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Application of the Stille coupling reaction to the synthesis of C2-substituted *endo–exo* unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs)

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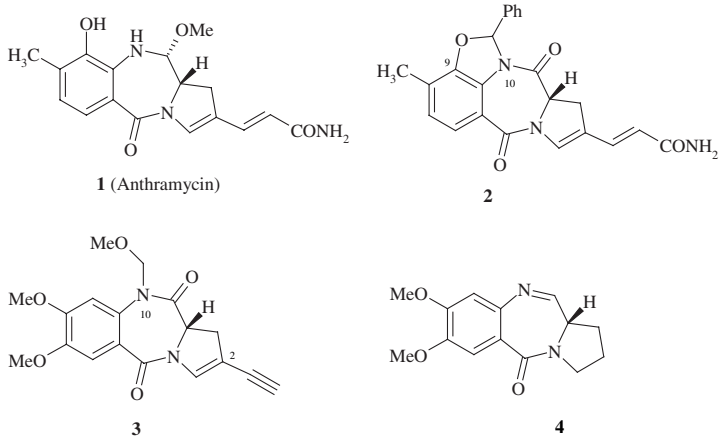
Abstract—The Stille coupling reaction has been used to introduce novel vinyl, alkynyl and heterocyclic substituents to the C2-position of pyrrolo[2,1-*c*][1,4]benzodiazepine dilactams. Sodium borohydride reduction followed by N10-SEM deprotection has provided five analogues (**6b**, **8a–d**) that contain C2-*endo*/*exo*-unsaturation and novel C2-substituents. These analogues have significant multilog cytotoxicity profiles in the NCI 60-Cell Line screen, and provide new SAR data for the PBD family.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of potent sequence-selective DNA-interactive antitumour antibiotics,[1] which are being exploited as components of a gene targeting strategy,[2] and as antitumour agents in the form of extended PBD dimers.[3–5] SAR studies[6–10] have shown that compounds with the greatest cytotoxicity and DNA-binding affinity usually possess *endo–exo* unsaturation in their C-rings combined with conjugated planar C2-substituents, such as the acrylamide side chain found in anthramycin (e.g., **1**, Fig. 1). However, the number of naturally occurring molecules possessing this structural feature is limited.[1] Therefore, as part of an ongoing investigation into the chemistry and SAR of the PBD ring system, we



1 (Anthramycin) **2** **3** **4**

Figure 1.

Keywords: PBD; Antitumour agent; Anticancer agent; Stille coupling; DNA-binding; Cytotoxic.
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have investigated the application of Stille coupling to the synthesis of C-ring *endo–exo* unsaturated C2-substituted PBDs. This work complements the recent study[10] of the use of the Heck reaction to achieve similar unsaturation/substitution patterns in the C-rings of PBDs.

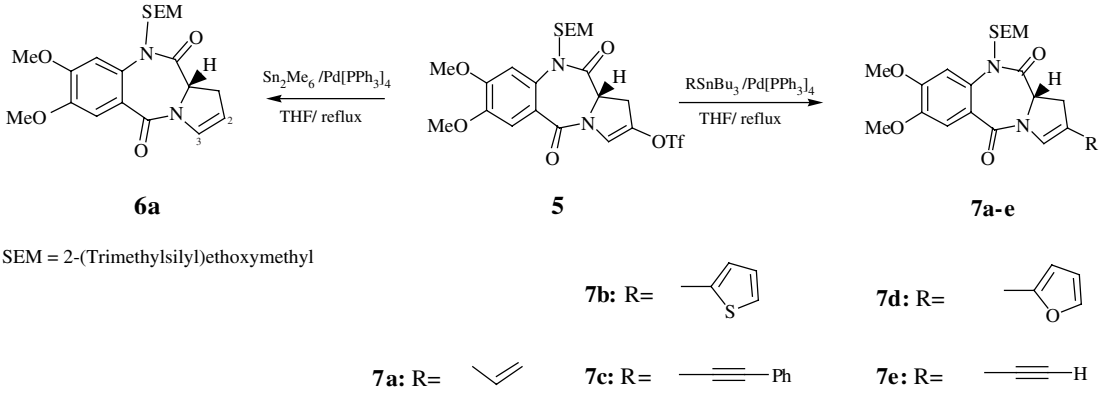
The Stille coupling reaction has been employed to great effect in modern organic chemistry.[11–13] In 1989, Pena and Stille reported the application of Stille coupling to a total synthesis of the naturally occurring PBD, anthramycin[14] (**1**). Initially they followed the strategy of Mori et al.,[15] which relies on N10-MOM protection to allow subsequent reduction of the dilactam C11-carbonyl. However, this was unsuccessful and the target anthramycin (**1**) was eventually obtained by returning to Leimgruber's original O9/N10-benzal protecting group strategy (e.g., **2**, Fig. 1).[16] Intriguingly, these workers also described a number of C2-alkynic and vinylic N10-MOM-protected PBD precursors prepared via Stille coupling (e.g., **3**, Fig. 1), but did not report their conversion to the N10-C11-imine containing final products. We report here the use of Stille coupling to produce five novel PBDs (**6b** and **8a–d**, Scheme 2), and explore the limitations of the final, critical, reduction/deprotection step.

The key enol triflate (**5**)[17] was coupled to a number of commercially available organotin reagents under Stille conditions[18] to provide **7a–e** (Scheme 1). Typically, yields of between 45% and 81% were obtained, with the best from the 2-furanyl coupling reaction (81% for **7d**[19]), and the lowest from the unsubstituted acetylene coupling (45% for **7e**). In the latter case, the modest yield was probably due to side reactions occurring at the unprotected terminal acetylenic position. In general, the reactions proceeded to completion within 2.5 h, although the 2-thiophenyl tin reagent was less reactive, taking 5 h to completely consume the triflate. In marked contrast to the other tin reagents, and previous results obtained from Suzuki reactions,[17] tributylphenyltin failed to afford any coupled product (see Table 1). An attempt to convert the enol triflate **5** to the more versatile trimethylstannyl alkene failed to afford the expected product, but yielded instead the simple unsubstituted *endo*-2,3-unsaturated product **6a** in 41% yield (Scheme 1).

The coupling products (**7a–e**) and **6a** were subjected to sodium borohydride reduction to transiently afford the SEM-protected carbinolamine intermediates, which converted to the PBD imines **8a–d** and **6b** upon exposure to moist silica gel (Scheme 2). Although sufficient quantities of these final products were obtained for biological evaluation, the yields for the final reduction/deprotection steps were disappointing (on average ca. 25%). The yields were particularly poor for the C2-alkynic compounds, where significant collateral reduction of the triple bond was observed. For example, reduction of the acetylene intermediate **7e** afforded mainly the 2-vinyl PBD (**8a**), while reduction of the phenylethynyl intermediate **7c** afforded a mixture of the anticipated PBD (**8c**) along with styryl and phenethyl by-products.

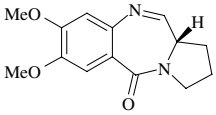
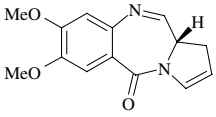
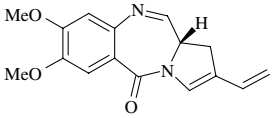
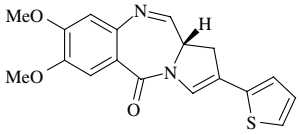
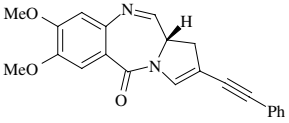
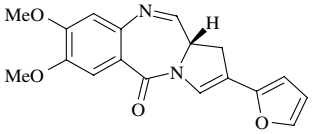
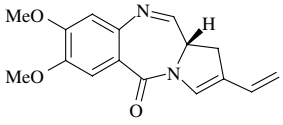
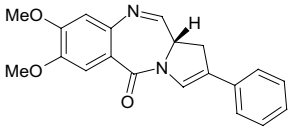
All novel PBDs were evaluated in the NCI 60-Cell Line screen. The mean GI_{50} data (Table 1) indicate that all five PBDs (**6b**, **8a–d**) are significantly cytotoxic with most GI_{50} values in the sub-micromolar range. Compound **4** (GI_{50} = 2.39 μM), a C-ring saturated/unsubstituted PBD[20] but with an A-ring identically substituted to **6b** and **8a–d**, is included in Table 1 for comparison. Interestingly, introduction of a 2,3-*endo* double bond as in **6b** results in a modest (i.e., 2-fold) increase in cytotoxicity. However, inclusion of a C2-vinyl substituent in addition to the double bond (i.e., **8a**) increases the activity by an order of magnitude (i.e., to 0.123 μM). Activity is further potentiated by up to 10-fold by incorporation of heteroaryl groups such as thiophenyl or furyl (e.g., 0.023 and <0.01 μM for **8b** and **8d**,[21] respectively). The results for the heteroaryl compounds **8b** and **8d** are thus similar in potency to the reported C2-aryl substituted PBDs.[17] Finally, isolating the aryl moiety from the C-ring with an alkyne functionality (e.g., **8c**) maintains the significant cytotoxicity of the directly conjugated C2-aryl[17] and C2-heteroaryl PBDs. These results are consistent with the hypothesis that the presence of planar C2-substituents enhance the fit of the PBD structure in the DNA minor groove.[22]

In summary, this study demonstrates that Stille coupling is complementary to the Suzuki reaction in being able to introduce heteroaromatic moieties to the C2 position of the PBD structure. However, it also demonstrates that, although C2-vinylic and acetylenic moieties can be effi-

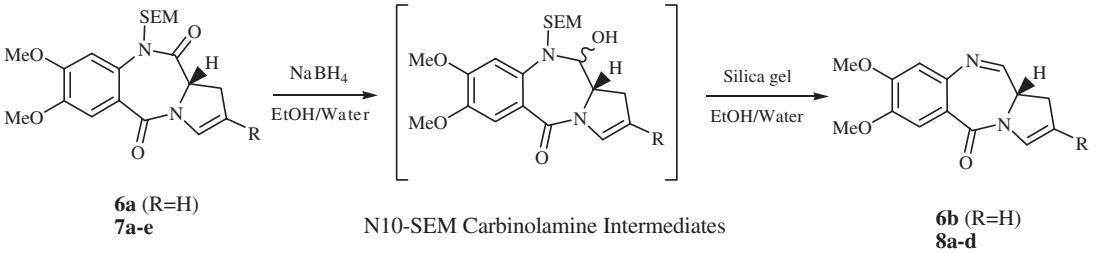


Scheme 1.

Table 1. Summary of tributyl tin coupling partners, yields of Stille coupling reactions and cytotoxicities of final products (**6b**, **8a–d**) compared to the C-ring saturated/unsubstituted PBD **4**

Tributyl tin coupling partner	Stille coupling yield for **7a–e** (%)	Final PBD products (**6b**, **8a–d**) and **4**		
		Structures	Number	Cytotoxicity: Mean GI_{50}[a] (μM)
—	—		**4**	2.39
Sn_2Me_6	40 (see Scheme 1)		**6b**	1.28
Bu_3Sn	72		**8a**	0.123
Bu_3Sn	66		**8b**	0.023
Bu_3Sn—≡—Ph	67		**8c**	<0.01
Bu_3Sn	81[19]		**8d**[21]	<0.01
Bu_3Sn—≡—H	45		**8a**	0.123
Bu_3Sn	No coupling observed		Ref. 17	<0.01

[a] Mean GI_{50}: Mean of concentrations of PBD that inhibits 50% of cell growth (measured using the Sulforhodamine B (SRB) assay) across each of the 60 cell lines of the NCI panel after incubation for 48 h at 37 °C. For details see: www.dtp.nci.nih.gov/branches/btb/ivclsp.html.



Scheme 2.

ciently introduced through Stille coupling, they are vulnerable to reduction later in the synthetic pathway. These observations suggest that reducible C2-substituents should be introduced to a N10-protected PBD carbinolamine intermediate (i.e., one that does not require a final reduction step) rather than to a N10-protected dilactam precursor such as **5**. The five novel compounds prepared (**6b**, **8a–d**) all possess potent cytotoxicity, and their SAR indicate the importance of planar C2-substituents in potentiating biological activity.

Acknowledgements

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18. General Stille coupling procedure: The enol triflate **5** (700 mg scale) was heated at reflux in dry THF (under nitrogen) in the presence of $Pd(PPh_3)_4$ (0.05 equiv), lithium chloride (3 equiv) and the appropriate tin reagent (1.3 equiv) for 2.5–5 h. Removal of THF afforded the crude product, which was partitioned between dichloromethane and 10% aqueous ammonium hydroxide. The separated organic phase was washed with water and brine, and dried over magnesium sulfate. Excess dichloromethane was removed by rotary evaporation and the residue purified by flash column chromatography (silica gel, gradient elution: ethyl acetate/hexane) to give the final product.
19. **7d**: ^{1}H NMR (400 MHz) ($CDCl_3$): δ 7.35 (s, 2H), 7.27 (s, 1H), 7.23 (s, 1H), 6.37 (dd, J_1 = 1.7 Hz, J_2 = 3.2 Hz, 1H), 6.26 (d, J_1 = 3.2 Hz, 1H), 5.52 (d, J = 10 Hz, 1H), 4.67 (d, J = 10 Hz, 1H), 4.58 (dd, J_1 = 3.3 Hz, J_2 = 10.6 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86–3.74 (m, 2H), 3.69–3.63 (m, 1H), 3.05 (ddd, J_1 = 2.0 Hz, J_2 = 10.6 Hz, J_3 = 15.8 Hz, 1H), 0.95 (m, 2H), 0.00 (s, 9H); ^{13}C NMR (100 MHz) ($CDCl_3$): δ 169.5, 163.0, 153.4, 150.6, 148.7, 143.7, 135.0, 122.6, 122.4, 117.7, 112.7, 108.6, 107.2, 79.7, 68.51, 58.75, 57.5, 31.8, 19.7, 0.0; IR (cm^{-1}, thin film): 2952, 1689, 1640, 1606, 1518, 1453, 1430, 1358, 1276, 1248, 1209, 1102, 1069, 1010, 858, 836, 787, 757; MS (ES+): 471.2 ($M^{+\cdot}$+H, 100%), 353.1 (65%); $[\alpha]_D^{19}$ +175 (*c* 0.02, $CHCl_3$).
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21. **8d**: ^{1}H NMR (400 MHz) ($CDCl_3$): δ 7.89 (d, J = 4Hz, 1H), 7.54 (s, 1H), 7.42 (d, J = 1.4 Hz, 1H), 7.38 (s, 1H), 6.84 (s, 1H), 6.44 (dd, J_1 = 1.7 Hz, J_2 = 3.2 Hz, 1H), 6.28 (d, J = 3.2 Hz, 1H), 4.41 (m, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.50 (m, 1H), 3.35 (ddd, J_1 = 1.6 Hz, J_2 = 5.2 Hz, J_3 = 16.2 Hz, 1H); ^{13}C NMR (100 MHz) ($CDCl_3$): δ 163.8, 162.8, 153.4, 150.7, 149.3, 143.9, 141.8, 124.3, 120.5, 115.8, 113.1, 112.9, 111.3, 108.2, 57.7, 57.6, 55.2, 36.0; IR (cm^{-1}): 3117, 2936, 1626, 1601, 1559, 1508, 1450, 1426, 1377, 1344, 1265, 1215, 1102, 1072, 1039, 1007, 956, 914, 882, 778, 730; MS (ES+): 325.1 ($M^{+\cdot}$+H, 100%); $[\alpha]_D^{20}$ +457 (*c* 0.15, $CHCl_3$).
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