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Total Synthesis of the Ramoplanin A2 and Ramoplanose Aglycon

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Ramoplanin is a unique lipoglycodepsipeptide isolated from the fermentation broths of *Actinoplanes* sp. ATCC 33076 (Figure 1).[1] The structure was established in 1989 and found to be composed of the three closely related compounds **1**−**3**, of which **2** is the most abundant, that differ only in the structure of the acyl group found on the Asn[1] N-terminus.[2] The ramoplanin complex was found to be 2−10 times more active than vancomycin against Gram-positive bacteria (500 strains), including methicillin-resistant enterococci (MIC = 0.5 μg/mL) and all known strains of methicillin-resistant *Staphylococcus aureus* (MRSA).[3] Shortly after its disclosure, ramoplanin was shown to disrupt bacterial cell wall biosynthesis where it inhibits the action of intracellular UDP-glcNAc transferase and the conversion of lipid intermediate I to lipid intermediate II.[4] This inhibition was proposed to arise by ramoplanin complexation of lipid intermediate I preventing its utilization as a substrate.[4,5] More recently, ramoplanin has been shown to also inhibit the subsequent and more accessible transglycosylase-catalyzed extracellular polymerization of lipid intermediate II and to form self-associating 1:1 complexes with close analogues of the substrate, lipid intermediate II.[6] These two steps immediately precede the transpeptidase-catalyzed cross-linking reaction and the site of action of vancomycin. Thus, mechanism-based cross resistance between ramoplanin and vancomycin is not observed and ramoplanin represents an excellent candidate for more expansive clinical use beyond its introduction for topical infections.[7] Ramoplanin is presently in Phase III clinical trials for the oral treatment of intestinal vancomycin-resisitant *Enterococcus faecium* (VREF) and Phase II trials for nasal MRSA.[3]

The structures were established by 2D NMR and found to consist of a 49-membered ring composed of 17 amino acids in which the C-terminal 3-chloro-4-hydroxyphenylglycine (Chp[17]) forms a lactone bond with the hydroxy group of β-hydroxyasparagine (β-OH-Asn[2]).[8] Twelve of the amino acids possess nonstandard side chains and seven possess the D-configuration. A related antibiotic ramoplanose, described by Williams in 1991,[9] contains the identical depsipeptide core and the ramoplanin A2 Asn[1] acyl side chain, but incorporates three versus two D-mannose units on Hpg[11]. The high-resolution solution structure of the well-defined conformation of ramoplanin A2, which served to correct the olefin stereochemistry of the acyl side chain and assign the Hpg[6] and Hpg[7] absolute stereochemistry, is characterized by two antiparallel β-strands (residues 2−7 and 10−14) stabilized by six transannular H-bonds and a connecting reverse β-turn (*a*Thr[8]-Phe[9]).[8] The conformation is further stabilized by a cluster of hydrophobic aromatic side chains (residues 3, 9, and 11) providing a U-shape topology to the β-sheet with the β-turn at one end and a flexible connecting loop at the other (residues 15−17).

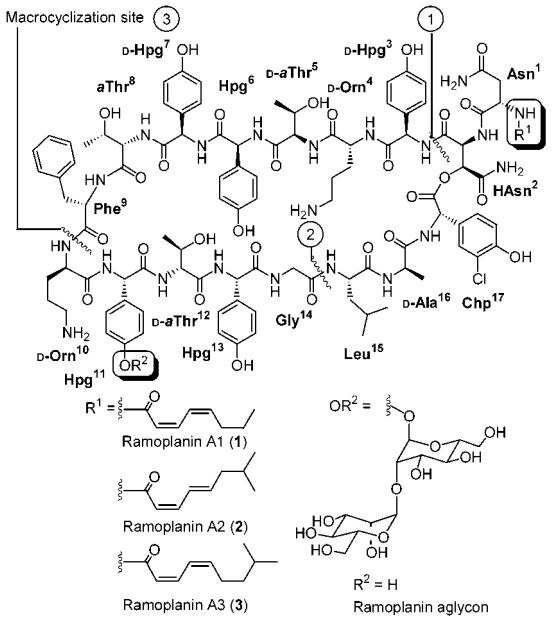

Figure 1.

Herein, we report the first total synthesis of the ramoplanin A2 and ramoplanose aglycon confirming the assigned structure.[10] Three key subunits composed of residues 3−9 (heptapeptide **15**), the pentadepsipeptide **26**, and pentapeptide **34** (residues 10−14) were sequentially coupled and cyclized to provide the 49-membered depsipeptide core of **1**−**3** (Figure 1). The indicated coupling sites were carefully chosen to maximize the convergency of the synthesis, including that of the three subunits, to minimize the use of protecting groups, to prevent late stage opportunities for racemization of carboxylate-activated phenylglycine-derived residues, and to enlist β-sheet preorganization of an acyclic macrocyclization substrate[11] for ring closure. As such, macrocyclization at the Phe[9]-D-Orn[10] site may benefit from both β-sheet preorganization as well as closure at a D-amine terminus.[12] Deliberate late stage incorporation of the subunit bearing the labile depsipeptide ester and a final stage Asn[1] side chain introduction provides future access to analogues of the aglycons which are reported to be equally potent or more potent than the natural products in antimicrobial assays.

Heptapeptide **15**, the first of the three key subunits, was assembled as shown in Scheme 1 from the tripeptide **7** and tetrapeptide **13** (EDCI, HOAt, DMF, 25 °C, 14 h, 76%)[13] followed by Boc deprotection (HCl−dioxane). In turn, **7** and **13** were prepared from the dipeptides **4**, **8**, and **10**. Notably, benzyl ester hydrogenolysis of **8** followed by coupling with **11** activated by DEPBT[14] ($NaHCO_3$, THF, 25 °C, 21 h, 83%) was accomplished with no detectable racemization of the sensitive D-Hpg residue

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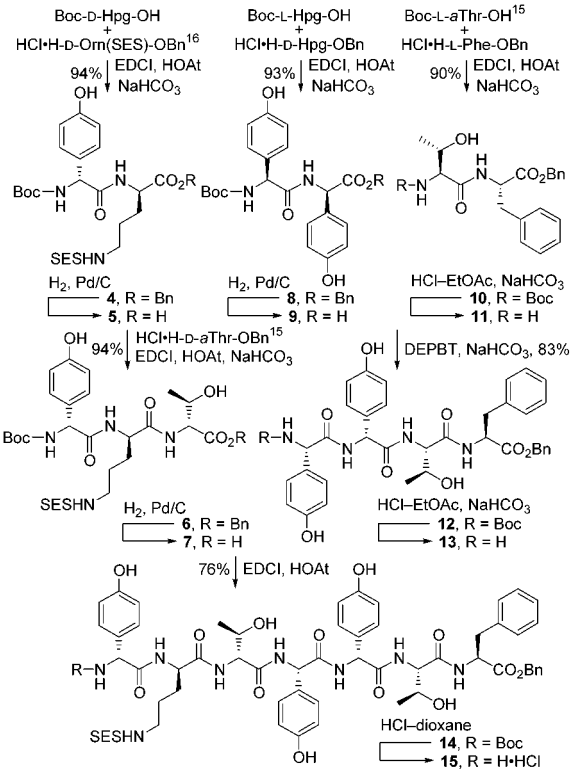
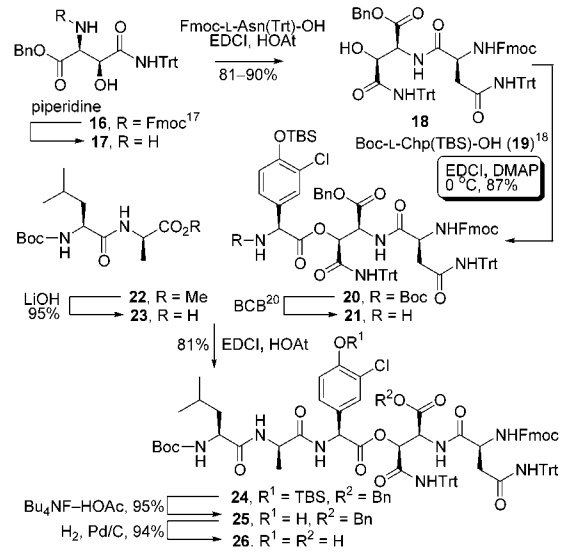
Scheme 1



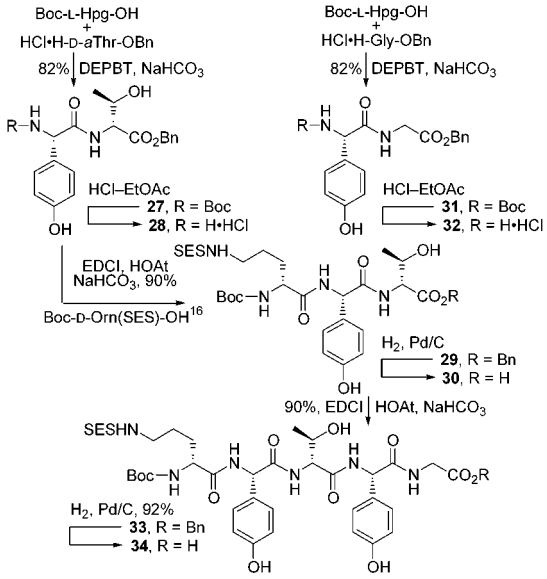
Scheme 2



(≥99% de) or β-elimination of the hindered L-*a*Thr whereas alternative coupling reagents gave lower conversions accompanied by substantial epimerization even when the reaction was conducted at 0 versus 25 °C [EDCI−HOAt (50%, 78% de); PyBop, $NaHCO_3$ (57%, 75% de); HATU, $NaHCO_3$ (80%, 95% de)].[13]

Key to preparation of the pentadepsipeptide **26** incorporating the sensitive backbone ester was the asymmetric synthesis of the orthogonally protected L-threo-β-hydroxyasparagine **16**[17] and the subsequent esterification of the hindered alcohol of **18** with **19**[18] activated with EDCI[13] conducted in the presence of DMAP[19] catalyst (CH_2Cl_2, 0 °C, 1 h, 87%, ≥10:1 diastereoselection), Scheme 2. Boc removal (BCB,[20] CH_2Cl_2, 0 °C, 2 h), coupling with **23** (EDCI, HOAt,[13] 20% DMF−CH_2Cl_2, 0 °C, 1.5 h, 81%), buffered TBS deprotection (Bu_4NF−HOAc, THF, 0 °C, 30 min, 91%),[21]

Scheme 3

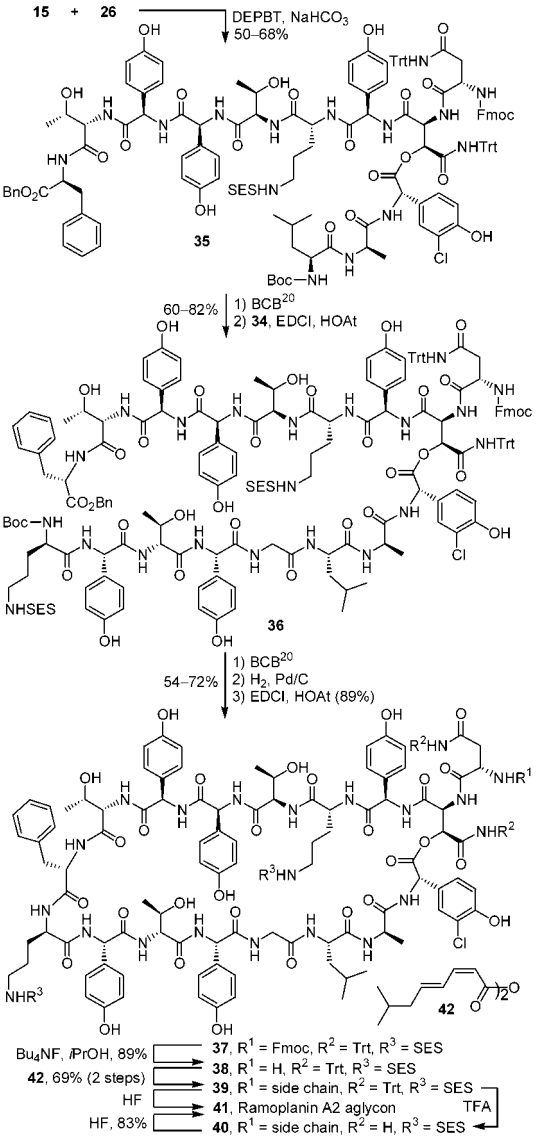


and benzyl ester hydrogenolysis (H_2, 10% Pd−C, EtOH, 94%) provided **26**.

The preparation of the final subunit **34** is detailed in Scheme 3. Notably, *C*-terminus protection of D-*a*Thr as its benzyl ester in **29** and elsewhere throughout the synthesis permitted hydrogenolysis deprotection avoiding base-catalyzed β-elimination. Similarly, coupling each sensitive Hpg-OH residue in **34**, like that throughout the synthesis, conducted upon activation with DEPBT[14] minimized epimerization especially when coupled with a hindered and sensitive *a*Thr amine terminus. For example, DEPBT promoted coupling to provide **27** proceeded in good yield with little or no epimerization (82%, 98% de) whereas EDCI−HOAt coupling was accompanied by significant racemization (80%, 80% de).

Assembly of the key fragments and completion of the total synthesis are detailed in Scheme 4. By far, the coupling of subunits **15** and **26** proved to be the most challenging step of the synthesis. Carboxylate activation of **26** typically resulted in preferential β-elimination of the acyloxy substituent. This can be attributed to the combination of a superb leaving group, the hindered nature of the activated carboxylate resulting from its α,β,β-trisubstitution and the large protecting groups (trityl and Fmoc), and the enhanced α-carbon acidity of the activated carboxylate derived from **26** resulting from the use of an *N*-acyl versus carbamate derivative. Only DEPBT[14] promoted the coupling to provide **35** in superb yields ($NaHCO_3$, DMF, 0 °C, 1 h, 50−68%) with *no* competitive β-elimination whereas all other alternative coupling reagents and conditions surveyed over several years provided predominantly β-elimination products. Boc removal was accomplished under mild conditions (BCB,[20] CH_2Cl_2, 0 °C, 30 min) that preserved the trityl protecting groups. Although unnecessary, their maintenance improved the detection, chromatographic, and solubility properties of subsequent intermediates. Coupling of the crude free amine with **34** (EDCI, HOAt, 25% DMF−CH_2Cl_2, 0 °C, 17 h, 60−82%)[13] provided the key acyclic depsipeptide **36**. Successive Boc removal (BCB,[20] CH_3CN, 0 °C, 3 h), benzyl ester hydrogenolysis (H_2, 10% Pd−C, EtOH, 2 h), and macrocyclization (EDCI, HOAt, 2−6:1 CH_2Cl_2−DMF, 1 mM, 0 °C, 18−20 h, 54−72% for 3 steps) afforded the cyclic depsipeptide core **37**. Presumably, β-sheet preorganization of the cyclization substrate[11] and closure at a D-amine terminus[12] contributes to the superb conversions for closure of the 49-membered ring (89% yield for macrocyclization using the purified amino acid). Fmoc removal under specially developed

Scheme 4



conditions (8 equiv of Bu_4NF, 10 equiv of *i*-PrOH, DMF, 25 °C, 1 h)[22] that do not promote competitive Asn^2 β-elimination, Asn^1 acyl side chain introduction (**42**,[23] 20% CH_2Cl_2−DMF, 69% for 2 steps), and a single step HF deprotection[24] of the trityl and SES groups (HF, anisole, 0 °C, 90 min) or sequential trityl (5% H_2O−TFA, 25 °C, 5 h) and SES deprotection (HF, 83%) provided the ramoplanin A2 and ramoplanose aglycon identical in all respects with an authentic sample (^{1}H NMR, HPLC, UV, MS).[25]

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Supporting Information Available: Full experimental details and characterization (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, PyBop = benztriazol-1-yl-oxytris-(pyrrolidino)phosphonium hexafluorophosphate, HATU = 2-(1*H*-7-aza-benzotriazol)-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate.
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(16) Boc-D-Orn(SES)-OH was prepared from Boc-D-Orn-OH as described in the Supporting Information. Further treatment with BnBr, $NaHCO_3$, DMF (92%); HCl−EtOAc (quant.), provided HCl·H-D-Orn(SES)-OBn. SES = 2-trimethylsilylethanesulfonyl.
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(25) Prepared by degradation of the ramoplanin complex (mixture of A1−A3, 2.5%, HCl−BuOH, DMF, 75 °C, 20−31%). Derivatization of **41** (SESCl, Et_3N, DMF, −20 °C, 3 h, 20%) provided a correlation sample of **40** also identical with synthetic material (^{1}H NMR, HPLC, UV, MS).

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