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Synthesis of a novel C2/C2′-aryl-substituted pyrrolo[2,1-*c*][1,4]benzodiazepine dimer prodrug with improved water solubility and reduced DNA reaction rate

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ABSTRACT

A prodrug form (**17**) of a novel C2/C2′-aryl-substituted pyrrolobenzodiazepine (PBD) dimer (**16**) has been synthesized by introducing sodium bisulfite groups to the C11/C11′-positions of the parent bis-imine. The prodrug form is highly water soluble, stable in aqueous conditions, and the rate of DNA cross-link formation is much slower compared to the parent bis-imine.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of naturally occurring antitumour antibiotics produced by *Streptomyces* species.[1–4] The PBD monomers (e.g., natural products: anthramycin, porothramycin and sibiromycin;[1] synthetic agents: DRH-417;[5] Fig. 1) exert their biological activity by binding sequence-selectively to Pu-G-Pu motifs within the minor groove of DNA followed by covalent bond formation between their C11-position and the C2–NH_2 functionality of a guanine base. The biological activity of these molecules can be potentiated by joining two PBD units together through their C8/C8′-positions via a flexible alkylene linker (e.g., DSB-120 and SJG-136; Fig. 1).[6,7] The PBD dimers are known to form sequence-selective DNA lesions such as the palindromic 5′-Pu-GATC-Py-3′ interstrand cross-link[8–10] which is thought to be mainly responsible for their biological activity. One example of a PBD dimer, SG2000 (SJG-136, **6**),[11–13] has recently completed Phase I clinical trials in the oncology area and is about to enter Phase II.

Many of the most potent, naturally occurring PBD monomers such as anthramycin (**1**),[14] porothramycin (**2**)[15,16] and sibiromycin (**3**)[17] have *endo/exo*-unsaturation at their C2-position. We have developed synthetic PBD monomers that retain this motif through the application of Suzuki coupling chemistry (e.g., DRH-417, **4**), and have established that C2-unsaturation enhances potency.[18] We have applied this finding to the synthesis of a PBD dimer series and report one example here, the C2/C2′-aryl-substituted dimer SG2202 (**16**, Scheme 1). Although this molecule had significantly greater in vitro cytotoxicity compared to the C2-unsubstituted parent, DSB-120[6] (**5**, Fig. 1), the additional C2/C2′-aryl substituents lowered the water solubility to a level that became problematic for in vivo evaluation. Therefore, we converted **16** to the C11/C11′-bisulfite adduct SG2285 (**17**)[19] based on methodologies reported for the synthesis of C11-bisulfite adducts of PBD monomers.[20,21] Importantly, we found that we could control the stereochemistry of the C11/C11′-bisulfite substituents through the reaction conditions, and the product formed was highly water soluble and stable in aqueous conditions thus allowing full in vitro and in vivo evaluation. Crucially, the bis-bisulfite **17** was found to be significantly slower at cross-linking DNA in vitro compared to the parent dimer **16**, suggesting that it is behaving as a prodrug form of the parent PBD dimer. It was also found to have significant antitumour activity across a range of in vivo models.

To prepare dimers **16** and **17**, the known 2-nitrobenzoic acid dimer core[11] **7** was converted to its acid chloride with oxalyl chloride and added as a solid to methyl 4-hydroxyprolinate hydrochloride in the presence of TEA to afford a quantitative yield of the bis-ester **8**. Reduction and spontaneous B-ring cyclisation to afford the tetralactam **9** was achieved with Raney nickel and hydrazine (CAU-

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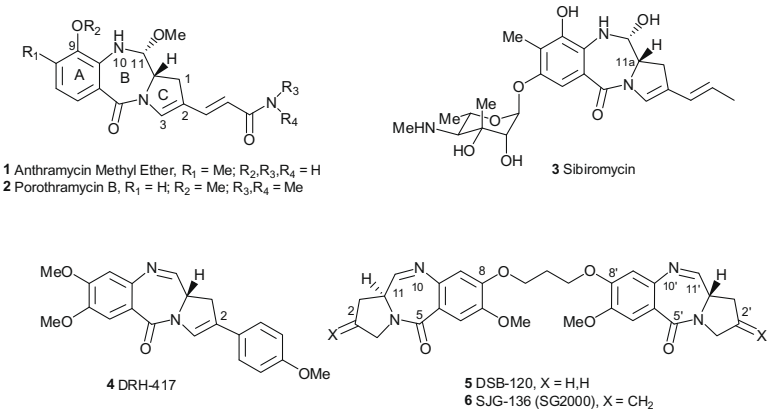
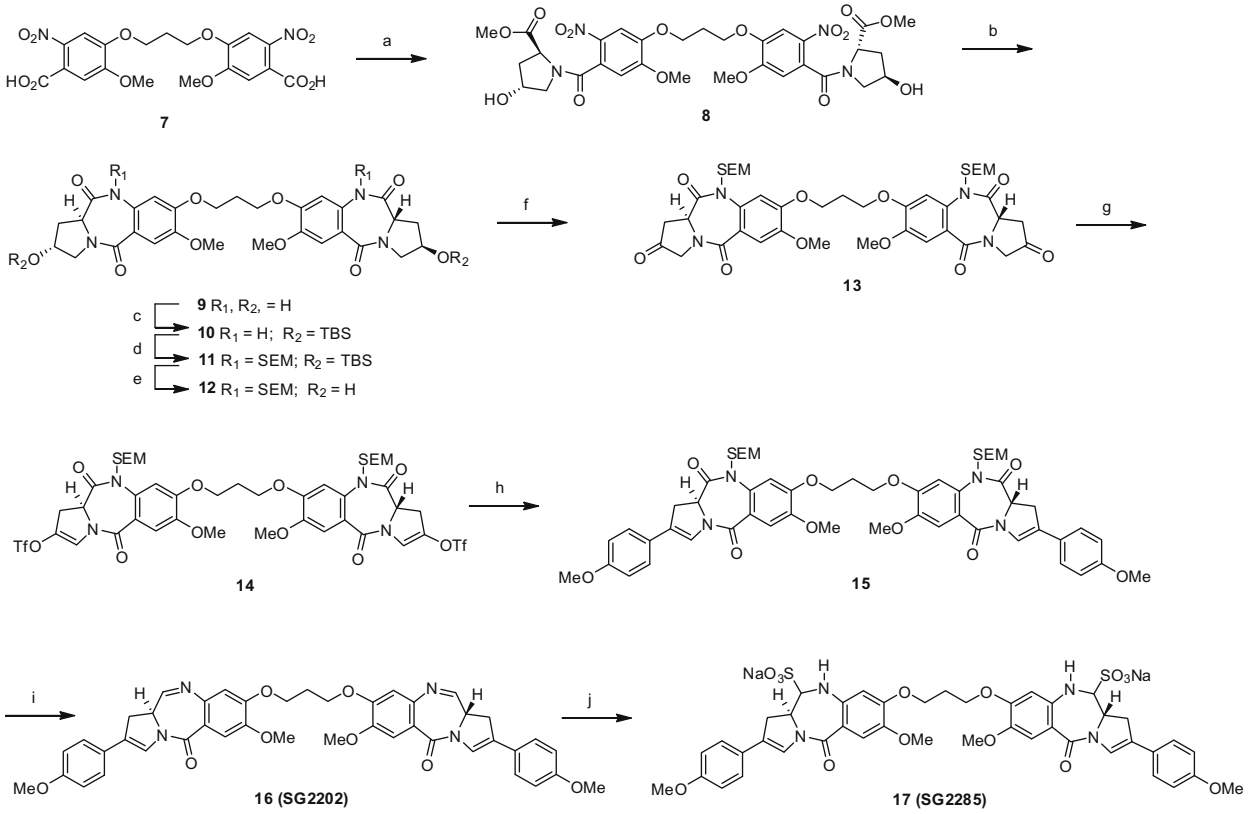


Figure 1. Structures of PBD monomers and dimers.

TION PYROPHORIC!) in 85% yield. Alternative reducing conditions (Pd/C, H_2 or H-cube) gave the uncyclized bis-amine, although subsequent treatment with hydrazine furnished the desired tetralactam **9**, thus offering a less pyrophoric approach to cyclisation in comparable yield (81%). The 2/2′-hydroxy groups were then protected as silyl ethers (**10**) under standard conditions in order to allow introduction of the SEM group (*n*-butyllithium, SEM-Cl) at the N10/N10′ positions (**11**). The silyl ethers could be selectively removed in the presence of the SEM group to afford the N10/N10′-protected tetralactam **12** (78% yield over three steps). The newly formed C2/C2′-secondary alcohol was oxidized under Swern conditions to give **13** in 56% yield, but a modified version of the method of Fey et al.[22] (TEMPO, NaOCl and KBr in DCM) afforded the same product in 75% yield on a 20 g scale. Triflation represented one of the most important steps in the synthesis, as the resulting bis-triflate intermediate **14** could be used to prepare a diverse set of C2/C2′-aryl substituted PBD dimers. Our original triflation conditions (pyridine, triflic anhydride) yielded **14** in reproducible but moderate yield (39%). However, replacing pyridine with the more-hindered 2,6-lutidine and performing the reaction at lower temperature raised the triflation yield to a more acceptable 70%. Next, Suzuki coupling with 4-methoxybenzeneboronic



Scheme 1. Synthesis of the C2/C2′-bis-aryl PBD dimer SG2202 (**16**) and the novel C11/C11′-bis-bisulfite prodrug SG2285 (**17**). Reagents and conditions: (a) (i) $(COCl)_2$, cat. DMF, DCM, rt, 16 h, (ii) (2*S*,4*R*)-methyl-4-hydroxyprolinate hydrochloride, TEA, −40 °C, 100%; (b) Raney Ni (CAUTION!), $N_2H_4{\cdot}H_2O$, MeOH, Δ, 85%, or (i) 10% Pd–C, H_2, DMF, 8 h, 45 psi, (ii) $N_2H_4{\cdot}H_2O$, EtOH, Δ, 50 min, 81%; (c) TBS-Cl, imidazole, DMF, rt, 3 h, 99%; (d) *n*-BuLi, SEM-Cl, THF, −30 °C, 100%; (e) TBAF, THF, rt, 1 h, 79%; (f) TEMPO, NaOCl, KBr, DCM, 0 °C, 1 h, 75%; (g) Tf_2O, 2,6-lutidine, DCM, −40 °C, 1 h, 70%; (h) 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, toluene, rt, 3 h, 87%; (i) (i) $LiBH_4$, THF, EtOH, 15–25 °C, 1 h, (ii) silica gel, EtOH, DCM, H_2O, 72 h, 94%; (j) $NaHSO_3$, IPA, H_2O, rt, 45 min, 82% (11*S*,11*S*′-diastereomer), or $NaHSO_3$, DCM, H_2O, rt, 25 h, 62% (11*S*,11*R*′-diastereomer).

acid proceeded smoothly at room temperature to give the C2/C2′-aryl-substituted PBD dimer **15** in 87% yield. The presence of the N10/N10′-SEM protecting groups allowed regioselective reduction of the C11/C11′-carbonyl functionalities in the presence of the C5/C5′-carbonyl groups. Previously, sodium tetraborohydride has been employed as a reducing agent for this step,[2] however we found that the extended reaction times required for this reagent could led to in situ removal of the SEM groups followed by reduction of the nascent PBD N10–C11/N10′–C11′ imines to secondary amine functionalities. However, use of the more powerful lithium tetraborohydride reduced the C11/C11′-carbonyl groups sufficiently rapidly so that premature N10-deprotection did not occur.[23] Subsequent treatment with silica gel afforded the free bis-imine **16** in 94% yield on a 12 g scale.

Compound **16** was then converted to the bis-C11/C11′-bisulfite adduct SG2285 (**17**) by treatment with sodium bisulfite. Initially, reaction in a two-phase DCM/water system afforded a 2:1 mixture of two diastereomers that could be separated by mass-directed preparative HPLC. 500 MHz NMR revealed the major and minor components to be the 11*S*,11*R*′ and 11*S*,11*S*′ isomers, respectively. The diastereomeric ratio was found to be very sensitive to reaction and work-up conditions. Performing the reaction in a miscible aqueous solvent system such as isopropanol/water (2:1) for 45 min followed by flash freezing of the reaction mixture and lyophilisation afforded almost exclusively the 11*S*,11*S*′ diastereomer (96.4:3.6; 11*S*,11*S*′:11*S*,11′*R*). Conversely, performing the reaction in an immiscible mixture of DCM and water (1:1) over 25 h, followed by separation and lyophilisation of the aqueous phase, afforded a (9:1) mixture in favour of the 11*S*,11′*R* diastereomer. Unlike the parent bis-imine **16** which was poorly soluble in water, the N10/N10′-bisulfite adducts of **17** were highly soluble (approximately 11 mg/ml).

Dimers **16** and **17** were evaluated in a panel of ten human tumour cell lines as shown in Table 1, with both exhibiting picomolar activity across the panel (continuous drug exposure, Alamar Blue assay), with the bis-bisulfite **17** only slightly less potent than the parent imine (**16**). Interestingly, the ratio of C11/C11′ diastereomers did not appear to influence the cytotoxicity of dimer **17**. Both **16** and **17** were more potent than the structurally-equivalent monomeric PBD **4** in the K562 leukemia cell line (360-fold for **16** and 250-fold for the bisulfite **17**) due to their ability to cross-link DNA. Compounds **16** and **17** were also significantly more potent than the C2-*exo*-unsaturated PBD dimer SG2000 (SJG-136, **6**) in the K562 leukemia cell line (86-fold for **16** and 40-fold for **17**).

The ability of **17** to cross-link naked DNA was investigated using a gel-based interstrand cross-linking assay with pUC18 plasmid DNA.[24] A longer incubation time was required to reach the peak of cross-linking for the bis-bisulfite **17** (18 h) compared to the parent N10–C11 bis-imine **16** (2 h). An XL_{50} value of 0.9 nM was achieved for **17**, indicating that it is a highly efficient cross-linking agent. Further investigations showed that while **17** alone is stable in phosphate buffer for at least six days, in the presence of double-stranded oligonucleotides containing Pu-GATC-Py motifs (e.g., duplex 5′-TATAGATCTATA-3′; drug/DNA molar ratio 4:1; 18 °C) in the same buffer it reacts within hours to form interstrand cross-linked adducts (e.g, 10.8%, 70.1% and 92.5% cross-linked in 4, 24 and 48 h, respectively).[25] Assuming that **17** does not react directly with DNA (which would require ejection of the relatively bulky C11/C11′-bisulfite groups within the minor groove), this suggests that in aqueous solution **17** may exist in equilibrium with a small amount of the N10–C11/N10′–C11′ bis-imine form (i.e., **16**), and it is this species that reacts with DNA, thus pulling the equilibrium towards **16** as the latter is consumed by DNA adduct formation.

In summary, the first example of a C2/C2′-aryl PBD dimer (**16**) is reported that can be converted into a stable, highly water soluble form (**17**) by conversion to C11/C11′-bisulfite diastereomers. Importantly, **17** appears to behave as a prodrug in that the rate of reaction with DNA is significantly delayed compared to the parent N10–C11-bis-imine (**16**), although a similar level of cross-linking is eventually achieved by both. **16** and **17** are cytotoxic at picomolar concentrations in a range of tumour cell lines, clearly demonstrating the potency-enhancing effect of the C2-aryl motif. Preliminary in vivo data show that **17** has significant antitumour activity across a wide range of human tumour xenograft models, and these data will be reported elsewhere. Finally, it is noteworthy that the versatile, late-stage, enol triflate intermediate **14** can be produced on a large scale and used to prepare a diverse set of C2/C2′-aryl PBD dimers.

Table 1
Cytotoxicity data (GI_{50}) for PBD dimers **16** and **17** across a panel of human tumour cell lines (Alamar Blue assay, continuous exposure)

Cell line	Cell type	SG2202 (**16**)[a] mean GI_{50} (nM)	SG2285 (**17**)[a] mean GI_{50} (nM)
K562[b]	CML	0.013	0.0185
CCRF-CEM	ALL	0.0001	0.0014
RPMI8226	Myeloma	0.0051	0.0278
A549	NSCL carcinoma	0.033	0.0194
DU145	Prostate carcinoma	0.0154	0.0064
LNCaP-FGC	Prostate carcinoma	0.0027	0.0007
A2780	Ovarian carcinoma	0.0024	0.0005
MCF7	Breast carcinoma	0.0186	0.031
LOXIMVI	Melanoma	0.0225	0.0535
LS174T	Colon	0.0007	0.0043

[a] GI_{50} = concentration required to reduce growth by 50% following continuous drug exposure using Alamar Blue.
[b] Comparative GI_{50} value for SJG-136 (**6**) in K562 under identical conditions = 0.419 nM.

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19. *Analytical data for the two diastereomers of* **17**: **11S/11S′**: $[\alpha]_D^{21}$ = +53 (*c* 0.12, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 (s, 2H, H-3), 7.38 (d, 4H, *J* = 8.7 Hz, H-2′ C2-aryl), 7.05 (s, 2H, H-6), 6.91 (d, 4H, *J* = 8.8 Hz, H-3′ C2-aryl), 6.51 (s, 2H, H-9), 5.26 (s, 2H, NH), 4.29 (td, 1H, *J* = 10.3, 3.6 Hz, H-11aS), 4.18–4.11 (m, 4H, CH_2O), 3.92 (d, 1H, *J* = 10.4 Hz, H-11), 3.75 (s, 6H, OMe), 3.71 (s, 6H, OMe), 2.28–2.18 (m, 2H, CH_2); *note*: H-1 signals obscured by residual buffer. ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 158.2, 151.3, 143.1, 140.1, 126.8, 126.0, 122.6, 122.1, 115.5, 114.1, 112.7, 106.6, 78.4, 65.1, 56.7, 56.0, 55.1, 35.5, 28.6; IR (ATR, neat) 3386 (weak), 2935 (weak), 1604, 1572, 1510, 1494, 1450, 1433, 1383, 1246, 1202, 1179, 1146, 1109, 1028, 970, 867, 822, 786, 758 cm^{-1}; **11S/11R′**: $[\alpha]_D^{22}$ = +291 (*c* 0.32, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6) d: 7.41 (s, 1H, H-3 'S'), 7.37 (d, 2H, *J* = 8.7 Hz, 2′-H C2-aryl, 'S'), 7.35 (s, 1H, H-3, 'R'), 7.32 (s, 1H, H-6, 'R'), 7.29 (d, 2H, *J* = 8.6 Hz, H-2′ C2-aryl, 'R'), 7.05 (s, 1H, H-6 'S'), 7.03 (d, 1H *J* = 6.4 Hz, NH 'R'), 6.91 (d, 2H, *J* = 8.9 Hz, H-3′ C2-aryl), 6.89 (d, 2H, *J* = 8.5 Hz, H-3′ C2-aryl), 6.52 (s, 1H, 9-H 'R'), 6.51 (s, 1H, 9-H 'S'), 5.25 (s, 1H, NH 'S'), 4.42 (dd, 1H, *J* = 12.4, 6.2 Hz, H-11aS 'R'), 4.29 (td, 1H, *J* = 10.3, 3.5 Hz, H-11aS 'S'), 4.17–4.11 (m, 3H, CH_2O 'S' and H-11 'R'), 4.06 (t, 2H, *J* = 6.4 Hz, CH_2O 'R'), 3.94 (d, 1H, *J* = 10.3 Hz, H-11 'S'), 3.76 (s, 3H, OMe 'S'), 3.75 (s, 3H, OMe 'S'), 3.72 (s, 3H, OMe 'R'), 3.66 (s, 3H, OMe 'R'), 3.66–3.62 (m, 1H, H-1 'R'), 3.52–3.45 (m, 1H, H-1 'S'), 3.29–3.16 (m, 2H, H-1 'S' and 'R'), 2.28–2.18 (m, 2H, CH_2) ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9, 158.2, 151.3, 143.1, 140.1, 126.8, 126.0, 122.5, 122.2, 115.4, 114.1, 112.7, 106.5, 78.4, 65.1, 56.7, 56.0, 55.1, 35.4, 28.6; IR (ATR, neat) 3370 (weak), 2935 (weak), 1605, 1572, 1512, 1450, 1434, 1383, 1241, 1200, 1178, 1143, 1107, 1028, 975, 872, 823, 786, 755 cm^{-1}.
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