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Synthesis and Biological Evaluation of an N10-Psec Substituted Pyrrolo[2,1-*c*][1,4]benzodiazepine Prodrug

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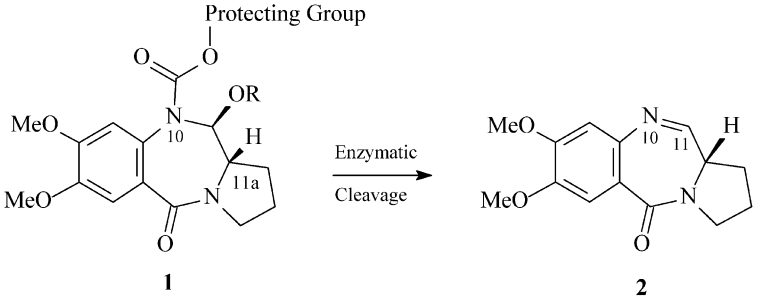
Abstract—The first example of an N10-protected (e.g., Psec, **15**) pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) analogue that retains significant cytotoxicity in a number of tumour cell lines is reported. This prototype could lead to a new generation of clinically useful N10-protected PBD prodrugs. © 2002 Published by Elsevier Science Ltd.

The pyrrolo[2,1-*c*][1,4]benzodiazepine antitumour agents are a family of biosynthetically derived tricyclic molecules produced by various *Streptomyces* species and include such members as DC-81 and anthramycin.[1] They bind selectively in the minor groove of DNA via a covalent aminal bond between the electrophilic C11-position of the PBD and the nucleophilic C2-amino group of a guanine base. This adduct formation is thought to lead to the observed biological activity.[1] The (*S*)-configuration at the chiral C11a-position provides the molecules with the necessary right-hand twist to fit snugly within the minor groove of DNA, spanning three base-pairs with a preference for 5′-Pu-G-Pu sequences.[1]

It has been shown previously that N10-protected PBDs (e.g., **1**, Scheme 1) are unable to interact with DNA and are devoid of cytotoxic activity.[2] However, they can act as prodrugs in that after enzymatic removal of the N10-protecting group, the free N10–C11 imine, carbinolamine or methyl ether forms (e.g., **2**) may interact with DNA and exert significant cytotoxicity against a range of cancer cell types.[2] This concept was exploited recently by Thurston and co-workers who developed N10-protected PBD prodrugs suitable for use in ADEPT- and GDEPT-type therapies.[2] These contained N10-[4-(nitrobenzyl)carbamate] protecting groups which could be enzymatically removed in the presence of nitroreductase and co-factor NADH to produce the parent cytotoxic PBD molecules.

Encouraged by the success of this general approach, we have now investigated the use of N10-protecting groups



Scheme 1. Activation of N10-protected PBDs (R = H or CH_3).

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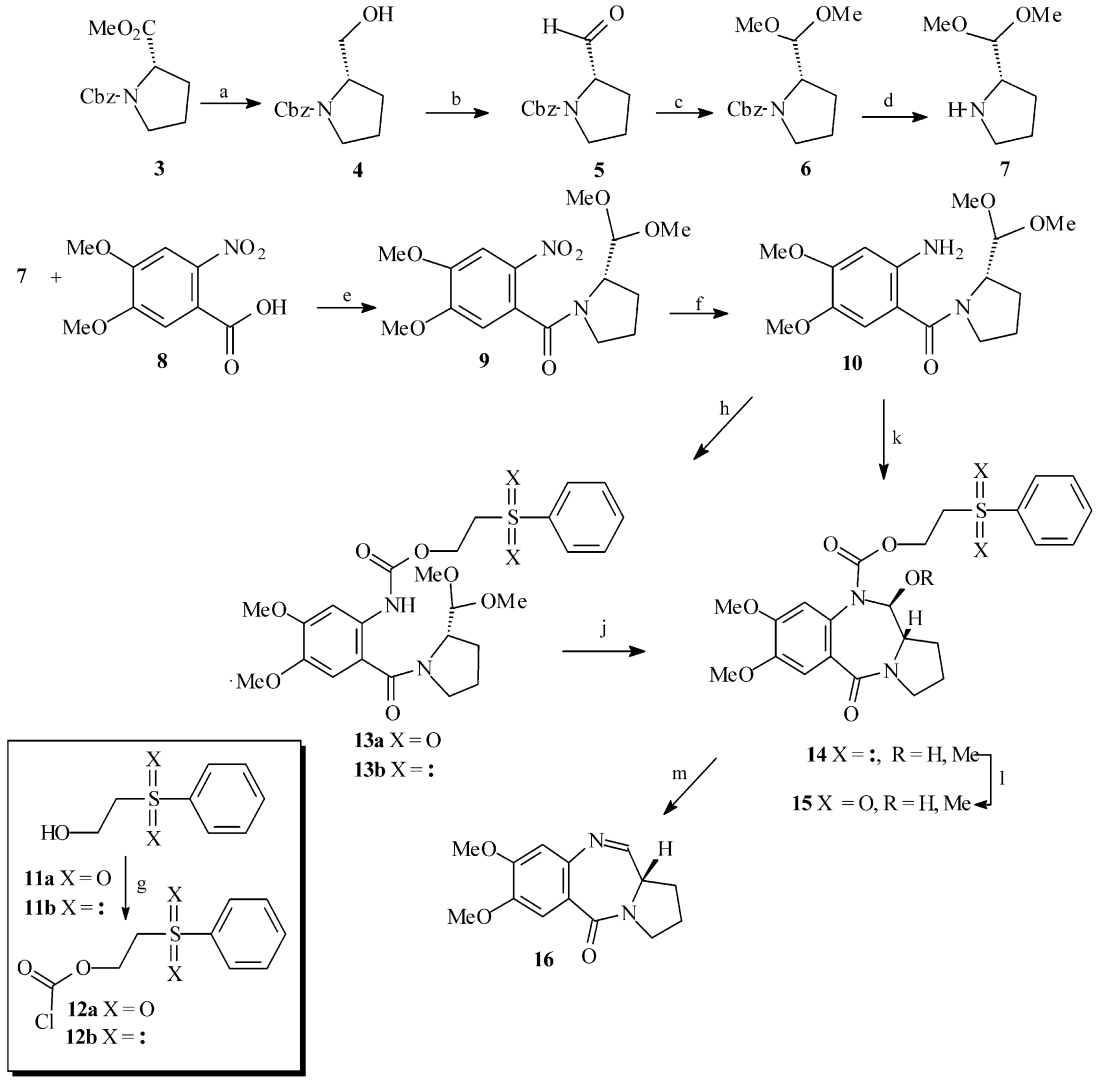
that are removable through alternative mechanisms. In particular, the 2-phenylsulphonylethyloxy (Psec) group has been used by Nicolaou and co-workers[3] as a triggering device for enediyne antitumour agents. Protected enediyne prodrugs can be converted into cytotoxic forms by in situ fragmentation of the Psec group through abstraction of the acidic protons at the α-position to the sulphonyl group. Enediynes fitted with the Psec triggering device display sub-nanomolar activity against promeocytic and T cell leukemia tumour lines. Similarly, Satyam and co-workers[4] have designed a phosphorodiamidate mustard analogue containing a 2-sulphonylethyloxycarbonyl linker which was found to be selectively cytotoxic in cells expressing glutathione transferase (GST). Molecular modelling studies suggested that a basic tyrosine phenoxide ion located in the active site of GST may aid deprotonation of the sulphonyl group, thus triggering fragmentation of the sulphonylethyloxycarbonyl linker to liberate the free mustard. Based on this approach, we designed the N10-Psec-protected PBD (**15**) along with the 2-phenylthioethyloxycarbonyl (Ptec)-protected control (**14**) which lacks the acidic sulphonyl protons, to test the potential of this triggering device in the PBD system.

The first stage of the synthesis was to produce C-ring acetal **7**, which was synthesised[5] in four steps from commercially available *N*-carbobenzyloxy-L-proline methyl ester **3** in 67% overall yield (Scheme 2). This involved reduction of the methyl ester to secondary alcohol **4** using lithium tetrahydridoborate in THF, followed by oxidation to aldehyde **5** using a 3-fold excess of SO_3·pyridine and triethylamine in $DMSO/CH_2Cl_2$ (4:5) at −10 °C. Acetal **6** was obtained by heating **5** with thionyl chloride and trimethyl orthoformate in methanol, followed by removal of the Cbz carbamate by hydrogenolysis to give **7**. This was joined to 4,5-dimethoxy-2-nitrobenzoic acid **8** by a standard coupling procedure using an equimolar ratio of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and diisopropylethylamine (DIPEA) in DMF to give **9** in 51% yield. Subsequent reduction of the nitro functionality to give aniline **10** was achieved using standard hydrogenolysis conditions.



Scheme 2. (a) $LiBH_4$, THF, 0 °C; (b) SO_3·pyridine, Et_3N, $DMSO/CH_2Cl_2$, −10 °C; (c) $SOCl_2$, $HC(OCH_3)_3$, MeOH, 60 °C; (d) (i) Raney nickel, EtOH or (ii) H_2, 10% Pd/C, EtOH; (e) TBTU, DIPEA, DMF; (f) H_2, 10% Pd/C, EtOH; (g) **11a/b**, triphosgene, pyridine, CH_2Cl_2, 0 °C; (h) **12a**, pyridine, CH_2Cl_2, 0 °C; (j) $(CH_3CN)_2PdCl_2$, Me_2CO; (k) **12b**, pyridine, CH_2Cl_2, 0 °C; (l) **14**, *m*-CPBA, CH_2Cl_2, 0 °C; (m) **15**, DBU, C_6H_6, 5 °C.

At this stage, an attempt was made to protect the amine as either the sulphone (**13a**) or sulphide (**13b**) carbamate, using the corresponding chloroformates (**12a**,**b**) which had been freshly prepared from 2-(phenylsulphonyl)ethanol (**11a**) or 2-(phenylthio)ethanol (**11b**) and triphosgene. However, on attempting to prepare the Ptec-protected PBD (**13b**) from **10** using this strategy, spontaneous cyclisation to the PBD sulphide **14**[6] occurred during the introduction of the Ptec group, and so **13b** could not be isolated. It is likely that HCl liberated during the protection reaction may have caused premature hydrolysis of the acetal leading to ring closure. Interestingly, this was not the case for the related Psec intermediate (**13a**), which could be isolated and then required acetal deprotection by treatment with trans-bis(acetonitrile)palladium(II) chloride[7] in acetone to give **15**.[8] Sulphone **15** could also be obtained in 87% yield by oxidation of sulphide **14** with 3-chloroperoxybenzoic acid (*m*-CPBA)[9] in CH_2Cl_2. The structure of **15** was confirmed by deprotection in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[9] in benzene to give PBD **16**[10] in 77% yield.

The sulphide (**14**), sulphone (**15**) and parent PBD (**16**) were evaluated for cytotoxicity in five ovarian cell lines, including the matched pairs A2780/A2780cisR and CH1/CH1cisR, and also SKOV-3 (Table 1). As anticipated, the Ptec-protected PBD (**14**) was essentially inactive in this panel (i.e., $IC_{50} = >25$ μM), as well as in the NCI's 60-cell line panel (data not shown). However, the N10-Psec prodrug **15** possessed significant sub-micromolar cytotoxicity in all cell lines examined. It had similar (e.g., A2780cisR and CH1cisR) or better (e.g., SKOV-3) activity compared to the parent PBD **16** in some cell lines, but was approximately 10-fold less active in A2780 and CH1. Sulphone **15** was also significantly active at the sub-micromolar level in a number of cell lines in the NCI's 60-panel screen (Table 2).

Table 1. Comparative cytotoxicity of **14**, **15** and **16** in five ovarian cell lines

Cell lines	Sulphide **14**[a]	Sulphone **15**[a]	PBD **16**[a]
A2780	>25	0.48	0.064
A2780cisR[b]	>25	0.49	0.155
RF[c]	NA	1	2.4
CH1	>25	0.4	0.082
CH1cisR[b]	>25	0.47	0.11
RF[c]	NA	1.2	1.3
SKOV-3	>25	0.56	1.7

[a]IC_{50} (μM), concentration required to inhibit cell growth by 50% on continuous exposure for 96 h.
[b]cisR denotes resistance to cisplatin.
[c]Resistance factor = cytotoxicity of cisR/normal cell line.

Table 2. In vitro cytotoxicity of **15** in a selection of NCI cell lines

Cell lines	GI_{50} (μM)[a]	LC_{50} (μM)[b]
Lung (NCI-H552)	0.11	1.38
Colon (Colo 205)	0.19	0.71
CNS (SNB-75)	0.72	9.00
Melanoma (SK-MEL-5)	0.29	6.78
Renal (RXF 393)	0.24	2.97
Breast (MDA-MB-435)	0.45	4.33

[a]GI_{50}, concentration required to restrict cell growth to 50% on continuous exposure for 48 h.
[b]LC_{50}, concentration required to decrease cell population by 50% on continuous exposure for 48 h.

In conclusion, this study has established that the N10-sulphone-protected PBD **15** possesses significant cytotoxicity in a number of cell lines, comparable or better in some cases (e.g., A2780cisR, CH1cis R and SKOV-3) to the parent unprotected PBD **16**. This represents the first example of inherent cytotoxicity in an N10-protected PBD. Although the precise mechanism of removal of the N10-Psec protecting group is presently unproven, the role of glutathione transferase is supported by the inactivity of the N10-Ptec PBD control **14**. Possible explanations for the greater cytotoxicity of **15** compared to **16** in SKOV-3 include favourable cellular penetration properties of **15** in this cell line and a higher cellular concentration of GST. Given the chemical interconvertibility of the N10-C11 imine, carbinolamine and carbinolamine methyl ether forms of a PBD, it is possible that the N10-Psec-protecting group may be useful in producing a single non-interconvertible PBD species for clinical use. Furthermore, compared to the PBD **16**, the similar activity of **15** in both parent and cisplatin-resistant cell lines suggests that N10-Psec protected PBDs may have potential use in drug resistant disease.

Acknowledgements

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6. Data for **14**: ^{1}H NMR (270 MHz, $CDCl_3$) where R = OH: δ 7.49–7.19 (m, 6H), 6.76 (s, 1H), 5.63 (d, 1H, J = 9.52 Hz), 4.38–4.33 (m, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.84–3.40 (m, 4H), 3.20–2.80 (m, 2H), 2.20–1.80 (m, 4H); ^{1}H NMR (400 MHz, $CDCl_3$) where R = OCH_3: δ 7.86–7.50 (m, 5H), 7.20 (s, 1H), 7.02 (s, 1H), 5.43 (d, 1H, J = 9.15 Hz), 4.66–4.63 (m, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93 (s, 3H), 3.60–3.20 (m, 8H), 2.20–1.90 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) where R = OH: δ 167.0, 156.0, 150.8, 148.4, 134.9, 129.9, 129.7, 129.1, 128.3, 126.7, 125.7, 112.6, 110.4, 86.1, 63.9, 59.9, 56.2,

46.4, 32.7, 28.7, 23.1, 14.1; ^{13}C NMR (100.5 MHz, $CDCl_3$) where R = OCH_3: δ 167.1, 155.5, 151.0, 148.7, 138.6, 134.1, 129.5, 128.9, 128.3, 127.9, 126.2, 113.4, 110.2, 93.3, 60.0, 58.6, 56.3, 54.7, 46.2, 28.9, 23.1, 14.2; MS (EI), *m*/*z* (relative intensity) 472 ($[M+1]^+$, R = OCH_3, 8), 458 ($[M+1]^+$, R = OH, 27), 336 (6), 304 (15), 292 (9), 278 (15), 275 (33), 260 (30), 250 (11), 245 (6), 223 (6), 206 (100), 192 (8), 180 (6), 164 (12), 154 (24), 150 (11), 137 (93), 123 (28), 109 (32), 70 (24).

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8. Data for **15**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.86–7.50 (m, 5H), 7.22 (s, 1H), 7.03 (s, 1H), 5.44 (d, 1H, J = 9.34 Hz), 4.69–4.65 (m, 1H), 4.11–3.94 (m, 9H), 3.80–3.40 (m, 5H), 3.22–3.16 (m, 1H), 2.05–1.78 (m, 4H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 167.4, 155.6, 151.2, 148.8, 138.6, 134.1, 129.5, 128.3, 128.1, 126.2, 113.3, 110.2, 93.3, 60.2, 58.6, 56.4, 54.7, 46.3, 28.9, 23.2, 21.1, 14.2; MS (EI), *m*/*z* (relative intensity) 504 ($[M]^+$, 36), 435 (64), 405 (17), 292 (8), 260 (100), 256 (7), 245 (21), 231 (14), 229 (14), 222 (18), 214 (9), 206 (20), 191 (24), 180 (8), 168 (20), 164 (29), 136 (28), 125 (64), 93 (12), 77 (53), 70 (16).

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10. Data for **16**: $[\alpha]_D^{23}$ +1481° (*c*1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.70 (d, 1H, J = 8.8 Hz), 7.43 (s, 1H), 6.85 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.90–3.48 (m, 3H), 2.40–2.29 (m, 2H), 2.12–2.05 (m, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 165, 163, 152, 148, 141, 121, 112, 110, 57, 55, 47, 30, 25.