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A novel approach to the synthesis of cytotoxic C2–C3 unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) with conjugated acrylyl C2-substituents

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Abstract—A concise synthesis of three novel C2–C3 unsaturated pyrrolo[2,1-*c*][1,4]benzodiazepine analogues (**18**–**20**) containing conjugated acrylyl C2-substituents is reported that utilises Heck coupling to install the C2-acrylyl side chains. These analogues possess significant cytotoxicity according to the NCI 60-cell line screen with **18** surpassing anthramycin (**1**) in potency.

There is currently interest in the pyrrolobenzodiazepine (PBD) group of antitumour antibiotics not only for their inherent antitumour activity, but also because of their ability to recognise GC base pairs and their potential as components of larger molecules that can regulate gene expression.[1–4] SAR studies have revealed that the presence of a C2-substituent along with *exo* and/or *endo* unsaturation in the C-ring are important for optimal DNA binding and antitumour activity.[5] Crucially, most published synthetic pathways to C-ring *endo*/*exo* unsaturated PBDs rely on the presence of a 9-hydroxy substituent in the A-ring (e.g. anthramycin, **1**, Fig. 1). This allows the formation of an O9–N10 benzal-protected dilactam intermediate of type **2** which can then be reduced to the target PBD containing the DNA-interactive electrophilic N10–C11 imine moiety.[6] However, it is now accepted that the presence of a 9-hydroxy group is therapeutically undesirable as it leads to acute cardiotoxicity.[7] Thus, a concise synthetic approach was required to allow the introduction of unsaturated C2-substituents to C2–C3 unsaturated *des*-9-hydroxy PBD targets.

Mori and co-workers have reported the synthesis of C2-*exo*-unsaturated PBD natural products such as tomaymycin via N10-MOM-protected dilactams of type **3**.[8] More recently we demonstrated that C2-aryl-substituted PBDs with C2–C3 *endo*-unsaturation could be prepared from N10-SEM-protected dilactam intermediates of type **4**.[9] However, in our hands, attempts to reduce C2-*N*,*N*′-dimethylacrylamide dilactams of type **5** to *endo*/*exo*-unsaturated PBDs with intact N10–C11 moieties have been unsuccessful.[10,11] Therefore, we have developed a strategy whereby the desired unsaturated C2 substituents are added by a Heck coupling reaction to an N10-protected PBD C11-carbinolamine, thus avoiding the need to reduce a dilactam intermediate.[12] This approach also has the advantage of directly introducing the complete C2-side chain thus avoiding the multiple functional group interconversions employed by Fukuyama[13] and Langlois[5] in their syntheses of porothramycin B (**6**), and by Leimgruber[6] in his synthesis of anthramycin (**1**).

The first task was to prepare a PBD C2–C3 enol triflate capable of participating in a Heck coupling reaction. The synthesis (Scheme 1) commenced with the coupling of an *o*-nitrobenzoic acid (**7**) to a TBS-protected C-ring fragment[14] employing oxalyl chloride/DMF to give **8**. The nitro group was then reduced with Raney nickel/hydrazine, and the resulting aniline (**9**) protected as a Troc carbamate (**10**). The Troc group was chosen for its compatibility with both palladium coupling chemistry and PBD N10–C11 imine formation.[15] The pro-C2-hydroxy group in **10** was oxidized to the ketone **11** under Swern conditions in excellent yield (99%). Removal of the TBS group in high yield (93%) provided

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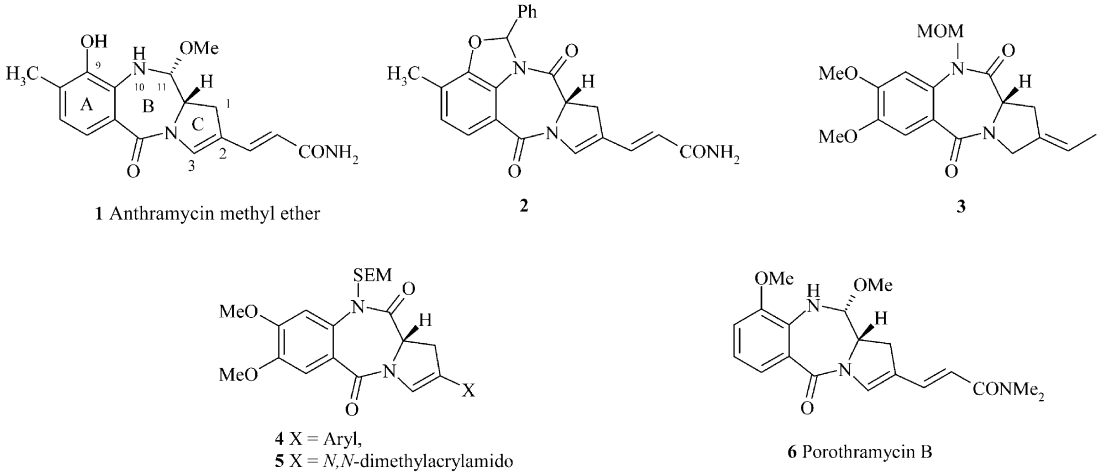
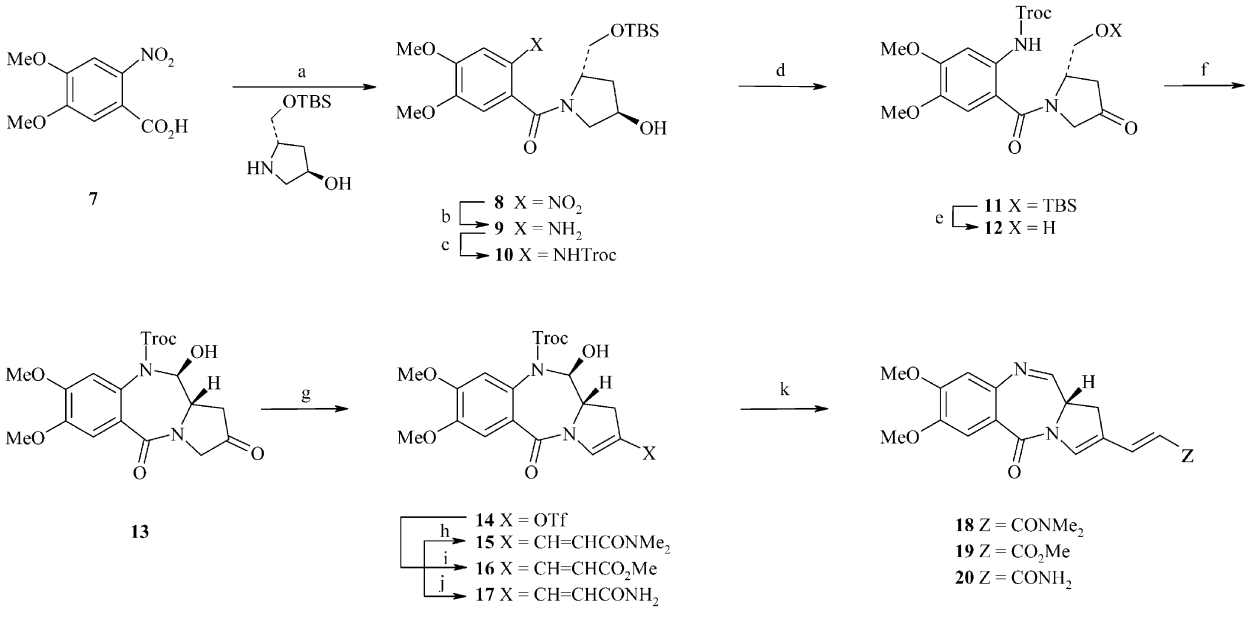


Figure 1. Some PBD natural products (**1**,**6**) and their synthetic precursors (**2**–**5**).



Scheme 1. (a) $(COCl)_2$, DMF, TEA, CH_2Cl_2, 55%; (b) Raney nickel, hydrazine, MeOH, 98%; (c) Troc–Cl, pyridine, DCM, 86%; (d) $(COCl)_2$, DMSO, TEA, DCM, −60 °C, 99%; (e) AcOH, H_2O, THF, 93%; (f) $(COCl)_2$, DMSO, TEA, DCM, −60 °C, 55%; (g) Tf_2O, pyridine, DCM, 30%; (h) $CH_2=CHCONMe_2$, DABCO, $(CH_3CN)_2Pd(II)Cl_2$, MeOH, 45–55 °C, 59%; (i) $CH_2=CHCOOMe$, $(PPh_3)_2Pd(II)Cl_2$, TEA, DMF, 75–78 °C, 10%; (j) $CH_2=CHCONH_2$, DABCO, $(CH_3CN)_2Pd(II)Cl_2$, MeOH, 45 °C, 48%; (k) 10% Cd–Pb, THF, NH_4OAc, 34% (**18**), 90% (**19**), 36% (**20**).

12 which could be cyclized to form the PBD B-ring (**13**) through Swern oxidation. The cyclized ketone **13** was treated with trifluoromethanesulphonic anhydride to afford the C2–C3 enol triflate **14** in 30% yield. This disappointing yield was due in part to a competing aldol reaction which lead to the formation of a C–C linked PBD dimer by-product. The triflate **14** was coupled to dimethyl acrylamide, methyl acrylate and acrylamide under Heck conditions to afford three N10-Troc-protected PBDs with unoptimised yields of 59% (**15**), 10% (**16**) and 48% (**17**), respectively. Removal of the Troc protecting group with Cd/Pb-couple afforded the target PBDs (**18**–**20**) in modest to good yields (34–90%).

Compounds **18**–**20** were evaluated in the NCI 60-cell line screen. Compared to the known cytotoxic agent anthramycin (**1**) which has an average GI_{50} value of 29.2 nM across the 60 cell lines, these analogues possessed potent cytotoxicity with average GI_{50} values of 12.0 nM (**18**), 12.6 nM (**19**) and 52.5 nM (**20**). Table 1 provides a summary of GI_{50}, TGI and LC_{50} data. In particular, compound **18** which possesses a *N,N*-dimethyl acrylamide C2-side chain is significantly more potent than anthramycin across all of these endpoints. It is remarkably cytotoxic towards certain cell lines (e.g., sub-nanomolar in CCRF-CEM and UACC-257), and a selection of these results in comparison to anthramycin

Table 1. Average GI_{50}, TGI and LC_{50} values for compounds **18**, **19** and **20** across the 60 cell lines of the NCI screen. Data for anthramycin methyl ether (**1**) are provided for comparison

Compd	GI_{50} (μM)[a]	TGI (μM)[b]	LC_{50} (μM)[c]
Anthramycin	0.029	0.61	12.7
18	0.012	0.096	0.65
19	0.013	1.32	55.0
20	0.053	3.72	69.2

[a] GI_{50}: Dose that inhibits 50% of cell growth.
[b] TGI: Dose that inhibits 100% of cell growth.
[c] LC_{50}: Dose that kills 50% of cells.

Table 2. Cytotoxicity data for **18** in selected sensitive cell lines from the NCI 60-cell line screen. Data for anthramycin methyl ether (**1**) are included for comparison

Tumour type	Cell Lines	GI_{50} (nM)[a]	
		Anthramycin	**18**
Leukaemia	CCRF-CEM	20.0	0.60
Non-small cell lung	HOP-92	20.0	2.34
Colon	HCT-116	39.8	11.7
Central nervous system	U251	20.0	3.33
Melanoma	UACC-257	63.1	0.25
Ovarian	OVCAR-5	50.1	3.21
Renal	RXF 393	31.6	1.65
Prostate	PC-3	20.0	18.6
Breast	MCF7	39.8	7.15

[a] GI_{50}: Dose that inhibits 50% of cell growth.

is shown in Table 2. It is noteworthy that the *des*-9-hydroxy analogue of anthramycin (**20**) retains a significant degree of the potency of the parent compound, thus indicating that cytotoxicity is essentially independent of the problematic 9-OH moiety.

In summary, the successful synthesis of the three novel PBDs **18–20** demonstrates the versatility of this new convergent route which should be applicable to a range of *des*-9-hydroxy C2-*endo*/*exo*-unsaturated PBDs otherwise difficult to access. The route is short compared to those previously reported for C-ring *endo*-*exo*-unsaturated PBDs. For example, Leimgruber employed six synthetic steps to elaborate the C2 side chain in his pioneering synthesis of anthramycin.[6] A further advantage is that the Heck approach avoids stereoselectivity issues potentially arising with alternative methods employing olefination reactions. The low yield of the triflation reaction was disappointing and efforts are currently underway to improve the efficiency of this critical step. Compounds **18–20** are presently undergoing in vivo evaluation and the results of these studies will be reported elsewhere.

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