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Efficient solid-phase synthesis of DNA-interactive pyrrolo[2,1-*c*][1,4]benzodiazepine antitumour antibiotics†

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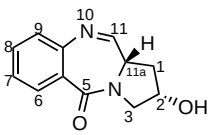
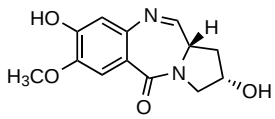
Abstract—The solid-phase synthesis of DNA-interactive pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) imines and biologically important pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones on Wang resin using a reduction/cyclization procedure is reported. © 2002 Elsevier Science Ltd. All rights reserved.

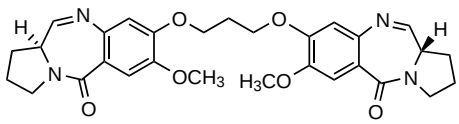
Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a group of potent, naturally occurring antitumour antibiotics produced by various *Streptomyces* species.[1] The cytotoxic and antitumour effects of these compounds are believed to arise from modification of DNA, which leads to inhibition of nucleic acid synthesis and production of excision-dependent single and double strand breaks in cellular DNA.[2] These antibiotics 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, resulting in the observed biological activity.[1] Anthramycin, tomaymycin, neothramycin, chicamycin, abbeymycin, DC-81 and its dimers are the well known examples of PBDs. The PBD imines are being used in the development of gene-targeting agents with the potential to down-regulate genes of therapeutic interest.[3] Among the well known methods for synthesis of these compounds the iminothioether approach has been extensively employed for the synthesis of naturally occurring PBD imines or their methyl ethers such as tomamycin, chicamycin[4] and also for the synthesis of structurally modified synthetic PBDs[5] wherein pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones are the intermediates. PBD-5,11-diones are also useful precursors for the PBD cyclic secondary amines which have recently been converted in our laboratory to PBD imines through a mild oxidative method.[6] Moreover, it is well known that PBD-5,11-diones are intermediates for the synthesis of compounds with a broad spectrum of biological activity such as antiphage activity,[7a] analgesic antagonist, antiinflammatory, psychomotor depressant activity,[7b] sedative activity,[7c] and even herbicidal properties.[7d]



Abbeymycin



Chicamycin A



DC-81 Dimer

There are many methods known for the solution phase synthesis of PBD imines.[8] However, there is only one report[9] on the solid-phase synthesis of these PBD antitumour antibiotics involving an oxidation/cyclization procedure based on the Fukuyama methodology employing *p*-nitrophenyl carbonate Wang resin. In continuation of our efforts in solid-phase synthesis,[10] we herein report an efficient solid-phase synthesis of N10–C11 imine containing PBDs and PBD-5,11-diones of biological interest particularly with 2-hydroxy substituents. It is well known in the literature, that C-ring hydroxy substitution plays an important role in their biological activity, examples being naturally occurring PBDs such as chicamycin A and B,[11a] neothramycin A and B,[11b,11c] and abbeymycin.[11d] Our synthetic strategy is partly based on the solution phase approach of Lown and Joshua,[12] which involves the reductive cyclization of *N*-(2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde with

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H_2/catalyst, while our approach is by reductive cyclization of *N*-(2-azidobenzoyl)pyrrolidine-2-carboxaldehyde with triphenyl phosphine (TPP).

In the first step, the Wang trichloroacetamidate resin (**2**), prepared by the Hanessian[13] protocol was coupled with Fmoc-protected 4-hydroxyproline methyl ester (**3**). The product, after Fmoc deprotection using 20% piperidine/DMF, was coupled with 2-azidobenzoic acid in the presence of DCC and DMAP to provide the amide **7**. Reductive cyclization of **7** using TPP afforded the PBD-5,11-dione (**11**), which upon cleavage from the resin yielded 2-hydroxy-7,8-substituted PBD-5,11-dione (**12**). Furthermore, the reduction of **7** by DIBAL-H followed by reductive cyclization and cleavage afforded 2-hydroxy-7,8-substituted PBD imine (**10**) (Scheme 1).

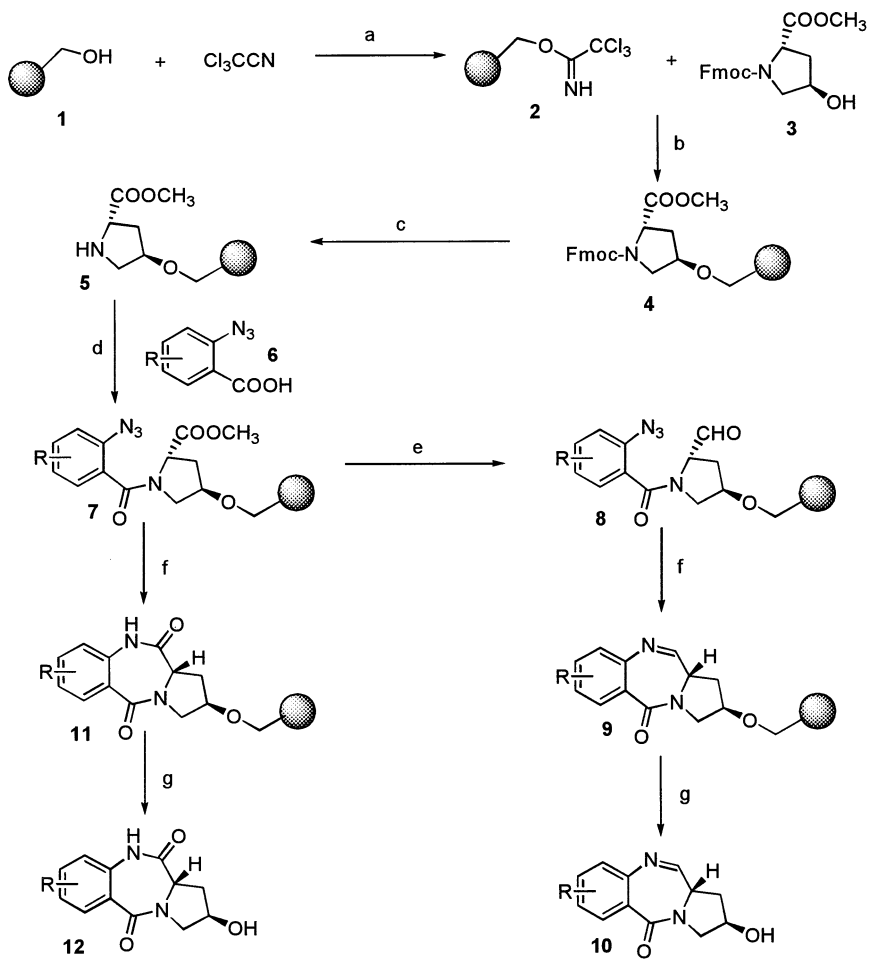
Similarly other 2-hydroxy 7,8-substituted PBD-5,11-diones and PBD imines have been prepared by employing the corresponding 2-azido benzoic acids as illustrated in Table 1.

In a typical synthesis, trichloroacetonitrile (3 mL) was added to a suspension of Wang resin (**1**, 1 g, 1.7 meq./g) in dry CH_2Cl_2 (20 mL), and the mixture was cooled to 0°C. DBU (0.2 mL) was then added dropwise to the suspension over a period of 5 min and the resin allowed to stir at 0°C for 40 min. The derivatized resin (**2**) was filtered and rinsed with CH_2Cl_2, DMSO, THF and CH_2Cl_2 and dried in vacuo. To the suspension of resin (**2**, 1.22 g, 1.39 meq./g) in dry CH_2Cl_2 (20 mL) was added Fmoc protected hydroxyproline methyl ester

Table 1. Yields and molecular ions observed for PBD analogues **10a–d** and **12a–d**

Entry	R	Yields (%)[a]	EIMS
10a	H	68	216
10b	7,8-$(OMe)_2$	62	277[b]
10c	8-Me	45	231[b]
10d	7-OMe, 8-OBn	56	352
12a	H	82	232
12b	7,8-$(OMe)_2$	74	292
12c	8-Me	60	246
12d	7-OMe, 8-OBn	70	368

[a] From initial loading of Wang resin.
[b] Molecular ion observed as M^++1 peak.



Scheme 1. (a) DBU, CH_2Cl_2; (b) $BF_3{\cdot}OEt_2$ or CF_3SO_3H, CH_2Cl_2; (c) 20% piperidine/DMF; (d) DCC, DMAP, CH_2Cl_2, 0°C; (e) DIBAL-H, CH_2Cl_2, –78°C, 2 h; (f) PPh_3, toluene; (g) TFA/CH_2Cl_2 (1:3).

(**3**, 2.50 g, 6.8 mmol) and $BF_3{\cdot}OEt_2$ (20 μL), and the resulting mixture stirred for 10–15 min. The ether (**4**) was filtered and rinsed with CH_2Cl_2, DMSO and THF. To the suspension of resin (**4**, 1.26 g, 0.94 meq./g) in DMF (16 mL), piperidine (4 mL) was added and allowed to stir for 2 h. The proline ether (**5**) was filtered and rinsed with DMF and CH_2Cl_2 and dried in vacuo. To a suspension of 2-azidobenzoic acid (**6**, 0.78 g, 4.76 mmol) in CH_2Cl_2 (20 mL), DCC (0.98 g, 4.76 mmol) and DMAP (10 mg) were added at 0°C and allowed to stir at the same temperature for 30 min. To the anhydride so generated, the resin (**5**, 1.10 g, 1.08 meq./g) was added and allowed to shake at room temperature for 10 h. The product resin (**7**) was filtered and rinsed with water, water/dioxane (1:9), MeOH and CH_2Cl_2 and dried in vacuo.

Synthesis of **12a**: To the suspension of resin (**7**, 0.60 g, 0.83 meq./g) in toluene, TPP (0.52 g, 2 mmol) was added and allowed to stir for 5 h at room temperature to afford the lactam resin derivative (**11a**) by reductive cyclization. The resin was filtered and rinsed with toluene and CH_2Cl_2. The suspension of **11a** in TFA/CH_2Cl_2 (1:3, 10 mL) was allowed to stir at 25°C for 2 h. The resin was filtered and washed with CH_2Cl_2 (3×10 mL). The combined filtrates were evaporated to afford the crude product **12a**.[14]

Synthesis of **10a**: To the suspension of resin (**7**, 0.60 g, 0.83 meq./g) in CH_2Cl_2 (10 mL) was added DIBAL-H (1 mL of 1 M solution in hexane, 1 mmol) dropwise at −78°C under dry nitrogen, and stirred at the same temperature for 2 h. The reaction was quenched by the addition of 10 mL of 0.5% HCl. The resin **8a** was filtered and rinsed with hexane, water, THF, CH_2Cl_2 and dried in vacuo. To the suspension of resin (**8a**, 0.59 g, 0.84 meq./g) in dry toluene was added TPP (0.52 g, 2 mmol) and allowed to stir for 5 h at room temperature. The product resin (**9a**) was filtered and rinsed with toluene and CH_2Cl_2. Finally, product (**10a**) was cleaved from the solid support using TFA/CH_2Cl_2 (1:3) to yield the crude product (**10a**),[15] which was further purified by column chromatography.

In summary, a practical solid-phase synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepines and their dilactams has been described from substituted 2-azido benzoic acids and Fmoc protected 4-hydroxyproline methyl ester. Diversity on the aromatic A-ring could be introduced by using various azido benzoic acids. This methodology allows the generation of a combinatorial library of not only PBD imines but also PBD-5,11-diones, particularly for the 2-hydroxy C-ring substituted compounds of biological importance.

Acknowledgements

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14. Spectral data for **12a**: ^{1}H NMR (200 MHz, $CDCl_3$+ DMSO-d_6) δ 2.00–2.10 (m, 1H, 1a), 2.78–2.95 (m, 1H, 1b), 3.50–3.62 (m, 1H, 3a), 3.85–3.95 (m, 1H, 3b), 4.10–4.26 (m, 1H, 11a), 4.40–4.55 (m, 1H, 2), 4.60–4.70 (m, 1H, OH), 7.08–7.26 (m, 2H, Ar), 7.40–7.50 (m, 1H, Ar),

7.85–8.00 (m, 1H, Ar), 10.20 (brs, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 35.0, 54.5, 55.6, 68.0, 121.8, 124.8, 126.5, 131.0, 133.0, 136.8, 165.6, 171.0; MS (EI) m/z 232.

15. Spectral data for **10a**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.70–1.80 (m, 1H, 1a), 1.95–2.10 (m, 1H, 1b), 3.50–3.75 (m, 2H, 3), 4.02 (m, 1H, 11a), 4.17–4.26 (m, 1H, 2), 4.92 (d, 1H, $J=4.8$ Hz, OH), 6.70–6.80 (m, 2H, Ar), 6.95 (d, 1H, $J=5$ Hz, Ar), 7.12–7.26 (m, 1H, Ar), 7.84 (d, 1H, $J=7.3$ Hz, imine); ^{13}C NMR (50 MHz, DMSO-d_6) δ 39.5, 52.0, 56.6, 71.0, 125.2, 128.6, 131.4, 131.7, 133.5, 144.6, 150.2, 166.5; MS (EI) m/z 216.