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Synthesis of a novel C2-aryl substituted 1,2-unsaturated pyrrolobenzodiazepine

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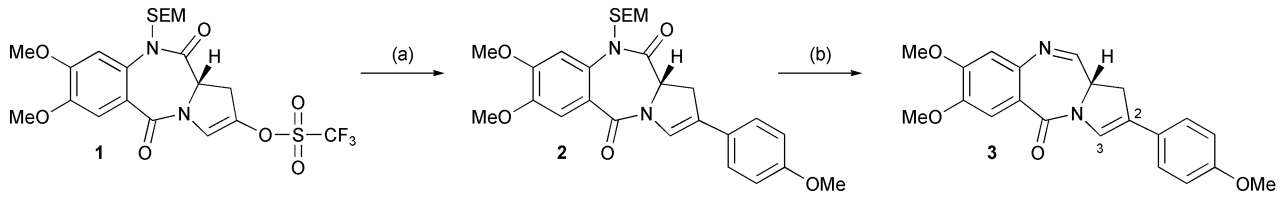
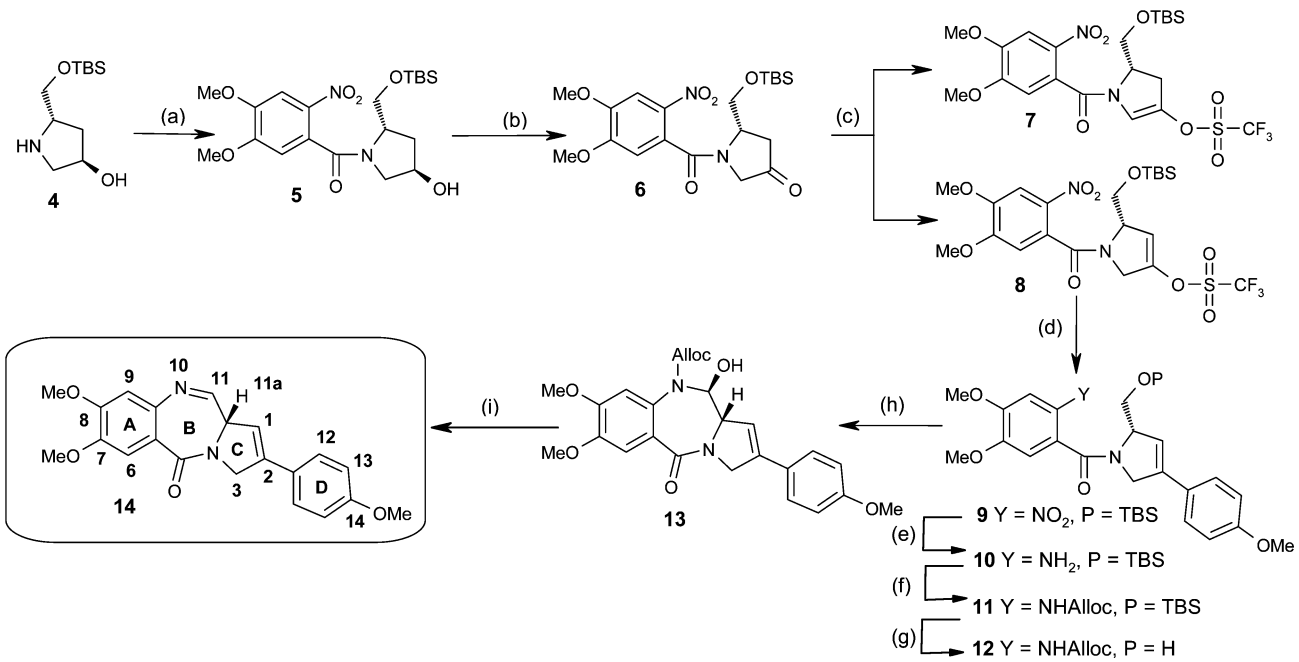
A novel C2-aryl 1,2-unsaturated PBD (14) has been prepared *via* an enol triflate intermediate (8). The regiochemistry of triflation is dependent upon the point at which the reaction is performed during the synthetic route.

Pyrrolobenzodiazepines (PBDs) are a family of low molecular weight antitumour antibiotics originally isolated from *streptomyces* species.[1,2] Following their discovery, there has been significant interest in their total synthesis and the preparation of novel analogs.[3–5] This interest stems from their ability to interact covalently with DNA in a sequence selective fashion which could lead to the synthesis of molecules capable of regulating gene expression.[2] For example, a pyrrolobenzodiazepine dimer, SJG-136,[6] with an affinity for Pu–GATC–Py sequences is presently under evaluation in clinical trials.

We recently reported a novel sub-class of synthetic C2-aryl substituted PBDs containing C2–C3 *endo* unsaturation which possess significant *in vitro* cytotoxicity and *in vivo* antitumour activity.[7] The original synthetic strategy led to exclusive formation of the C2–C3 unsaturated product (e.g. **3**, Scheme 1). However, we now report the synthesis of the complementary C1–C2 unsaturated PBD *via* an alternative synthetic strategy.[8] Previously, C2-aryl PBDs of type **3** had been obtained by performing the Suzuki reaction on a PBD dilactam triflate of type **1**. The resulting dilactam **2** was then reduced with sodium tetraborohydride leading to loss of the SEM protecting group to



Scheme 1 (a) 4-Methoxybenzeneboronic acid (1.0 equiv), 2 M Na_2CO_3 (1.0 equiv), tetrakis(triphenylphosphine)palladium(0) (0.01 equiv), toluene : EtOH = 1 : 1, 2 h, 90%, (b) $NaBH_4$ (2.0 equiv), EtOH : THF = 7 : 3, wet silica gel, RT, 12 h, 72%.



Scheme 2 (a) 6-Nitroveratric acid (1.2 equiv), DCC (1.2 equiv), HOBt (1.2 equiv), DMF (cat), DCM, 48 h, 70%; (b) BAIB (1.1 equiv), TEMPO (0.1 equiv), DCM, 2 h, 90%; (c) LDA (1.1 equiv) or NaHMDS (1.1 equiv), THF, −78 °C, 1 h; then 5-chloro-*N*,*N*-bis(trifluoromethanesulfonyl)aminopyridine (1.1 equiv), −30→25 °C, 4 h, 50%; (d) 4-methoxybenzeneboronic acid (1.0 equiv), 2 M Na_2CO_3 (1.0 equiv), tetrakis(triphenylphosphine)palladium(0) (0.01 equiv), toluene : EtOH = 1 : 1, reflux, 1 h, 80%; (e) $Na_2S_2O_4$ (5.0 equiv), THF : H_2O = 5 : 1, 5 h, 80%; (f) allyl chloroformate (1.1 equiv), pyridine (2.2 equiv), DCM, 4 h, 82%; (g) TBAF (1.2 equiv), THF, 1 h, 75%; (h) BAIB (1.1 equiv), TEMPO (0.1 equiv), DCM, 5 h, 84%; (i) pyrrolidine (1.1 equiv), tetrakis(triphenylphosphine)palladium(0) (0.01 equiv), DCM, 0→25 °C, 1 h, 80%; BAIB = iodobenzene diacetate.

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afford the C2–C3 unsaturated C2-aryl PBD **3**. In the new synthetic strategy the triflation/Suzuki coupling reactions are carried out prior to B-ring closure (Scheme 2) thus avoiding the dilactam intermediate and the risk of over-reduction to the biologically inactive N10–C11 secondary amine.

The PBD backbone was established by coupling 4,5-dimethoxy-2-nitrobenzoic acid to the amine **4** derived from 4-L-*trans*-hydroxyproline[9] in the presence of DCC/HOBt in 70% yield. The critical pro-C2 ketone (**6**) was obtained by oxidising the 2-hydroxy group with BAIB/TEMPO in 90% yield.[10] At this stage a number of triflation strategies were explored. Trifluoromethanesulfonic anhydride with pyridine failed to give appreciable amounts of enol triflate with little consumption of the starting ketone. The use of LDA and 5-chloro-*N*,*N*-(trifluoromethanesulfonyl)aminopyridine[11] afforded a mixture of 1,2 (**8**) and 2,3 (**7**) unsaturated enol triflates in a 4 : 1 ratio. However, use of the same triflating agent with NaHMDS as base afforded almost exclusively the 1,2-unsaturated product **8** in 50% yield. The NMR spectra of the isomeric triflates were quite distinct with the two H3 signals clearly visible in the spectrum of **8**.[12] The 1,2-product **8** was subjected to Suzuki coupling conditions in the presence of 4-methoxybenzeneboronic acid. The reaction proceeded in excellent yield (> 80%) but required heating unlike the Suzuki reactions involving the dilactam-based enol triflate reported previously.[7] The nitro group was then reduced with sodium dithionite in 80% yield without jeopardising the C1–C2 double bond. The newly formed amine **10** was protected as an allyl (Alloc) carbamate (**11**) and the TBDMS group removed (**12**) in order to permit a Fukuyama B-ring cyclization. Normally, this reaction is performed under Swern conditions, but we found that oxidation with BAIB/TEMPO[10] achieved B-ring closure to **13** in 84% yield. This is a significant improvement as, unlike the Swern reaction, the TEMPO/BAIB method does not require anhydrous conditions or an inert atmosphere, thus making the cyclization easier to perform and monitor. The ^{1}H spectrum of **13** revealed the diagnostic H-11 signal at 5.7 ppm. Removal of the allyl carbamate group under Deziel conditions[9] afforded the novel C2-aryl substituted 1,2-unsaturated PBD **14**[13] in 80% yield.[14]

The NMR spectrum of **14** was compared to that of the previously reported **3**. In addition to the expected aromatic and methoxy signals, it revealed a doublet at δ 7.82 for the 11-H imine proton, a singlet for 1-H at 6.19 with some evidence of fine coupling, two overlapping doublets at 4.81–4.78 for 3-H/H′ and a broad singlet for H-11a at 4.63 ppm.[15] The orientation of C-ring unsaturation was confirmed by a 2D proton–proton NMR experiment. The diagnostic H-11 imine signal correlated with the multiplet at 4.63 ppm, revealing it as H-11a. This signal correlated in turn with the alkenic multiplet at 6.19 ppm confirming it as H-1 and supporting assignment of C1–C2 unsaturation. These experiments showed that the double bond orientation set-up in the enol triflate forming reaction was not isomerised by subsequent steps involving palladium catalysis.

In conclusion, altering the sequence of synthetic events by triflating prior to B-ring closure affords a novel type of C2-aryl substituted PBD with 1,2-*endo* unsaturation, thus complementing the C2–C3 unsaturated compounds produced by the reverse sequence of events.[7] A further advantage is that this synthetic route avoids a reduction step[16] thus removing the risk of N10–C11 secondary amine formation and allowing reduction-sensitive groups to be included on the C2-aryl ring. Furthermore, a convenient, new, and high yielding oxidation method has been discovered for B-ring cyclization, which should be applicable to other PBD systems.

Preliminary modelling studies suggest that there is an appreciable difference in C-ring conformation between the 2,3- and 1,2-unsaturated structures. In the former case, the molecule has a crescent shape with each of the four rings closely following the curve. However, with the new 1,2-unsaturated arrangement reported here, the molecule is more linear in shape with the component rings much less aligned, suggesting that it does not fit so well into the minor groove of DNA. Comparison of the biophysical and biological properties of the two structures is presently underway and the results will be reported elsewhere.

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12 The 2,3-unsaturated product **7** exhibited spin systems for H-1 and H-1′ at 2.9 and 3.1 ppm, and an H-3 signal was observed at 6.0 ppm. In contrast, the 1,2-unsaturated compound **8** revealed two 3-H protons at 4.1 and a H-1 alkenic signal at 5.85 ppm.
13 Selected data for **14**: (250 MHz, $CDCl_3$): δ 7.82 (d, 1H, J = 4.1, H11), 7.56 (s, 1H, H6), 7.43 (d, 2H, J = 8.7, H12), 6.93 (d, 2H, J = 8.7, H13), 6.88 (s, 1H, H9), 6.19 (m, 1H, H1), 4.81–4.78 (m, 2H, H3), 4.63 (m, 1H, H11a), 3.98 (s, 3H, 8-OCH_3), 3.94 (s, 3H, 7-OCH_3), 3.84 (s, 3H, 14-OCH_3); m/z (ES+): 383 ([M$^{\cdot+}$ carbinolamine form, 65%), 365 ([M + H]$^{+}$, imine form, 100%); IR ($CHCl_3$): 3307, 2931, 2842, 1643, 1604, 1513, 1454, 1433, 1380, 1254, 1218, 1179, 1098, 1033, 911, 827, 768, 728 cm^{-1}.
14 Exposure of **14** to heat during work-up should be avoided as this can provoke C-ring aromatization.
15 In contrast the spectrum of **3** clearly shows two distinct spin systems for H1 and H1′ at 3.4 and 3.6 ppm, and the diagnostic enamide 3-H at 7.4 ppm.
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