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Total Synthesis of the Ramoplanin A2 and Ramoplanose Aglycon

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Abstract: Full details of a convergent total synthesis of the ramoplanin A2 and ramoplanose aglycon are disclosed. Three key subunits composed of residues 3−9 (heptapeptide **15**), pentadepsipeptide **26** (residues 1, 2 and 15−17), and pentapeptide **34** (residues 10−14) were prepared, sequentially coupled, and cyclized to provide the 49-membered depsipeptide core of the aglycon. Key to the preparation of the pentadepsipeptide **26** incorporating the backbone ester was the asymmetric synthesis of an orthogonally protected L-threo-β-hydroxyasparagine and the development of effective and near-racemization free conditions for esterification of its hindered alcohol (EDCI, DMAP, 0 °C). The coupling sites were chosen to maximize the convergency of the synthesis including that of the three subunits, to prevent late stage racemization of carboxylate-activated phenylglycine-derived residues, and to enlist β-sheet preorganization of an acyclic macrocyclization substrate for 49-membered ring closure. By altering the order of final couplings, two macrocyclization sites, Phe^9−D-Orn^{10} and Gly^{14}−Leu^{15}, were examined. Macrocyclization at the highly successful Phe^9−D-Orn^{10} site (89%) may benefit from both β-sheet preorganization as well as closure at a D-amine terminus within the confines of a β-turn at the end of the H-bonded antiparallel β-strands. A more modest, but acceptable macrocyclization reaction at the Gly^{14}−Leu^{15} site (40−50%) found at the other end of the H-bonded antiparallel β-strands within a small flexible loop may also benefit from preorganization of the cyclization substrate, is conducted on a substrate incapable of competitive racemization, and accommodates the convergent preparation of analogues bearing depsipeptide modifications. 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 themselves are equally potent or more potent than the natural products in antimicrobial assays.

Ramoplanin is a novel 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 (MurG) and the conversion of lipid intermediate I to lipid intermediate II (Figure 2).[4] This inhibition was proposed to arise by ramoplanin complexation of lipid intermediate I preventing its utilization as a substrate.[4,5] More recently, Walker has shown that ramoplanin also inhibits the subsequent and more accessible transglycosylase-catalyzed extracellular polymerization of lipid intermediate II and forms self-associating 1:1 complexes with close analogues of the substrate, lipid intermediate II, suggesting this may represent the more relevant biological site of action.[6] Further supporting this premise, Walker has shown that ramoplanin binds a lipid intermediate II analogue better than the corresponding lipid intermediate I analogue, that ramoplanin also binds MurG (1:1, $K_d = 4 \times 10^{-6}$ M), that the inhibition of MurG cannot be overcome with additional substrate inconsistent with a mechanism simply involving substrate depletion, and that a ramoplanin analogue incapable of binding lipid intermediate I still inhibits MurG but does not exhibit antimicrobial activity.

(1) Cavalleri, B.; Pagani, H.; Volpe, G.; Selva, E.; Parenti, F. *J. Antibiot.* **1984**, *37*, 309. Pallanza, R.; Berti, M.; Scotti, R.; Randisi, E.; Arioli V. *J. Antibiot.* **1984**, *37*, 318.
(2) Ciabatti, R.; Kettenring, J. K.; Winters, G.; Tuan, G.; Zerilli, L.; Cavalleri, B. *J. Antibiot.* **1989**, *42*, 254. Kettenring, J. K.; Ciabatti, R.; Winters, G.; Tamborini, G.; Cavalleri, B. *J. Antibiot.* **1989**, *42*, 268. Review: Parenti, F.; Ciabatti, R.; Cavalleri, B.; Kettenring, J. *Drugs Exptl. Clin. Res.* **1990**, *16*, 451.
(3) Review: Espersen, F. *Curr. Opin. Anti-Infect. Invest. Drugs* **1999**, *1*, 78.
(4) Somner, E. A.; Reynolds, P. E. *Antimicrob. Agents Chemother*. **1990**, *34*, 413. Brötz, H.; Bierbaum, G.; Reynolds, P. E.; Sahl, H.-G. *Eur. J. Biochem.* **1997**, *246*, 193. Review: Reynolds, P. E.; Somner, E. A. *Drugs Exptl. Clin. Res.* **1990**, *16*, 385.
(5) Brötz, H.; Josten, M.; Wiedemann, I.; Schneider, U.; Götz, F.; Bierbaum, G.; Sahl, H.-G. *Mol. Microbiol.* **1998**, *30*, 317.
(6) Lo, M.-C.; Men, H.; Branstrom, A.; Helm, J.; Yao, N.; Goldman, R.; Walker, S. *J. Am. Chem. Soc.* **2000**, *122*, 3540. Helm, J. S.; Chen, L.; Walker, S. *J. Am. Chem. Soc.* **2002**, *124*, 13970. For dimerization of ramoplanin, see: Lo, M.-C.; Helm, J. S.; Sarngadharan, G.; Pelczer, I.; Walker, S. *J. Am. Chem. Soc.* **2001**, *123*, 8640.

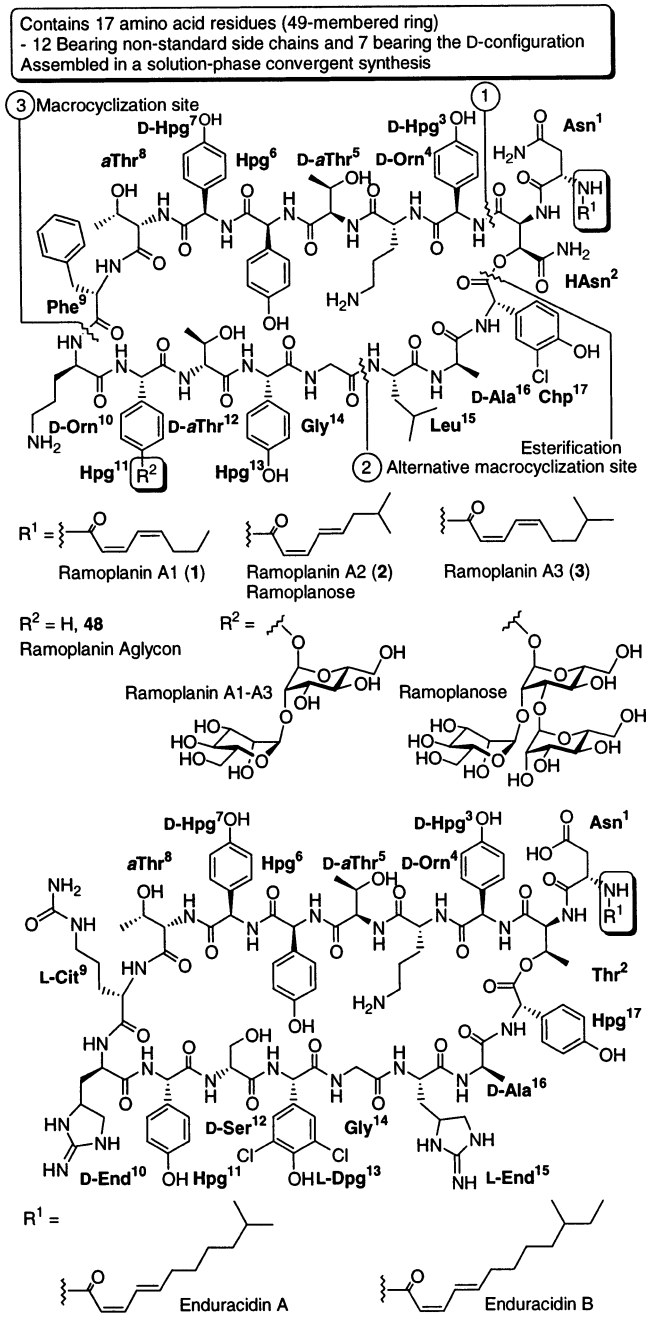

Figure 1.

These results suggest substrate recognition (lipid II > lipid I), while unnecessary for MurG inhibition, is important for in vivo antimicrobial activity. They also support the contention that inhibition of extracellular transglycosidation ($IC_{50} = 0.25\ \mu M$, $MIC = 0.1\ \mu M$) with lipid II as the substrate is the more relevant site of action for observation of antimicrobial activity. These two steps immediately precede the transpeptidase-catalyzed cross-linking reaction and a principal 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]

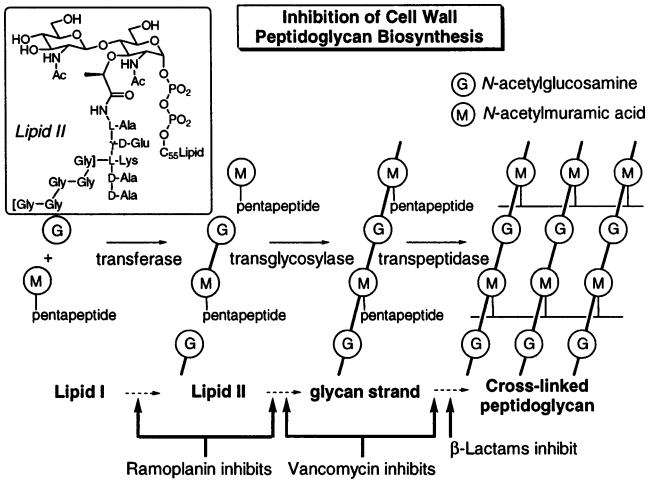

Figure 2.

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 ($HAsn^2$).[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 on the acyl side chain and to allow for assignment of 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 ($a\text{Thr}^8-\text{Phe}^9$).[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 3.

Although less well characterized, the enduracidins[10] represent closely related antibiotics acting by inhibition of bacterial cell wall biosynthesis[11] that had been disclosed much earlier than the ramoplanins. Notably, they do not bear a Hpg^{11} di- or trisaccharide and contain only subtle structural changes in the stretch of residues 9−17 as well as at residues 1 (lipid side chain) and 2 (Figure 1). In addition, the uncharacterized antibiotic janiemycin has been reported to bear an amino acid composition and biological properties suggesting it represents an additional member of this class of natural products.[12,13]

Structural details of the interaction of ramoplanin with peptidoglycan precursors are beginning to emerge from the

(7) Jones, R. N.; Barry, A. L. *Diagn. Microbiol. Infect. Dis.* **1989**, *12*, 279.
(8) Kurz, M.; Guba, W. *Biochemistry* **1996**, *35*, 12570.
(9) Skelton, N. J.; Harding, M. M.; Mortishire-Smith, R. J.; Rahman, S. K.; Williams, D. H.; Rance, M. J.; Ruddock, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7522.
(10) Hori, M.; Iwasaki, H.; Horii, S.; Yoshida, I.; Hongo, T. *Chem. Pharm. Bull.* **1973**, *21*, 1175. Iwasaki, H.; Horii, S.; Asai, M.; Mizuno, K.; Ueyanagi, J.; Miyake, A. *Chem. Pharm. Bull.* **1973**, *21*, 1184.
(11) Higashide, E.; Hatano, K.; Shibata, M.; Nakazawa, K. *J. Antibiot.* **1968**, *21*, 126. Asai, M.; Muroi, M.; Sugita, N.; Kawashima, H.; Mizuno, K. *J. Antibiot.* **1968**, *21*, 138. Tsuchiya, K.; Takeuchi, Y. *J. Antibiot.* **1968**, *21*, 426.
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(13) McCafferty, D. G.; Cudic, P.; Yu, M. K.; Behenna, D. C.; Kruger, R. G. *Curr. Opin. Chem. Biol.* **1999**, *3*, 672.

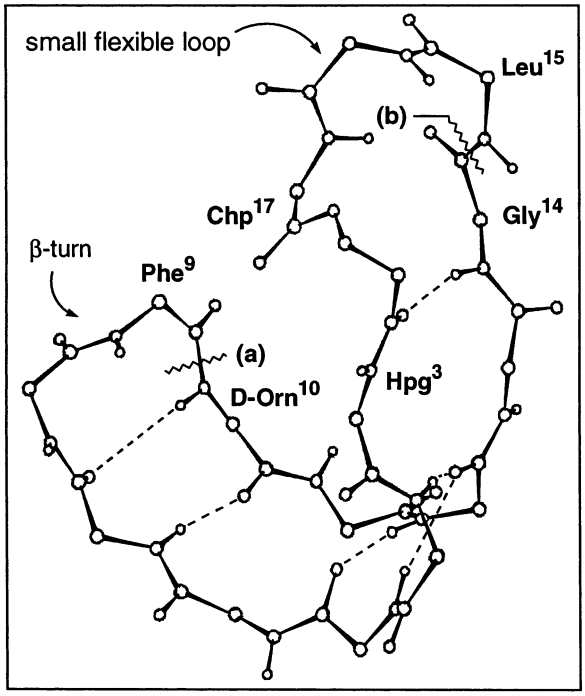

Figure 3. Macrocyclization sites.

studies of McCafferty[14] and these are defining the structural features and sites within **2** (Figure 1) that contribute to recognition, affinity, and selectivity for lipid intermediate I and II binding.[15] Most notably, the octapeptide sequence of Hpg^3−Orn^{10}, which is highly conserved between the ramoplanins and enduracidins, and the muramyl carbohydrate and adjacent pyrophosphate of soluble lipid intermediate derivatives (PG monomer) exhibit the greatest chemical shift changes and majority of intramolecular nOe's in a complex generated in vitro. Insights derived from such studies[14,15] and those of Walker[6] combined with the work detailed herein provide the opportunity to conduct detailed structure−function studies on this unique class of natural products.[16]

Herein, we provide full details of the first total synthesis of the ramoplanin A2 and ramoplanose aglycon.[17] Three key subunits composed of residues 3−9 (heptapeptide **15**), the pentadepsipeptide **26** (residues 1, 2 and 15−17), and pentapeptide **34** (residues 10−14) were sequentially coupled and cyclized in a solution phase, convergent approach to the 49-membered depsipeptide core of **1**−**3** (Figure 1). The indicated coupling sites were 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[18] for ring closure. Two macrocyclization sites, Phe^9−D-Orn^{10} and Gly^{14}−Leu^{15}, were chosen for examination. Macrocyclization at the Phe^9−D-Orn^{10} site, which was disclosed in our earlier communication,[17] proved unusually successful and represents a site found at the corner of the β-turn at the end of the H-bonded antiparallel β-strands (Figure 3). Consequently, closure at this site may benefit from both β-sheet preorganization of the substrate as well as closure at a D-amine terminus.[19] The additional closure at the Gly^{14}−Leu^{15} site detailed herein lies within a small flexible loop at the other end of the H-bonded antiparallel β-strands (Figure 3). In addition to also potentially benefiting from β-sheet preorganization of the macrocyclization substrate, it represents closure at a nonhindered site incapable of racemization. Pertinent to projected future studies, this closure site may prove especially useful for introducing alterations in the sensitive depsipeptide ester found in the pentapeptide **26** which contributes to the instability of the natural product. Deliberate late stage incorporation of this subunit bearing the labile depsipeptide ester and a final stage Asn^1 side chain introduction potentially provides access to analogues of the aglycons which themselves have been reported to be equally potent or more potent than the natural products in antimicrobial assays. Also key to the implementation of the approach was the judicious choice of the Orn^4/Orn^{10} SES protection and the Asn^1 Fmoc protection providing orthogonal protecting groups stable to Boc, Cbz, and benzyl ester deprotections, yet capable of sequential and selective removal in the presence of the depsipeptide ester using conditions we introduced in these or earlier unrelated studies.

Synthesis of the Hpg^3−Phe^9 Subunit (15). Heptapeptide **15**, the first of the three key subunits which contains all but Orn^{10} of the putative Hpg^3−Orn^{10} recognition sequence,[15] was assembled as shown in Scheme 1 from the tripeptide **7** and tetrapeptide **13** (EDCI, HOAt, DMF, 25 °C, 14 h, 76%)[20] followed by Boc deprotection (HCl−dioxane). This coupling was found to be sensitive to the reaction concentration (<40% at <0.2 M vs >65% at ≥0.33 M) and cleanly provided **14** without deliberate protection of the free alcohols, and with no evidence of competitive β-elimination or trace racemization. Moreover, the heptapeptide **14** was sufficiently insoluble in EtOH that a trituration recrystallization could be utilized for its large scale purification albeit with a recovery somewhat lower than a conventional chromatography. Tripeptide **7** was prepared

(14) Cudic, P.; Kranz, J. K.; Behenna, D. C.; Kruger, R. G.; Tadesse, H.; Wand, A. J.; Veklich, Y. I.; Weisel, J. W.; McCafferty, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7384.
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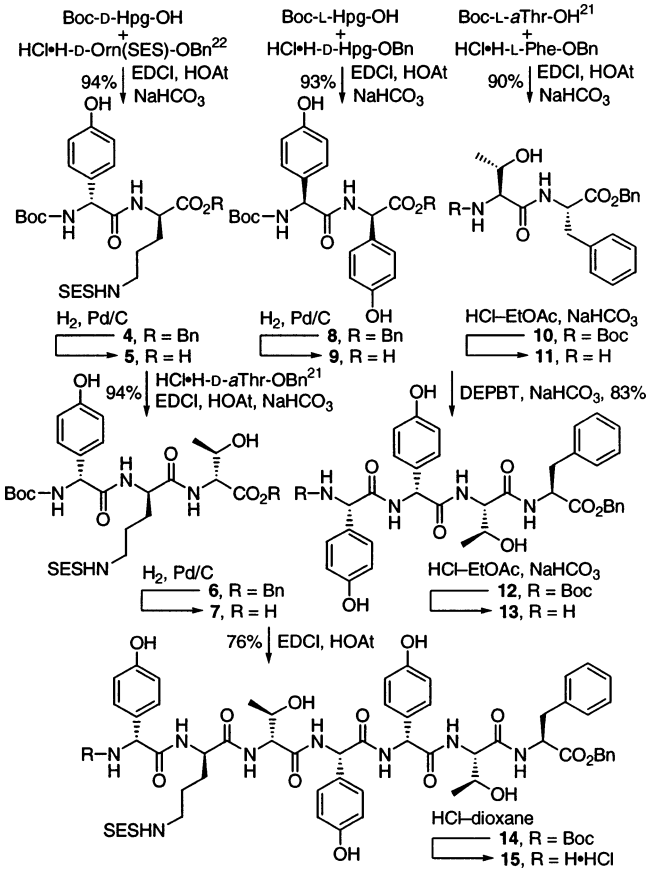
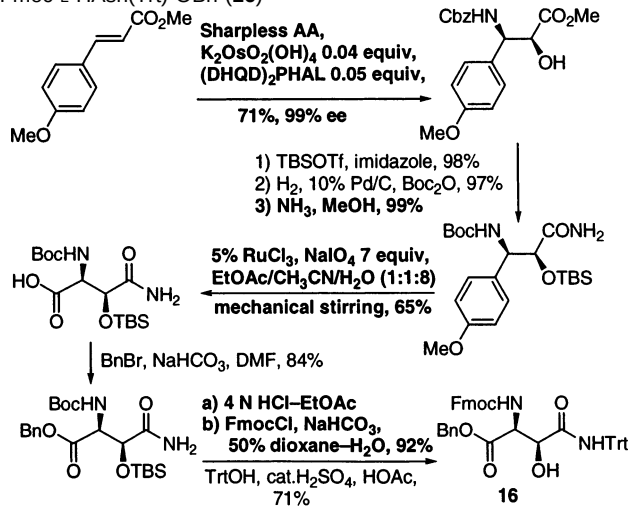
(16) Review: Chu, D. T. W.; Plattner. J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853.
(17) Jiang, W.; Wanner, J.; Lee, R. J.; Bounaud, P.-Y.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5288. Review: Boger, D. L. *Med. Res. Rev.* **2001**, *21*, 356.
(18) Maplestone, R. A.; Cox, J. P. L.; Williams, D. H. *FEBS Lett.* **1993**, *326*, 95.
(19) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101. Rich, D. H.; Bhatnagar, P.; Mathiaparanam, P.; Grant, J. A.; Tam, J. P. *J. Org. Chem.* **1978**, *43*, 296.
(20) EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole, HOBt = 1-hydroxybenzotriazole, PyBop = benzotriazol-1-yl-oxytris-(pyrrolidino)phosphonium hexafluorophosphate, HATU = 2-(1*H*-7-azabenzotriazol)-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate, DCC = 1,3-dicyclohexylcarbodiimide, DPPA = diphenylphosphoryl azide.

Scheme 1



by benzyl ester deprotection of **6** (H_2, 10% Pd/C, MeOH, 25 °C, 2 h, 100%) obtained by coupling D-*a*Thr-OBn[21] with the dipeptide **5** (EDCI, HOAt, 20% DMF−CH_2Cl_2, 0−10 °C, 3 h, 94%).[20] In turn, **5** was obtained by benzyl ester deprotection of **4** (H_2, 10% Pd/C, MeOH, 25 °C, 2 h, 98%) derived from coupling Boc-D-Hpg-OH and D-Orn(SES)-OBn (EDCI, HOAt, 25% DMF−CH_2Cl_2, 0 °C, 6 h, 94%).[20,22] Tetrapeptide **13** was prepared from the dipeptides **8** and **10** secured by the coupling of Boc-L-Hpg-OH with D-Hpg-OBn (EDCI, HOAt, 25% DMF−CH_2Cl_2, 0−25 °C, 8 h, 93%)[20] and Boc-L-*a*Thr-OH[21] with L-Phe-OBn (EDCI, HOAt, 20% DMF−CH_2Cl_2, 0−25 °C, 18 h, 90%),[20] respectively. Notably, benzyl ester hydrogenolysis of **8** (H_2, 10% Pd/C, MeOH, 25 °C, 2 h, 99%) followed by coupling with **11** activated by DEPBT[23] ($NaHCO_3$, THF, 25 °C, 20 h, 83%) was accomplished with no detectable racemization of the sensitive D-Hpg residue (>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)].[20] This convergent and efficient synthesis of the Hpg[3]−Phe[9] subunit which is ultimately capped with Orn[10] bearing a basic amine side chain lends itself to systematic modification of the putative Hpg[3]−Orn[10] recognition domain in efforts that could establish the importance and potential role of each residue.

Scheme 2. Improvements in the Preparation of Fmoc-L-HAsn(Trt)-OBn (**16**)[24]



Synthesis of the Depsipeptide Subunit (26). 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**[24] and the subsequent esterification of the hindered alcohol of **18** with **19**.[25] Since our disclosure of the preparation of **16** which relied on a key Sharpless asymmetric aminohydroxylation (AA) reaction,[24] we have improved its preparation in several subtle ways. These are detailed in Scheme 2 highlighting the improvements which entail conducting the Sharpless AA with 0.04 equiv of $K_2OsO_2(OH)_4$/0.05 equiv of $(DHQD)_2PHAL$ (lower amounts can result to lower ee), increasing the reaction time of the amidation reaction, and conducting the oxidative cleavage of the substituted anisole in an EtOAc−CH_3CN−H_2O (1:1:8) solvent system with vigorous mechanical stirring (vs magnetic stir bar) of the heterogeneous reaction mixture for larger scale reactions. The yield of the amidation reaction utilized for the conversion of the methyl ester to primary amide improved simply by increasing the reaction time (sat. NH_3−MeOH, 25 °C, 7−10 d, 62−77% vs 14 d, 99%). Attempts to conduct this transformation by hydrolysis of the methyl ester (LiOH, THF/MeOH/H_2O) followed by amidation (PyBop, HOBt, *i*-Pr_2NEt, 10 equiv NH_4Cl, 58%)[20,26] proceeded faster, but suffered competitive epimerization and TBS deprotection especially when conducted on large scales. Finally, a single step Boc and TBS group removal was accomplished by treatment of Boc-L-HAsn(OTBS)-OBn with 4 N HCl−EtOAc (30 equiv). This was followed by treatment of the amine with FmocCl (1.5 equiv) and $NaHCO_3$ (2 equiv) in 50% H_2O−dioxane to provide Fmoc-L-HAsn-OBn in an improved 92% yield and an intermediate that can be purified by simple recrystallization on large scale.

(21) Beaulieu, P. L. *Tetrahedron Lett.* **1991**, *32*, 1031. Beaulieu, P. L.; Schiller, P. W. *Tetrahedron Lett.* **1988**, *29*, 2019. Boc-L-*a*Thr-OH was prepared from L-*a*Thr-OH by treatment with Boc_2O, Na_2CO_3, THF/H_2O (87%). HCl•H−D-*a*Thr-OBn was prepared from Boc-D-*a*Thr-OH by treatment with BnBr, $NaHCO_3$, DMF (89%); 4 N HCl-EtOAc (quant.).

(22) SES = 2-trimethylsilylethanesulfonyl: Weinreb, S. M.; Demko, D. M.; Lessen, T. A. *Tetrahedron Lett.* **1986**, *27*, 2099. Huang, J.; Widkanski, T. S. *Tetrahedron Lett.* **1992**, *33*, 2657. Boc-D-Orn(SES)-OH, Boc-D-Orn(SES)-OBn, and D-Orn(SES)-OBn were prepared as described in the Supporting Information.

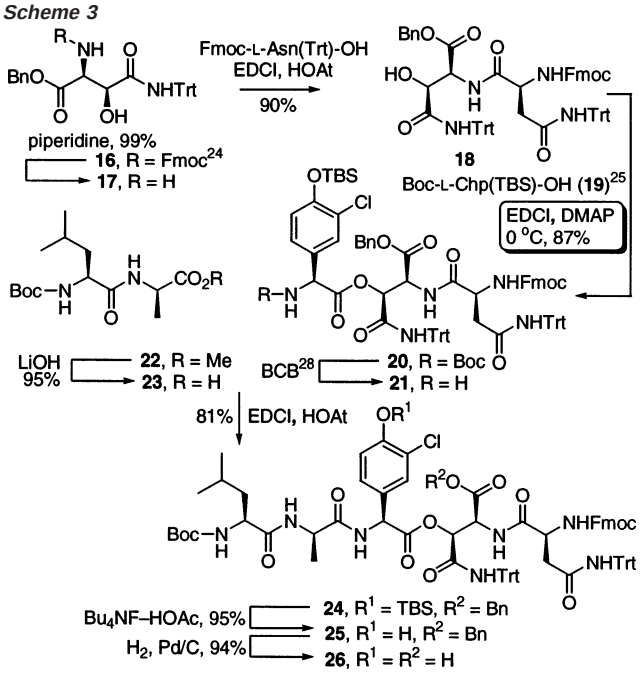
(23) DEPBT = 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one: Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455. Li. H.; Jiang, X.; Ye, Y.-H.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91.

(24) Boger, D. L.; Lee, R. J.; Bounaud, P.-Y.; Meier, P. *J. Org. Chem.* **2000**, *65*, 6770.

(25) Prepared from L-Chp-OH (Holdrege, C. T. U.S. Patent 3646024; *Chem. Abstr*. **1972**, *77*, 34543) by treatment with Boc_2O, Na_2CO_3, 25% H_2O−THF (98%); BnBr, $NaHCO_3$, DMF (98%); $CF_3CONMeTBS$, THF, 40−45 °C (100%); H_2, 10% Pd/C, EtOH (quant.).

(26) Wang, W.; McMurray, J. S. *Tetrahedron Lett.* **1999**, *40*, 2501.

Scheme 3



Fmoc deprotection of **16** and coupling of **17** with Fmoc-L-Asn(Trt)-OH (EDCI, HOAt, 17% DMF−CH_2Cl_2, 25 °C, 2.5 h, 81−90%)[20] provided **18**, and an intermediate that can purified by simple recrystallization on large scale, Scheme 3. The key esterification of **18** with **19**[25] required activation with EDCI[20] conducted in the presence of DMAP[27] catalyst (CH_2Cl_2, 0 °C, 1 h, 87%, >10:1 diastereoselection). A wide range of alternative esterification protocols were examined including the use of reactive, unhindered mixed anhydride activations, an acyl fluoride activation, Mitsunobu esterification, Yamaguchi esterification, and the Corey−Nicolaou 2-pyridylthioester activation with metal catalysis. These and EDCI or DCC[20] promoted couplings in the absence of DMAP provided no product or resulted in much lower conversions, lower diastereomeric ratios, or suffered from competitive β-elimination. Even the EDCI−DMAP, or a less satisfactory DCC−DMAP,[20,27] reaction required carefully controlled reaction conditions with higher reaction temperatures (25 vs 0 °C), longer reaction times, or more DMAP (0.5−2 equiv vs 0.15−0.2 equiv) offering less satisfactory conversions. Boc removal (BCB,[28] CH_2Cl_2, 0 °C, 2 h), coupling with **23** (EDCI, HOAt, 20% DMF−CH_2Cl_2, 0 °C, 1.5 h, 81%),[20] buffered TBS deprotection (Bu_4NF−HOAc, THF, 0 °C, 30 min, 95%),[29] and benzyl ester hydrogenolysis (H_2, 10% Pd/C, EtOH, 25 °C, 4.5 h, 94%) provided **26**. Efforts to shorten this sequence by first coupling **23** with L-Chp(OTBS)-OBn followed by benzyl ester deprotection and esterification with **18** have not been successful and an unbuffered Bu_4NF deprotection of **24** (no HOAc) led to competitive epimerization of the Chp α-aminoester center. Given the effectiveness of this preparation of **26**, its simple adoption to incorprate amide or even more stable carbon replacements of the sensitive depsipeptide ester can be easily envisioned.

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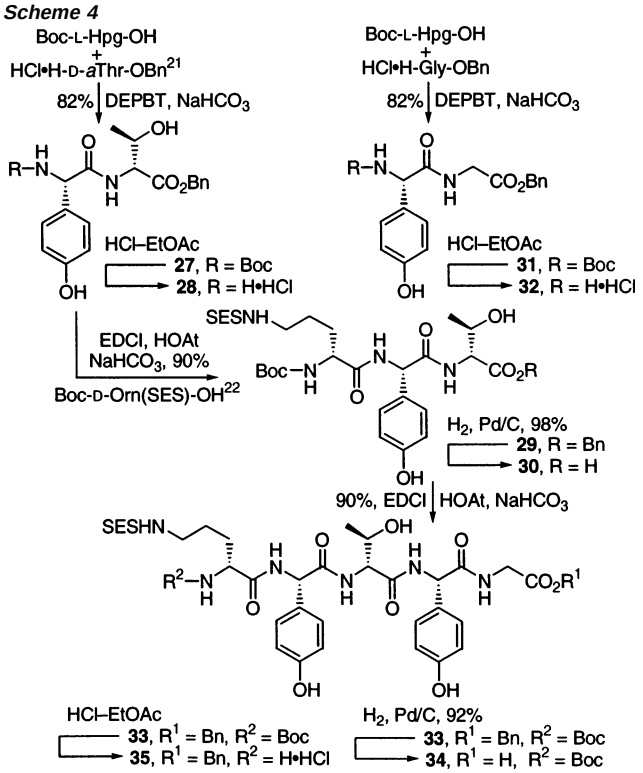
(28) BCB = *B*-bromocatecholborane: Boeckman, R. K., Jr.; Potenza, J. C. *Tetrahedron Lett.* **1985**, *26*, 1411.

(29) Boger, D. L.; Miyazaki, S.; Kim. S. H.; Wu, J. H.; Loiseleur, O.; Castle, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 3226. Boger, D. L.; Miyazaki, S.; Kim, S. H.; Wu, J. H.; Castle, S. L.; Loiseleuer, O.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 10004.

Scheme 4

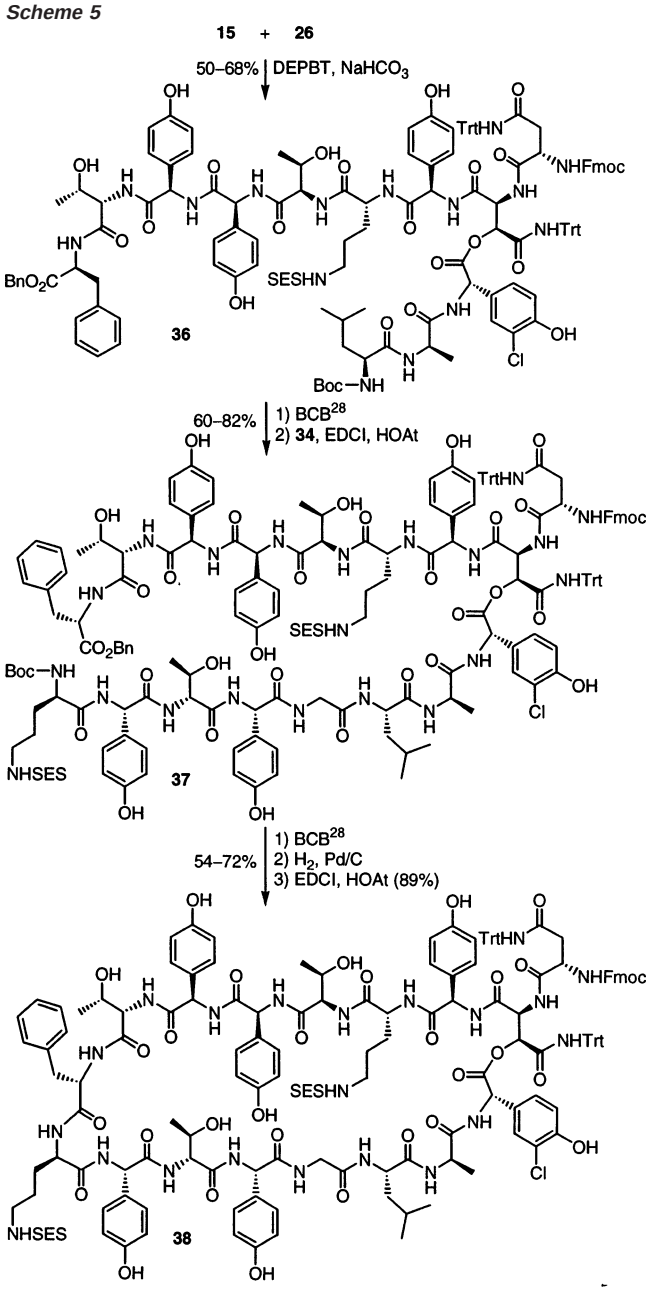


Synthesis of the Orn[10]−Gly[14] Subunit (34). The preparation of the final subunit **34** is detailed in Scheme 4. D-*a*Thr-OBn[21] was directly coupled with Boc-L-Hpg-OH to give dipeptide **27** (DEPBT,[23] $NaHCO_3$, THF, 0−25 °C, 18 h, 82%) devoid of any diastereomeric contaminants. Boc deprotection (HCl−EtOAc) and direct coupling of **28** with Boc-D-Orn(SES)-OH[22] provided tripeptide **29** (EDCI, HOAt, $NaHCO_3$, 20% DMF−CH_2Cl_2, 0−15 °C, 4 h, 90%).[20] Benzyl ester deprotection (H_2, 10% Pd/C, MeOH, 25 °C, 2 h, 98%) and coupling of **30** with **32** gave pentapeptide **33** (EDCI, HOAt, $NaHCO_3$, 50% DMF−CH_2Cl_2, 0−25 °C, 25 h, 90%).[20] In turn, the dipeptide **32** was obtained in two steps by coupling Boc-L-Hpg-OH and Gly-OBn hydrochloride to give dipeptide **31** (DEPBT,[23] $NaHCO_3$, THF, 0−25 °C, 19 h, 82%), followed by Boc deprotection (HCl−EtOAc). 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[23] minimized epimerization especially when coupled with a hindered and sensitive *a*Thr amine terminus. For example, DEPBT[23] promoted coupling to provide **27** proceeded in good yield with little or no epimerization (82%, 98% de) whereas an EDCI−HOAt[20] coupling was accompanied by significant racemization (80%, 80% de). Finally, the Orn[10]−Gly[14] subunit was initially prepared as the methyl ester[30] but ultimately implemented as the benzyl ester **33** permitting a cleaner ester deprotection conducted on a substrate (i.e., **41**) leading to Gly[14]−Leu[15] macrolactamization.

Assembly of Three Subunits and Macrocyclizations at the Phe[9]−Orn[10] and Gly[14]−Leu[15] Sites. One of the strategic advantages of a convergent synthesis employing three key fragments was the opportunity to examine macrocyclization at

(30) Experimental details are provided in the Supporting Information.

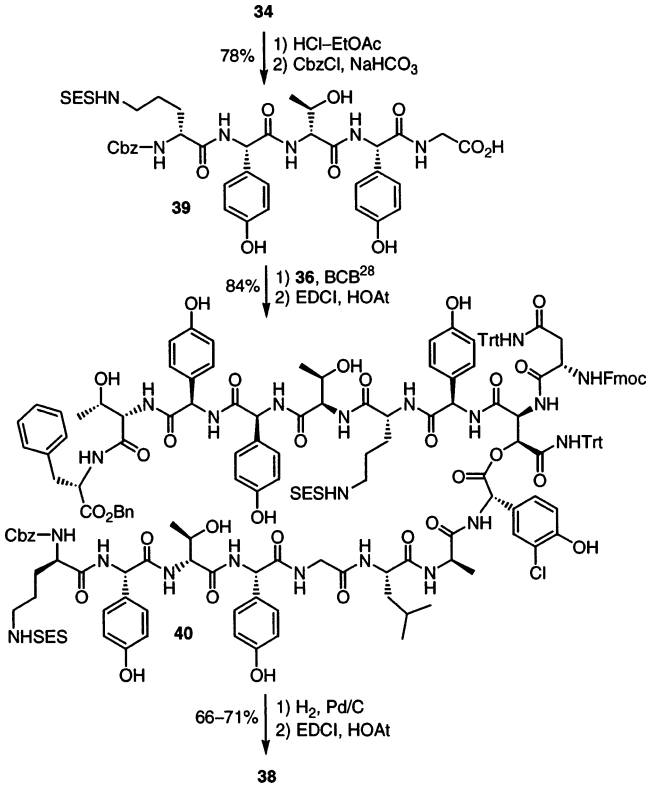
Scheme 5



either the Phe[9]−Orn[10] or the Gly[14]−Leu[15] site enlisting common precursors altering only the order or site of fragment assembly. At the onset of our work and despite the well-founded macrocyclization site selections, the closure to provide the 49-membered ring was unprecedented. Consequently, the convergent fragment assembly provided maximum flexibility in examining the macrocyclization in addition to providing the strategic advantages commonly attributed to a convergent versus linear (i.e., solid phase) synthesis. Assembly of three key fragments and macrocyclization at the Phe[9]−Orn[10] site, which we judged most attractive to initially examine, are detailed in Scheme 5. 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 a *N*-acyl versus carbamate derivative. Only DEPBT[23] promoted the coupling to provide **36** in superb yields ($NaHCO_3$, DMF, 0 °C, 20 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. By way of contrast, the best alternative to this coupling enlisted EDCI (HOAt, 0−85% CH_2Cl_2−DMF, 0 °C, 18−40 h)[20] and provided **36** in variable conversions of 0−19%. Little or no reaction was observed when this DEPBT coupling was run in the absence of added $NaHCO_3$ (1.5 equiv with 1.5 equiv of DEPBT), whereas the use of stronger tertiary amine bases led to competitive and extensive β-elimination or hydrolysis of the desired product. In addition, in early studies we found that exposure of isolated **36** to MeOH to effect transfers, especially on small scale, led to hydrolysis (methanolysis) of the labile ester. As a consequence, the exposure of **36** and all subsequent intermediates to MeOH or tertiary amine bases was avoided although we do not know whether these precautions are necessary. However, consistent with these observations and expectations, we have found that treatment of either ramoplanin (**2**) or the ramoplanin aglycon (**48**) with 1% Et_3N-H_2O results in rapid hydrolysis of the depsipeptide ester to cleanly provide the linear acyclic derivative[18] (>90% isolated yield, 20 min; 80%, 5 min, 30%, 1 min)[30] with little or no difference in the rates of reaction. Boc removal was accomplished under mild conditions (BCB,[28] 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%)[20] provided the key acyclic depsipeptide **37**. Successive Boc removal (BCB,[28] CH_3CN, 0 °C, 3 h), benzyl ester hydrogenolysis (H_2, 10% Pd/C, EtOH, 25 °C, 2 h), and macrocyclization (EDCI, HOAt, 66−85% CH_2Cl_2−DMF, 1 mM, 0 °C, 18−20 h, 54−72% for 3 steps) afforded the cyclic depsipeptide core **38**. Presumably, β-sheet preorganization of the cyclization substrate[18] and closure at a D-amine terminus[19] at the corner of a β-turn contributes to the superb conversions for closure of the 49-membered ring (89% yield for macrocyclization using the purified amino acid). Given the unique success of an initial ring closure attempt enlisting EDCI,[20] the subsequent optimization studies were conducted with the examination of very few alternative reagents. Thus, although this was not investigated in detail, small scale reactions with either DEPBT[23] (lower conversion) or DPPA[20] (no reaction) did not suggest they would be useful alternatives to EDCI. Notably, the linear peptide **37** proved insoluble in most nonpolar, aprotic solvents (CH_2Cl_2, $CHCl_3$, EtOAc), slightly soluble in CH_3CN, and soluble in protic solvents (MeOH, EtOH). The choices of solvents for the Boc deprotection (CH_3CN) and benzyl ester deprotection (EtOH) were dictated by this solubility and the latter (EtOH vs MeOH) minimizes competitive ester cleavage by the solvent under the reaction conditions.

In an effort to further streamline the synthesis, the *N*-terminus Boc group of the Orn[10]−Gly[14] subunit **33** was exchanged for a Cbz protecting group such that the ester and amine deprotections of **40** could be conducted in a single operation. Thus, Boc deprotection of **34** (4 N HCl−EtOAc) followed by Cbz

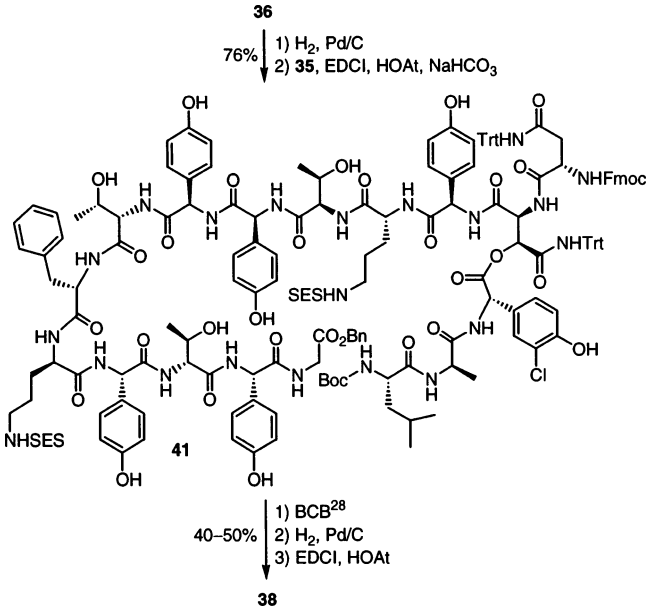
Scheme 6



protection (CbzCl, $NaHCO_3$, 50% dioxane−H_2O, 25 °C, 30 min, 78%) provided **39**, Scheme 6. BCB[28] Boc deprotection of **36** (CH_2Cl_2, 0 °C, 30 min) with isolation and purification of the free amine followed by coupling with **39** provided **40** (EDCI, HOAt, DMF, 0 °C, 26 h, 84%).[20] Single step Cbz and benzyl ester deprotection (H_2, 10% Pd/C, 50% DMF−MeOH, 25 °C, 4 h) followed by EDCI promoted macrocyclization under the conditions describe earlier provided **38** (66−71%) in competitive conversions. However, the conversions were challenging to reproducibly observe resulting in occasional competitive Fmoc and Trt deprotections and ester cleavage under the hydrogenolysis conditions especially if conducted for extended reaction periods. Although these were perceived as solvable problems complicated only by the insolubility properties of **40**, this coupled with the challenges of purifying **39** provided the incentive for us to carry forward with material prepared as detailed in Scheme 5.

Finally, the results of a brief study on macrocyclization at the alternative Gly[14]−Leu[15] site are shown in Scheme 7. Although unnecessary for the completion of the total synthesis of the ramoplanin aglycon, the examination of closure at this site was of interest for several reasons. First, it opens the opportunity to prepare analogues of the sensitive depsipeptide region of **2** (Figure 1) enlisting an alternative coupling order first assembling Hpg[3]−Gly[14] as a common precursor, followed by coupling with modified depsipentapeptide subunits and a final key macrocyclization at the Gly[14]−Leu[15] site. Just as interestingly, it constitutes closure at the other end of the H-bonded antiparallel β-strands at a site within a small flexible loop and should benefit from β-sheet preorganization of the cyclization substrate like closure at the Phe[9]−Orn[10] site. Unlike the Phe[9]−Orn[10] site, it does not benefit from closure at a D-amine terminus although the carboxylate terminus now lacks a L-side chain substituent that may decelerate such ring closures. It also

Scheme 7

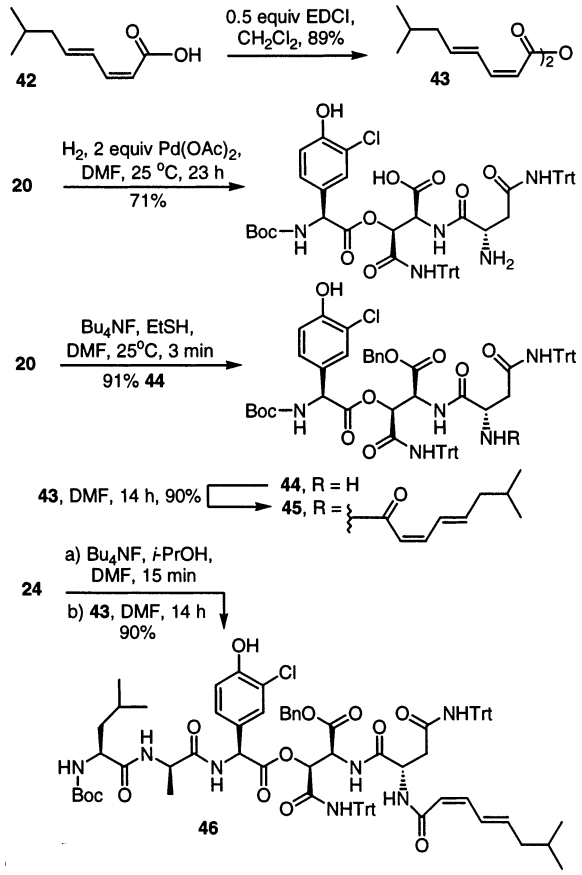


precludes competitive racemization of the activated carboxylate. As such, and based on these criteria alone, the two closures might be expected to perform comparably. The distinction between the two sites rests with Phe[9]−Orn[10] lying at the corner of a conformationally defined β-turn adjacent to the H-bonded antiparallel β-strands whereas the Gly[14]−Leu[15] site is embedded in what appears to be a conformationally more flexible region of the molecule. Benzyl ester deprotection of **36** (H_2, 10% Pd/C, EtOH, 25 °C, 4 h) followed by coupling with **35** provided **41** (EDCI, HOAt, $NaHCO_3$, 2:1 CH_2Cl_2−DMF, 0 °C, 18−20 h, 76%).[20] Boc deprotection (BCB,[28] CH_3CN, 0 °C, 3 h), benzyl ester deprotection (H_2, 10% Pd/C, EtOH, 25 °C, 2 h) followed by macrocyclization (EDCI, HOAt, 75% CH_2Cl_2−DMF, 1 mM, 0 °C, 18−20 h) provided **38** in good conversions (40−50%). This preliminary observation, which has not been subjected to optimization efforts, suggests that closure at the Gly[14]−Leu[15] site, while perfectly acceptable, may not be as facile as closure at the Phe[9]−Orn[10] site.

Acyl Side Chain Introduction and Completion of the Total Synthesis. Having served their purpose admirably, the removal of the orthogonal Fmoc protecting group with introduction of the unsaturated acyl side chain followed by sequential or simultaneous trityl and SES deprotections remained for completion of the total synthesis. Both were viewed as formidable challenges to conduct in the presence of the sensitive depsipeptide ester. The latter SES deprotection in the presence of a sensitive depsipeptide ester was a challenge we had addressed earlier in the course of a total synthesis of the luzopeptins and quinoxapeptins and solved by introducing a HF deprotection.[31] Our selection of the orthogonal SES protecting group was based on this experience and we assumed, but did not know, that the Trt protecting groups would also be removed under these conditions and tentatively hoped that the acyl side chain olefin stereochemistry would be unaffected. As anticipated based on a related experience in the total synthesis of thiocoraline and

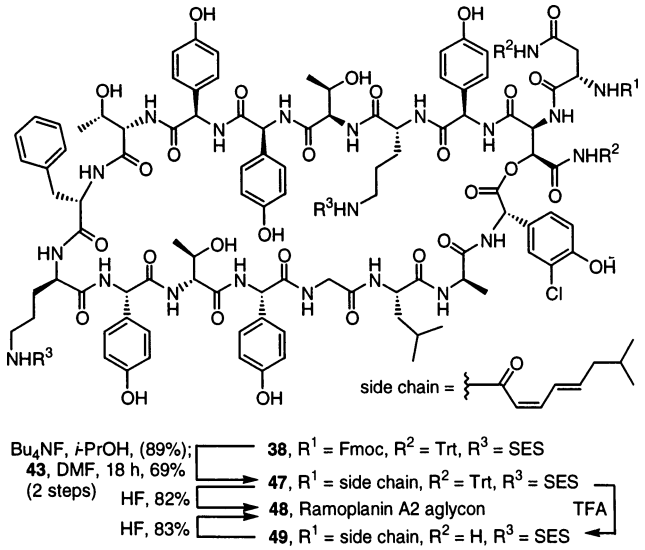
(31) Boger, D. L.; Ledeboer, M. W.; Kume, M. *J. Am. Chem. Soc.* **1999**, *121*, 1098. Boger, D. L.; Ledeboer, M. W.; Kume, M.; Searcey, M.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 11 375.

Scheme 8



BE-22179 which bear a sensitive depsipeptide thioester,[32] attempted Fmoc deprotection of **38** with piperidine (5% piperidine−DMF, 0 °C, 20 min) simply led to multiple products including cleavage of the depsipeptide ester by β-elimination and/or ester aminolysis without generating perceptible amounts of the desired macrocyclic free amine or the subsequent acylation product **47** (5 equiv of **42**, EDCI, HOAt, DMF−CH_2Cl_2, 0 °C, 18−24 h).[20] Consequently, we examined a number of alternatives to a conventional Fmoc deprotection and the subsequent *N*-acylation reaction using advanced intermediates bearing the sensitive depsipeptide ester (Scheme 8). On the basis of the report[33] that the Fmoc protecting group is sensitive to certain hydrogenation conditions and our own observation that **24**, **37**, and **40** may have suffered slow competitive Fmoc deprotection under extended reaction times for the benzyl ester deprotection, the deprotection of **24** with H_2 and $Pd(OAc)_2$ was examined and found satisfactory providing both benzyl ester and Fmoc deprotection, albeit requiring a prolonged reaction time (DMF, 16 h) notably without competitive Trt deprotection. However, this Fmoc deprotection proved capricious to reproducibly implement and provided only minor amounts of **45** or the corresponding unstable amine when applied to **38** (H_2, 2 equiv $Pd(OAc)_2$, DMF, 25 °C, 14−46 h). In part, this may be attributed to the unrecognized instability of the product free amine and the use of subsequent nonoptimal acylation conditions (5 equiv **42**, EDCI, HOAt, DMF−CH_2Cl_2, 0 °C, 24 h).[20] We

Scheme 9



subsequently examined the Fmoc deprotection utilizing Bu_4NF in the presence of EtSH.[34] These conditions (2 equiv Bu_4NF, 10 equiv EtSH, DMF, 25 °C, 3 min) did provide the desired amine from **20** and **24**, but the rapid reaction times made it difficult to cleanly isolate the product free amine without promoting cleavage of the depsipeptide and were not successful when implemented with the macrocyclic precursor **38**. Although the inclusion of EtSH in this reaction mixture serves to trap the released dibenzofulvene,[35] we viewed its inclusion as one that also serves to buffer the basicity of the reagent mixture. As such, we examined several variations on such a buffered Bu_4NF promoted Fmoc cleavage (e.g., PhSH, HOAc, CF_3CH_2OH) and found that inclusion of the relatively hindered alcohol, *i*-PrOH, in place of the thiol provided a superb new method of Fmoc deprotection. Thus, treatment of **20** and **24** with Bu_4NF (2 equiv, 10 equiv *i*-PrOH, DMF, 25 °C, 15 min, sonication) followed by acylation of the crude free amines with anhydride **43** (2 equiv, DMF, 25 °C, 16 h) provided **45** and **46** (90%) in superb overall conversions. Through the course of these studies, we established that this subsequent *N*-acylation was most effective when conducted with the anhydride **43** permitting a relatively easy purification of the products from the reaction byproducts compared to acylation reactions conducted with the corresponding carboxylic acid **42** (EDCI).[20]

Fmoc removal of **38** under these specially developed conditions (8 equiv of Bu_4NF, 10 equiv of *i*-PrOH, DMF, 25 °C, 1 h) that do not promote competitive Asn^2 β-elimination followed by side chain acylation of crude free amine with anhydride **43** (2 equiv, 20% CH_2Cl_2−DMF, 69% for 2 steps) provided **47** in excellent overall conversions, Scheme 9. Notably, efforts to purify and characterize the intermediate free amine were not successful in early efforts on the Fmoc deprotection which we tentatively ascribe to its instability. A single step HF deprotection[31] of the trityl and SES groups (HF, anisole, 0 °C, 90 min, 82%) or sequential trityl (5% H_2O−TFA, 25 °C, 5 h) and SES deprotection (HF, 83%) provided the ramoplanin A2 and ramoplanose aglycon **48** identical in all respects with an authentic sample (^1H NMR, HPLC, UV, MS).[36,37]

(32) Boger, D. L.; Ichikawa, S.; Tse, W. C.; Hedrick, M. P.; Jin, Q. *J. Am. Chem. Soc.* **2001**, *123*, 561. Boger, D. L.; Ichikawa, S. *J. Am. Chem. Soc.* **2000**, *122*, 2956.
(33) Atherton, E.; Bury, C.; Sheppard, R. C.; Williams, B. J. *Tetrahedron Lett.* **1979**, 3041.
(34) Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617. Ueki, M.; Nishigaki, N.; Aoki, H.; Tsurusaki, T.; Katoh, T. *Chem. Lett.* **1993**, 721.
(35) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401.

In our hands, and unlike the conformational heterogeneity alluded to by McCafferty,[15] the ramoplanin aglycon **48** adopts a single, rigid conformation in solution indistinguishable from that of the natural product (^{1}H NMR) although this was not explored with 2D techniques. Similarly, we have not observed stability differences between the natural product and the aglycon. We do, like McCafferty, observe losses in material upon reverse phase chromatography of **2** or **48**. This poor recovery (ca. 50%), in our assessment, is not derived from degradation (no other products detected), but rather the poor physical properties of the compounds including their complete and irretrievable adherence to plastic and related materials. Consequently, we do not view the role of the disaccharide subunit of ramoplanin A2 as one that conveys stability or conformational rigidity to the molecule,[15] but rather one that, among other roles, may alter and potentially improve solubility or physical properties. Thus, the final, single step HF deprotection of **47** to provide the aglycon **48** was most effectively conducted with a trituration purification (EtOAc) of the final product avoiding a reverse phase chromatography purification and the simple recovery losses. Notably, cyclic depsipeptide intermediates bearing the Fmoc, Trt, and SES protecting groups (e.g., **38**) do not suffer these same recovery properties and could be purified by standard versus reverse phase chromatography, and **47** bearing the Asn^1 acyl side chain and Trt/SES protecting groups could be purified by reverse phase chromatography without analogous apparent recovery losses.

Deglycosidation of Ramoplanin and Functionalization of the Ramoplanin A2 Aglycon. In an effort to secure comparison samples of authentic **48** and preceding synthetic intermediates, we examined methods for the deglycosidation of ramoplanin and the subsequent functionalization of the ramoplanin A2 aglycon. Past protocols for the deglycosidation of ramoplanin have relied on the treatment of the ramoplanin complex with either: (1) trimethylsilyl iodide or trimethylsilyl chloride in the presence of sodium iodide followed by hydrolysis, or (2) a strong mineral acid (HCl) in the presence of a lower alcohol (e.g., BuOH) under anhydrous conditions (entry 1).[36] Typically, pure ramoplanin A2 aglycon is obtained in 20−30% yield using optimized conditions for these methods. In addition to low conversions, the isolation of pure ramoplanin aglycon requires a tedious HPLC purification that results in some loss of material and contributes to the low conversions. We have found that the use of anhydrous HF cleanly cleaves the dimannose sugar of ramoplanin, without affecting the sensitive ester linkage or acyl side chains.[37] Thus, treatment of the ramoplanin complex with anhydrous HF cleanly provided the A1−A3 aglycons contaminated only with mannose-derived reaction byproducts, Scheme 10. Reverse phase HPLC purification of the mixture which serves to separate the A1−A3 aglycons (20−50% CH_3CN−$HCOONH_4$ (aq, 0.05 M)) provided pure A1 (3%), A2 (46%), A3 (3%), albeit with a recovery loss due to the physical properties of the natural product aglycons. Enlisting pure ramoplanin A2 (**2**), the HF deglycosidation followed by a simple EtOAc trituration to remove the deglycosidation byproducts provided the pure A2 aglycon (**48**) in 92% yield avoiding the material loss that accompanies HPLC purification. Thus, the use of anhydrous HF for deglycosidation of ramoplanin A1−A3 is exceptionally effective providing the corresponding aglycons in conversions as high as 92% and offers a superb improvement over existing methods.

Antimicrobial Activity, *Staphylococcus aureus* (ATCC 25923)

compound	MIC (μg/mL)
2, ramoplanin A2	0.5
48, ramoplanin A2 aglycon	0.25
acycloramoplanin A2	>128
acycloramoplanin A2 aglycon	>128
49, Orn^4,Orn^{10}-diSES ramoplanin A2 aglycon	>128
50, Orn^{10}-SES ramoplanin A2 aglycon	4

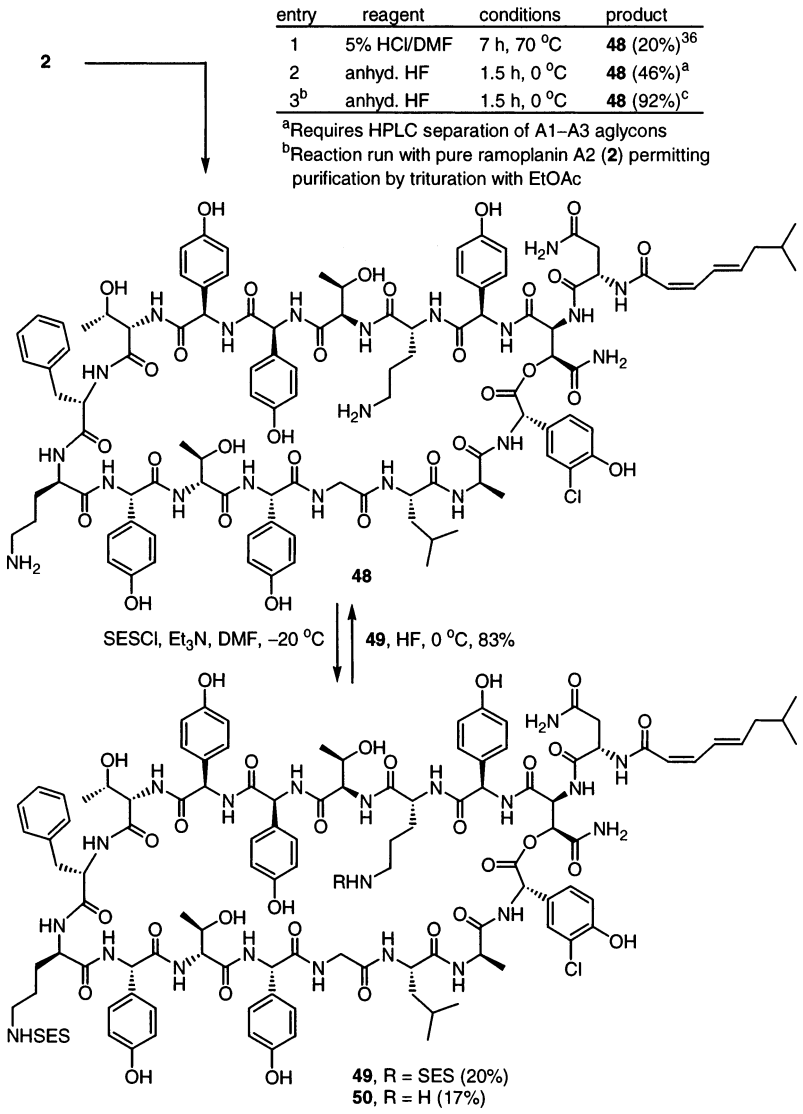
Figure 4.

Reaction of **48** with SESCl (Et_3N, DMF, −20 °C) provided a mixture of **49** and **50** with the Orn^4 amine reacting only sluggishly under a wide range of reaction conditions, Scheme 10. Importantly, authentic **49** prepared in this manner proved identical (^{1}H NMR, HPLC, UV, MS) with the synthetic material described above. Notably, **49** and **50** have proven to be key derivatives of **2** useful in the definition of the site of action of ramoplanin and the details of these and related studies will be disclosed in due time.

Antimicrobial Activity of Key Derivatives. The results of an antimicrobial evaluation of several of the key derivatives of ramoplanin A2 that were prepared herein are summarized in Figure 4 using *Staphylococcus aureus* (ATCC 25923) which is among the least sensitive wild-type bacteria. Consistent with the original[36] and more recent subsequent observations,[15] the antimicrobial potency of ramoplanin A2 (**2**) and its aglycon **48** were not distinguishable with the latter typically displaying slightly greater potency in our single assay. Consequently, glycosidation does not contribute to intrinsic in vitro antimicrobial potency. Similarly, acycloramoplanin A2 and its aglycon, in which the macrocyclic lactone was hydrolyzed, were inactive (MIC > 128 μg/mL) and found to be >250- to 500-fold less potent. Previous studies detailed an approximate 2000-fold loss in antimicrobial activity upon hydrolysis of the macrocyclic lactone of ramoplanin A2[15,18] and we see analogous drops in the activity of not only this derivative, but its aglycon as well. Similarly, **49**, in which both the Orn^4 and Orn^{10} δ-amino groups are SES protected, was found to be inactive (MIC >128 μg/mL, >500-fold loss in activity). Analogous observations were recently disclosed by McCafferty[15] with Orn^4, Orn^{10}-diacetyl ramoplanin A2 which was found to be 500-fold less potent than ramoplanin A2 highlighting the importance of these two basic amines which are conserved among all members of this class of antimicrobial natural products. Much more interestingly, the mono SES derivative **50** (MIC = 4 μg/mL), in which only the Orn^{10} δ-amino group is protected and the Orn^4 amine is not, was 16-fold less potent than the free aglycon **48**, but >32-fold more potent than the diSES derivative **49**. Clearly, both the Orn^4 and Orn^{10} amines contribute to the antimicrobial activity and the latter result suggests, but does not require, that the Orn^4 free amine is more important than the Orn^{10} amine. Recent work of Walker's[6] enlisting alanine derivatives of the Orn^4 and Orn^{10} δ-amines, which maintain but move or extend the position of the free amines, found that the Orn^4−Ala was an active antimicrobial agent (MIC = 0.8 μg/mL) and bound peptidogly-

(36) Ciabatti, R.; Cavalleri, B. (BioSearch Italia S.p. A.) US patent 5491128; *Chem. Abstr.* **1990**, *112*, 179 893.
(37) Wanner, J.; Tang, D.; McComas, C. C.; Crowley, B. M.; Jiang, W.; Moss, J.; Boger, D. L. *Bioorg. Med. Chem. Lett.*, in press. See also: Boger, D. L.; Menezes, R. F.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 959.

Scheme 10



can lipid I analogues whereas the Orn^{10}−Ala derivative was nearly inactive (MIC = 100 μg/mL) and did not bind to lipid I peptidoglycan analogues implicating Orn^{10} versus Orn^{4} as the more important of the two residues. Interestingly, the positively charged amine of Orn^{4} flanks one side of the ramoplanin solution structure whereas that of Orn^{10} flanks the other and they lie at the opposite ends of the conserved putative peptidoglycan binding domain Hpg^{3}−Orn^{10}. Our observation of a significant, but not complete loss of activity with **50** (16-fold loss) suggests that the Orn^{10} free amine is important, but not absolutely essential for activity. Further studies on these and additional synthetic and semisynethtic derivatives should serve to clarify the role of the Orn^{4} and Orn^{10} amines, the putative Hpg^{3}−Orn^{10} recognition domain, and offer insights into the site and mechanism of action of ramoplanin.

Conclusions

A solution phase convergent total synthesis of the ramoplanin A2 and ramoplanose aglycon was developed defining two strategically effective macrocyclization sites. In addition to the design elements inherent in a convergent synthesis that were highlighted in the Introduction, unexpected extensive epimerization at many of the coupling sites were observed even when enlisting some of the most effective coupling reagents and unanticipated challenges associated with forming and maintaining the depsipeptide ester were addressed as the key subunits were assembled. In light of these findings, it is unlikely that a linear solid phase synthesis of the ramoplanin aglycon could be effectively implemented without the knowledge derived from the work detailed herein. In anticipation of a continuation of our efforts, the modular assemblage of **38** from three key fragments makes the approach amenable to the systematic preparation of analogues in which the role of each residue and each side chain structural feature may be assessed without conducting a complete linear solid-phase synthesis of each individual compound. This superimposition of the parallel, divergent synthesis of a library of ramoplanin analogues onto a convergent total synthesis[38] is in progress and will be disclosed in due course.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA41101) and The Skaggs Institute for Chemical Biology. We wish to thank Dr. Inkyu Hwang for conducting the antimicrobial assays, Dr. S.

(38) Chen, Y.; Bilban, M.; Foster, C. A.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5431.

Ichikawa for interim studies on the coupling of **15** and **26** and macrocyclization studies of **38**, Dr. P. Meier for contributing to material supplies in interim studies, Dr. Mark O′Neil-Johnson (Sequoia Sciences, San Diego, CA) for microscale ^{1}H NMR spectra, and Dr. Ciabatti (Biosearch Italia S.p.A.) for a supply of the natural ramoplanin complex and a correlation sample of the ramoplanin A2 aglycon. We wish to especially thank Professor K. D. Janda for the use of the anhydrous HF apparatus and Jason Moss for the training and assistance with each anhydrous HF reaction.

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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