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A facile intramolecular azido/amido reductive cyclization approach: synthesis of pyrrolobenzodiazepines and their dimers

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Abstract

A new synthetic pathway has been developed for the preparation of imine-containing pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) and their dimers. Selective reduction of aromatic azides as well as aliphatic amides in a single step leading to an intramolecular reductive cyclization process by employing $LiAlH_4$ or $LiBH_4$ provides the cyclized imines.

Keywords: Pyrrolo[2,1-*c*][1,4]benzodiazepines; Intramolecular reductive cyclization; Azides; Amides; $LiAlH_4$; $LiBH_4$

Recently, there has been growing interest in developing and discovering small nitrogen-containing heterocyclic compounds capable of binding to DNA in a highly sequence-selective manner.[1] The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of tricyclic, low molecular weight, naturally occurring DNA-interactive antitumour antibiotics isolated from various *Streptomyces* species. Well-known examples include anthramycin, chicamycin A, abbeymycin, DC-81 dimers and SJG-136 (Fig. 1). These compounds exert their cytotoxic potency by covalently binding between the electrophilic C11-position of the PBD B-ring and the C2-amino group of a guanine base in the minor groove of DNA[2] in a sequence specific fashion preferentially with Pu–G–Pu motifs.[2a] In recent developments, viable synthetic routes[3] have allowed many analogues of PBD monomers to be explored, including the joining of two DC-81 units together through their C8-positions using alkane spacers to create a new family of PBD dimers.[4,5] These bisfunctional-alkylating agents are capable of cross-linking DNA and one of the C8[6]-linked dimers, DSB-120 ($n = 1$), is a remarkably efficient interstrand DNA cross-linker, being approximately 300- and 50-fold more efficient than the clinically used cross-linking agents, melphalan and cisplatin, respectively. DSB-120 is highly cytotoxic in a number of murine and human cell lines with IC_{50} values as low as 0.5 nM. Another PBD dimer, SJG-136 shows markedly superior in vitro cytotoxic potency and interstrand DNA cross-linking reactivity and is presently under clinical evaluation.[7] Recently, a number of efficient solid-supported[8] as well as solid-phase[9] combinatorial synthetic approaches have been developed in this laboratory for the preparation of different types of PBDs.

There has been considerable effort from a number of research groups towards developing new synthetic routes for the preparation of the PBD ring system.[10] However, due to the difficulties associated with the synthesis of substantial quantities of these compounds, little has been achieved in structure–activity relationship (SAR) aspects.[11] Kaneko and co-workers[12] have developed a method for the reduction of PBD dilactams to the carbinolamine through iminothioether reduction using aluminium amalgam (Al–Hg). Similarly, Thurston and co-workers[13] developed

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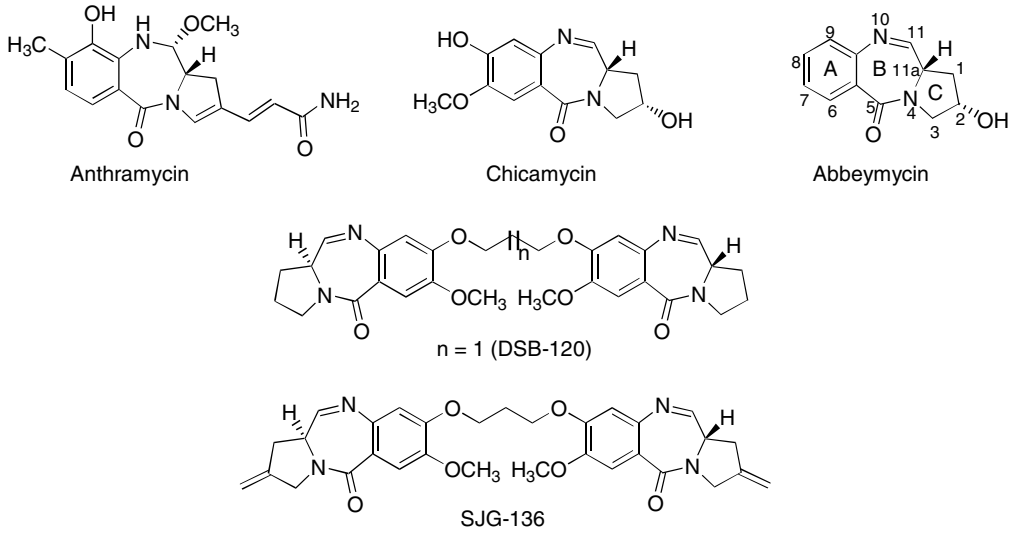
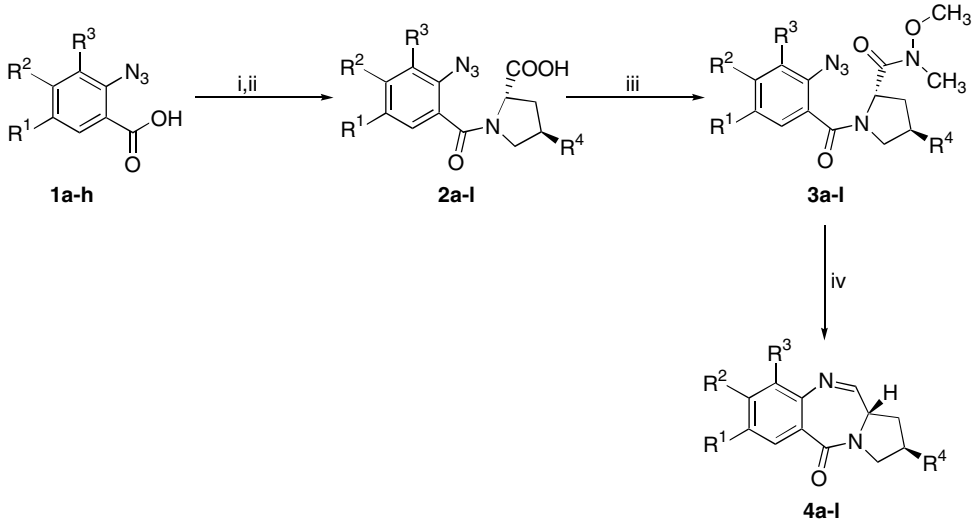


Fig. 1.

a deprotective-cyclization method employing $HgCl_2$ and $CaCO_3$, which has been employed quite successfully for the preparation of a variety of PBDs. However, some of these reported synthetic pathways involve a large number of steps, and expensive and hazardous reagents such as $SnCl_2$, $HgCl_2$ and thiols. In spite of the advantages of tin and mercury in synthetic organic chemistry, the presence of traces of these in the final products has proved to be disadvantageous towards most of the cell lines screened. Recently, some new methods for the synthesis of PBDs have also been developed in this laboratory, for example, bismuth triflate deprotective-cyclization[14] and azido-reductive cyclizations.[15] In continuation of these efforts, a versatile and inexpensive strategy has been developed for the reduction of aromatic azides and aliphatic amides in one-step leading to intramolecular reductive cyclization as a convenient route for the preparation of imine-containing PBDs.

Synthesis of substituted 2-azidobenzoic acids **1a**–**h** has been accomplished by using an established procedure,[16] which involves the reduction of commercially available 2-nitrobenzoic acids with $SnCl_2{\cdot}2H_2O$ followed by azidation with $NaNO_2$, HCl (aq), NaN_3 and NaOAc to afford the starting materials. These were then treated with oxalyl chloride and coupled to 2(*S*)-proline hydrochloride or 2(*S*),4(*R*)-hydroxy L-proline hydrochloride in the presence



Scheme 1. Reagents and conditions: (i) $(COCl)_2$, 1–3 drops DMF, CH_2Cl_2, 0 °C–rt, 2 h; (ii) Et_3N, L-proline hydrochloride or hydroxy L-proline hydrochloride, THF/H_2O, 0 °C–rt, 2 h, 75–80%; (iii) *N*,*O*-dimethylhydroxylamine hydrochloride, EDCI, NMM, CH_2Cl_2, −15 °C, 1 h, 80–85%; (iv) $LiAlH_4$, dry THF, −15 to −20 °C, 40–50 min or $LiBH_4$ dry THF 0 °C–rt, 120–140 min, 50–68%.

Table 1
Synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepine analogues **4a–l** through intramolecular (azido/amido) reductive cyclization employing $LiAlH_4$ or $LiBH_4$

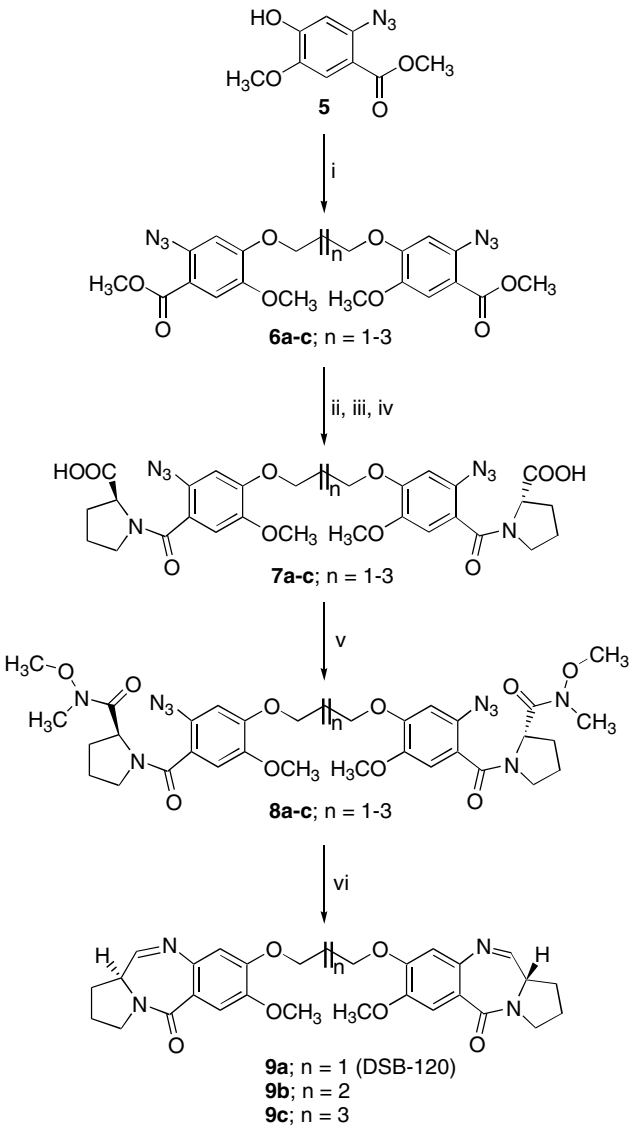
Product	R^1	R^2	R^3	R^4	$LiAlH_4$ Time (min)/yield[a] (%)	$LiBH_4$ Time (min)/yield[a] (%)	EIMS
4a	H	H	H	H	40/65	120/55	200
4b	Me	H	H	H	45/60	125/50	214
4c	Cl	H	H	H	40/62	130/60	235
4d	OMe	H	H	H	45/68	120/65	230
4e	H	H	OBn	H	45/60	125/52	306
4f	Br	H	Br	H	40/65	120/60	358
4g	OMe	OMe	H	H	50/58	135/58	260
4h	OMe	OBn	H	H	45/58	140/62	336
4i	H	H	H	OH	45/52	130/58	216
4j	H	Me	H	OH	40/56	135/56	230
4k	OMe	OMe	H	OH	50/52	130/50	276
4l	OMe	OBn	H	OH	50/58	140/68	352

[a] Isolated yields.

of Et_3N to give amides **2a–l** in excellent yields. The carboxylic acid groups of **2a–l** were protected with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of NMM (*N*-methylmorpholine) and EDCI (1-ethyl-3-(3-dimethylamino-propyl)carbodiimide) to afford the azidobenzoyl pyrrolidine Weinreb amides **3a–l**[17] in good yields. In this approach, apart from novelty and high yields, other significant advantages are simple work-up and ease of purification. The azide as well as amide functionalities were reduced with $LiAlH_4$ or $LiBH_4$ to provide the cyclized target compounds **4a–l**[18] as illustrated in Scheme 1 and Table 1. This deprotective-cyclization has been investigated under different conditions by varying the temperature and time. However, prolonged reaction times and higher temperatures led to a minor amount of the secondary amine form of the PBD being obtained (10–15%).

Further, this approach has also been extended towards the preparation of PBD dimers **9a–c**. The required precursor, methyl 2-azido-4-hydroxy-5-methoxybenzoate (**5**) was prepared by a reported method.[19] Upon dimerization with different alkane spacers in the presence of K_2CO_3 followed by hydrolysis with LiOH (2 N), the acid groups were treated with oxalyl chloride, and then coupled with 2(*S*)-proline hydrochloride in the presence of Et_3N to afford dimeric acids **7a–c**. The acid groups were then protected with *N*,*O*-dimethylhydroxylamine hydrochloride followed by reductive cyclization to give the target dimers **9a–c** as shown in Scheme 2.

In conclusion, a new versatile method has been developed for the synthesis of PBD monomers and their dimers. In this method we have utilized an intramolecular reductive cyclization approach for simultaneous reduction of azido and amido functionalities by employing $LiAlH_4$ or $LiBH_4$. This new synthetic pathway is highly amenable for the generation of libraries of pyrrolo[2,1-*c*][1,4]benzodiazepines and their dimers with diversity in both the A- and C-rings.



Scheme 2. Reagents and conditions: (i) K_2CO_3, dibromoalkane, dry DMF, rt, 12 h, 85%; (ii) LiOH (2 N) THF/MeOH/H_2O (3:1:1), rt, 4 h, 85–88%; (iii) $(COCl)_2$, 1–3 drops DMF, CH_2Cl_2, 0 °C–rt, 2 h; (iv) Et_3N, L-proline hydrochloride, THF/H_2O, 0 °C–rt, 2 h, 75%; (v) *N*,*O*-dimethylhydroxylamine hydrochloride, EDCI, NMM, CH_2Cl_2, −15 °C, 1 h, 85%; (vi) $LiAlH_4$, dry THF, −15 to −20 °C, 40–50 min or $LiBH_4$ dry THF 0 °C–rt, 120–140 min, 50–68%.

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17. *Typical procedure for the synthesis of compound* **3a**: The azidobenzoyl proline acid (**2a**, 276 mg, 1 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and cooled to −15 °C followed by the addition of *N*,*O*-dimethylhydroxylamine hydrochloride (107 mg, 1.1 mmol) and NMM (0.08 mL, 1.1 mmol). Then EDCI (210 mg, 1.1 mmol) was added portionwise as a solid over 30 min. The reaction mixture was stirred at the same temperature for 1 h and then ice-cold HCl (1 M, 5 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layer was washed with aqueous $NaHCO_3$ (3 × 30 mL) solution, dried over anhydrous Na_2SO_4, and evaporated under vacuum to give **3a** (Scheme 1) in good yield. Compounds **3a–l** and **8a–c** were synthesized in the same manner. Compound **3a** (FT-IR): cm^{-1} 2128; 1636; 1486; 1449; 1420; 1296; ^1H NMR (300 MHz, $CDCl_3$): δ 7.35–7.45 (m, 2H); 7.11–7.21 (m, 2H); 5.03–5.10 (m, 1H); 3.91 (s, 3H); 3.40–3.50 (m, 1H); 3.30–3.39 (m, 1H); 3.25 (s, 3H); 2.22–2.34 (m, 1H); 1.82–2.12 (m, 3H); ESI-MS: *m*/*z* 326 (M^++Na); HRMS calcd for $C_{14}H_{17}N_5O_3$ (M^+) 303.1949, found 303.1919. Compound **3e**: (FT-IR): cm^{-1} 2963; 2125; 1670; 1636; 1578; 1456; 1423; 1385; 1310; 1265; ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.42 (m, 5H); 7.06 (t, 1H, $J = 7.55$ Hz); 6.90–6.94 (m, 2H); 5.15 (s, 2H); 5.03–5.07 (m, 1H); 3.90 (s, 3H); 3.31–3.44 (m, 2H); 3.24 (s, 3H); 2.24–2.31 (m, 1H); 1.84–2.09 (m, 3H); LC-MSD: *m*/*z* 432 (M^++Na); HRMS calcd for $C_{21}H_{23}N_5O_4$ (M^+) 409.2739, found 409.2742. Compound **8a**: (FT-IR): cm^{-1} 2114; 1631; 1514; 1460; 1430; 1385; 1248; 1210; ^1H NMR (300 MHz, $CDCl_3$): δ 6.88 (s, 2H); 6.69 (s, 2H); 5.05–5.09 (m, 2H); 4.21–4.28 (m, 4H); 3.88 (s, 6H); 3.83 (s, 6H); 3.36–3.50 (m, 4H); 3.25 (s, 6H); 2.24–2.44 (m, 4H); 1.83–2.10 (m, 4H); 1.68–1.73 (m, 2H); ESI-MS: *m*/*z* 739 (M^++H); 761 (M^++Na); HRMS calcd for $C_{33}H_{42}N_{10}O_{10}$ (M^+) 738.4472, found 738.4450.
18. *Typical procedure* ($LiAlH_4$) *for compound* **4a**: To azide **3a** (200 mg, 0.66 mmol) in dry THF (15 mL) was added $LiAlH_4$ (52 mg, 1.45 mmol) portionwise over 5 min at −15 to −20 °C and the reaction stirred at the same temperature for 40 min. This was then quenched with ethyl acetate, then ice-cold water was added and the reaction mixture filtered through a sintered funnel. The combined layers were extracted with ethyl acetate (3 × 30 mL), the organic layers dried over anhydrous Na_2SO_4 and evaporated under vacuum to give product **4a**. This was purified by column chromatography (silica gel 100–200 mesh) using ethyl acetate/hexane (9:1) as eluent. Procedure for $LiBH_4$: Compound **3a** (200 mg, 0.66 mmol) in dry THF (15 mL) was treated with $LiBH_4$ (42 mg, 2.00 mmol) at 0 °C and the reaction stirred at room temperature for 130 min. Quenching with ice-cold water, filtration through a sintered funnel, extraction with ethyl acetate (3 × 30 mL), and purification by column chromatography gave **4a**. Both procedures were also applied for the preparation of PBD dimers by utilizing varying amounts (2 equiv) of pyrrolobenzodiazepine followed by purification by column chromatography (silica gel 100–200 mesh) using $CHCl_3$/MeOH (95:5) as eluent to produce **9a–c** as shown in Scheme 2. Compound **4a**: ^1H NMR (200 MHz, $CDCl_3$): δ 8.05 (d, 1H, $J = 7.43$ Hz); 7.79 (d, 1H, $J = 4.46$ Hz); 7.53 (t, 1H, $J = 6.69$ Hz); 7.28–7.38 (m, 2H); 3.36–3.94 (m, 3H); 2.26–2.38, (m, 2H); 2.02–2.16 (m, 2H); MS (EI): *m*/*z* 200 [M^+]; $[\alpha]_D^{26}$ +343 (*c* 0.4, $CHCl_3$); HRMS calcd for $C_{12}H_{12}N_2O$ (M^+) 200.1506, found 200.1513. Compound **4e**: ^1H NMR (200 MHz, $CDCl_3$): δ 7.64 (d, 1H, $J = 6.68$ Hz); 7.28–7.38 (m, 6H); 6.89–7.03 (m, 2H); 5.03–5.15 (m, 2H); 3.67–3.95 (m, 2H); 2.54–2.62 (m, 1H); 1.61–2.13 (m, 3H); LC-MSD: *m*/*z* 337 (m+CH_3O)$^+$; $[\alpha]_D^{26}$ +298 (*c* 0.4, $CHCl_3$); HRMS calcd for $C_{19}H_{18}N_2O_2$ (M^+) 306.2297, found 306.2301. Compound **9a**: ^1H NMR (200 MHz, $CDCl_3$): δ 7.57 (d, 2H, $J = 2.34$ Hz); 7.50 (s, 2H); 7.10 (s, 2H); 4.16–4.35 (m, 4H) 3.96 (s, 6H); 3.40–3.87 (m, 6H); 2.05–2.48 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.8, 28.6; 29.0; 31.8; 33.9; 46.5; 56.1; 65.3; 105.5; 108.0; 113.5; 148.1; 158.1; 160.3; 177.0; FABMS: *m*/*z* 533 (M^++H); HRMS calcd for $C_{29}H_{32}N_4O_6$ (M^+) 532.3587, found 532.3522. Compound **9b**: ^1H NMR (200 MHz, $CDCl_3$): δ 7.68 (d, 2H, $J = 4.68$ Hz); 7.50 (s, 2H); 6.82 (s, 2H); 4.10–4.20 (m, 4H) 3.93 (s, 6H); 3.51–3.84 (m, 4H); 2.27–2.38 (m, 4H); 2.06–2.10 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.6; 162.4; 150.6; 147.8; 140.6; 120.3; 111.6; 110.7; 65.4; 56.1; 53.7; 46.7; 29.6; 25.7; 24.2; FABMS: *m*/*z* 547 (M^++H); HRMS calcd for $C_{30}H_{34}N_4O_6$ (M^+) 546.3710, found 546.3737. Compound **9c**: ^1H NMR (200 MHz, $CDCl_3$): δ 7.67 (d, 2H, $J = 4.68$ Hz); 7.50 (s, 2H); 6.80 (s, 2H); 4.04–4.23 (m, 4H) 3.93 (s, 6H); 3.44–3.84 (m, 6H); 2.27–2.37 (m, 4H); 1.92–2.11 (m, 8H); 1.66 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.7; 162.4; 150.8; 147.8; 140.6; 120.1; 111.5; 110.4; 68.5; 56.2; 53.7; 46.7; 29.7; 28.6; 24.2; 22.5; FABMS: *m*/*z* 561 (M^++H); HRMS calcd for $C_{31}H_{36}N_4O_6$ (M^+) 560.3833, found 560.3812.
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