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Synthesis of the First Examples of *A*-C8/*C*-C2 Amide-Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimers

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Abstract—We report the synthesis of novel *A*-**C8**/*C*-**C2** amide-linked pyrrolo[2,1-*c*][1,4]benzodiazepine dimers (**4a** and **4b**) via convergent routes. These compounds lack the potent DNA interstrand cross-linking ability and resultant pronounced cytotoxicity of the known *A*-**C8**/*A*-**C8′** linked dimers (e.g., **2a**–**b**).

The pyrrolobenzodiazepines are a family of tricyclic antitumour antibiotics that interact in the minor groove of DNA forming monocovalent adducts.[1–4] The first PBD dimer (**1**) comprising two unsubstituted PBD units joined through their *A*-**C7**/*A*-**C7′** positions was reported by Suggs and co-workers in 1988[5,6] (Fig. 1). Dimers with this linkage had only weak DNA cross-linking activity and no cytotoxicity data were reported. The first dimer with an *A*-**C8**/*A*-**C8′** linkage (**2a**) was reported[7–9] in 1992. Dimers of this type have significant DNA interstrand cross-linking activity, and pronounced in vitro cytotoxicity and in vivo antitumour activity. One example (**2b**, SJG-136)[10,11] has been selected for clinical evaluation. More recently, Lown and co-workers reported[12] three *C*-**C2**/*C*-**C2′** linked dimers (**3a**–**c**). These are less cytotoxic than the *A*-**C8**/*A*-**C8′** dimers but their cross-linking efficiency has not been reported. The examples cited above spurred us to explore the *C*-**C2**/*A*-**C8′** linkage by targeting the novel dimers **4a** and **4b** to obtain further SAR data and to potentially allow the synthesis of longer PBD oligomers by coupling a series of PBD units together in this orientation.

In order to construct prototype *C*–*A* amide-linked PBD dimers of type **4**, it was first necessary to synthesise the C8-amino and C2-methylenecarboxy PBD building

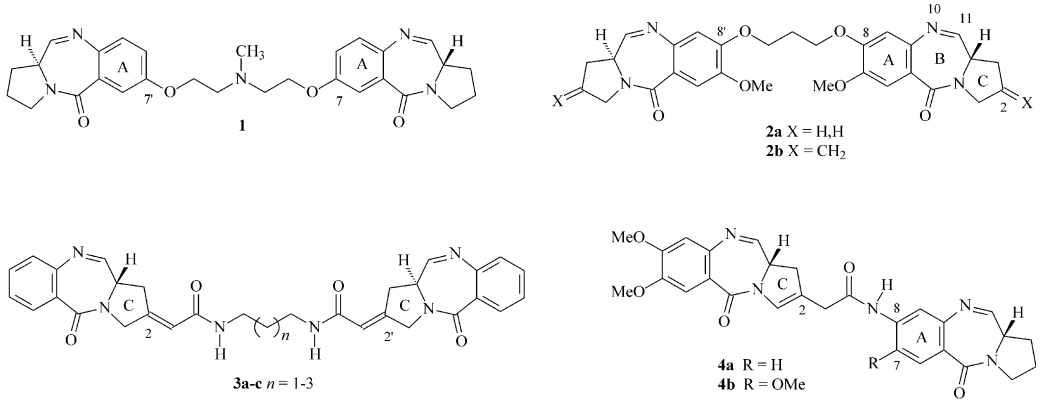

Figure 1.

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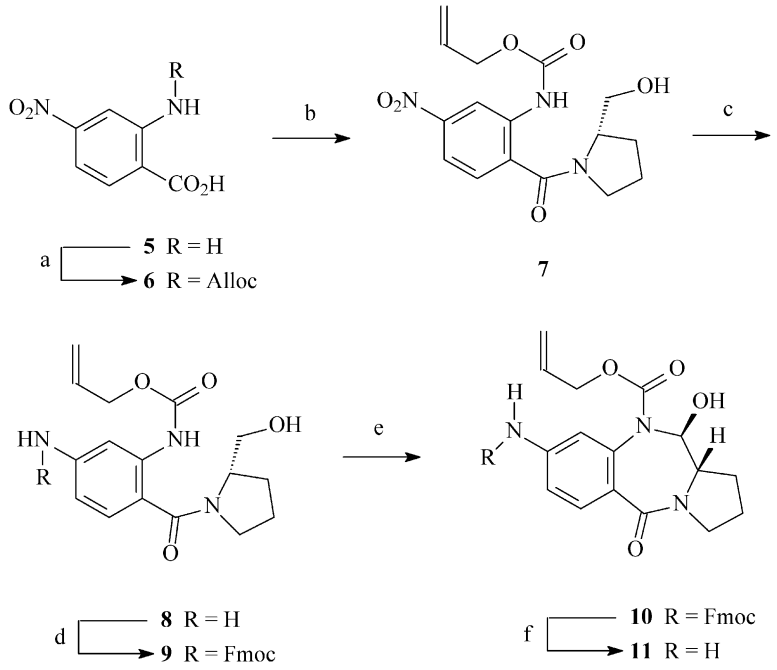
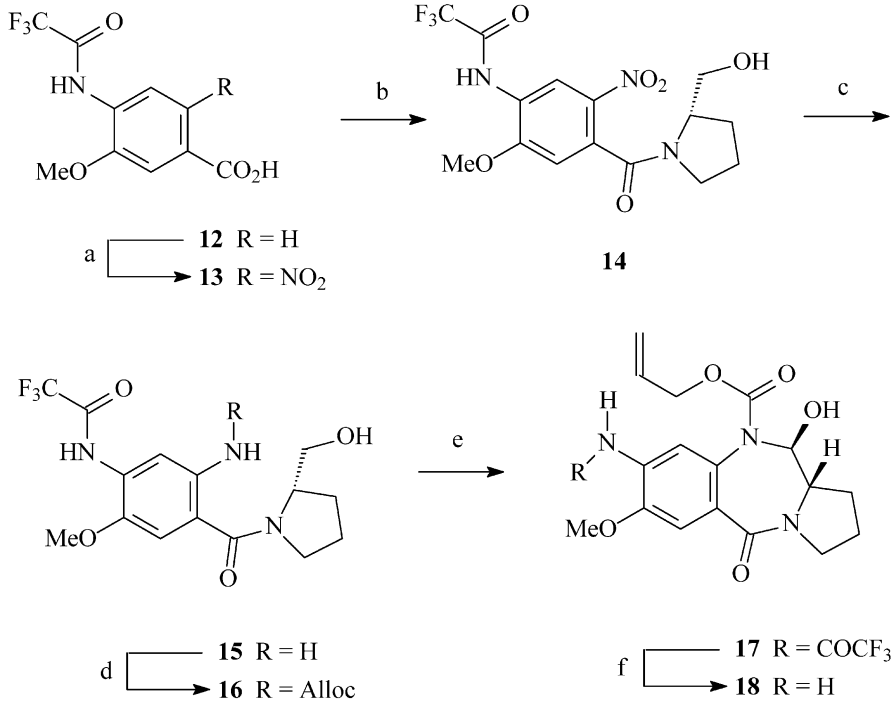
blocks. Synthesis of the right-hand portion of the C7-*des*-methoxy dimer **4a** began by *N*-protecting commercially available 4-nitroanthranilic acid **5** as its allyl carbamate **6** (Scheme 1). This was coupled without further purification to (*S*)-(+)-2-pyrrolidinemethanol to provide **7** which was reduced using $SnCl_2$ in refluxing methanol. The resulting aniline **8** was then *N*-Fmoc protected in good yield using 9-fluorenylmethyl chloroformate in the presence of aqueous sodium carbonate and THF. The orthogonal dicarbamate **9** was subjected to Swern conditions to provoke B-ring cyclisation in excellent yield. Treatment of the cyclised compound **10** with *N*,*N*-dimethylamine in MeOH removed the Fmoc protecting group to provide the key C7-*des*-methoxyaniline **11**.

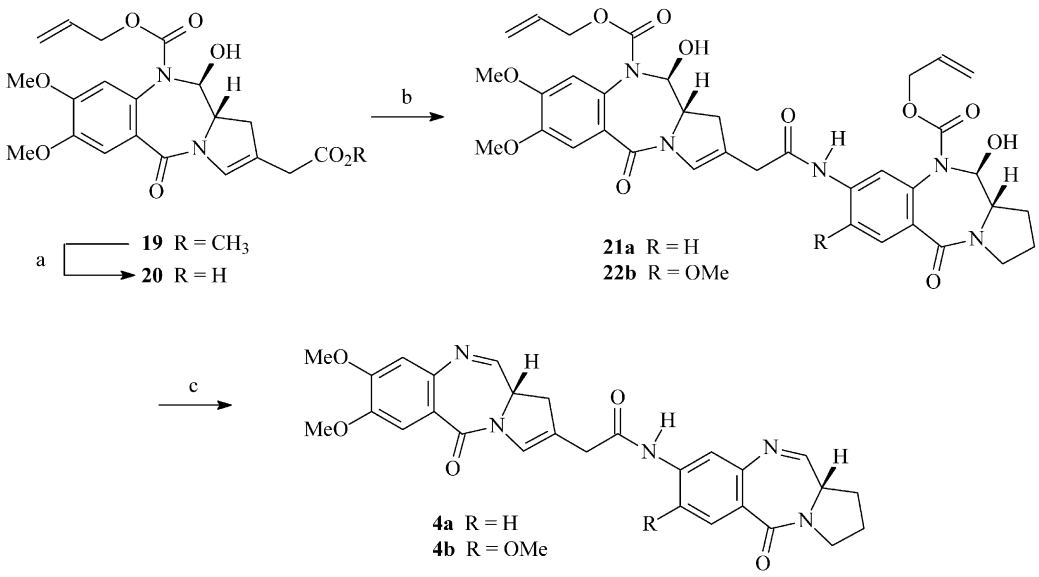
The aniline coupling partner (**18**) for the C7-methoxy dimer **4b** was synthesised via a similar route. Known



Scheme 1. (a) AllocCl, pyridine, CH_2Cl_2, 0 °C, 75%; (b) TBTU, (*S*)-(+)-2-pyrrolidinemethanol, DIPEA, DMF, 40%; (c) $SnCl_2.H_2O$, MeOH, Δ, 74%; (d) FmocCl, aq Na_2CO_3, THF, 0 °C, 76% (e) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −45 °C, 76%; (f) $HNMe_2$, MeOH, 80%.



Scheme 2. (a) *f*-HNO_3, 0 °C, 90%; (b) DCC, HOBt, CH_2Cl_2, 0 °C then (*S*)-(+)-2-pyrrolidinemethanol, CH_2Cl_2, −20 °C, 78%; (c) H_2, 45 psi, 10% Pd/C, EtOH, quant; (d) AllocCl, pyridine, CH_2Cl_2, 0 °C, 81%; (e) pyridinium dichromate, 4 Å sieves, CH_2Cl_2, 51%; (f) aq K_2CO_3, MeOH, CH_2Cl_2, 70%.



Scheme 3. (a) LiOH.H_2O, MeOH, H_2O, 0 °C, 89%; (b) DIC, HOBT, CH_2Cl_2, 0 °C then **11** or **18**, CH_2Cl_2, 40% (**21a**), 67% (**21b**); (c) $Pd(PPh_3)_4$, PPh_3, pyrrolidine, CH_2Cl_2, 70% (**4a**), 77% (**4b**).

trifluoroacetylamino benzoic acid **12**[13] was nitrated in high yield using fresh *f*-HNO_3 at 0 °C (max^m 3 g scale; 5 min reaction time) to provide nitro acid **13** (Scheme 2). This was coupled to (*S*)-(+)-2-pyrrolidinemethanol using DCC/HOBt to provide the amide **14** in good yield. Reduction of the nitro group furnished aniline **15** which was subsequently *N*-protected (**16**) upon treatment with allyl chloroformate in the presence of pyridine. The best yield for oxidation of **16** was achieved using pyridinium dichromate to give the N10-Alloc protected PBD **17** in 51% yield. Key C7-methoxy aniline **18** was obtained in high yield after cleavage of the trifluoroacetlyl protecting group.

The PBD C2-tethered acid **20** is common to both dimers and was synthesised by hydrolysing known PBD-ester **19**[14] in good yield (Scheme 3). The Alloc protected *C–A* linked PBD dimers were synthesised in moderate (40% for C7-*des*-methoxy **21a**) and good (67% for C7-methoxy **21b**) yields. Although several coupling methods were attempted for **21a**, the yield could not be improved upon. Treatment of **21a–b** with palladium(0) in the presence of pyrrolidine cleaved the Alloc protecting groups to give the novel N10–C11 imine PBD dimers **4a**[15] and **4b**.[16]

4a and **4b** were evaluated for in vitro cytotoxicity and DNA cross-linking ability. Neither molecule demonstrated significant cytotoxicity in a number of human tumour cell lines (e.g., **4a**: $IC_{50} > 25$ μM in A2780, A2780*cis*, CH1, CH1*cis* and SKOV-3). Similarly, both molecules are poor cross-linking agents (e.g., **4a** approximately 200-fold less efficient than **2b**). These observations suggest that the *A*-**C8**/*A*-**C8′** linkage is optimal for DNA cross-linking and cytotoxicity compared to *A*-**C7**/*A*-**C7′**, *C*-**C2**/*C*-**C2′** or *C*-**C2**/*A*-**C8** linkages. Although the *C*-**C2**/*A*-**C8** linkage of **4a** and **4b** may be inherently sub-optimal for DNA interaction, preliminary modelling studies have suggested an alternative explanation. Due to the relatively short linker in molecules of type **4a** and **4b** compared to **1**, **2** and **3**, they may be forced into a non-isohelical conformation not conductive to binding in the DNA minor groove. To investigate this possibility we are currently synthesising *C*-**C2**/*A*-**C8** linked dimers where the nitrogen moiety of the amide group is separated from the A-ring by an alkoxy chain, thus allowing more flexibility between the PBD units.

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15. Data for **4a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, 1H, $J = 8.24$ Hz), 7.78–7.44 (m, 3H), 7.40–7.25 (m, 2H), 6.91 (s, 1H), 6.77 (s, 1H), 4.28–4.10 (m, 1H), 3.89 and 3.85 (s×2, 6H), 3.83–3.60 (m, 2H), 3.55–3.46 (m, 1H), 3.34–3.10 (m, 4H), 2.36–1.60 (m, 4H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 168.4, 164.8, 162.9, 161.5, 152.0, 147.8, 146.7, 141.2, 140.6, 131.2, 126.3, 119.6, 118.8, 117.9, 111.4, 109.9, 56.2, 54.0, 53.8, 46.7, 37.5, 37.2, 29.6, 24.2; MS (FAB), *m/z* (relative intensity) 514 ($M^{+\cdot} + H$, 97), 279 (43), 271 (19), 216 (23), 192 (53), 180 (100), 149 (21), 135 (17), 112 (28), 91 (59), 80 (36), 73 (91), 57 (80); IR (NUJOL) 3315 (br), 2924, 2854, 1682, 1624, 1598, 1511, 1455, 1378, 1246, 1214, 1128, 1071, 1010, 965, 872, 827, 722, 665 cm^{-1}; HRMS $[M+H]^{+\cdot}$ calcd for $C_{28}H_{28}N_5O_5$ *m/z* 514.2090, Found (FAB) *m/z* 514.2096; $[\alpha]_D^{20} = +624.0$ (*c* 0.03, $CHCl_3$).
16. Data for **4b**: ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.28 (s, 1H), 7.86 (d, 1H, $J = 3.94$ Hz), 7.69 (d, 1H, $J = 4.46$ Hz), 7.53 (s, 1H), 7.50 (s, 1H), 7.05 (s, 1H), 6.82 (s, 1H), 4.61–4.45 (m, 1H), 4.38–4.21 (m, 1H), 3.95–3.78 (m, 9H), 3.60–3.48 (m, 2H), 3.34–3.00 (m, 4H), 2.37–2.18 (m, 2H), 2.10–1.78 (m, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 167.2, 164.4, 162.5, 161.4, 151.9, 147.8, 146.1, 140.6, 140.4, 129.9, 127.1, 122.9, 118.9, 117.9, 111.5, 110.3, 109.8, 56.2, 56.1, 53.9, 53.6, 46.7, 37.6, 37.4, 29.6, 24.1; MS (FAB), *m/z* (relative intensity) 544 ($M^{+\cdot} + H\cdot$, 100), 413 (83), 391 (89), 329 (54), 307 (91), 289 (81), 272 (77), 246 (56); IR ($CHCl_3$) 3329 (br), 3011, 2930, 2867, 1725, 1691, 1602, 1511, 1454, 1434, 1387, 1343, 1264, 1215, 1128, 1073, 1019, 969, 874, 664 cm^{-1}; HRMS $[M+H]^{+\cdot}$ calcd for $C_{29}H_{30}N_5O_6$ *m/z* 544.2196, Found (FAB) *m/z* 544.2209; $[\alpha]_D^{23} = +405.9$ (*c* 0.10, $CHCl_3$).