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An efficient solid-phase synthesis of biologically important DNA-interactive pyrrolo[2,1-*c*][1,4]benzodiazepine dimers (DSB-120) and their C2-fluorinated analogues

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Abstract—A facile method for the solid-phase synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepine dimers has been developed. *p*-Nitrophenyl carbonate Wang resin attached to 2-amino-5-methoxy-methyl benzoate has been utilized as the resin-bound starting material and these reactions are monitored by FT-IR spectroscopy of resin beads.

Solid-phase combinatorial chemistry has recently emerged as a useful tool for the generation of libraries of new molecules with different biological profiles.[1] In recent years, solid-phase heterocyclic chemistry has expanded rapidly, and numerous methods have been reported.[2] As part of our continuing effort to develop new heterocyclic solid-phase strategies for synthesizing nitrogen-rich heterocyclic compounds based on pyrrolobenzodiazepines, we required libraries of the dimers of pyrrolobenzodiazepines for lead generation against certain disease targets, particularly cancer.[3]

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD)s are a family of sequence-selective DNA-interactive antitumour antibiotics derived from *Streptomyces* species.[4] These compounds bind within the minor groove of DNA, forming a covalent aminal bond between the C11-position of the central B-ring and the N2-amino group of guanine base.[5,6] The cytotoxic and antitumour activity of PBDs are attributed to their ability to form covalent DNA adducts. Molecular modelling, solution NMR, fluorometry and DNA footprinting experiments have shown that these molecules have preferred selectivity for Pu–G–Pu sequences,[7,8] and can be oriented with their A-rings pointed either towards the 3′- or the 5′-end of the covalently bonded DNA strand. Many members of the PBD family such as DC-81 **1**, tomaymycin **2**, SJG-136 **3**, DSB-120 **4a** ($n = 1$) and C2-fluoro-substituted PBD dimers **4d–f** exert their biological activity through covalent binding via their guanine residue within the minor groove of DNA (Fig. 1). Some of these compounds have reached various stages of clinical trials but have not progressed due to problems including cardiotoxicity or lack of efficacy.[9]

A number of antiviral and antitumour agents have been developed in which a fluorine substituent has played a

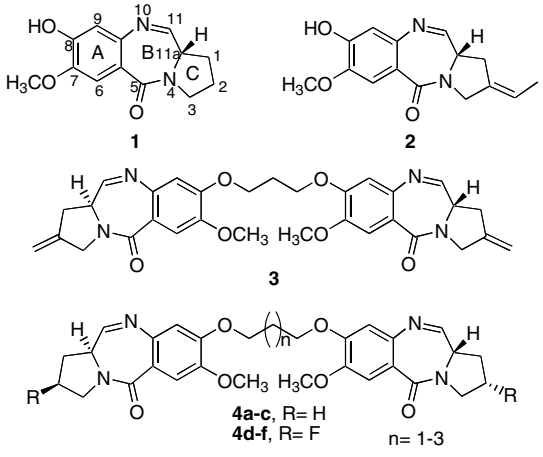

Figure 1.

Keywords: Solid-phase synthesis; Pyrrolo[2,1-*c*][1,4]benzodiazepine dimers; DSB-120; Swern oxidation.
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key role in their biological activity.[10] In earlier studies, attempts were made to enhance the sequence selectivity and antitumour potency by synthesizing C7- or C8-linked PBD dimers,[11,12] for example, DC-81 dimer (DSB-120), which is one of the most potent irreversible interstrand cross-linking agents. Recently, SJG-136 **3**, an analogue of DSB-120 was reported to be significantly more cytotoxic than DSB-120 across a number of cancer cell lines and this PBD dimer is presently under phase I clinical trials.[13] In continuation of our earlier efforts on the structural modifications of PBDs[14] and their dimers,[15] we were interested in the development of solid-phase methodologies, particularly for the PBD dimers.

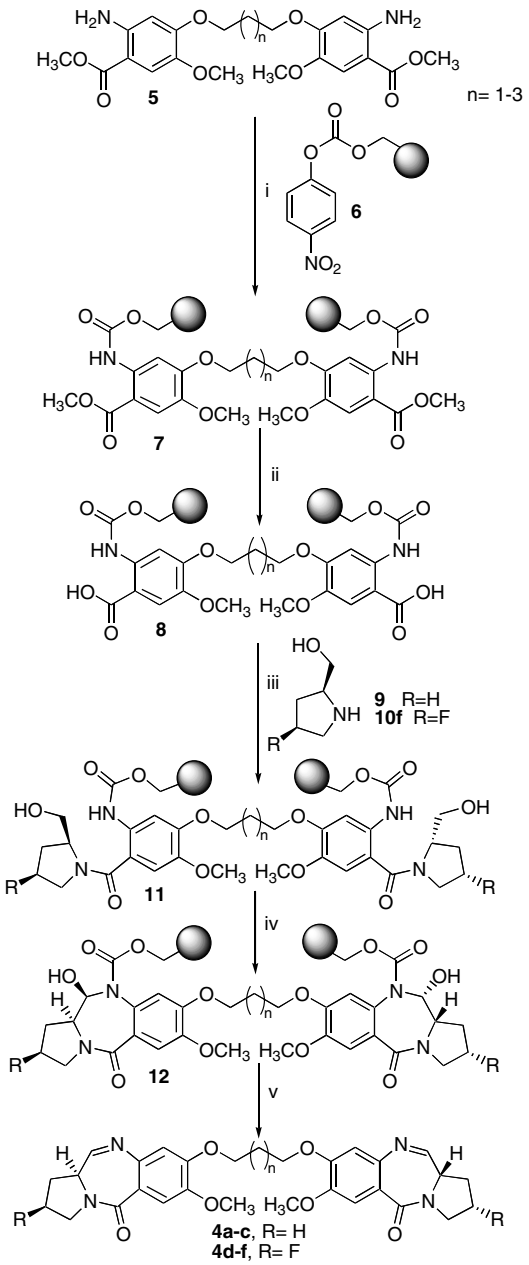
In this letter, we report for the first time, the solid-phase synthesis of PBD dimers such as DSB-120 and its C2-fluoro-substituted analogues. In our previous investigations we developed solid-phase procedures for the synthesis of DC-81 and other related PBD monomers in addition to several solution-phase methodologies.[16] It has been observed that in most solution-phase procedures for the preparation of PBD dimers there are problems relating to solubility. Therefore, the development of solid-phase protocols for PBD dimers is of immense importance as it could address the solubility difficulties encountered during work-up.

The starting material **5** was prepared by employing the procedure described in our earlier study,[17] which involved bis-coupling 2-nitro-4-hydroxy 5-methoxymethyl benzoate with a dihaloalkane and subsequent reduction of the nitro group. It is interesting to note from the literature that there are site–site interactions on using resins, which depend on the percentage amount of cross-linking polystyrene.[18] Usually, as the percentage of cross-linking decreases and as the functionalization increases, the site–site interactions also increase. In the present study, we have employed (1% DVB) polystyrene for the synthesis of the target compound based on pyrrolo[2,1-*c*][1,4]benzodiazepine dimers. A stirred solution of **5** was treated with *p*-nitrophenyl carbonate Wang resin **6** using 1-hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIPEA) in CH_2Cl_2–DMF (2:1) to give **7**. After loading, the unreacted free hydroxyl groups of the Wang resin were capped with acetic anhydride/triethylamine in dichloromethane. Hydrolysis of the methyl esters afforded the corresponding acid,[19] **8**, which was coupled with pyrrolidinemethanol **9** in the presence of EDCI and HOBt to provide **11**. Swern oxidation[20] of **11** then gave **12** via an oxidative cyclization process. However, Thurston et al. had reported a similar oxidation method using Dess–Martin periodinane for the preparation of PBD monomers.[21] Finally, the resin was cleaved[22] using TFA (50%) to afford the target product **4**[23] via loss of the hydroxyl groups and the products were formed in moderate yields as shown in Table 1. Similarly, C2-fluoro-substituted pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) dimers were also synthesized as shown in Scheme 1, employing fluoro-substituted pyrrolidinemethanol **10f**, which was itself prepared from hydroxy L-proline **10a** as shown in Scheme 2.

Table 1. Yields and molecular ions observed for PBD dimers **4a**–**f**

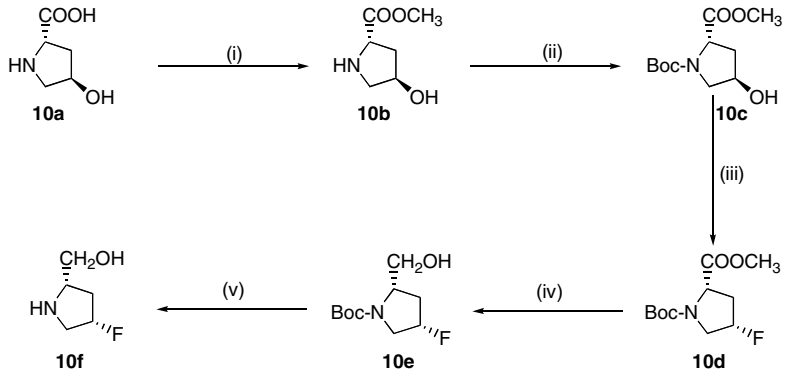
Entry	*n*	R	Yield (%)[a]	FABMS[b]
4a	1	H	62	533
4b	2	H	68	547
4c	3	H	63	561
4d	1	F	56	569
4e	2	F	58	583
4f	3	F	55	597

[a] Based on initial loading of the *p*-nitrophenyl carbonate Wang resin.
[b] Parent ion observed as $(M+H)^+$.



Scheme 1. Reactions and conditions: (i) HOBt, DIPEA, CH_2Cl_2/DMF (2:1) 6 h, rt; (ii) 1 N NaOH, 1,4-dioxane, 80 °C, 12 h; (iii) EDCI, HOBt, 15–24 h, rt; (iv) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 2 h; (v) TFA/CH_2Cl_2 (1:1), 2 h, rt.

In a typical synthesis, a suspension of *p*-nitrophenyl carbonate Wang resin **6** (2.00 g, 1.2 mmol/g, 100–200 mesh



Scheme 2. Reagents and conditions: (i) $SOCl_2$, MeOH, 0 °C, rt, 6 h; (ii) Boc-anhydride, Et_3N, DMAP, CH_2Cl_2, 0 °C, rt, overnight; (iii) DAST, CH_2Cl_2, −78 °C, 12 h; (iv) $LiBH_4$, THF, 0 °C, 12 h; (v) TFA, CH_2Cl_2, rt, 6 h.

and 1% DVB) in CH_2Cl_2–DMF (2:1, 20 mL) was stirred for 30 min. A solution of **5** (0.360 g, 0.80 mmol), HOBt (0.649 g, 4.80 mmol) and DIPEA (1.39 mL, 4.80 mmol) in CH_2Cl_2–DMF (2:1, 10 mL) was added to the swollen resin and stirring was continued at room temperature for 6 h. The derivatized resin **7** was then filtered, rinsed with DMF (2 × 15 mL), CH_2Cl_2 (2 × 15 mL), MeOH (2 × 15 mL), ether (2 × 15 mL) and dried in vacuo. To a suspension of **7** in dioxane (30 mL) was added 1 N NaOH solution (10 mL) and the reaction heated at 100 °C for 12 h. On cooling, the resin **8** was filtered and rinsed with water (2 × 15 mL), water/dioxane (1:9, 2 × 15 mL), MeOH (2 × 15 mL), CH_2Cl_2 (2 × 15 mL), Et_2O (2 × 15 mL) and dried in vacuo. To a suspension of resin **8** in DMF (20 mL), EDCI (0.918 g, 4.80 mmol), HOBt (0.649 g, 4.80 mmol) and pyrrolidinemethanol **9** (0.32 mL, 129.25 mmol) were added and the reaction mixture was stirred for 15–24 h at room temperature. The resin was then filtered and washed with DMF (2 × 15 mL), DMF/water (8:2, 2 × 15 mL), MeOH (2 × 15 mL), MeOH/water (9:1, 2 × 15 mL), MeOH (2 × 15 mL), CH_2Cl_2 (2 × 15 mL), Et_2O (2 × 15 mL) and dried in vacuo. To a suspension of resin **11** in CH_2Cl_2 (15 mL) was added DMSO (0.91 mL, 12.80 mmol), $(COCl)_2$ (0.57 mL, 6.40 mmol), Et_3N (2.23 mL, 16.00 mmol) and the reaction stirred at −78 °C for 2 h. The oxidized resin was filtered, rinsed with CH_2Cl_2 (2 × 15 mL), DMSO (2 × 15 mL), MeOH/water (8:2, 2 × 15 mL), MeOH (2 × 15 mL), CH_2Cl_2 (2 × 15 mL) and Et_2O (2 × 15 mL), then dried in vacuo. A suspension of resin **12** in (15 mL) TFA/CH_2Cl_2 (50%) was allowed to stir for 2 h then filtered. This procedure was repeated once again to ensure complete cleavage of the product from the resin. The combined supernatant was diluted with water (20 mL) and neutralized by the addition of solid $NaHCO_3$. The organic phase was separated and washed with water, brine and dried over Na_2SO_4 and evaporated under reduced pressure. The crude products **4a**–**f** were purified by column chromatography through silica gel (60–120 mesh) with $CHCl_3$/MeOH (95:5) to give 55–68% yields of dimers **4a**–**f**. The product from each step was confirmed by NMR and FT-IR and also by comparison with the products obtained in solution-phase.

In conclusion, an efficient and practical solid-phase synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepine dimers and their C2-flourinated analogues has been demonstrated. This methodology is also highly amenable for the generation of combinatorial libraries of PBD dimers with diversity in the C-rings.

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23. Analysis of PBD dimer **4b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.09–1.96 (m, 10H), 2.40–2.25 (m, 4H), 3.88–3.48 (m, 4H), 3.93 (s, 6H), 4.28–4.10 (m, 4H), 6.82 (s, 2H), 7.50 (s, 2H), 7.66 (d, $J = 4.68$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.2, 25.7, 29.6, 46.7, 53.7, 56.1, 65.4, 110.7, 111.6, 120.3, 140.6, 147.8, 150.6, 162.4, 164.6; FABMS, *m/z* for $C_{30}H_{34}N_4O_6$ $[M+H]^+$: 547.