



# Total Synthesis of Polydiscamides B, C, and D via a Convergent Native Chemical Ligation–Oxidation Strategy

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**(5)** Supporting Information

**ABSTRACT:** The first total syntheses of the marine spongederived cyclic depsipeptide natural products Polydiscamides B, C, and D are described. The molecules were constructed through the convergent fusion of cyclic and linear fragments via an unprecedented native chemical ligation—oxidation protocol.



P olydiscamides B, C, and D (1-3) were isolated by Quinn and co-workers from a marine sponge *Ircinia sp.*, collected from the Great Barrier Reef in Australia (Figure 1).<sup>1</sup> These



Figure 1. Polydiscamides B–D (1–3).

natural products were shown to exhibit potent agonist activity against a human neuron-specific G-protein coupled receptor involved in the modulation of pain. Key structural features of 1-3 include a hydrophilic cyclic depsipeptide fragment that contains two D-amino acids (valine and aspartic acid) with a lactone functionality occurring between the side chain of Lthreonine and the  $\alpha$ -carboxylate of the D-aspartic acid residue. The cyclic depsipeptide of 1-3 is linked through the  $\alpha$ -amine of L-threonine to a linear hydrophobic peptide chain possessing p-bromo-L-phenylalanine and D-cysteic acid residues. In addition, polydiscamides B and C have a  $\beta$ -methyl-L-isoleucine in this linear portion, while polydiscamides B and D contain tert-D-leucine and tert-L-leucine residues respectively, notably at different sites in the linear chain. The polydiscamides belong to a family of sponge-derived natural products that includes polydiscamide A,<sup>2</sup> discodermins A–H,<sup>3-6</sup> halicylindramides A– D,<sup>7,8</sup> and microspinosamide,<sup>9</sup> and to date, only halicylindramide A has succumbed to total synthesis.<sup>10</sup> In addition to the

interesting structural features described above, a number of members of this family have been shown to exhibit promising antimicrobial<sup>3,4</sup> and antifungal activity.<sup>7,8</sup> Discodermin A has also been shown to exhibit a permeabilizing effect on the plasma membrane,<sup>11</sup> while polydiscamide A has been reported to possess inhibitory activity against a human lung cancer cell line.<sup>2</sup>

In this study, we set out to develop a robust and convergent synthetic strategy to access all three cyclic depsipeptides 1-3 and thus facilitate profiling of the antimicrobial activity of the natural products against a range of clinically relevant Gram positive and Gram negative bacterial strains.

From the outset, we envisaged that all three natural products could be accessed via a novel native chemical ligation—oxidation strategy (Scheme 1). Specifically, it was proposed that a common cyclic depsipeptide fragment bearing a pendant D-cysteine moiety (7) could be fused to linear peptide thioesters 4-6 (unique to each natural product) under native chemical ligation conditions.<sup>12</sup> Following the ligation event, oxidation of the cysteine residue would then afford polydiscamides B–D (1-3). For this synthetic strategy we deemed that protection of the side chain of tryptophan was crucial to prevent overoxidation of the indole ring,<sup>13,14</sup> and therefore peptide thioesters 4-6 were targeted with side chain protecting groups intact.

Synthesis of the cyclic depsipeptide fragment 7 commenced with loading of 2-chlorotrityl chloride resin with Fmoc-Pro-OH (Scheme 2). Elongation to the linear resin-bound peptide 8 was achieved via Fmoc-solid phase peptide synthesis (SPPS), whereby Fmoc-deprotection was facilitated through treatment with 10 vol % piperidine in DMF, and amino acids were coupled using (benzotriazol-1-yloxy)tripyrrolidinophospho-

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nium hexafluorophosphate (PyBOP) as the coupling agent and *N*-methylmorpholine as the base (see Supporting Information for full details). Notably, coupling of Fmoc-L-Thr-OH to the Nmethylated L-glutamine residue required modified conditions of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) as the coupling reagent and iPr2NEt as the base. Following the assembly of resin-bound peptide 8 the free side-chain hydroxyl moiety of the L-threonine residue was esterified with Fmoc-D-Asp(OtBu)-OH en bloc using Steglich esterification conditions [N,N']diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), DMF]. Fmoc-deprotection followed by cleavage of the side-chain protected peptide from the resin by treatment with 30 vol % hexafluoroisopropanol (HFIP) in CH<sub>2</sub>Cl<sub>2</sub> yielded the crude, suitably protected peptide 9, ready for cyclization. Macrolactamization between the free carboxylic acid of the Cterminal L-proline residue and the free N-terminal amine of the D-aspartic acid residue was facilitated by HATU and *i*Pr<sub>2</sub>NEt in CH<sub>2</sub>Cl<sub>2</sub> at a final concentration of 0.001 M.<sup>15</sup> The cyclization reaction was monitored by HPLC-MS and upon completion (16 h) was subjected to an acidic cocktail comprising TFA/  $iPr_3SiH/H_2O$  (90:5:5 v/v/v) to remove the side-chain protecting groups. Following reversed-phase HPLC purification the cyclic pentapeptide 7 was isolated in 12% yield over 14 steps (based on the initial resin loading).

Synthesis of the linear peptide thioester fragments 4-6 began with loading of Fmoc-L-Arg(Pbf)-OH onto 2-chlorotrityl chloride resin (Scheme 3). Fmoc-SPPS of the linear sequence included incorporation of several nonproteinogenic amino acids, namely, Fmoc-L-*tert*-Leu-OH 10, Fmoc-D-*tert*-Leu-OH 11, Fmoc-L- $\beta$ -MeIle 12<sup>16</sup> (see Supporting Information for the synthetic details), and Fmoc-L-p-Br-Phe-OH 13. After assembly

Scheme 2. Synthesis of Cyclic Depsipeptide Fragment 7



of the linear peptide fragments, on-resin formylation of the *N*-terminal D-alanine residue was effected by treatment with *p*-nitrophenyl formate in DMF. Mild acidolytic cleavage of the resin-bound peptide with 30 vol % HFIP in CH<sub>2</sub>Cl<sub>2</sub> yielded the crude side-chain protected peptides (**14–16**). These were not purified but rather subjected to low temperature thioester-ification with ethyl-3-mercaptopropionate using PyBOP as the coupling reagent and *i*Pr<sub>2</sub>NEt as the base at -30 °C.<sup>17,18</sup> Following reversed-phase HPLC purification the target side-chain protected peptide thioester fragments **4–6** were isolated in good yields (21–26% over 15 steps, based on the original resin loading).

With the cyclic depsipeptide 7 and linear peptide thioester fragments **4–6** in hand, efforts now turned to the assembly of the natural products via the proposed ligation—oxidation strategy. A mixed solvent system [1:1 v/v 6 M Gn·HCl, 1.0 M 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES)/N-methylpyrrolidone (NMP), pH 7.2]<sup>19</sup> was used to ensure complete solubility of both fragments. To a solution of cyclic peptide 7 and peptide thioesters **4**, **5**, or **6** was added thiophenol as an aryl thiol catalyst. After 16 h at 37 °C the reactions were judged by HPLC-MS analysis to be complete and purification by flash column chromatography on normal phase silica yielded **17–19** in good yields (51–72%). These compounds were then subjected to oxidation conditions to convert the thiol functionality of the cysteine side chains (used Scheme 3. Synthesis of Linear Peptide Thioester Fragments 4–6



as an auxiliary in the ligation reactions) to the corresponding cysteic acid residues found in the natural products. Optimal conditions involved treatment with a 1 vol % aqueous performic acid solution<sup>20</sup> for 1 h at 0 °C which effected clean oxidation as judged by HPLC-MS (Scheme 4; see Supporting Information). Subsequent treatment with an acidic cocktail of TFA/ $iPr_3SiH/H_2O$  (90:5:5 v/v/v) to remove the side-chain protecting groups, followed by reversed-phase HPLC purification, afforded polydiscamide B (1), polydiscamide C (2), and polydiscamide D (3) in good yields over the three steps (54-70%). The spectroscopic data obtained for synthetic polydiscamides B-D(1-3) were in complete accordance with those reported for the isolated natural products<sup>1</sup> (see Supporting Information for NMR spectral comparsions). Furthermore, the measured specific rotations were also in agreement in terms of both magnitude and sign. These data validate the full stereochemical assignment of polydiscamides B-D reported by Quinn and co-workers.<sup>1,21</sup> Having prepared polydiscamides B-D (1-3) we were next interested in investigating the antimicrobial activity. To this end, the compounds were screened against a panel of 15 clinically relevant Gram positive and Gram negative bacterial strains using a high-throughput screening methodology recently reported by Linington and co-workers (see Supporting Information).<sup>22</sup> Interestingly, despite the structural similarities with the discodermin family of natural products that possesses activity against strains of Bacillus subtilis and Pseudomonas aeruginosa, 1-3 showed no antibacterial activity against any of



the 15 strains up to a concentration of 100  $\mu$ M. As such, future work in our laboratory will focus on the application of our native chemical ligation—oxidation strategy to access these natural products, and others in the family, to identify the subtle structural features that lead to antimicrobial activity.

In summary, we have successfully completed the first total syntheses of the cyclic depsipeptide natural products polydiscamides B, C, and D (1–3). A key feature of our synthetic approach involved the late stage, high yielding assembly of the natural products by native chemical ligation of a common cyclic depsipeptide fragment to a variety of linear peptide thioesters, followed by chemoselective oxidation of a D-cysteine residue to a D-cysteic acid moiety. Using this strategy the natural products were isolated in 30-50% yield following a three-step ligation—oxidation—deprotection sequence. Given the efficient and convergent nature of the synthetic strategy described herein, it is anticipated that it will find future utility in the total synthesis of structurally related natural products

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including the discodermins,  $^{3,4}$  halicylindramides,  $^7$  and microspinosamide.  $^9$ 

## ASSOCIATED CONTENT

## **Supporting Information**

Full characterization of polydiscamides B–D and all novel intermediates. <sup>1</sup>H and <sup>13</sup>C NMR spectra, analytical HPLC chromatograms, and high resolution mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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