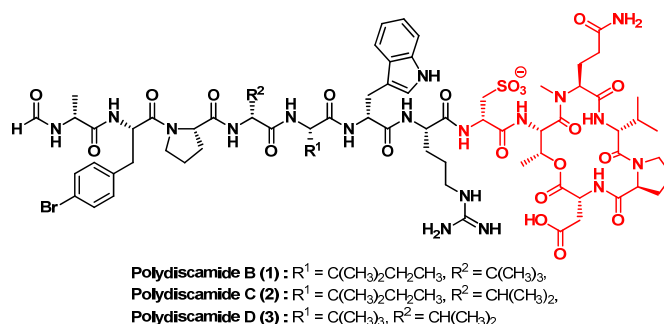


Total Synthesis of Polydiscamides B, C and D *via* a Convergent Native Chemical Ligation-Oxidation Strategy.

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Supporting Information Placeholder



ABSTRACT: The first total syntheses of the marine sponge-derived 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.

Polydiscamides 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).¹ These 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 L-threonine and the α-carboxylate of the D-aspartic acid residue. The cyclic depsipeptide of **1-3** is linked through the α-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 β-methyl-L-isoleucine in this linear portion, whilst 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,² discodermins A-H,³⁻⁶ halicyclindramides A-D^{7,8} and microspinosamide⁹ and, to date, only halicyclindramide A has succumbed to total synthesis.¹⁰ In addition to the interesting structural features described above, a number of members of this family have been shown to exhibit promising antimicrobial^{3,4} and antifungal activity.^{7,8} Discodermin A has also been shown to exhibit a permeabilizing effect on the plasma membrane,¹¹ while polydiscamide A has been reported to possess inhibitory activity against a human lung cancer cell line.²

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.

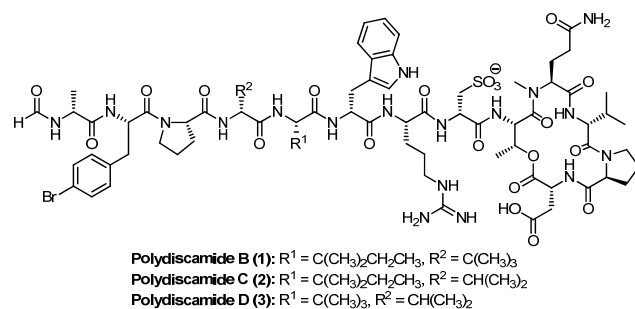
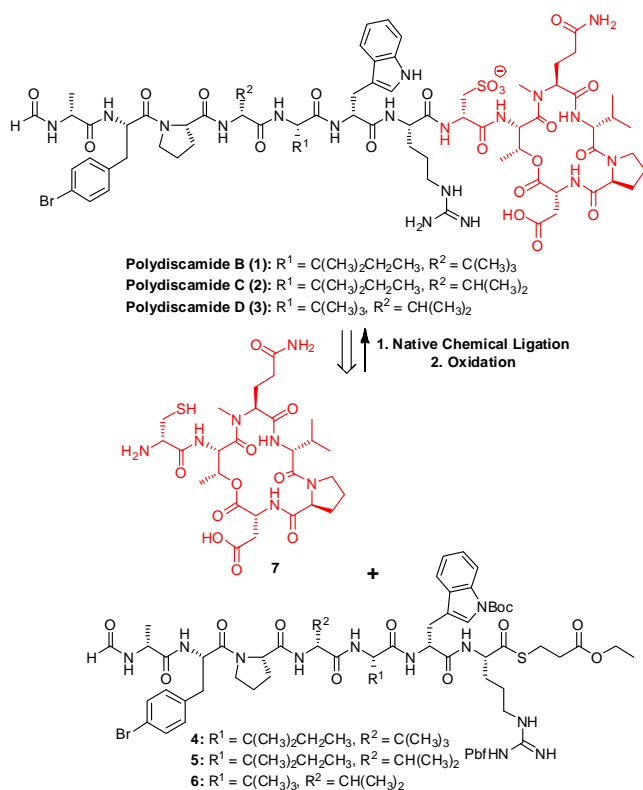


Figure 1. Polydiscamides B-D (1-3**).**

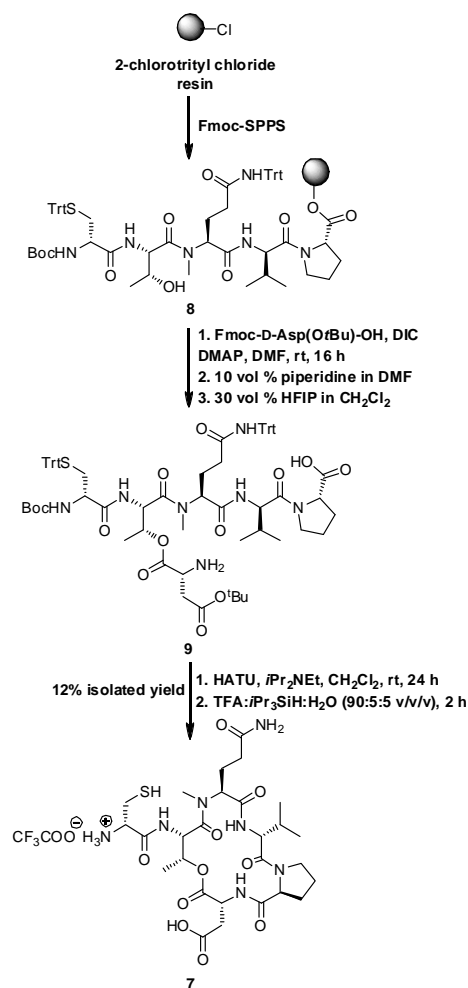
From the outset, we envisaged that all three natural products could be accessed *via* a novel native chemical ligation-oxidation strategy (Scheme 1). Specifically, it was proposed that a common cyclic depsipeptide fragment bearing a pendant D-cysteine moiety (**7**) could be fused to linear peptide thioesters **4-6** (unique to each natural product) under native chemical ligation conditions.¹² 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 over-oxidation of the indole ring^{13,14} and therefore peptide thioesters **4-6** were targeted with side-chain protecting groups intact.



Scheme 1. Retrosynthetic Analysis of Polydiscamides B-D (1-3).

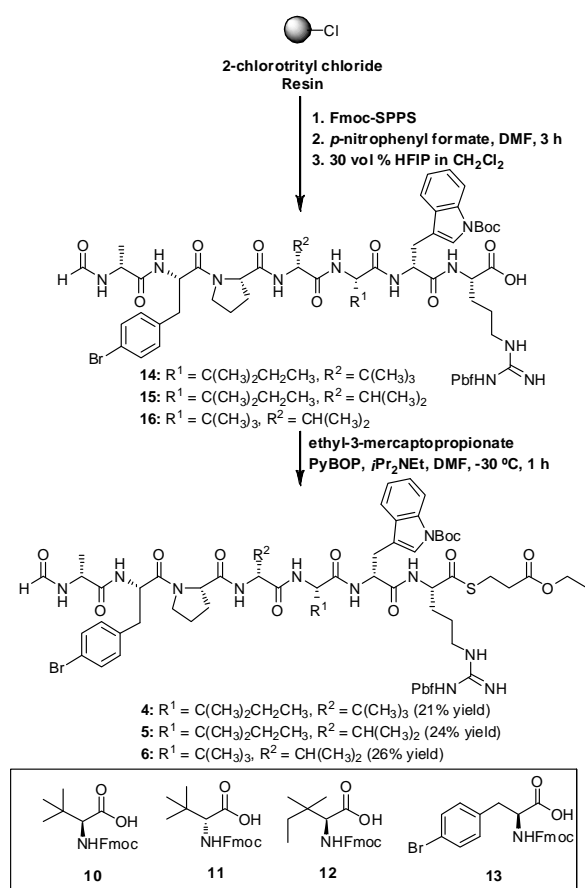
Synthesis of the cyclic depsipeptide fragment **7** commenced with loading of 2-chlorotrityl chloride resin with Fmoc-Pro-OH (Scheme 1). Elongation to the linear resin-bound peptide **8** was achieved *via* Fmoc-solid phase peptide synthesis (SPPS), whereby Fmoc-deprotection was facilitated through treatment with 10 vol % piperidine in DMF, and amino acids were coupled using (benzotriazol-1-yloxy)tripyrrolidinophosphonium 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 *N*-methylated L-glutamine residue required modified conditions of 1-[*bis*(dimethylamino) methyl]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) as the coupling reagent and *i*Pr₂NEt as 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(OrBu)-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 CH₂Cl₂ yielded the crude, suitably protected peptide **9**, ready for cyclization. Macrolactamization between the free carboxylic acid of the C-terminal L-proline residue and the free N-terminal amine of the D-aspartic acid residue was facilitated by HATU and *i*Pr₂NEt in CH₂Cl₂ at a final concentration of 0.001 M.¹⁵ The cyclization reaction was monitored by HPLC-MS and upon completion (16 h) was subject-

ed to an acidic cocktail comprising TFA:*i*Pr₃SiH:H₂O (90:5:5 v/v/v) to remove the side-chain protecting groups. Following reverse-phase HPLC purification the cyclic pentapeptide **7** was isolated in 12% yield over 14 steps (based on the initial resin loading).



Scheme 2. Synthesis of cyclic depsipeptide fragment 7.

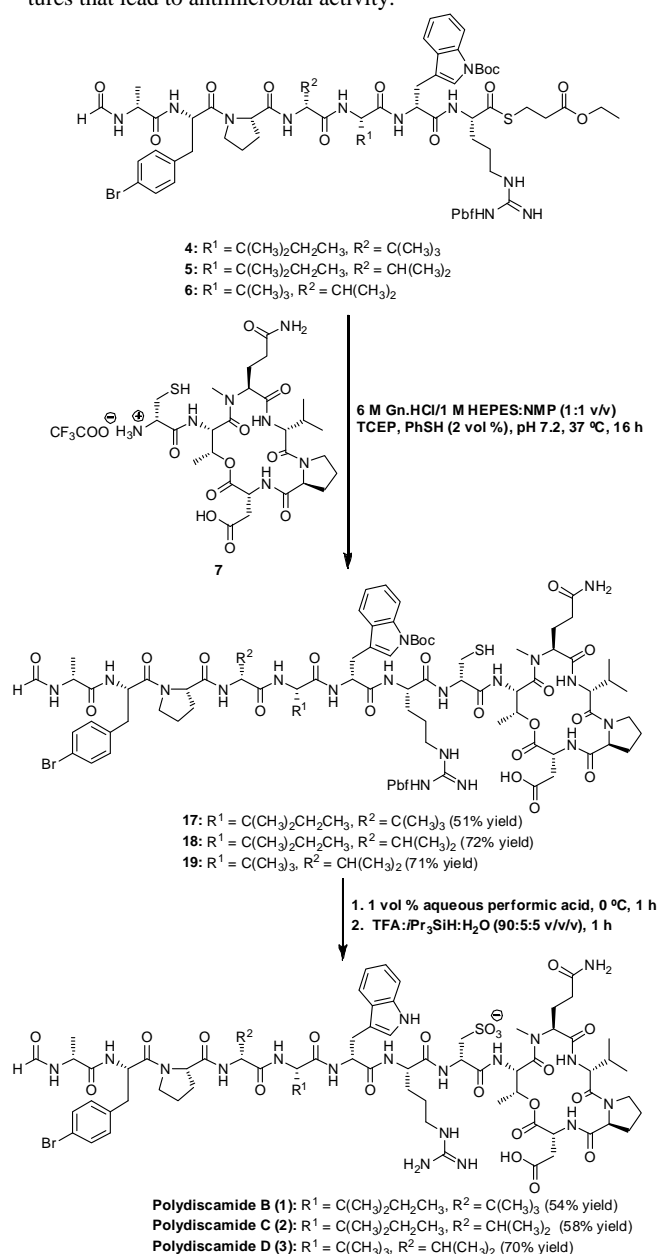
Synthesis of the linear peptide thioester fragments **4-6** began with loading of Fmoc-L-Arg(Pbf)-OH onto 2-chlorotrityl chloride resin (Scheme 3). Fmoc-SPPS of the linear sequence included incorporation of several non-proteinogenic amino acids, namely, Fmoc-L-*tert*-Leu-OH **10**, Fmoc-D-*tert*-Leu-OH **11**, Fmoc-L-β-Melle **12**¹⁶ (see Supporting Information for the synthetic details) and Fmoc-L-*p*-Br-Phe-OH **13**. After assembly 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 CH₂Cl₂ yielded the crude side-chain protected peptides (**14-16**). These were not purified but rather subjected to low temperature thioesterification with ethyl-3-mercaptopropionate using PyBOP as the coupling reagent and *i*Pr₂NEt as base at -30 °C.^{17,18} Following reverse-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).



Scheme 3. Synthesis of linear peptide thioester fragments 4-6.

With the cyclic depsipeptide **7** and linear peptide thioester fragments **4-6** in hand, efforts now turned to 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]¹⁹ was used to ensure complete solubility of both fragments. To this 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 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 solution²⁰ 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 $\text{TFA}:\text{Pr}_3\text{SiH}:\text{H}_2\text{O}$ (90:5:5 v/v/v) to remove the side-chain protecting groups, followed by reverse-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 products¹ (see Supporting Information for NMR spectral comparisons). Furthermore, the measured specific rotations were also in agreement both in terms of magnitude and sign. These data

validate the full stereochemical assignment of polydiscamides **B-D** reported by Quinn and co-workers.^{1,21} 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).²² Interestingly, despite the structural similarities with the discodermin family of natural products that possess 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 $100\ \mu\text{M}$. 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.



Scheme 4. Total synthesis of Polydiscamides B-D (1-3).

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 including the discodermins,^{3,4} halicylindramides⁷ and microspinosamide.⁹

ASSOCIATED CONTENT

Supporting Information

Full characterization of polydiscamides B-D and all novel intermediates. ¹H and ¹³C 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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REFERENCES

- (1) Feng, Y.; Carroll, A. R.; Pass, D. M.; Archbold, J. K.; Avery, V. M.; Quinn, R. J. *J. Nat. Prod.* **2007**, *71*, 8.
- (2) Gulavita, N. K.; Gunasekera, S. P.; Pomponi, S. A.; Robinson, E. V. *J. Org. Chem.* **1992**, *57*, 1767.
- (3) Ryu, G.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1994**, *50*, 13409.
- (4) Ryu, G.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 8251.
- (5) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, *25*, 5165.
- (6) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1985**, *26*, 855.
- (7) Li, H.-Y.; Matsunaga, S.; Fusetani, N. *J. Med. Chem.* **1995**, *38*, 338.
- (8) Li, H.-Y.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 163.
- (9) Rashid, M. A.; Gustafson, K. R.; Cartner, L. K.; Shigematsu, N.; Pannell, L. K.; Boyd, M. R. *J. Nat. Prod.* **2001**, *64*, 117.
- (10) Seo, H.; Lim, D. *J. Org. Chem.* **2009**, *74*, 906.
- (11) Sato, K.; Horibe, K.; Amano, K.; Mitusi-Saito, M.; Hori, M.; Matsunaga, S.; Fusetani, N.; Ozaki, H.; Karaki, H. *Toxicon* **2001**, *39*, 259.
- (12) Dawson, P.; Muir, T.; Clark-Lewis, I.; Kent, S. *Science* **1994**, *266*, 776.
- (13) Dai, J.; Wang, J.; Zhang, Y.; Lu, Z.; Yang, B.; Li, X.; Cai, Y.; Qian, X. *Anal. Chem.* **2005**, *77*, 7594.
- (14) Dai, J.; Zhang, Y.; Wang, J.; Li, X.; Lu, Z.; Cai, Y.; Qian, X. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1130.
- (15) Wen, S.; Packham, G.; Ganesan, A. *J. Org. Chem.* **2008**, *73*, 9353.
- (16) Pigza, J. A.; Molinski, T. F. *Org. Lett.* **2010**, *12*, 1256.
- (17) Kajihara, Y.; Yoshihara, A.; Hirano, K.; Yamamoto, N. *Carbohydr. Res.* **2006**, *341*, 1333.
- (18) Wilkinson, B. L.; Stone, R. S.; Capicciotti, C. J.; Thaysen-Andersen, M.; Matthews, J. M.; Packer, N. H.; Ben, R. N.; Payne, R. J. *Angew. Chem. Int. Ed.* **2012**, *51*, 3606.
- (19) Payne, R. J.; Ficht, S.; Greenberg, W. A.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2008**, *47*, 4411.
- (20) Cao, R.; Liu, Y.; Chen, P.; Lv, R.; Song, Q.; Sheng, T.; He, Q.; Wang, Y.; Wang, X.; Liang, S. *Anal. Biochem.* **2010**, *407*, 196.
- (21) It should be noted that while the spectroscopic data of synthetic polydiscamide B, C and D was consistent with that reported for the isolated natural products there was no natural material still available for any of the natural products and therefore a direct spectroscopic comparison could not be made.
- (22) Wong, W. R.; Oliver, A. G.; Linington, R. G. *Chem. Biol.* **2012**, *19*, 1483.