# The Synthesis of Functionalized Pyrrolo-[2,1-*c*][1,4]-Benzodiazepines

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**Abstract:** Two concise and high yielding routes to the antitumour antibiotic pyrrolo-[2,1-*c*][1,4]-benzodiazepine ring system are described. Thus, condensation of prolinol with 2-azidobenzoylchloride gives the tertiary amide (3). Oxidation to the aldehyde (4) followed by generation of the phosphoroimine by Staudinger reaction results in ring closure *via* an aza-Wittig reaction to yield the desired ring system. Alternatively, coupling of prolinol with the appropriate isatoic anhydride yields the corresponding amino alcohol. Oxidation with Dess-Martin periodinane yields the PBDs in moderate to good yield.

The pyrrolo-[2,1-*c*][1,4]-benzodiazepine (PBD) ring system is found in a number of natural products that can recognize and bind to specific sequences of DNA. Such compounds have potential as therapeutic agents in the treatment of certain genetic disorders including some cancers[1]. They also have potential as affinity cleavage reagents in molecular biology. The family of natural products, collectively known as the anthramycins, includes compounds such as anthramycin, prothracarcin and porothramycin (figure 1). This area has recently been the subject of an excellent review[2].

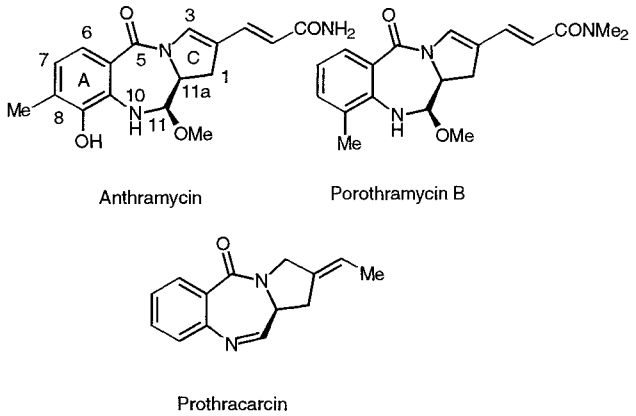
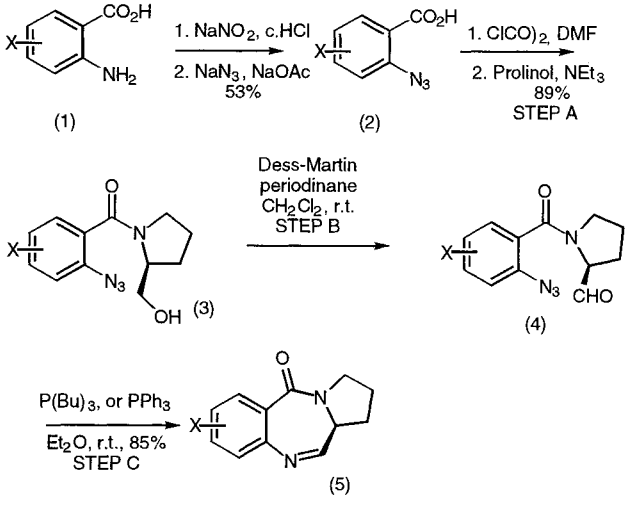



**Figure 1**

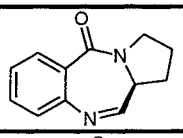
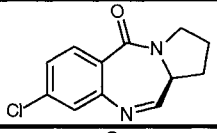
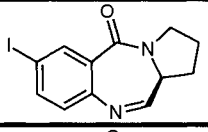
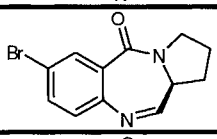
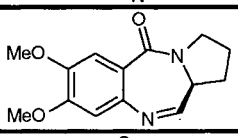
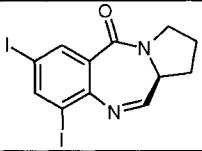
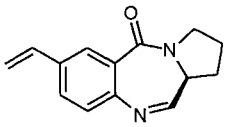
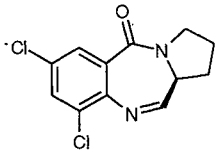
The application and exploitation of PBDs has been hampered by the lack of a short, high yielding route to the parent ring system and modified analogues. The major problems encountered in the synthesis of PBDs is the installation of the sensitive N10-C11 imine or carbinolamine functionality and racemization at the C11a position. It is known that the C11a configuration is essential to the ability of PBDs to bind to the minor groove of DNA. Recent publications prompt us to disclose our results in this area[3].

We were interested in preparing compounds which possessed novel functionality on the aromatic A ring. In particular, the incorporation of halogens would open up the possibility of utilizing palladium chemistry in the preparation of compounds bearing additional minor groove binding sequences. We chose to leave the installation of the N10-C11 imine to the final step and elected to use a Staudinger reaction to generate a phosphoroimine followed by an intramolecular aza-Wittig reaction to effect this transformation[4]. This approach has recently been reported by Molina and Eguchi independently[3]. The synthetic sequence for X = H is outlined in scheme 1. Thus, treatment of anthranilic acid (1) with sodium nitrite and conc. HCl followed by addition of buffered sodium azide yielded the 2-azidobenzoic acid (2) in 53% yield[5]. Conversion of (2) to the acid chloride by treatment of the acid with oxalyl chloride and catalytic DMF,[6] followed by addition of prolinol gave the amidoazide (3) in excellent overall yield (step A). The use of thionyl chloride to generate the acid chloride as reported by Molina gave considerably reduced yields of the coupled product. Molina had then utilized PCC to convert the alcohol (3) to the aldehyde (4). However in our hands the yields were low, particularly with halogen substituents on the A ring. A variety of different oxidants were investigated, including, PDC[8], Swern[10] and TPAP[11], although again none proved satisfactory. We then found use of the Dess-Martin periodinane[7] effected conversion of the primary alcohol (3) to the aldehyde (4) in excellent yield and without detectable racemization (step B). Finally, treatment of aldehyde (4) with either tributylphosphine or triphenylphosphine generated the phosphoroimine which underwent an intramolecular aza-Wittig cyclization to give the desired PBD (5) in 85% yield (step C). The only other product in the reaction was the phosphine oxide which was readily separated by flash chromatography[12]. We have also been able to convert the azido-aldehyde (4) to the imine (5) in 50% yield by heating with tributyltin hydride and AIBN in toluene. The use of a phosphine was preferred due to the lower cost and toxicity of this reagent system.




**Scheme 1**

**Table 1.** The preparation of A ring modified pyrrolo-[1,4]-benzodiazepines

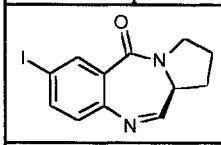
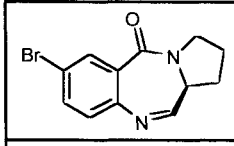
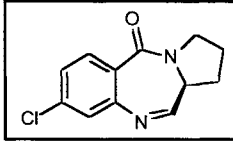
| Entry | % yield step A | % Yield step B | % Yield step C | Product |
|---|---|---|---|---|
| 1 | 90 | 81 | 85 |  |
| 2 | 71 | 100 | 55 |  |
| 3 | 91 | 90 | 57 |  |
| 4 | 40 | 65 | 69 |  |
| 5 | 51 | 56 | 90 |  |
| 6 | 75 | 65 | 72 |  |
| 7 | 91 | 45 | 80 |  |
| 8 | 72 | 61 | 58 |  |

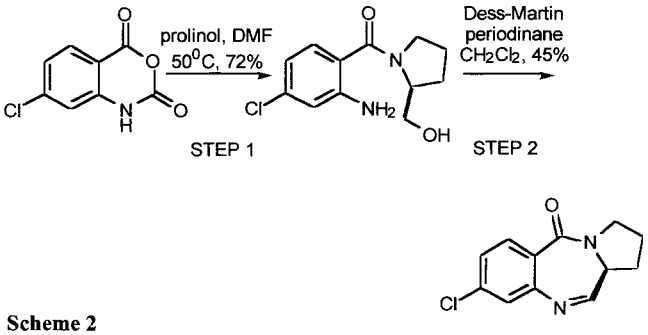
We have applied the above synthetic sequence to a number of anthranilic acid derivatives which possess substituents on the aromatic A ring. The results are summarized in table 1 .

The iodo substituted series of intermediates proved particularly valuable as they could be converted into PBDs with carbon substituents on the A ring. Thus, coupling of (*2S*)-*N*-(2-azido-5-iodobenzoyl)hydroxymethylpyrrolidine with vinyltributyltin, in the presence of Pd(0), gave the corresponding azido alcohol in excellent yield. Oxidation with periodinane and Staudinger/aza-Wittig ring closure furnished the PBD (entry 7, table 1). Selected novel PBDs, entries 2–4 in Table 1, were screened for *in vitro* cytotoxicity ($IC_{50}$) against two human A2780 ovarian carcinoma cell lines.[13,14] The results (table 2) show a clear rank order for activity: 7-bromo > 7-iodo >> 8-chloro, with the bromo derivative having significant cytotoxic potency. Interestingly, the three PBD monomers retain partial or complete activity towards cisplatin-resistant A2780cisR cells, where this cell line has acquired ~11-fold resistance toward this clinical antitumour agent.

Since the Dess-Martin periodinane reagent was found to be far superior to any of the other oxidants in the above synthetic route, we decided to investigate this reagent in the direct preparation of the PBD ring system from an amino-alcohol precursor. This approach has been investigated before[2], however, over-oxidation of the initial amino aldehyde (via the hemiaminal) to the amide has prevented this route being used synthetically. The amino alcohols are readily available from the condensation of prolinol with the appropriate isatoic anhydride (scheme 2). This would eliminate the need to prepare the azido alcohol and to carry out the Staudinger/aza-Wittig cyclization, considerably shortening the synthetic sequence.

**Table 2.** *In vitro* cytotoxicity of selected novel PBDs

| Compound | Cytotoxicity IC 50 (μM) A2780 | Cytotoxicity IC 50 (μM) A2780 cisR | Resistane factor |
|---|---|---|---|
|  | 2.9 | 13 | 4.5 |
|  | 0.54 | 1.65 | 3.1 |
|  | >100 | 76 | < 0.8 |



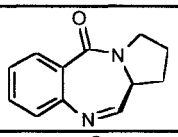
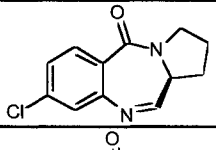
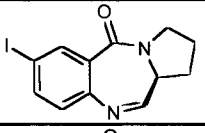
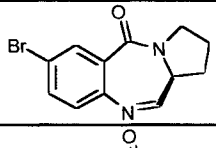
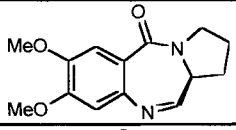
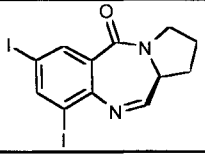
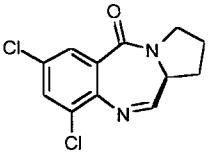

**Scheme 2**

Thus, treatment of 5-chloroisatoic anhydride with prolinol in DMF gave the amino alcohol in 72%. Oxidation with 1 equivalent of the Dess-Martin periodinane gave the imine directly in 45%. Although the yield for oxidation reaction is modest, this is the first example of such an oxidative ring closure for the preparation of the PBD ring system. Since a wide range of isatoic anhydrides are readily available we examined this novel approach further. The results are summarized in table 3.

Coupling of prolinol with the appropriate isatoic anhydride proceeded smoothly in all cases. Oxidation of amino-alcohols gave the corresponding PBD directly. The only case where this reaction failed was the dimethoxy amino-alcohol (entry 5). In general the yields for this oxidative cyclization are modest to good, the conditions are mild and coupled with the brevity of this sequence represents a novel and highly expedient route to PBDs. We are currently investigating the scope of this reaction with related systems.

These rapid and concise approaches to functionalized PBDs should allow the practical synthesis of a number of analogues which show enhanced specificity for binding to DNA and potentially improved anticancer activity. In conclusion we have shown that the Dess Martin periodinane is a superior reagent for the oxidation step in the Staudinger/aza-Wittig approach to PBDs and that it effects a novel oxidative cyclization of appropriate amino alcohols to PBDs.

**Table 3.** The preparation of A ring modified pyrrolo-[1,4]-benzodiazepines by the oxidation of amino alcohols with Dess-Martin periodinane

| Entry | % yield step 1 | % Yield step 2 | Product |
|---|---|---|---|
| 1 | 81 | 39 |  |
| 2 | 72 | 45 |  |
| 3 | 65 | 53 |  |
| 4 | 62 | 30 |  |
| 5 | 85 | 0 |  |
| 6 | 60 | 84 |  |
| 7 | 63 | 93 |  |

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**References and Notes**

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Eguchi, S.; Yamashita, K.; Matsushita, Y.; Kakehi, A. *J.Org. Chem.*, **1995**, 60, 4006.
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12. Preparation of (2*S*)-*N*-(2-azido-5- iodobenzoyl) pyrrolidine-carboxaldehyde:

To a solution of the iodo-alcohol (0.2g, 0.54 mmol) in $CH_2Cl_2$ (8 ml.) under an atmosphere of $N_2$ was added a suspension of Dess-Martin periodinane (0.51g, 0.81 mmol) in $CH_2Cl_2$ (5 ml.) at room temperature and the reaction mixture was stirred for 1 hour. The reaction mixture was washed with 1:1 sat. $NaHCO_3$:10% aq. $Na_2S_2O_4$, water and brine. The crude product was purified by flash chromatography (EtOAc) to give the title compound (0.16g, 80%) as a pale yellow oil, $[\alpha]_D$ = - 86.5 (c = 0.32 in MeOH); $\upsilon_{max}$. (film) 2129, 1732 and 1629 $cm^{-1}$. $\delta_H$ (300MHz, $CDCl_3$) 1.93 (1H, m, ring $CH_2$), 2.12 (3H, m, ring $CH_2$), 3.39 (8/5H, m, ring $CH_2$), 3.80 (2/5H, m, ring $CH_2$), 4.14 (1/5H, m, ring CH), 4.63 (4/5H, m, ring CH), 6.96 (1H, m, aromatic), 7.17 (2H, m, aromatic), 9.33 (1/5H, s, CHO) and 9.68 (4/5, s, CHO); m/z (EI) 370 ($M^+$, 1%), 342 ($M^+$ - $N_2$, 2%), 325 ($M^+$ - $N_2$ - OH, 6%), 313 ($M^+$ -$N_2$ - CHO, 100%).

Preparation of (11a,*S*)-8-iodo-1,2,3,11a-tetrahydro-*5H*-pyrrolo [1,4](2,1-c) benzodiazepin-5-one:

To a solution of the iodo-aldehyde (0.10g, 0.27 mmol) in $CH_2Cl_2$ (2 ml.) under an atmosphere of nitrogen at room temperature was added triphenylphosphine (0.08g, 0.30 mmol). The reaction was stirred at room temperature overnight and then concentrated in *vacuo*. The crude residue was purified by flash chromatography on silica gel (EtOAc) to give the title compound (0.05g, 57%) as a white solid, m.pt. 129-130°C; $[\alpha]_D$ = + 120 (c = 1.0 in DMF); $\upsilon_{max}$. (film) 3260, 1627, and 1607 $cm^{-1}$; $\delta_H$(200MHz, $CDCl_3$) 2.06 (2H, m, ring $CH_2$), 2.34 (2H, m, ring $CH_2$), 3.54 (1H, m, ring CH), 3.79 (2H, m, ring $CH_2$), 4.83 (1H, d, J = 9.0 Hz, carbinolamine 11S, H-11), 5.14 (1H, s, carbinolamine, 11R, H-11), 7.05 (1H, d, J = 8.4 Hz, aromatic), 7.60 (2H, m, aromatic) and 7.83 (1H, d, J = 4.4Hz, imine); $\delta_C$ (50MHz, $CDCl_3$) 164.6, 140.2, 139.07, 132.2, 132.0, 128.64, 128.37, 58.54, 46.81, 29.61 and 24.11; m/z (EI) 326 ($M^+$, 100%), 216 (2%), 202 (89%), 89 (4%) and 70 (38%).

Preparation of (2*S*)-*N*-(2-azido-5- vinylbenzoyl) hydroxy-methylpyrrolidine:

A solution of the iodide (0.10g, 0.26mmol), $Pd(PPh_3)_4$ (0.06g, 5 mol%), CuI (0.0015g, 2.5 mol%) and $Ph_3As$ (0.0085g, 10 mol%) in *N*methylpyrrolidinone (4.0 ml.) was degassed. To this was added vinyltributyltin (0.07 ml., 0.26 mmol) at room temperature. The reaction mixture was stirred overnight and then concentrated *in vacuo*. The resulting residue was washed with aqueous KF solution (1M), extracted with EtOAc and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product purified by flash chromatography on silica gel (EtOAc/pet. ether, 1/5) to give the title compound as a pale yellow oil, (0.06g, 85%); $[\alpha]_D$ = -152 (c = 1.05 in MeOH) $\upsilon_{max}$(film) 3384, 2121 and 1615 $cm^{-1}$. $\delta_H$ (200MHz, $CDCl_3$) 1.95 (3H, m, ring $CH_2$), 2.19 (1H, m, ring $CH_2$), 3.28 (2H, m, ring $CH_2$), 3.78 (2H, m, $CH_2OH$), 4.39 (1H, m, ring CH), 4.78 (1H, br.s, OH), 5.28 (1H, d, J = 10.9Hz, C*H*$CH_2$), 5.73 (1H, d, J = 17.6Hz, CHC*H*$_2$), 6.68 (1H, dd, J = 17.6, 10.9Hz, CHC*H*$_2$), 7.14 (1H, d, J = 8.4Hz, aromatic), 7.36 (1H, s, aromatic) and 7.46 (1H, d, J = 8.4Hz, aromatic); $\delta_C$ (50MHz, $CDCl_3$) 157.6, 135.1, 128.35, 125.54, 118.75, 114.85, 66.57, 61.31, 49.31, 49.56, 28.61, 24.51; m/z (EI) 272 ($M^+$, 2%), 244 (34%), 172 (15%), 116 (45%) and 102 (4%).

13. *In vitro* cytotoxicity assays:

Drug-induced cytotoxic activity toward human A2780 ovarian carcinoma cells and a derived cisplatin-resistant (A2780cisR) cell line was determined using the sulforhodamine B assay as detailed previously.[14] Cells were plated at between 0.5–1.0¥$10^4$ in 96-well microtitre plates and left overnight for cells to adhere prior to drug treatment. Aqueous PBD solutions at pH 7.0 were then added to the cells at various concentrations following dilution of a stock solution. After 96-h continuous drug exposure at 37°C, growth inhibition was assessed using sulforhodamine B protein staining. $IC_{50}$ values (drug dose required for 50% growth inhibition compared to drug-free controls) were determined by comparing treated with untreated wells. Resistance factors[14] are given by the $IC_{50}$ ratio (A2780cisR/A2780) for the resistant and parent cell lines.

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