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Design and Synthesis of Novel Pyrrolobenzodiazepine (PBD) Prodrugs for ADEPT and GDEPT

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Abstract—Three N10-(4-nitrobenzyl)carbamate-protected PBD prodrugs (**9a**, **9b** and **15**) have been synthesized and evaluated for potential use in nitroreductase-based ADEPT and GDEPT therapies. An approximately 100-fold activation was observed for the DC-81 prodrug **9a**.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) belong to a group of antitumour antibiotics that exert their biological activity through sequence-selective covalent bonding via their electrophilic N10-C11 imine functionality to the N2-position of guanine within the minor groove of DNA.[1] More recently, members of the PBD family such as DC-81 (**1**) and tomaymycin (**2**) have been used as templates for the design of PBD dimer analogues such as DSB-120 (**3a**)[2–5] and SJG-136 (**3b**)[6] which are among the most potent DNA interstrand cross-linking agents known to date (Fig. 1).

The ability to deliver such agents selectively to a tumour site could lead to an effective treatment for solid tumours. Antibody-directed enzyme prodrug therapy (ADEPT) is a two-stage therapeutic approach with the objective of selectively generating a cytotoxic agent from its non-toxic prodrug form at the tumour site.[7] A tumour-specific monoclonal antibody–enzyme conjugate is administered and time allowed for localization at the tumour site followed by subsequent clearance of the conjugate from healthy tissues. This is followed by administration of a non-toxic prodrug which is converted enzymatically to the active agent only at the tumour site thus avoiding collateral toxicity. Gene directed enzyme prodrug therapy (GDEPT) is a related approach but relies on the selective delivery to the tumour site of a gene coding for the relevant enzyme.[7]

Nitroreductase is an enzyme used in ADEPT and GDEPT that requires either NADH or NADPH to reduce aromatic nitro groups to hydroxylamines,[8] a process which can induce self-immolation of a 4-nitrobenzylcarbamate functionality. This observation led to the design and synthesis of the N10-(4-nitrobenzyl) carbamate-protected prodrugs of benzyl DC-81, benzyl tomaymycin and DSB-120 reported here (i.e. **9a**, **9b** and **15**). These molecules are unable to interact with DNA and do not exert their cytotoxic effect until the N10-protecting groups are removed to give **10a**, **10b** and **16**, respectively.

The N10-protected PBD molecules were synthesized as shown in Schemes 1 and 2 using a modified Fukuyama approach[9] involving TPAP oxidation to cyclize appropriately substituted pro-N10-4-(nitrobenzyl)carbamate-protected amino alcohols of type **8a**, **8b** and **14**. This route was found to have a number of advantages over previous approaches to PBD synthesis.[10] In the case of the monomer prodrugs (**9a** and **9b**), the previously reported[11] nitrobenzoic acid fragment (**4**) was coupled to the appropriate C-ring fragments **5a** or **5b** using oxalyl chloride in the presence of potassium carbonate or TEA to provide intermediates **6a** and **6b** (Scheme 1). The tomaymycin C-ring (**5b**) was produced by a variation of a method reported by Gregson.[12] Compound **6a** could be conveniently reduced to the amino alcohol **7a** using Raney Ni and hydrazine hydrate, however, **6c** (prepared

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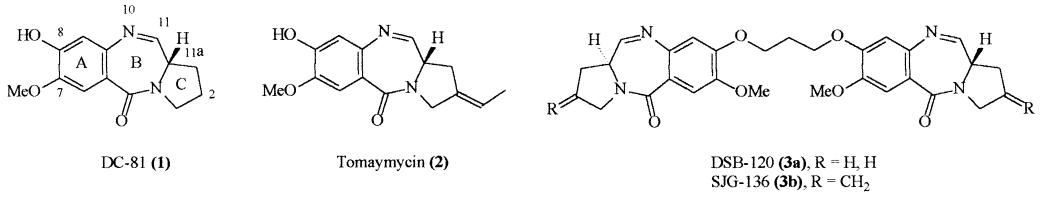
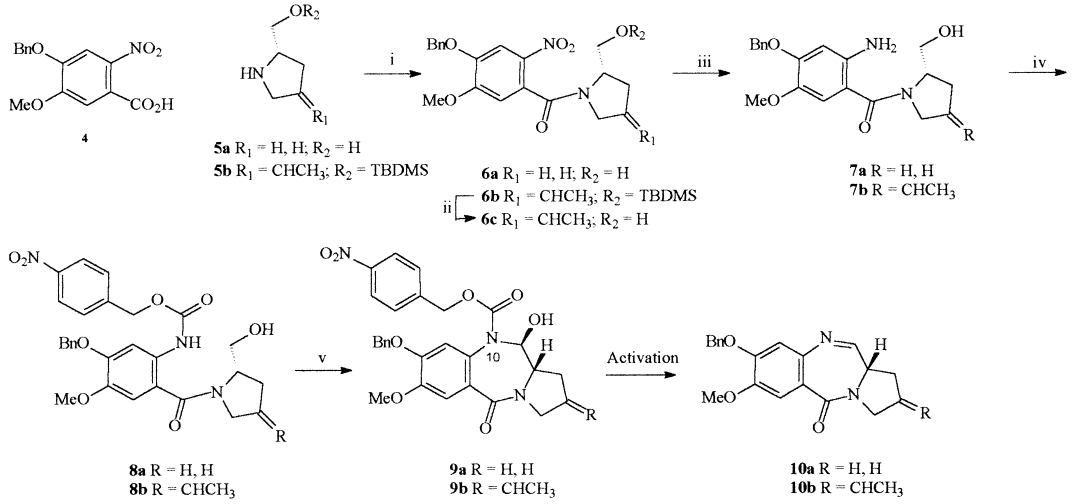
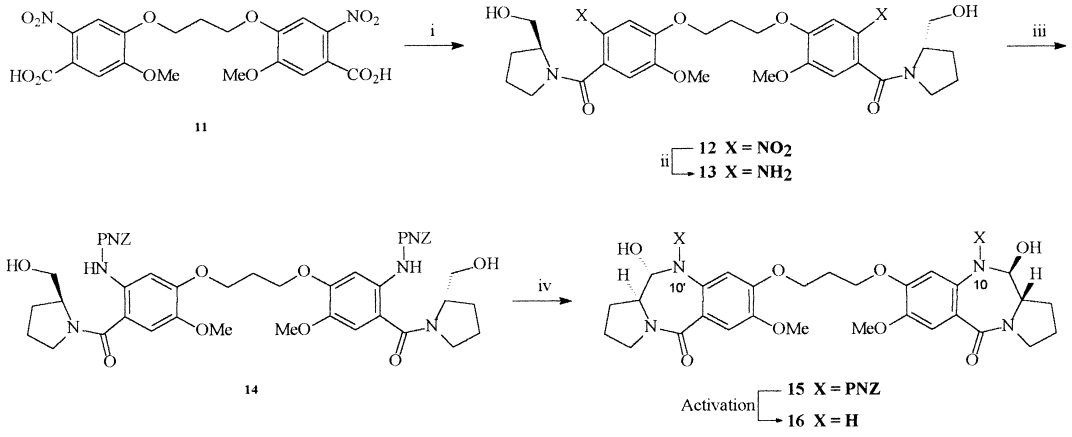


Figure 1. Structures of representative pyrrolobenzodiazepines.



Scheme 1. (i) $(COCl)_2$, DMF, CH_3CN, 24 h then **5a** or **5b**, K_2CO_3, CH_3CN, −25 °C, 88% for **6a**, 66% for **6b**; (ii) TBAF, THF, 0 °C, quant. for **6c**; (iii) Raney Ni, NH_2NH_2, MeOH, Δ, 81% for **7a**,; $Sn(II)Cl_2$, MeOH, Δ, 66% for **7b**; (iv) 4-nitrobenzyl chloroformate, pyridine, CH_2Cl_2, 0 °C, 77% for **8a**, 76% for **8b**; (v) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2:CH_3CN (3:1), 31% for **9a**, 45% for **9b**.



Scheme 2. (i) $(COCl)_2$, DMF, CH_3CN:THF (1:1), 24 h then **5a**, K_2CO_3, CH_3CN, −25 °C, 65%; (ii) Raney Ni, NH_2NH_2, MeOH, Δ, 70%; (iii) 4-nitrobenzyl chloroformate, pyridine, CH_2Cl_2, 0 °C, 81%; (iv) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2:CH_3CN (3:1), 42% (PNZ = *p*-nitrobenzyloxycarbonyl).

from **6b**) which contains unsaturation had to be reduced to **7b** with tin(II) chloride which gave a slightly lower yield. The amino alcohols **7a** and **7b** were treated with 4-nitrobenzyl chloroformate and pyridine to afford the pro-N10-protected intermediates (**8a**, **8b**) which were cyclized with TPAP/NMO to give **9a**[13] and an *E*/*Z* mixture of **9b**.

Similarly, the previously reported nitro dimer core[4] (**11**) was coupled to pyrrolidinemethanol to give the bis(nitro amide) **12** (Scheme 2). This was reduced (**13**), bis-*N*-protected (**14**) and oxidized to afford the bis(N10,N10′-[4-nitrobenzylcarbamate]) PBD dimer prodrug **15**.[14]

Prodrug **9a** was established to be non-toxic in the human adenocarcinoma cell-line LS174T (IC_{50} = >500 μM), whereas in the presence of nitroreductase and NADH co-factor the IC_{50} was found to be between 5.0 and 6.0 μM (Fig. 2 and Table 1), suggesting an activation factor of ~100-fold or greater. This appears to represent only partial release of the parent C8-*O*-benzyl-DC-81 (**10a**), as an authentic sample of **10a** was shown

Table 1. Cytotoxicity of PBD prodrugs before and after activation with nitroreductase/NADH in the LS174T cell line

Prodrug	IC_{50} (μM)[a] of prodrug in LS174T cells	IC_{50} (μM)[a] in presence of enzyme and NADH	Activation factor	IC_{50} (μM)[a] of parent PBD
9a	>500	5.0–6.0	100 (or >)	0.008
9b[b]	86.2	6.4	13.5	0.001–0.01
15	215.3	13.7	15.7	0.0005

[a]Dose of prodrug required to inhibit cell growth by 50% compared to drug-free controls.
[b]Evaluated as mixture of *E*/*Z* isomers.

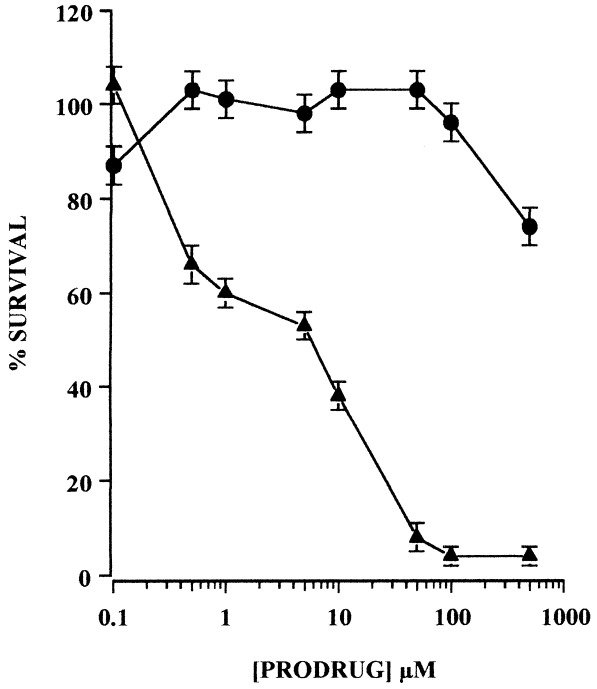


Figure 2. Cytotoxicity results for prodrug **9a** before and after activation with nitroreductase/NADH in LS174T cells. 2000 cells/well were treated with different concentrations of prodrug in the presence and absence of enzyme for 1 h. Cells were then washed and incubated for 3 days (-●- without enzyme; -▲- with enzyme).

to have an IC_{50} of 0.008 μM in the same cell line. Similarly, a 13.5-fold activation was observed for the benzyl tomaymycin prodrug *E*/*Z* mixture **9b** in LS174T cells after addition of the enzyme, again suggesting incomplete activation given that the IC_{50} of authentic benzyltomaymycin (**10b**) is 0.001–0.01 μM in the same cell line. However, it is possible that either the pure *E* or *Z* version of **9b** may have a higher activation value than the mixture, and this will be addressed in future studies. A similar result was observed for the dimer prodrug **15** which was relatively non-toxic towards LS174T cells (IC_{50} = 215.3 μM) but produced an IC_{50} value of 13.7 μM after the addition of enzyme, representing a >15-fold activation. The IC_{50} of authentic DSB-120 in the same cell line was found to be 0.0005 μM, suggesting a less efficient activation compared to the monomer analogue **9a**.

These results provide evidence that N10-protected analogues of PBD monomers and dimers have potential use as prodrugs in ADEPT- and GDEPT-type therapies. However, the finding that activation results in a lower cytotoxicity than equivalent concentrations of the authentic parent molecules suggests that only partial deprotection may be occurring. This could be due to a number of factors including sub-optimal activity of the enzyme or detrimental steric and/or electronic interactions between structural features of the prodrugs and the active site of the enzyme. Alternatively, the parent PBDs **10a**, **10b** and **16** may have enhanced cellular penetration in LS174T cells under the conditions of the experiment. Nevertheless, these results establish the principle that PBDs can be modified for use as prodrugs in ADEPT- or GDEPT-type therapies, and that an activation factor of at least 100-fold can be achieved. Based on this, the design and synthesis of a second generation of PBD prodrugs with potentially higher activation factors is currently underway.

Acknowledgements

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13. For **9a**: $[\alpha]_D^{20}$:−62.3 (c = 0.4497, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) (rotamers): δ = 8.11 (d, $2H_{arom}$, J = 8.43 Hz),

7.27–7.53 (m, $6H_{arom}$), 7.21 (d, $2H_{arom}$, $J=8.4$ Hz), 6.70 (s, $1H_{arom}$), 5.62 (d, 1H, $J=10.2$ Hz), 5.07 (d, 4H, $J=10.7$ Hz), 3.94 (s, 3H), 3.52–3.72 (m, 3H), 2.04–2.10 (m, 4H); ^{13}C NMR (68.7 MHz, $CDCl_3$): $\delta=166.8$, 149.3, 142.9, 136.0, 128.8–127.2, 126.4, 114.6, 112.5, 111.0, 105.9, 86.2, 71.1, 66.2, 59.9, 56.2, 47.3, 46.4, 28.7, 23.1; IR (neat): ν (cm^{-1}): 3600–3200, 2820–3000, 1710, 1600, 1510, 1450, 1430, 1400, 1370, 1350, 1305, 1270, 1220, 1120–1100, 1050, 1030, 1010; MS (FAB): (*m*/*z*, relative intensity) = 535 ($M^{+\cdot}+2$, 2), 353 (6), 337 (10), 286 (5), 256 (3), 241 (2), 228 (2), 192 (3), 136 (7), 91 (100); HRMS: calcd 533.1798; found 533.1813.

14. For **15**: ^{1}H NMR (270 MHz, $CDCl_3$) (rotamers): $\delta=8.18$ (d, 4H, $J=8.8$ Hz), 7.74 (s, 2H), 7.47 (d, 4H, $J=8.8$ Hz), 6.71 (s, 2H), 5.65 (d, 2H, $J=10$ Hz), 5.26 (s, 4H), 4.29 (t, 4H, $J=6$ Hz), 4.07–4.16 (m, 2H), 3.83 (s, 6H), 3.42–3.75 (m, 8H), 2.25–2.32 (m, 2H), 2.10–2.22 (m, 4H), 1.68–1.75 (m, 4H); ^{13}C NMR (68.7 MHz, $CDCl_3$): $\delta=166.9$, 153.2, 147.6, 143.8, 142.8, 128.2, 123.9, 113.9, 110.9, 108.3, 86.2, 69.1, 66.3, 65.6, 60.0, 56.4, 46.4, 29.7, 28.7, 23.1, 14.8; IR (neat): ν (cm^{-1}): 3500–3000, 2933, 2253, 1728, 1599, 1523, 1465, 1431, 1409, 1348, 1270, 1206, 1174, 1111, 1060; MS (FAB): (*m*/*z*, relative intensity) = 925 ($M^{+\cdot}-1$, 1), 889 (5), 711 (6), 501 (3), 286 (10), 252 (7), 213 (15), 192 (32), 197 (11), 185 (22), 181 (42), 165 (15), 149 (47), 131 (18), 119 (16), 105 (29), 91 (96), 73 (100), 57 (54).