin.[17] The IC_{50} value for **1** in the A2780 cell line is only 23 pM, representing a 320-fold increase in cytotoxicity compared to DSB-120 (IC_{50} = 7.2 nM). Interestingly, whereas DSB-120 has a reduced potency towards A2780cisR (IC_{50} = 0.21 μM), SJG-136 is almost 9000-fold more potent in this cell line with a similar IC_{50} value (24 μM) to that in the parent cells, giving a Resistance Factor of 1.1. The fact that DSB-120 and cisplatin give RF values of 29.2 and 32, respectively, for this pair of cell lines suggests that SJG-136 may have potential in cisplatin-refractory disease.

In summary, the synthesis of SJG-136 **1** reported here demonstrates the importance of C2/C2′-*exo*-unsaturation in enhancing the DNA-binding affinity and cytotoxicity of the PBD dimers, and in overcoming cisplatin resistance. The sequence selectivity and cross-linking ability of **1** will be reported elsewhere.

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Notes and references

† *Selected data* for **1**: δ_H(270 MHz, $CDCl_3$) 7.68 (d, 2H, *J* 4.4, H11/H11′), 7.49 (s, 2H, H6/H6′), 6.85 (s, 2H, H9/H9′), 5.20 and 5.17 (2 × br s, 4H, H12/H12′), 4.46–4.19 (m, 8H, H3/H3′ and H13/H13′), 3.93 (s, 6H, 2 × OCH_3 at C7/C7′), 3.89–3.80 (m, 2H, H11a/H11a′), 3.12 (dd, 2H, J_1 = 16.2, J_2 8.6, H1b/H1b′), 2.94 (d, 2H, *J* 16.3, H1a/H1a′), 2.45–2.38 (m, 2H, 2 × H14); *m/z* (FAB) 773 ([M + H + 2 × thioglycerol]$^{\cdot+}$, 3%), 665 ([M + H + thioglycerol]$^{\cdot+}$, 7), 557 ([M + H]$^{\cdot+}$, 9), 464 (3), 279 (12), 257 (5), 201 (5), 185 (43), 166 (6), 149 (12), 93 (100); ν_{max}(Nujol)/cm^{-1} 3600–3100 (br), 2923, 2849, 1599, 1511, 1458, 1435, 1391, 1277, 1228, 1054, 1011, 870, 804, 761, 739; $[\alpha]_D^{21}$ + 357.7 (*c* 0.07, $CHCl_3$).

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