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Total Synthesis and Examination of Three Key Analogues of Ramoplanin: A Lipoglycodepsipeptide with Potent Antibiotic Activity

Yosup Rew, Dongwoo Shin, Inkyu Hwang, and Dale L. Boger*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received November 18, 2003; E-mail: boger@scripps.edu

Ramoplanin is a novel lipoglycodepsipeptide with potent antibacterial activity that was isolated from the fermentation broth of *Actinoplanes* sp. ATCC 33076 as a mixture of three closely related compounds **1**−**3**, of which **2** is the most abundant (Figure 1).[1] The structure was determined in 1989, and it was established that the three compounds differ only in the acyl group attached to the Asn[1] N-terminus.[2] The ramoplanin complex is 2−10 times more active than vancomycin against Gram-positive bacteria[3] with a distinct mode of action,[4,5] and the ramoplanin A2 aglycon (**4**) is equally or slightly more potent than the natural products in antimicrobial assays.[6] Ramoplanin disrupts bacterial cell wall biosynthesis where it inhibits both the intracellular MurG-catalyzed (UDP-glcNAc transferase) conversion of lipid intermediate I to II and the more accessible extracellular transglycosylase-catalyzed polymerization of lipid intermediate II to provide the cell wall peptidoglycan backbone. It binds and sequesters the substrates, lipid intermediates I and II (lipid II > lipid I),[5] and the structural details of this interaction are only now beginning to emerge.[5,7,8] These two steps immediately precede the transpeptidase-catalyzed cell wall cross-linking reaction and a principal site of action of vancomycin. Thus, cross-resistance between ramoplanin and other antibiotics including vancomycin is not observed, and ramoplanin represents an excellent candidate for more expansive clinical use (e.g., VREF and MRSA) beyond its present introduction for topical infections. However, the intrinsic instability of ramoplanin derived from a rapid hydrolysis of the depsipeptide ester (IV administration) and its poor absorption characteristics (oral administration) have limited its clinical potential.[3] Nonetheless, ramoplanin is currently in Phase III clinical trials for the oral treatment of intestinal vancomycin-resistant *enterococcus faecium* (VREF) and Phase II trials for nasal MRSA, neither of which require absorption.

The three-dimensional structure of ramoplanin A2 adopts a U-shaped topology,[9] forming two antiparallel β-strands (residues 2−7 and 10−14) connected by six transannular H-bonds and a reverse β-turn (*a*Thr[8] and Phe[9]). The conformation is stabilized by a hydrophobic cluster of aromatic side chains on residues 3, 9, and 11, providing a U-shape topology to the β-sheet with the β-turn at one end (*a*Thr[8] and Phe[9]) and a flexible connecting loop at the other (residues 15−17). We recently reported the first total synthesis of the ramoplanin A2 aglycon designed to permit access to analogues containing deep-seated changes in the natural product structures.[10] In addition to defining solutions to key challenges presented by the preparation of synthetic **4**, its deliberate late stage convergent assemblage from three key subunits was anticipated to facilitate the synthesis of an extensive series of analogues required to probe the features that contribute to lipid intermediate I and II binding and are critical for antimicrobial activity. Prior to embarking on a comprehensive dissection of each structural feature where each residue is examined, we identified three key analogues whose examination would facilitate subsequent studies. Herein, we report the total synthesis and examination of these three analogues **5a**−**5c**.

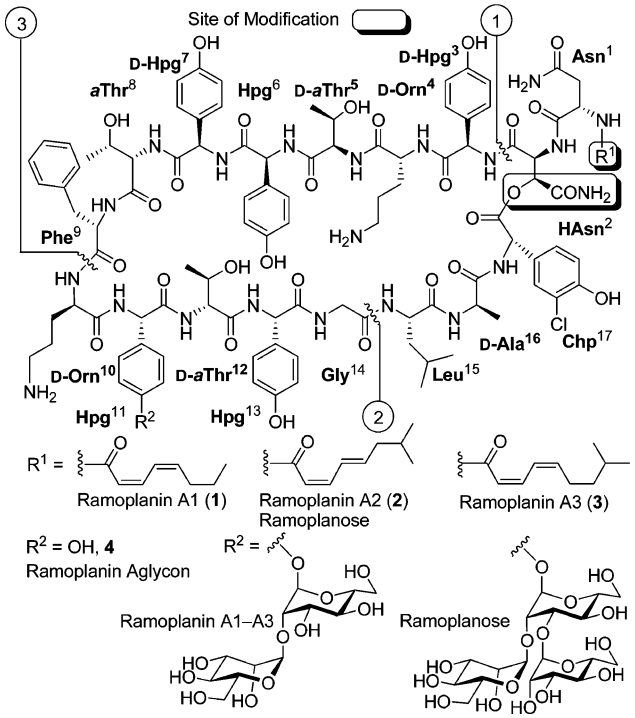


Figure 1. Structure of ramoplanin.

Not only is the HAsn[2] residue the most lengthy to secure,[11] but its incorporation into and maintenance throughout the late stages of the synthesis constitute the most difficult challenge limiting the ease of total synthesis.[10] More significantly, it is the most fragile linkage in the molecule and is central to the instability of the natural products. Williams described the mild hydrolysis of ramoplanose providing the inactive linear peptide.[12] McCafferty and co-workers reported the time-dependent decomposition of ramoplanin A2 aglycon in acidic solutions,[7] and we have described depsipeptide cleavage that occurs rapidly even under mild basic conditions (1% Et_3N-H_2O, 25 °C, 40% hydrolysis in 2 min).[10] Because it lies at the end of the antiparallel β-sheet with the ester adopting a transoid carbonyl eclipsed conformation analogous to that of an amide and forms the link to the short flexible loop (residues 15−17),[9] its replacement with L-2,3-diaminopropionic acid (Dap) or L-2,4-diaminobutyric acid (Dab) to provide **5a** and **5b** was expected to have a minimal impact on the conformation but would preclude hydrolysis (**5a** and **5b**) and significantly reduce (**5a**) or preclude (**5b**) β-elimination derived cleavage. Both would simplify the synthetic challenges associated with assembling a library of analogues and would be expected to improve in vivo stability.

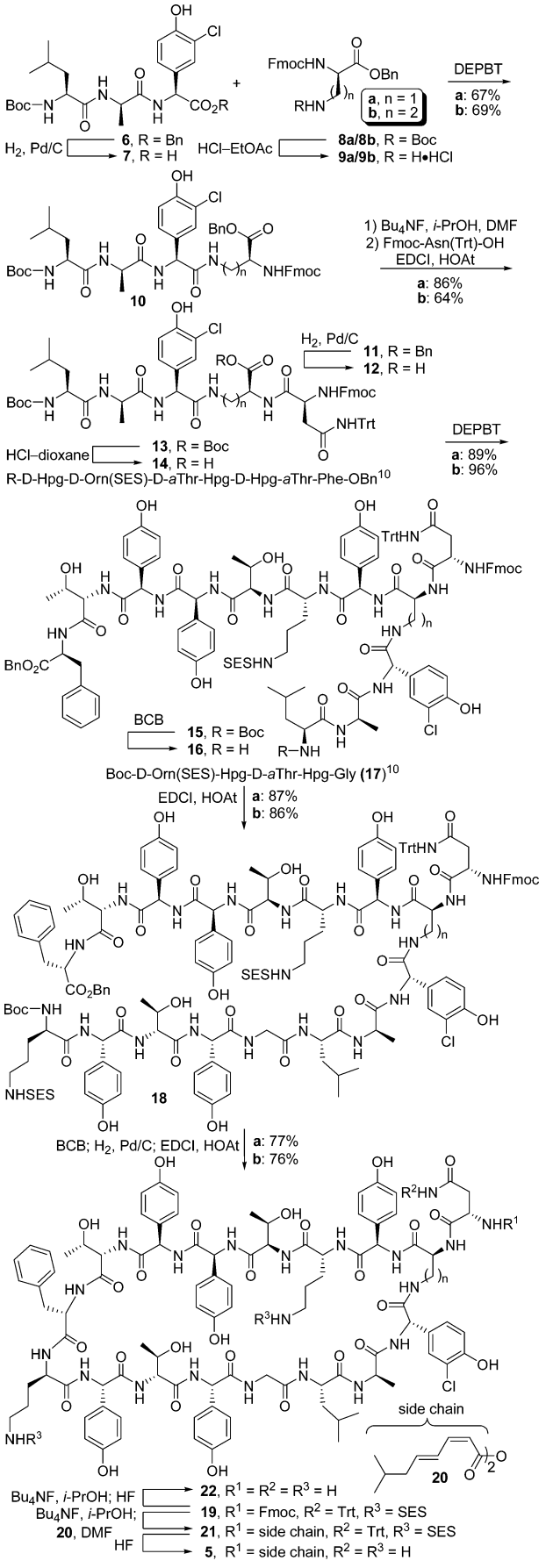
The simplified pentapeptide subunits **12a** and **12b**, incorporating the Dap[2] and Dab[2] amides in place of HAsn,[2] were synthesized from tripeptide **6** (Scheme 1),[13] which in turn was obtained by coupling Boc-Leu-D-Ala-OH[10] with Chp-OBn[10] (EDCI, HOAt, 20% DMF−CH_2Cl_2, 0 °C, 12 h, 96%) followed by deprotection of the benzyl ester (H_2, 10% Pd/C, EtOH, 25 °C, 1 h). Fmoc-Dap-OBn (**9a**)/Fmoc-Dab-OBn (**9b**), obtained from Fmoc-Dap(Boc)-OH/Fmoc-Dab(Boc)-OH, were coupled with **7** using DEPBT ($NaHCO_3$, 16% DMF−THF, 25 °C, 40 h, 67% for **10a**/$NaHCO_3$, THF, 25 °C, 36 h, 69% for **10b**) to give only a single diastereomer of each tetrapeptide **10a**/**10b**, whereas pairs of diastereomers (>8:1), resulting from competitive epimerization, were obtained with EDCI/HOAt. Fmoc removal (Bu_4NF, *i*-PrOH, DMF, 25 °C, 10 min, sonication),[10] coupling with Fmoc-Asn(Trt)-OH (EDCI, HOAt, DMF, 0 °C, 48 h, 86% for **11a**/20% DMF−THF, 64% for **11b**), and benzyl ester hydrogenolysis (H_2, 10% Pd/C, EtOH, 25 °C, 1 h, 82−87%) provided **12**.

The coupling of **12a**/**12b** with heptapeptide **14**[10] provided **15a**/**15b** (DEPBT, $NaHCO_3$, DMF, 0 °C, 3 days, 72−89% for **15a**/68−96% for **15b**). The coupling reactions were carried out with excess **12a**/**12b**, leading to complete consumption of **14** because residual **14** could not be removed from the product by either an acid wash or chromatography. The intermediates **15a**/**15b** were isolated by successive washing with ice cold aqueous 0.5 N HCl, water, and EtOAc, and were used for the next step without further purification. Boc removal was accomplished under the mild conditions (BCB, CH_3CN, 0 °C, 3 h) that preserve the trityl protecting group. In turn, the crude amines **16a**/**16b** were coupled with pentapeptide **17**,[10] to yield the macrocyclization substrates **18a**/**18b** (DEPBT, $NaHCO_3$, DMF, 0 °C, 96 h, 75−87% for **18a**/EDCI, HOAt, $NaHCO_3$, DMF, 0 °C, 72 h, 75−86% for **18b**). The conversion of **12** to **18** and the subsequent macrocyclization could often be conducted without deliberate purification of the intermediates or even **18**. Because conventional purification techniques (i.e., chromatography) typically resulted in substantial material losses attributable to the poor physical properties of the 12−17 residue peptides, the overall yields were much higher without the conventional purifications. In instances when the macrocyclization precursors **18a**/**18b**, isolated in the same manner described for **15a**/**15b**, were not sufficiently pure for further reaction, we found that membrane dialysis (MWCO = 2000) in DMSO was the most efficient and effective way to purify **18a**/**18b**.

Successive Boc removal (BCB, CH_3CN, 0 °C, 3 h), benzyl ester hydrogenolysis (H_2, 10% Pd/C, 10% DMF−EtOH, 25 °C, 10 h for **18a**/50% DMF−EtOH for **18b**), and macrocyclization (EDCI, HOAt, $NaHCO_3$, 70% CH_2Cl_2−DMF, 0 °C, 3 days for **19a**/EDCI, HOAt, $NaHCO_3$, DMF, 0 °C, 3 days for **19b**) afforded the cyclic peptide core **19a**/**19b**. Fmoc removal under mild conditions (8 equiv of Bu_4NF, 10 equiv of *i*-PrOH, DMF, 25 °C, 1 h, sonication),[10] Asn[1] acyl side chain introduction (**20**,[10] 20% CH_2Cl_2−DMF, 90% for two steps for **21a**), and global deprotection of the trityl and SES groups upon HF treatment (HF, anisole, 0 °C, 90 min, >75%) provided **5a**/**5b**. The amide analogues lacking the Asn[1] side chain, **22a**/**22b**, were also prepared by successive Fmoc removal (8 equiv of Bu_4NF, 10 equiv of *i*-PrOH, DMF, 25 °C, 1 h)[10] and HF deprotection of the trityl and SES groups (HF, anisole, 0 °C, 90 min).

A third key analogue **5c** was prepared and assessed in which the variable Asn[1] lipid *N*-acyl group was replaced with a minimal *N*-acetyl group. Semisynthetic modifications of the natural products and their aglycons have established that removal of the side chain unsaturation (hydrogenation) appears to have a minimal effect,[6] but the impact of the lipid side chain presence and its potential role

Scheme 1



Scheme 2

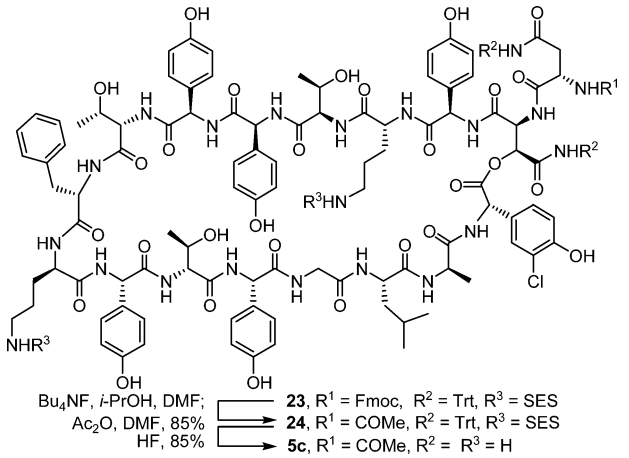


Table 1. Antimicrobial Activity, *Staphylococcus aureus*

compound	MIC (μg/mL)
2, ramoplanin A2	1.56
4, ramoplanin A2 aglycon	0.78
5a	0.39
22a	6.25
5b	>50
22b	>100
5c	12.5

was not known. This was addressed with the total synthesis of **5c** in Scheme 2. Removal of the Fmoc protecting group from **23**[10] (8 equiv of Bu_4NF, 10 equiv of *i*-PrOH, DMF, 25 °C, 1 h, sonication), the advanced synthetic intermediate we prepared enroute to the ramoplanin aglycon **4**,[10] followed by treatment with Ac_2O (DMF, 85% for two steps) provided the fully protected *N*-acetyl analogue **24**. Global deprotection using HF (HF, anisole, 0 °C, 90 min) furnished **5c** (85%).

The antimicrobial activity of the key analogues **5a**−**5c** and the corresponding Asn[1] free amines **22a**/**22b** were compared with ramoplanin A2 and its aglycon against *Staphylococcus aureus* (ATCC 25923), which is among the least sensitive wild-type bacteria, and the results are summarized in Table 1. The analogue **5a** in which the backbone depsipeptide ester was replaced with an amide retained or exhibited a slightly increased antimicrobial potency (MIC = 0.39 μg/mL) relative to the ramoplanin A2 aglycon (MIC = 0.78 μg/mL). In contrast, the depsipeptide amide analogue **5b** containing the single additional methylene in the macrocycle was inactive (MIC > 50 μg/mL), exhibiting a >100-fold loss in activity relative to **4** and **5a**. Interestingly, both **22a**, the active ramoplanin amide analogue lacking the Asn[1] side chain, and the ramoplanin aglycon *N*-acetyl analogue **5c** were approximately 16-fold less potent than the corresponding compounds containing the natural side chain. Thus, the depsipeptide ester of **1**−**4** may be replaced with a more stable amide without impacting the in vitro antimicrobial activity, the HAsn[2] carboxamide found in **1**−**4** but is absent in **5a** does not appear to contribute directly to the properties, and the lipid side chain of **1**−**4** contributes significantly but is not essential, whereas even a simple methylene insertion into the Dap[2] residue of **5a** abolishes activity.

Just as importantly, **5a** proved to be completely stable to mildly basic conditions (1% Et_3N-H_2O, 25 °C, 24 h, 0% disappearance) that rapidly consume the ramoplanin aglycon (80% within 5 min at 25 °C).[10] The enhanced antimicrobial potency and physical stability of **5a** make it a promising lead compound in the search for ramoplanin analogues with improved profiles and a more stable and accessible macrocyclic template on which to conduct structure−function studies.

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Supporting Information Available: Full experimental details and compound characterizations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole, DEPBT = 3-(diethoxylphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one, BCB = *B*-bromocatecholborane.

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