# Total Synthesis of Marformycins A and D

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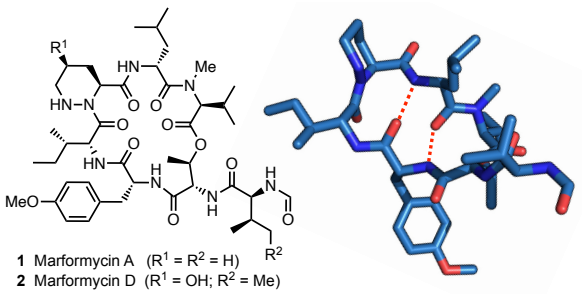
**ABSTRACT:** We report the synthesis of the antimicrobial cyclodepsipeptides marformycin A (**1**) and marformycin D (**2**) using a solid-phase approach. A scalable solution-phase synthesis of the γ-hydroxypiperazic acid subunit in **2**, starting from *cis*-hydroxyproline, is also described. Structural analysis of **1** and its Leu-*epi* congener demonstrate conformational differences that may underlie their divergent antimicrobial activities. The described approach enables further development of conformation-activity relationships within this class of depsipeptide natural products.




The unique conformational and physicochemical properties of naturally occurring depsipeptides make them attractive targets for chemical synthesis and drug design.[1] The combination of ester and amide bonds within these structures is often attended by the presence of other unusual peptide modifications that are important for bioactivity. Daptomycin and romidepsin, both of which feature non-canonical amino acid residues embedded within their structures, are notable examples of cyclodepsipeptide natural products that have reached the clinic.[2,3]

The marformycins are a class of macrocyclic heptadepsipeptides isolated from a marine-derived microbial strain of *Streptomyces drozdowiczii*.[4] Although the planar structure of marformycin D was first elucidated in 2006,[5] full configurational assignments were not established until the isolation of marformycins A–D in 2014 by Zhou and coworkers.[4] In addition to an ester linkage between Thr and (*N*-Me)Val, each of the marformycins features backbone N-oxidation in the form of a piperazic acid (Piz) or γ-hydroxypiperazic acid ((γ-OH)Piz) residue.[6,7] Despite their potent and selective growth inhibitory activity against *Micrococcus luteus* and *Propionibacterium sp.*, the marformycins have not yet been the subject of a reported chemical synthesis. Here, we describe the total synthesis of **1**, **2**, and an unnatural epimer of marformycin A using a solid-phase approach.

Our retrosynthesis of **1** and **2** relied on peptide macrocyclization in solution to form the Leu-(*N*-Me)Val amide bond (Figure 1). The linear precursor is in turn derived from on-resin esterification of the Thr side chain with a protected (*N*-Me)Val derivative. We envisioned peptide elongation using standard Fmoc protocols and incorporation of Piz or (γ-OH)Piz as orthogonally-protected dipeptide building blocks. We previously showed that similar N-amino dipeptide fragments can be used in automated solid-phase peptide synthesis (SPPS) without significant epimerization of the C-terminal Cα.[8,9] The Piz and (γ-OH)Piz subunits were traced back to δ-hydroxynorvaline and *cis*-hydroxyproline as chiral progenitors.

The synthesis of the Piz dipeptide fragment required for marformycin A commenced with δ-hydroxynorvaline (**3**), as shown in Scheme 1.[10] Protection of **3** as the TBS ether, Fmoc deprotection, and electrophilic amination with TBDOT[9,11] afforded hydrazino ester derivative **4** in 80% yield over three steps. Acylation of **4** with Fmoc-D-*allo*-Ile-Cl[12] in the presence of sodium bicarbonate provided dipeptide **5** in 88% yield. Silyl ether deprotection with mild acid was followed by diazinane ring formation under Mitsunobu conditions to obtain protected Piz dipeptide **6**. Benzyl ester hydrogenolysis afforded carboxylic acid **7**, suitable for standard Fmoc SPPS on 2-cholorotrityl chloride (2-CTC) resin. Following elongation, the *N*-terminus was formylated with *p*-nitrophenyl formate in the presence of DIEA, providing resin-bound peptide **8**.

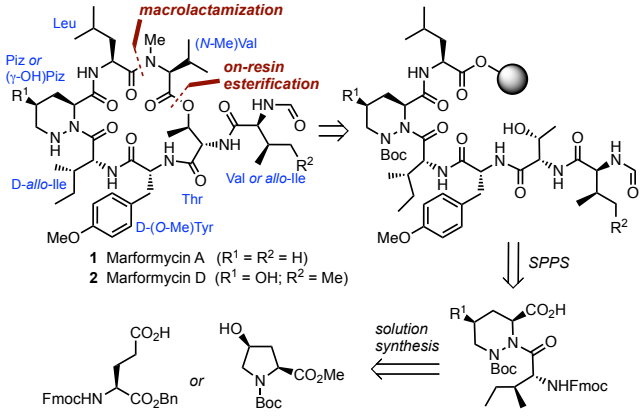
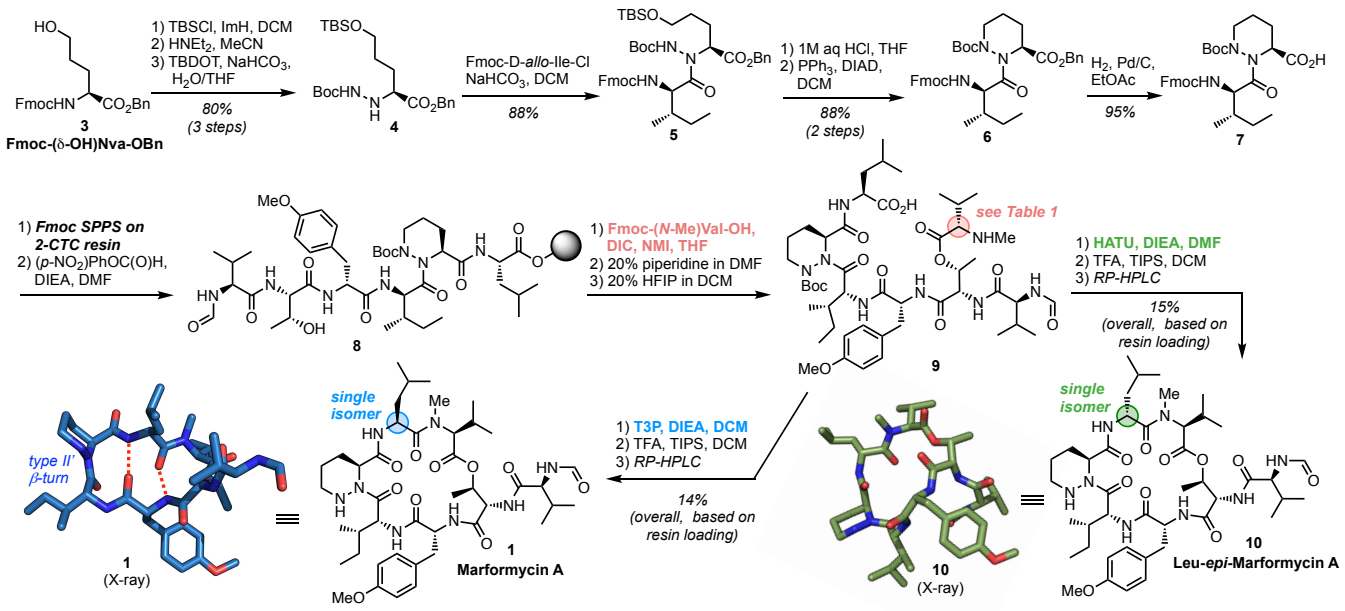



**Figure 1**. Retrosynthetic plan for marformycins A and D.




**Scheme 1**. Synthesis of marformycin A (**1**) and Leu-*epi*-marformycin A (**10**).

Initial attempts to form the ester bond in **9** using DIC and catalytic DMAP in DMF led to complete racemization of the Val active ester prior to condensation (entry 1, Table 1). We then subjected **8** to several esterification conditions, followed by Fmoc deprotection, cleavage, and analysis of diastereomeric ratios (d.r.) of **9** by RP-HPLC. As shown in Table 1, the use of DCM or THF as solvent led to a modest improvement in d.r. (entries 2–3). Treatment of resin-bound **8** with the symmetric anhydride of Fmoc-(*N*-Me)Val-OH and catalytic DMAP provided the desired diastereomer of **9** in a 75:25 ratio in DMF (entry 4) and a 95:5 ratio in THF (entry 5). Further suppression of epimerization was achieved in THF by using N-methylimidazole (NMI) as the base catalyst in the presence of 1 equiv of DIC (entry 8).

**Table 1**. Diastereomeric ratio (d.r.) of **9** following esterification of **8**, Fmoc deprotection and cleavage (as determined by RP-HPLC peak integrations at 220 nm).

| entry | DIC (equiv) | base (0.1 equiv) | solvent | d.r. (9:Leu-*epi*-9) |
|---|---|---|---|---|
| 1 | 1 | DMAP | DMF | 51:49 |
| 2 | 1 | DMAP | DCM | 57:43 |
| 3 | 1 | DMAP | THF | 78:22 |
| 4 | 0.5 | DMAP | DMF | 75:25 |
| 5 | 0.5 | DMAP | THF | 95:5 |
| 6 | 1 | NMI | DMF | 72:28 |
| 7 | 1 | NMI | DCM | 76:24 |
| 8 | 1 | NMI | THF | 97:3 |

With linear substrate **9** in hand, we proceeded with macrocyclization in the presence of HATU/DIEA in DMF and isolated a single major product following Boc deprotection and RP-HPLC purification. A comparison of the $^1$H NMR spectra for this product and that reported for natural marformycin A showed chemical shift discrepancies indicative of a configurational isomer. X-ray crystal diffraction revealed that the Leu Cα center had been epimerized during cyclization, resulting in the formation of **10** in 15% overall yield based on initial resin loading. After screening several macrocyclization conditions we found that T3P/DIEA in DCM afforded the desired diastereomer (**1**) as a single product in 14% overall yield following acidolysis and purification. NMR spectral data for synthetic **1** matched those of the natural product in every respect. X-ray crystal diffraction confirmed the configuration of the Leu Cα center and revealed that **1** adopts a β-sheet-like conformation featuring a D-*allo*-Ile-Piz type II' β-turn.

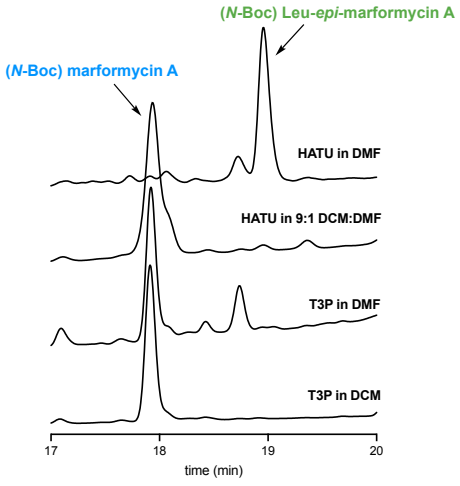
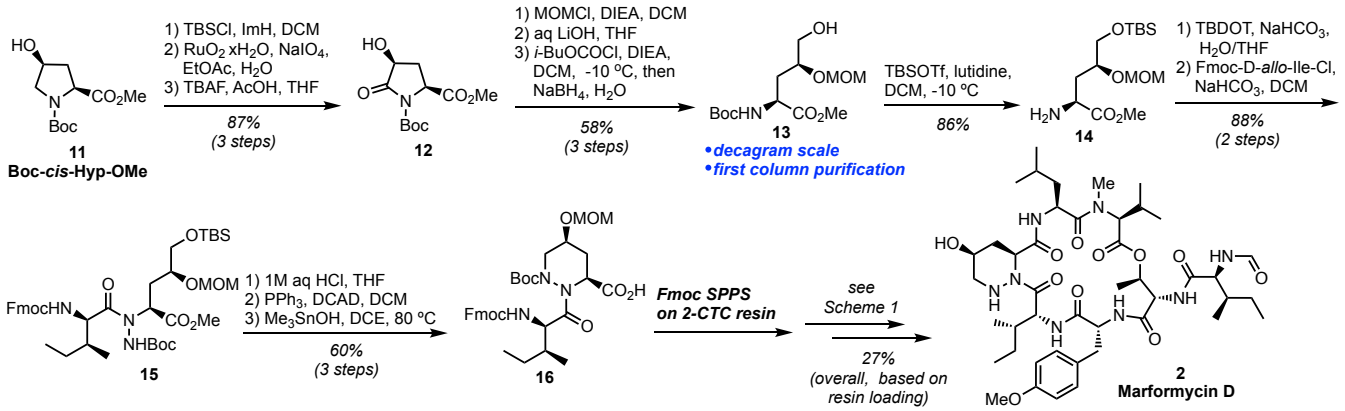



**Figure 2**. Analytical RP-HPLC traces of crude reaction mixtures following macrocyclization of **9** under various conditions (UV monitored at 220 nm).




**Scheme 2**. Total synthesis of marformycin D (**2**).

While partial C-terminal epimerization during peptide cyclization is a common occurrence, examples of dominant Cα inversion are rare.[13–16] Analysis of the crude reaction mixture by RP-HPLC confirmed nearly complete stereochemical inversion at Leu Cα following macrocyclization of **9** with HATU/DIEA in DMF (Figure 2). Surprisingly, **1** was the only diastereomer observed when 9:1 DCM:DMF was used as solvent (HATU required a small amount of DMF to dissolve). These results are consistent with facile epimerization in the more polar medium, and a lower transition state barrier for cyclization of **Leu-*epi*-9** versus **9** (Curtin-Hammett conditions). Given the solid-state structures of **1** and **10**, we speculate that DMF may disrupt preorganization of the active ester of **9** into a β-sheet conformation, thus slowing its cyclization relative to that of its epimer. In contrast to HATU, use of T3P as the condensation reagent provided **1** as the only diastereomer in both DMF and DCM. In addition to effectively suppressing epimerization, the crude purity of T3P-mediated cyclization in DCM made it the preferred condition for generating **1**.

Having secured entry into the natural diastereomer of **1**, we turned our attention to the synthesis of marformycin D (**2**) using an analogous approach. Previous syntheses of the (γ-OH)Piz residue in **2** have typically relied on diastereoselective installation of the Cα or Cγ substituent using chiral induction or auxiliaries.[17–23] As an alternative, we developed a scalable route in which both stereocenters are already present in the starting material. As shown in Scheme 2, conversion of *cis*-4-hydroxyproline derivative **11** into pyroglutamate **12** was carried out via TBS protection, Ru(IV) oxidation, and silyl ether cleavage in 87% overall yield.[24] The hydroxyl group of **12** was then protected as its MOM ether and the resulting imide selectively hydrolyzed with aqueous LiOH. The side chain carboxylic acid was then reduced via its asymmetric anhydride to provide **13** in 58% yield over 3 steps. Notably, this sequence was routinely carried out on decagram scale and required only a single column purification over six steps.

N-Boc deprotection and concomitant TBS etherification of **13** was achieved in 86% yield under Ohfune's conditions.[25] Electrophilic amination of **14** was followed by acylation with Fmoc-D-*allo*-Ile-Cl to provide fully protected N-amino dipeptide **15**. As with **5**, mild silyl ether deprotection and Mitsunobu cyclization afforded the diazinane ring of (γ-OH)Piz. Methyl ester hydrolysis in the presence of the base-labile Fmoc group was then effected with $Me_3SnOH$, providing **16** in 60% yield over three steps. This dipeptide building block was used to synthesize **2** in 27% overall yield following the same procedure described for **1**. The $^1H$ and $^{13}C$ NMR spectra obtained for synthetic **2** were in agreement with those reported for marformycin D isolated from natural sources.

Finally, we tested compounds **1**, **2**, and **10** for their ability to inhibit the growth of *M. luteus* as well as strains of the ESKAPE pathogens. Consistent with previously reported assays, both **1** and **2** were highly selective against *M. luteus*, with **2** displaying remarkable potency (MIC = 0.098 μg/mL, Table 2). Substitution of Val for *allo*-Ile and addition of the Piz hydroxyl group in **2** resulted in an eight-fold enhancement in *M. luteus* growth inhibitory activity relative to **1**. The loss of activity observed for **10** suggests that β-sheet-like conformation is important for function. Several antimicrobial macrocyclic peptide natural products that adopt β-sheet folds suffer from non-specific membranolytic activity.[26,27] We therefore tested **1**, **2**, and **10** for hemolytic activity toward defibrinated red blood cells, alongside the control cyclopeptide gramicidin S.[28] No significant hemolytic activity was observed for **1**, **2**, or **10** up to 100 μM, while gramicidin S exhibited an $HD_{50}$ of approximately 50 μM (see Supporting Information).

**Table 2**. MIC values determined for **1**, **2**, and **10** against selected pathogens.

| | MIC (μg/mL) | | |
|---|---|---|---|
| **bacterial strain** | **1** | **2** | **10** |
| *M. luteus* ATCC 4698 | 0.78 | 0.098 | > 100 |
| *E. faecium* ATCC 19434 | > 100 | > 100 | > 100 |
| *S. aureus* ATCC 12600 | > 100 | > 100 | > 100 |
| *K. pneumoniae* ATCC 13883 | > 100 | > 100 | > 100 |
| *A. baumannii* ATCC 19606 | > 100 | > 100 | > 100 |
| *P. aeruginosa* ATCC 10145 | > 100 | > 100 | > 100 |
| *E. cloacae* ATCC 13074 | > 100 | > 100 | > 100 |

In summary, we describe the total synthesis of marformycins A and D using a Piz dipeptide fragment condensation strategy on solid support. As part of this study, we also developed a new, scalable synthesis of (γ-OH)Piz starting from readily available *cis*-hydroxyproline. Notably, macrocyclization en route to marformycin A led to unusually high selectivity for **1** or its Leu Cα

epimer (**10**), depending on choice of solvent and condensation reagent. Structural analysis of **1** and **10** by X-ray diffraction suggest that a β-sheet-like conformation may be required for the antimicrobial activity of the marformycins. With an efficient synthetic route in hand, elucidation of conformation-activity relationships within this family of depsipeptides are currently underway in our laboratory.

## ASSOCIATED CONTENT

### Data Availability Statement

The data underlying this study are available in the published article and its online supplementary material.

### Supporting Information

Detailed experimental procedures, characterization data for novel compounds, copies of RP-HPLC, HRMS, NMR spectra (PDF), crystal data, and biological assay data.

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