Total Synthesis of Dehydrodidemnin B. Use of Uronium and Phosphonium Salt Coupling Reagents in Peptide Synthesis in Solution†

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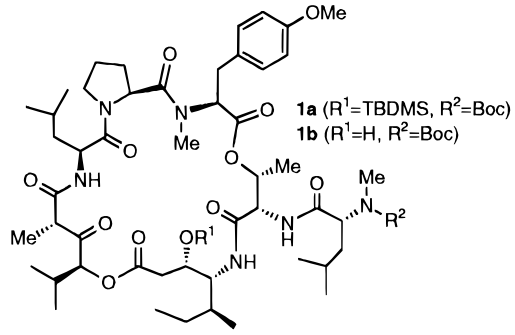
New total syntheses of didemnin A and of dehydrodidemnin B are described. The latter didemnin has the highest antiproliferative activity of all members of this family of macrocyclic depsipeptides. It was produced on coupling the side chain Pyr-Pro-OH to didemnin A, which was itself synthesized by two novel routes. One of these was based on the elaboration of a linear heptadepsipeptide incorporating the first amino acid of the didemnin side chain, (*R*)-N(Me)-Leu. Deprotection of the amino and carboxyl terminii of this linear precursor followed by macrocyclization gave a protected derivative of didemnin A. The second route involved synthesis of the Boc-protected didemnin macrocycle from a linear hexadepsipeptide lacking (*R*)-N(Me)-Leu. Removal of the Boc group from the macrocycle followed by its coupling with Boc-(*R*)-N(Me)-Leu-OH then gave Boc-didemnin A. The overall yield was much higher for the second strategy (27% compared to 4% for the first synthesis), but both allowed synthetic didemnin A, identical with a natural sample, to be prepared. Extensive use was made of phosphonium and uronium salt-based coupling reagents, such as BOP, PyBrOP, PyAOP, HBTU, and HATU for the formation of both the secondary and tertiary amide bonds present in these complex depsipeptides.

Introduction

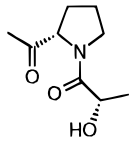
The didemnins[1] are macrocyclic depsipeptides, first isolated in 1981 from the marine tunicate *Tridemnum solidum* by Rinehart.[2,3] Subsequent studies established that they all have have the same basic structure (**1**, R^1 = H), consisting of a 23-membered macrocycle with an attached side chain. The macrocycle is made up of six subunits, (*S*)-Leu, (*S*)-Pro, (1*S*,2*R*)-Thr, (*S*)-N(Me)-O(Me)-Tyr, (3*S*,4*R*,5*S*)-isostatine (Ist), and (2*S*,4*S*)-3-oxo-4-hydroxy-2,5-dimethylhexanoic acid, also known as α-(α-hydroxyisovaleryl)propionic acid (HIP). The side chain, whose first amino acid is always (*R*)-N(Me)-Leu, is joined to the Thr of the macrocycle, and its structure differentiates the various didemnins: in the simplest member of the family, didemnin A (**1c**), it consists only of (*R*)-N(Me)-Leu, whereas in other cases it can be appreciably more complex.



Didemnin A, **1c** R^1=R^2=H

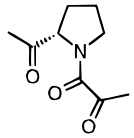
Didemnin B, **1d** [R^1=H, R^2=(S)-Pro-(S)-Lac]

1d R^1=H, R^2=



Dehydrodidemnnin B, **1e** [R^1=H, R^2=(S)-Pro-Pyr]

1e R^1=H, R^2=



Certain didemnins exhibit significant antiproliferative and immunosuppressive activity, and didemnin B (**1d**), the most active of those hitherto isolated, was the first marine natural product to enter phase I/II clinical trials as a candidate drug at the U.S. National Cancer Institute.[4−9] The encouraging physiological activity of this intriguing class of compounds together with their structural complexity has provided the impetus for

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† Abbreviations for amino acids and peptides used in this paper are those recommended by the IUPAC-IUPAB Commission of Biochemical Nomenclature, and published in *J. Biol. Chem.* **1972**, *247*, 977−983. Additional abbreviations used are as follows: Boc, *tert*-butoxycarbonyl; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BOP-Cl, *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; Bzl, benzyl; CI, chemical ionization; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; ES, electrospray; FAB, fast atom bombardment; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IR, infrared; Lac, lactyl; NMR, nuclear magnetic resonance; PyAOP, [(7-azabenzotriazol-1-yl)oxy]tris(pyrrolidino)-phosphonium hexafluorophosphate; PyBrOP, bromotripyrrolidinophosphonium hexafluorophosphate; Pyr, pyruvyl; RP, reversed phase; SEM, [(trimethylsilyl)ethoxy]methyl; TBAF, tetra-*n*-butylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; Tce, 2,2,2-trichloroethyl, TFA, trifluoroacetic acid; THF, tetrahydrofuran; t_R, retention time; TTFH, tetramethylfluoroformamidinium hexafluorophosphate; Z, benzyloxycarbonyl.

⊗ Abstract published in *Advance ACS Abstracts*, December 15, 1996.

(1) Li, W.-R.; Joullié, M. M. In *Studies in Natural Products*; Attaur-Rahman, Ed.; Elsevier Science Publishers: New York, 1992; Vol. 10, Part F; pp 241−302.

(2) Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857−1859.

(3) Rinehart, K. L.; Gloer, J. B.; Hughes, R. G.; Renis, H. E.; McGovren, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science* **1981**, *212*, 933−935.

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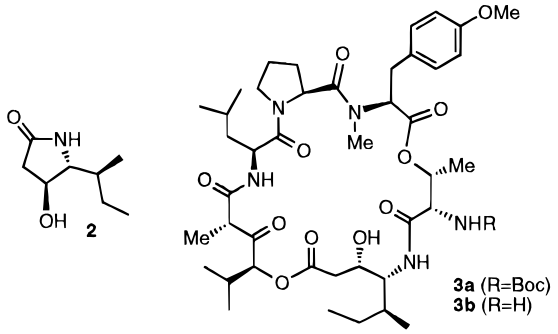
synthetic studies, culminating in the total synthesis of several didemnins[10−14] and of some analogues.[15,16]

More recently, while the biological activity of other newly isolated didemnins[17,18] still awaits full evaluation, dehydrodidemnin B (**1e**), from *Aplidium albicans*,[16,19,20] has shown even higher antineoplastic activity than didemnin B, both *in vitro* and *in vivo*. It is currently the most active didemnin known but, unfortunately, is only present in tiny quantities in extracts of the tunicate. Although a preliminary evaluation of its therapeutic usefulness can be done with material obtained by semisynthesis from the relatively abundant natural didemnin A (**1c**), a more satisfactory source of the molecule for biological testing would be chemical synthesis. Here we report on two new total syntheses of the didemnins that could, in principle, provide useful quantities of dehydrodidemnin B.

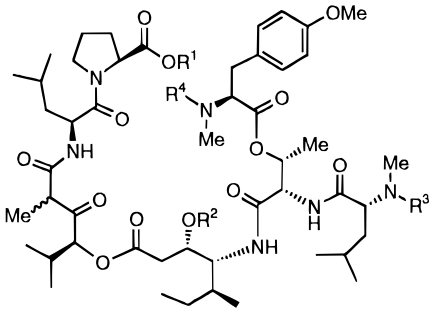
Strategical and Tactical Considerations

Didemnin A (**1c**) is the primary synthetic objective of any total synthesis of this family of compounds, since all other members can be obtained from it by attachment of the requisite side chain to the amino terminal of (*R*)-N(Me)-Leu. Two general synthetic strategies to **1c** can be envisaged, the difference between them residing in whether or not the linear precursor to the macrocycle incorporates the first amino acid of the side chain. Both approaches have formed the basis of successful total syntheses.

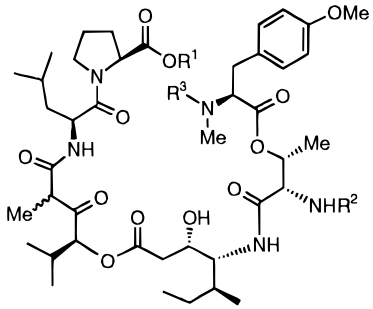
The key step in the synthesis of these molecules, regardless of whether (*R*)-N(Me)-Leu is present in the linear precursor, is the formation of the macrocycle. The greater facility with which amide bonds can be formed, a consequence of the superior nucleophilicity of the amine over the hydroxyl group, makes macrolactamization the preferred mode for ring closure. Successful cyclization at all of the four possible amide bonds has been achieved in previous syntheses of the didemnins.[21] However, in the present work, that between Pro and Leu was rejected because of the possibility of cyclo-[(*S*)-Pro-(*S*)-N(Me)-O(Me)-Tyr] diketopiperazine formation. That between (*S*)-Leu and the HIP unit was rejected because of the well-known tendency of β-keto acids to decarboxylate, and that between Ist and Thr was also rejected because of the possible cyclization of Ist to give the five-membered γ-lactam **2**. In consequence, the bond linking N(Me)-O(Me)-Tyr and Pro was selected as the point for macrocyclization.[12] Although formation of an amide here is, at first sight at least, more demanding, owing to the steric effect of the N-methylated amino group, it has the advantages that the risks of epimerization are reduced since the C-terminal amino acid is Pro and, more importantly, that no other obvious side reactions are associated with it.



Consideration of these factors led to the choice of the two linear precursors, **4a** and **5a**, as primary synthetic objectives. Linear precursor **4a** incorporates (*R*)-N(Me)-Leu whereas **5a** does not, but both are diastereomeric mixtures at C-2 of the HIP unit. Previous syntheses of the didemnins have indicated that optically pure macrocyclic products can be obtained from such mixtures.[10,12,13,22] Removal of the protecting groups from the amino and carboxyl functions of **4a**, followed by cyclization leads to the formation of **1a**, a protected derivative of didemnin A. Similar operations on **5a** give **3a**, a protected derivative of the didemnin macrocycle; attachment of (*R*)-N(Me)-Leu or more complex side-chains is then necessary to obtain didemnins.



4a (R^1=Tce, R^2=TBDMS, R^3=Boc, R^4=Z)
4b (R^1=H, R^2=TBDMS, R^3=Boc, R^4=Z)
4c (R^1=H, R^2=TBDMS, R^3=Boc, R^4=H)



5a (R^1=Bzl, R^2=Boc, R^3=Z)
5b (R^1=H, R^2=Boc, R^3=H)

(4) Dorr, F. A.; Kuhn, J. G.; Phillips, J.; von Hoff, D. D. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1699−1706.
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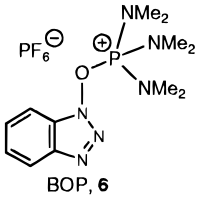
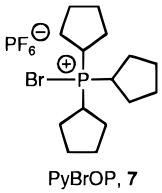
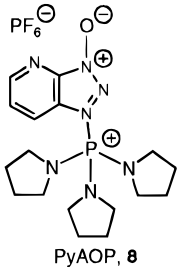
In addition to standard peptide (secondary amide) bonds, these complex depsipeptides contain ester and tertiary amide linkages between subunits. Peptide bond formation between the DNA-encoded amino acids is a highly optimized procedure that can be brought about repetitively and reproducibly in very high yield. However, the formation of esters or of secondary amides is more demanding, as a consequence of the poorer nucleophile in the former case and of steric hindrance in the latter. The new generation of phosphonium and uronium salt-based coupling reagents have been used to great effect in the solid-phase synthesis of peptides, in particular for the coupling of N-alkylated or otherwise sterically-hindered amino acids. The synthesis of the didemnins provided an opportunity for assessing their suitability for the synthesis of peptides in solution, where they have been much less frequently employed. Among the reagents that have been used in this work are the phosphonium and uronium[23−28] reagents BOP (**6**), PyBrOP (**7**), PyAOP (**8**), HBTU (**9**), and HATU (**10**). Additionally, the recently-described[29,30] additive HOAt (**11**), a structural variant of the well-known[31] HOBt (**12**), was also used.



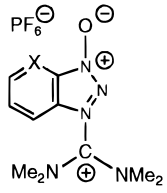
BOP, **6**



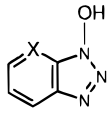
PyBrOP, **7**



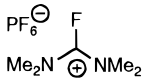
PyAOP, **8**



HBTU, X=CH, **9**
HATU, X=N, **10**



HOAt, X=N, **11**
HOBt, X=CH, **12**

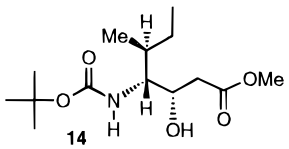


TTFH, **13**

The protection scheme for depsipeptide synthesis was based upon the use of the Boc- and Z-groups as amino protection, Bzl or TBDMS ethers for the protection of alcohols, and Bzl, Tce, or SEM esters for the protection of carboxylic acids.

Synthesis of the Subunits

Of the constituents of the didemnins employed in these syntheses, Boc-(*S*)-Pro-OH, Boc-(*S*)-Leu-OH, and Boc-(1*S*,2*R*)-Thr(Bzl)-OH are available commercially. The N-methylated amino acids Boc-(*R*)-N(Me)-Leu-OH and Boc-(*S*)-N(Me)-O(Me)-Tyr-OH were synthesized using methods described by Joullié.[14] We have previously described[32] the synthesis of protected derivatives of Ist, **14** and **15**, from Boc-(1*R*,2*S*)-Ile-OH.



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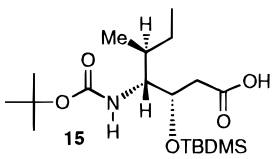

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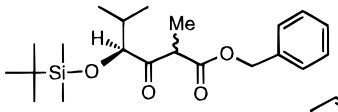

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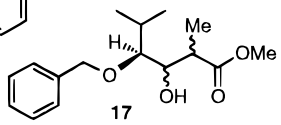

17

The most challenging subunit to manage is the HIP residue, and two protected derivatives, **16** and **17**, were employed. Both were used as mixtures of diastereomers, and both were synthesized from H-(*S*)-Val-OH, as reported by us.[33] β-Keto ester **16** is prone to undergo decarboxylation on hydrogenolytic deprotection of its carboxylic acid terminus, which can complicate its synthetic manipulation. Although this side reaction can normally be controlled, alcohol **17** was prepared as an alternative in which, after ester hydrolysis, decarboxylation occurs much less readily.[33]

Synthesis of Didemnin A

Strategy 1. The key step in the synthesis of linear precursor **4a**, whose deprotection and cyclization lead directly to the didemnin A derivative **1a**, was the union of the eastern and western segments **21** and **29**, respectively, by formation of an ester bond. The synthesis of segment **21** is outlined in Scheme 1.

Removal of the Tce group from the Bzl-HIP-Leu-OTce unit **18**, prepared from alcohol **17**, as previously described by ourselves,[33] furnished carboxylic acid **19** that was coupled with H-Pro-OTce using HBTU (**9**) in the presence of HOBt (**12**), giving **20**. Hydrogenolysis then gave eastern segment **21**.

The corresponding western segment **29** was synthesized as outlined in Scheme 2.

Removal of the Boc group from Ist derivative **14** followed by coupling to Boc-Thr(Bzl)-OH, using HBTU (**9**) in the presence of HOBt (**12**), gave dipeptide **22**. Boc group removal, followed by coupling with Boc-(*R*)-N(Me)-Leu-OH, using BOP (**6**), in the presence of HOBt (**12**),

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(16) Rinehart, K. L. US Patent No. *5294603*; 1994; pp 1−25.
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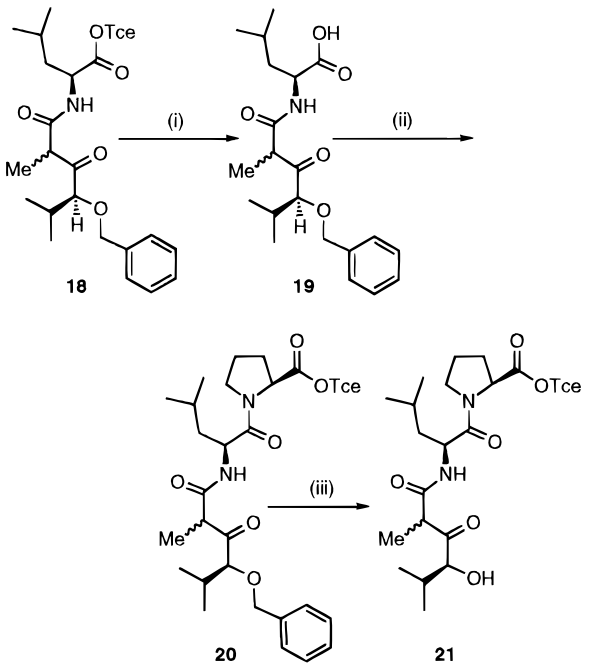
(33) González, I.; Jou, G.; Caba, J. M.; Albericio, F.; Lloyd-Williams, P.; Giralt, E. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1427−1434.

Scheme 1[a]



[a] Reagents and conditions: (i) Zn dust, 1 M NH_4Ac, 84%; (ii) H-Pro-OTce, HBTU, HOBt, DIEA, 76%; (iii) H_2/Pd−C, 78%.

gave tripeptide **23**. Saponification of the methyl ester and protection of the hydroxyl group of **24** as the TBDMS ether afforded carboxylic acid **25** that was protected as its Tce ester **26**. Catalytic hydrogenolysis of **26** gave alcohol **27** that was esterified with Z-N(Me)-O(Me)-Tyr-OH using DCC in the presence of DMAP, giving **28**. Removal of the Tce group then furnished segment **29**.

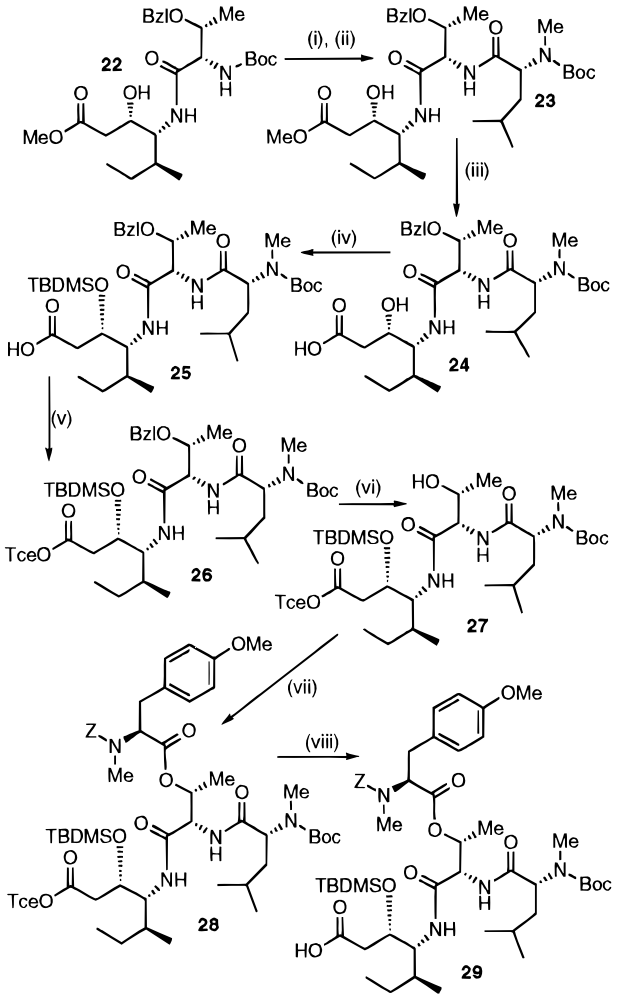
Formation of an ester bond between eastern and western segments **21** and **29** proved to be challenging and required very careful control of the reaction conditions. Use of DCC or DIPCDI in the presence of DMAP at room temperature led only to the formation of the corresponding *N*-acylisourea derivatives of **29** and recuperation of unchanged **21**. Esterification could, however, be brought about by treatment of a mixture of **21** and **29** with DIPCDI and DMAP in the presence of DMAP·CF_3-COOH in refluxing chloroform. This is a variation of the procedure decribed by Boden[34] and allowed the formation of **4a** in 60% yield, after chromatography.

The transformation of linear precursor **4a** into didemnin A derivative **1a** was accomplished by sequential deprotection of its carboxyl and amino terminii, giving **4b** and then **4c**, respectively, followed by macrocyclization, in 28% yield, using HATU (**10**) in the presence of HOAt (**11**). Nuclear magnetic resonance studies together with HPLC data of the product indicated that only one stereoisomer was present. Treatment of **1a** with 5 M HCl−dioxane gave material whose physical data were identical with those of a natural sample of didemnin A (**1c**).

Strategy 2. An alternative approach to the didemnins is based upon the synthesis of linear precursor **5a**, by formation of an amide bond between segments **34** and **38**. Simultaneous deprotection of the amino and carboxyl terminii of **5a** gives **5b**, whose cyclization provides the protected didemnin macrocycle **3a**. Removal of the amino protecting group from **3a** then allows the attachment of

(34) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394−2395.

Scheme 2[a]



[a] Reagents and conditions: (i) 40% TFA−DCM (ii) Boc-(*R*)-N(Me)-Leu-OH, BOP, HOBt, DIEA, 81%; (iii) 1 M NaOH, MeOH, 96%; (iv) TBDMS-Cl, imidazole, 72%; (v) Tce-OH, DCC, 80%; (vi) H_2, Pd−C, 95%; (vii) Z-(*S*)-N(Me)-O(Me)-Tyr-OH, DCC, DMAP, 79%; (viii) Zn dust, 1 M NH_4Ac, 66%.

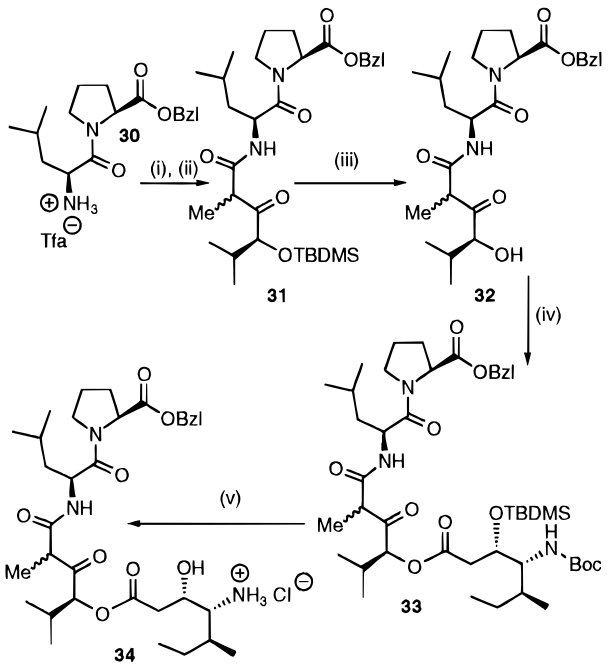
side chains and the formation of didemnins. The synthesis of segment **34** is outlined in Scheme 3.

Dipeptide **30** was prepared by DCC-mediated coupling between Boc-Leu-OH and H-Pro-OBzl followed by Boc group removal. Amide bond formation between **30** and the HIP unit was brought about by catalytic hydrogenolysis of **16**, furnishing the corresponding β-keto acid that was coupled rapidly *in situ* by treatment with HBTU (**9**) in the presence of HOBt (**12**), giving **31**. Removal of the TBDMS group then gave alcohol **32** that was esterified with Ist derivative **15**, using DCC in the presence of DMAP, affording ester **33**. Simultaneous acidolytic deprotection of the Boc and TBDMS groups then gave segment **34**.

The complementary didepsipeptide segment **38** required to form linear precursor **5a** was prepared in a similar manner to that reported by Joullié,[14] according to Scheme 4.

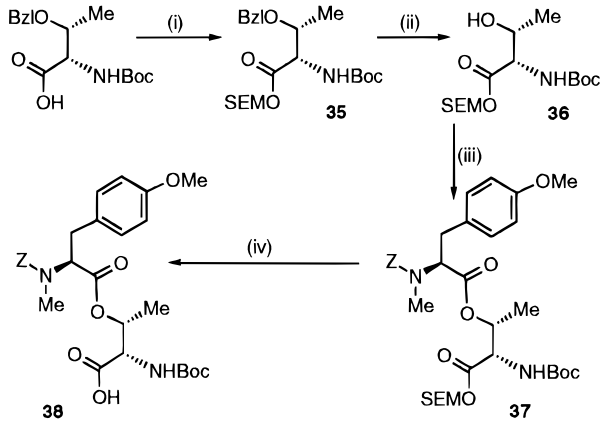
Protection of the carboxyl group of Boc-Thr(Bzl)-OH as the SEM ester **35** followed by catalytic hydrogenolysis gave alcohol **36**, which was esterified with Z-N(Me)-O(Me)-Tyr-OH using DCC in the presence of DMAP, giving **37**. Removal of the SEM group from **37** could not be carried out using TBAF because of unavoidable decomposition of the didepsideptide, presumably initiated

Scheme 3[a]



[a] Reagents and conditions: (i) TBDMS-Hip-OH (ii) HBTU, HOBt, DIEA, 83%; (iii) TBAF, 97%; (iv) Boc-Ist(TBDMS)-OH, DCC, DMAP, −20 °C, 84%; (v) 5.7 M HCl−dioxane, 99%.

Scheme 4[a]



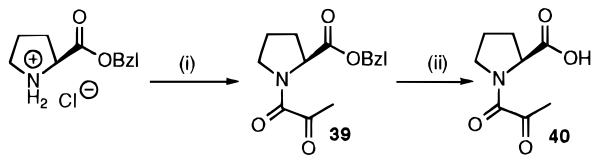
[a] Reagents and conditions: (i) SEM-Cl, Li_2CO_3, 78%; (ii) H_2, Pd−C, 97%; (iii) DCC, DMAP, 88%; (iv) 6% aqueous HF−MeCN, 72%.

by β-elimination, and was consequently carried out acidolytically by treatment with HF at −20 °C, furnishing **38**. Careful control of the reaction conditions was necessary here in order avoid concomitant removal of the Boc group.[14]

The formation of precursor **5a** by amide bond formation between **34** and **38** was complicated by the formation of the γ-lactam **2**, derived from cyclization of Ist, but was achieved in 82% yield using HBTU (**9**) in the presence of HOBt (**12**), using DIEA as base. Catalytic hydrogenolysis of **5a** to **5b** occurred quantitatively and was followed by macrocyclization, using HATU (**10**) in the presence of HOAt (**11**), which gave macrocycle **3a** in 76% yield after chromatography. Nuclear magnetic resonance studies and HPLC data of the product again indicated the presence of only one stereoisomer.

After removal of the Boc group, by treatment with a 5.7 M HCl−dioxane solution, macrocycle **3b** was coupled in 92% yield to Boc-(*R*)-N(Me)-Leu-OH using BOP (**6**) in the presence of HOBt (**12**), giving Boc-didemnin A (**1b**). Treatment of this with 5.7 M HCl−dioxane then gave material identical both with a natural sample of didemnin A (**1c**) and with that obtained in the previously described synthesis.

Scheme 5[a]



[a] Reagents and conditions: (i) pyruvic acid, DCC, HOBt, DIEA, 36%; (ii) H_2, Pd−C, 72%.

Synthesis of Dehydrodidemnin B

Preparation of dehydrodidemnin B requires the coupling of the (*S*)-Pro-Pyr side chain **40** to the amino group of the (*R*)-N(Me)-Leu residue of didemnin A. Side chain **40** was synthesized as shown in Scheme 5.

Amide bond formation between H-Pro-OBzl and pyruvic acid was brought about using DCC in the presence of HOBt (**12**), giving ester **39**, which was subsequently submitted to catalytic hydrogenolysis, producing **40**. Coupling of didemnin A (**1c**) with **40** using PyBrOP (**7**) afforded dehydrodidemnnin B (**1e**) in a yield of 62%. The synthetic material obtained had physical properties identical with those of an authentic sample.

Discussion

Several interesting observations emerge from the results obtained in these syntheses. With regard to the formation of the different types of linkages between the subunits of the didemnins, it appears that different types of reagents are better suited to the formation of different types of linkages. Of those examined, the most useful for the formation of esters were the carbodiimides DIPCDI and DCC, usually in the presence of DMAP. Occasionally, in the most demanding esterifications, such as the union of **21** and **29**, the use of DMAP·CF_3COOH in addition to DMAP and the carbodiimide was required.[34] Even here, however, ester formation could be brought about under mild conditions and in good yield. Other reagents such as isopropenyl chloroformate[35] or BOP-Cl[36,37] were much less effective in these situations, as were the phosphonium and uronium reagents tested.

The formation of secondary amides (standard peptide bonds) could be carried out equally efficiently using either carbodiimides, such as DCC or DIPCDI, or phosphonium or uronium reagents, such as BOP (**6**) or HBTU (**9**), respectively. However, for the formation of tertiary amides, phosphonium and uronium reagents such as PyBrOP (**7**), HBTU (**9**), and HATU (**10**) consistently gave higher yields than other reagents, including carbodiimides, isopropenyl chloroformate, and even BOP-Cl, whose use has been recommended for the formation of this type of amide.[38] When the uronium reagents HBTU (**9**) or

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HATU (**10**) are used to form *tertiary* amides they can be added directly to the mixture of amino and carboxyl components, functioning as true coupling reagents, without the need for separate preactivation steps. The formation of Schiff base analogues[39,40] does not occur when secondary amines are involved.

The yield for the macrolactamization step using HATU (**10**) in the presence of HOAt (**11**) is, in the case of linear precursor **5a**, very high indeed (76%), and although the corresponding yield for **4a** is much lower, scarcity of material did not permit this key step to be optimized. Slightly lower, but still excellent, yield (70%) for the cyclization of **5a** was obtained using PyAOP (**8**) in the presence of HOAt (**11**), whereas the use of PyBrOP (**7**) gave significantly poorer results (maximum yield of cyclized product, 37%). Rapid (less than 12 h) cyclization of **5a** using the acid-fluoride-forming[41] reagent TTFH (**13**) was also observed, but mass spectrometry of the product formed indicated that fluorination of the macrocycle had occurred. The use of this reagent was not investigated further.

In the syntheses of didemnin A (**1c**) described here, linear precursors **4c** and **5b**, both of which were mixtures of diastereomers at C-2 of the HIP unit, were cyclized to give optically pure macrocycles in which this stereogenic carbon atom had the correct (*S*) configuration. This may indicate that, in both these cases, the diastereomer of the linear precursor having the (*S*) configuration at C-2 of the HIP unit undergoes macrocyclization more rapidly than that having the (*R*) configuration. The (*R*) diastereomer of the linear precursor remaining after cyclization of the (*S*) diastereomer could then, under the basic conditions of the macrocyclization reaction, undergo epimerization, generating more (*S*) diastereomer that would then cyclize. The overall effect is to convert a diastereomeric mixture of linear precursors into a single stereoisomer of the macrocycle with the natural (*S*) configuration at C-2 of the HIP unit. An alternative possibility is that both diastereomers of the macrocycle are formed initially but that epimerization occurs to provide the more thermodynamically stable stereoisomer under the conditions of the cyclization reaction. However, no evidence for the production of diastereomeric macrocycles was obtained at any time in this work. Joullié has suggested that epimerization at C-2 of the HIP unit in the macrocycle is not possible because of conformational constraints.[14]

Both synthetic and natural dehydrodidemnin B (**1e**) show two peaks in their analytical HPLC profiles, in an approximate proportion of 55:45. Mass spectrometric analysis of the two fractions obtained by collecting the HPLC effluent indicates a molecular ion corresponding to that of dehydrodidemnin B in both cases. Reinjection of each of the isolated fractions gave identical chromatograms, each showing the same two peaks observed in the initial natural or synthetic sample. Peak coalescence is observed on heating the HPLC column to 50 °C, but subsequent reinjection at room tempereature of the collected analytical HPLC effluent again gives chromatograms showing the same two components. These data are consistent with the existence of slow *cis*−*trans* rotational isomerism about one of the amide bonds of the molecule. Such rotational isomerism is not commonly observed in chromatography, although the phenomenon is not without precedent,[42] particularly for tertiary amide-containing peptides.[43−45]

Rotational isomerism is, however, common enough on the time scale of NMR experiments and is often observed in spectroscopic studies on peptides and proteins.[46] High-field NMR spectroscopy of dehydrodidemnin B (**1e**) indicates the presence of two predominant conformations in $CDCl_3$, in a ratio of approximately 55:45, consistent with the HPLC data for **1e**. Additionally, two other minor conformations are detected in DMSO-d_6. Similar conformational equilibria have been observed in NMR spectroscopic studies on other didemnins.[47,48] The possibility that such behavior plays a part in the pronounced biological activity of dehydrodidemnin B cannot be discounted.

Conclusions

The total synthesis of dehydrodidemnin B, the most active didemnin discovered to date, has been carried out by coupling the Pyr-Pro-OH unit **40** to didemnin A (**1c**), itself synthesized by two novel routes. One of these, based on linear precursor **5a**, is clearly superior, taking place in an overall yield of 27% based on H-(*S*)-Pro-OBzl. The other strategy, based on linear precursor **4a**, has an overall yield of 4% based on HIP unit **17**. The more efficient synthesis could, in principle, be used to provide sufficient quantities of dehydrodidemnin B for clinical trials.

Extensive use has been made of several of the newer phosphonium and uronium salt coupling reagents, more usually applied in solid phase peptide synthesis. The results obtained highlight the power and versatility of these in peptide synthesis in solution.

Experimental Section

General Procedures. All reagents were obtained commercially and were purified where appropriate. Tetrahydrofuran was distilled from sodium/benzophenone. Dichloromethane and chloroform were distilled from calcium hydride and filtered through an alumina column prior to use. Chromatographic purification of products was carried out by "flash" chromatography[49] using Merck silica gel 60 (230−400 mesh). Thin layer chromatography was carried out on Merck silica gel 60F plates, and visualization of the developed chromatogram was effected by ultraviolet absorption, iodine, or phosphomolybdic reagent (7% w/v) in absolute ethanol. Organic solutions were concentrated by rotatory evaporation at ~25 mmHg (water aspirator) and were dried over sodium sulfate.

Chemical shifts are quoted in δ values downfield from tetramethylsilane, and J values are given in Hz. Fast atom bombardment (FAB) and electrospray (ES) mass spectrometry were performed on a VG Quattro apparatus. All melting

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points are uncorrected. Reversed-phase HPLC was performed using a Shimadzu apparatus. Detection was by ultraviolet absorption at 220 nm, and Nucleosil C_{18} (25 × 0.4 cm, 10 μm, 120 Å) or Nucleosil C_4 (25 × 0.4 cm, 10 μm, 120 Å) columns were used as indicated.

Boc-Pro-OTce. DCC (1.51 g, 5.58 mmol) in CH_2Cl_2 (5 mL) was added over 5 min to a solution of Boc-Pro-OH (1.01 g, 4.65 mmol), DMAP (110 mg, 0.93 mmol) and 2,2,2-trichloroethanol (0.53 mL, 5.58 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After being stirred for 7 h at 0 °C, the mixture was filtered, the solvent removed, and the residue taken up in AcOEt (70 mL). This solution was washed with 10% citric acid (2 × 15 mL), 5% aqueous $NaHCO_3$ solution (2 × 15 mL), and saturated aqueous NaCl solution (2 × 15 mL). Drying and filtration, followed by solvent removal, gave an oil (1.87 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:6), giving Boc-Pro-OTce as white prisms (1.61 g, 99%): mp 50−52 °C; $[\alpha]_D$ −45.0 (*c* 1.4, $CHCl_3$); IR (film) ν 3000−2800, 1770, 1700, 1390, 1370, 1265, 1150, 1090 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (9H, s), 1.47 (9H, s), 1.90−2.40 (4H, m), 3.35−3.66 (2H, m), 4.34−4.50 (1H, m), 4.65 (2H, d, J = 12.9), 4.76 (2H, s), 4.94 (2H, d, J = 12.9); ^{13}C NMR (50 MHz, $CDCl_3$) 23.97, 24.74, 28.88, 30.35, 31.34, 46.78, 47.00, 59.23, 59.43, 74.58, 80.03, 80.07, 154.00, 172.00; *m*/*z* (FAB) 371.9 [(M″ + Na)$^+$, 5], 369.9 [(M′ + Na)$^+$, 19], 367.9 [(M + Na)$^+$, 20], 349.9 [(M″ + H)$^+$, 7], 348.0 [(M′ + H)$^+$, 20], 346.0 [(M′ + H)$^+$, 22], 289.9 (100); *m*/*z* (FABHRMS) 346.039 351, $C_{12}H_{18}Cl_3NO_4$ requires (M + H)$^+$, 346.037 967. Anal. Calcd for $C_{12}H_{18}Cl_3NO_4$: C, 41.74; H, 5.22; N, 4.06%. Found: C, 41.73; H, 5.31, N, 3.81.

Bzl-Hip-Leu-OH (19). Zinc dust (90 mg, 13.70 mmol) was added, portionwise, to a solution of ester **18** (150 mg, 0.29 mmol) in THF (2 mL), followed by 1 M aqueous NH_4OAc solution (0.40 mL), and the mixture was stirred vigorously under Ar for 24 h. After filtration and solvent removal the residue was taken up in AcOEt (50 mL), and this solution was washed with 5% aqueous $KHSO_4$ (5 mL) and with saturated aqueous NaCl solution (5 mL). Drying and filtration followed by solvent removal gave the two diastereomers of **19** (90 mg, 85%) as a clear oil: IR (film) ν 3314, 2962, 2950, 2875, 1719, 1654, 1540, 1457, 1389, 1369, 1208, 1071, 1028 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85−0.98 (12H, m), 1.20−1.40 (1H, m), 1.36 (3H, d, J = 7.2), 1.40−1.80 (2H, m), 2.00−2.20, (1H, m), 3.70 (1H, d, J = 6.0), 3.90 (1H, q, J = 7.1), 3.92 (1H, q, J = 7.1), 4.38 (1H, d, J = 11.0), 4.41 (1H, d, J = 11.6), 4.40−4.58 (1H, m), 4.59 (1H, d, J = 11.4), 4.63 (1H, d, J = 11.6), 6.71 (1H, d, J = 7.2), 6.75 (1H, d, J = 8.5), 7.25−7.45 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.88, 16.11, 17.56, 17.66, 18.94, 21.47, 21.58, 22.79, 24.80, 24.86, 30.36, 30.44, 40.46, 40.61, 48.89, 49.39, 50.89, 50.97, 72.88, 73.37, 89.13, 89.86, 127.64, 127.74, 127.93, 127.96, 128.20, 128.28, 128.46, 137.26, 169.51, 176.95, 210.35, 210.85; *m*/*z* (FAB) 400.2 [(M + Na)$^+$, 10], 378.2 [(M + H)$^+$, 100]; *m*/*z* (FABHRMS) 378.229 519. $C_{21}H_{31}NO_5$ requires: (M + H)$^+$, 378.228 048.

Bzl-Hip-Leu-Pro-OTce (20). TFA (0.89 mL) was added to Boc-Pro-OTce (140 mg, 0.41 mmol) in CH_2Cl_2 (2 mL), and after 20 min, the solvent was removed and chased with Et_2O. The solid obtained was dissolved in dry MeCN (1 mL) at 0 °C and added to a solution of **19** (72 mg, 0.19 mmol) in MeCN (1 mL). HOBt (29 mg, 0.19 mmol), HBTU (73 mg, 0.19 mmol), and DIEA (0.10 mL, 0.60 mmol) were then added successively to the mixture, and after 3 h stirring at rt, the solvent was removed and the residue taken up in AcOEt (50 mL). This solution was washed with 5% aqueous $KHSO_4$ (2 × 5 mL), with 5% aqueous $NaHCO_3$ (2 × 5 mL), and with saturated aqueous NaCl solution (2 × 5 mL). Drying and filtration followed by solvent removal gave an oil (160 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 2:3), giving the two diastereomers of **20** (88 mg, 76%) as a clear colorless oil: IR (film) ν 3303, 2960, 2950, 2873, 1765, 1725, 1634, 1530, 1453, 1368, 1269, 1153, 1095, 1070 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85−0.98 (12H, m), 1.32 (3H, d, J = 7.1), 1.34 (3H, d, J = 6.9), 1.40−1.70 (3H, m), 1.95−2.40 (5H, m), 3.50−3.95 (4H, m), 4.33−4.42 (1H, m), 4.51−4.75 (1H, m), 4.36−4.90 (4H, m), 6.63 (1H, d, J = 8.4), 6.75 (1H, d, J = 9.0), 7.27−7.36 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.97, 15.50, 17.61, 17.73, 18.93, 19.09, 21.63, 21.69, 23.34, 24.61, 24.86, 28.93, 30.49, 30.60, 41.52, 41.66, 46.69, 46.75, 49.03, 49.10, 49.23, 49.60, 58.58, 58.63, 72.84, 73.25, 74.00, 89.18, 89.66, 127.67, 127.73, 127.91, 128.35, 128.42, 137.62, 137.46, 168.94, 170.23, 171.00, 208.82, 209.97; *m*/*z* (FAB) 611.1 [(M‴ + H)$^+$, 5], 608.8 [(M″ + H)$^+$, 30], 607.2 [(M′ + H)$^+$, 100], 605.3 [(M + H)$^+$, 100].

H-Hip-Leu-Pro-OTce (21). Ester **20** (110 mg, 0.30 mmol) in dry THF (4 mL) was hydrogenolyzed in the presence of 10% Pd−C (110 mg) for 60 min at rt and atmospheric pressure. Filtration through Celite and solvent removal gave an oil that was purified by chromatography (silica gel, AcOEt−hexanes, 1:1), giving the two diastereomers of **21** (approximate proportions, 1:1) as a white solid (80 mg, 78%): mp 100−103 °C; RP-HPLC t_R 16.82 and 17.70 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film) ν 3313, 2962, 2921, 2871, 1763, 1719, 1636, 1540, 1449, 1369, 1153, 1097, 1022 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73 (3H, d, J = 6.6), 0.82 (3H, d, J = 6.9), 0.94 (6H, d, J = 6.6), 0.95 (6H, d, J = 6.6), 0.96 (6H, d, J = 6.6), 0.99 (6H, d, J = 6.3), 1.01 (3H, d, J = 6.9), 1.09 (3H, d, J = 6.9), 1.31 (3H, d, J = 6.9), 1.38 (3H, d, J = 7.2), 1.54−1.78 (3H, m), 2.04−2.16 (5H, m), 3.58−3.68 (3H, m), 3.72−3.84 (3H, m), 3.66 (1H, q, J = 6.9), 3.91 (1H, q, J = 7.2), 3.96−4.00 (1H, m), 4.22−4.26 (1H, m), 4.60−4.82 (2H, m), 4.67 (1H, d, J = 12.1), 4.68 (1H, d, J = 12.1), 4.86 (1H, d, J = 12.1), 4.87 (1H, d, J = 12.1), 6.63−6.68 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.54, 13.71, 15.21, 16.00, 19.45, 19.91, 21.43, 21.68, 23.36, 23.41, 24.78, 24.89, 28.87, 28.94, 30.97, 31.44, 40.62, 41.80, 46.78, 46.80, 49.30, 50.18, 50.31, 50.45, 58.70, 58.79, 74.04, 80.44, 81.29, 94.71, 168.80, 170.08, 170.12, 171.04, 172.11, 209.18, 210.01; *m*/*z* (FAB) 521.6 [(M‴ + H)$^+$, 15], 519.1 [(M″ + H)$^+$, 25], 517.1 [(M′ + H)$^+$, 80], 515.2 [(M + H)$^+$, 85] 497.2 (100); *m*/*z* (FABHRMS) 515.145 730, $C_{21}H_{33}Cl_3N_2O_6$ requires (M + H)$^+$ 515.148 245.

Boc-Thr(Bzl)-Ist-OMe (22). A 40% solution of TFA in CH_2Cl_2 (10 mL) was added to **14** (970 mg, 3.36 mmol) in CH_2Cl_2 (5 mL), and after 20 min, the solvent was removed and chased with Et_2O. The solid obtained was dissolved in dry MeCN (8 mL) and was added to a solution of Boc-Thr-(Bzl)-OH (1.56 g, 5.04 mmol), HOBt (770 mg, 5.04 mmol), and HBTU (1.91 g, 5.04 mmol) in dry MeCN (8 mL). DIEA (1.43 mL, 8.39 mmol) was added over 40 min, and the mixture was stirred for 24 h at rt. The solvent was removed and the residue taken up in AcOEt (125 mL), and this solution was washed with 5% aqueous $KHSO_4$ (3 × 15 mL), 5% aqueous $NaHCO_3$ (3 × 15 mL), and saturated aqueous NaCl solution (3 × 15 mL). Drying and filtration followed by solvent removal gave an oil (3.41 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:2), giving **22** as a colorless semisolid (1.10 g, 69%): $[\alpha]_D$ −5.3 (*c* 0.7, $CHCl_3$); IR (film), 3500−3100, 3000−2800, 1720, 1700, 1630, 1610, 1530, 1490, 1450, 1430, 1380, 1365, 1170 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.71 (3H, d, J = 6.7), 0.88 (3H, t, J = 7.2), 1.20 (3H, d, J = 6.8), 1.15−1.35 (2H, m), 1.46 (9H, s), 1.83−2.00 (1H, qd, J_1 = 6.7, J_2 = 2.6); 2.35−2.55 (2H, m), 3.67 (3H, s), 3.85 (1H, td, J_1 = 7.8, J_2 = 3.9), 3.99 (1H, td, J_1 = 8.8, J_2 = 3.1), 4.50−4.63 (1H, m), 4.52 (1H, d, J = 11.1), 4.62 (1H, d, J = 11.1), 5.50 (1H, m), 6.43 (d, J = 9.4), 7.32 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.64, 13.04, 15.32, 27.02, 28.24, 33.66, 38.26, 51.72, 51.72, 55.31, 57.80, 68.70, 71.50, 74.52, 80.07, 127.47, 127.63, 127.87, 127.96, 128.20, 128.30, 128.39, 137.61, 155.51, 170.16, 173.57; *m*/*z* (FAB) 481.4 [(M + H)$^+$, 51], 381.4 (100).

Boc-(*R*)-N(Me)-Leu-Thr(Bzl)-Ist-OMe (23). A 5 M solution of HCl in dioxane (12 mL) was added to **22** (880 mg, 1.83 mmol) in dioxane (3 mL), and after 50 min, the solvent was removed and chased with Et_2O. The solid obtained was dissolved in DMF (4 mL) and added to a solution of Boc-(*R*)-N(Me)-Leu-OH (670 mg, 2.75 mmol) in DMF (8 mL) at 0 °C. HOBt (460 mg, 3.03 mmol), BOP (1.34 g, 3.03 mmol), and DIEA (1.29 mL, 7.62 mmol) were added successively, and this mixture was stirred for 24 h at rt. The solvent was removed and the residue taken up in AcOEt (150 mL). This solution was washed with 5% aqueous $KHSO_4$ (3 × 20 mL), 5% aqueous $NaHCO_3$ (3 × 20 mL), and saturated aqueous NaCl solution (3 × 20 mL). Drying and filtration followed by solvent removal

gave an oil (1.50 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:1), giving **23** as a white solid (910 mg, 81%): mp 94−96 °C; RP-HPLC t_R 19.67 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +22.8 (*c* 1.2, $CHCl_3$); IR (film) ν 3441, 3338, 2960, 2950, 2900, 2900, 1671, 1497, 1456, 1387, 1368, 1322, 1254, 1158 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.62−0.72 (3H, m), 0.82−0.85 (3H, t, J = 7.2), 0.90 (3H, d, J = 6.6), 0.93 (3H, d, J = 6.6), 1.12 (3H, d, J = 5.4), 1.07−1.30 (2H, m), 1.39−1.54 (1H, m), 1.39 (9H, s), 1.64−1.74 (1H, m), 1.84 (1H, qd, J_1 = 6.9, J_2 = 3.6), 2.30−2.45 (2H, m), 2.80 (3H, bs), 3.30 (1H, bs), 3.63 (3H, s), 3.75−3.85 (1H, m), 3.96 (1H, td, J_1 = 9.2, J_2 = 2.7), 4.12−4.20 (1H, m), 4.40−4.45 (1H, m), 4.47−4.65 (3H, m), 6.36 (1H, d, J = 10.8), 6.47 (1H, d, J = 9.2), 6.93 (1H, bs) , 7.00 (1H, d, J = 6.8), 7.26−7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.68, 13.23, 14.97, 15.50, 21.32, 21.63, 23.22, 24.60, 24.94, 27.03, 28.21, 29.50, 31.72, 33.76, 36.77, 38.03, 51.74, 55.47, 56.06, 56.61, 57.51, 57.85, 68.75, 71.63, 72.03, 73.98, 80.62, 81.00, 127.90, 128.00, 128.44, 168.00, 169.78, 171.82, 171.5, 173.57; *m/z* (FAB) 630.6 [$(M + Na)^+$, 9], 608.6 [$(M + H)^+$, 50], 508.6 (100); *m/z* (FABHRMS) 608.391 288, $C_{32}H_{53}N_3O_8$ requires $(M + H)^+$ 608.391091. Anal. Calcd for $C_{32}H_{53}N_3O_8$: C, 63.26; H, 8.73; N, 6.91. Found: C, 63.02; H, 8.77; N, 6.63.

Boc-(*R*)-N(Me)-Leu-Thr(Bzl)-Ist-OH (24). A 1.1 M NaOH solution (1.76 mL, 1.93 mmol) was added dropwise to **23** (470 mg, 0.77 mmol) in MeOH (3.4 mL) at 0 °C, and after 4 h the mixture was allowed to attain rt over 60 min and was diluted with H_2O (50 mL). The solution was extracted with AcOEt (2 × 20 mL), and the combined organic phases were extracted with saturated aqueous $NaHCO_3$ (2 × 15 mL). The combined aqueous phases were taken to pH 3 by addition of 2 M aqueous $KHSO_4$ and extracted with AcOEt (4 × 50 mL). The combined organic phases were washed with saturated aqueous NaCl solution (2 × 10 mL), dried, and filtered. Solvent removal gave **24** as an oil (440 mg, 96%): RP-HPLC t_R 18.41 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +26.0 (*c* 0.7, $CHCl_3$); IR (film) ν 3492, 2959, 2866, 1734, 1635, 1456, 1425, 1200, 1170 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.69 (3H, d, J = 6.9), 0.81−0.88 (3H, t, J = 7.0), 0.91 (3H, d, J = 6.1), 0.94 (3H, d, J = 6.2), 1.14 (3H, d, J = 6.2), 1.10−1.60 (3H, m), 1.41 (9H, s), 1.65−1.75 (2H, m), 1.80−1.97 (1H, m), 2.25−2.50 (2H, m), 2.78 (3H, s), 2.84 (3H, s), 3.75−3.88 (1H, m), 3.92−4.04 (1H, m), 4.10−4.18 (1H, m), 4.50−4.70 (4H, m), 6.50−6.70 (2H, m), 7.27−7.31 (5H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.15, 13.74, 16.04, 16.34, 21.81, 22.16, 23.68, 25.13, 25.45, 27.56, 28.74, 30.19, 32.00, 34.29, 37.25, 37.61, 38.83, 56.35, 57.28, 57.97, 68.98, 72.31, 74.61, 81.34, 128.62, 129.00, 170.00, 172.50, 176.01; *m/z* (FAB) 616.4 [$(M + Na)^+$, 27], 594.4 [$(M + H)^+$, 30], 494.4 (100); *m/z* (FABHRMS) 616.352 980, $C_{31}H_{51}N_3O_8$ requires $(M + Na)^+$ 616.357 380.

Boc-(*R*)-N(Me)-Leu-Thr(Bzl)-Ist(TBDMS)-OH (25). A suspension of **24** (110 mg, 0.19 mmol), TBDMS-Cl (170 mg, 1.16 mmol), and imidazole (210 mg, 3.09 mmol) in DMF (0.10 mL) was stirred for 36 h under nitrogen. The resulting solution was diluted with AcOEt (50 mL) and washed with cold 1 M $KHSO_4$ solution (2 × 5 mL) and saturated aqueous NaCl solution (2 × 5 mL). After drying, filtration, and solvent removal, the residue was taken up in dioxane (2 mL). This solution was taken to pH 9.5 by addition of aqueous 1 M NaOH and after 0.5 min the pH was adjusted to 7.0 by addition of 1 M $KHSO_4$ solution. The solvent was removed and the residue taken up in H_2O (20 mL) and extracted with AcOEt (2 × 10 mL). The aqueous phase was taken to pH 3 by addition of 2 M $KHSO_4$ solution and extracted with AcOEt (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl solution (1 × 10 mL), dried, and filtered. Solvent removal gave an oil (170 mg) that was purified by chromatography (silica gel, 1% MeOH in CH_2Cl_2 → 5% MeOH in CH_2Cl_2, gradient elution), giving **25** as an oil (100 mg, 72%): RP-HPLC t_R 24.30 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +31.7 (*c* 0.9, $CHCl_3$), IR (film) ν 3332, 2960, 2933, 2900, 1867, 1684, 1507, 1457, 1472, 1389, 1368, 1322, 1254, 1154, 1096 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (3H, s), 0.07 (3H, s), 0.65 (3H, d, J = 6.9), 0.87 (9H, s), 0.88 (3H, t, J = 7.5), 0.92 (3H, d, J = 6.9), 0.94 (3H, d, J = 6.9), 1.12 (3H, d, J = 6.6), 1.10−1.25 (2H, m), 1.45 (9H, s), 1.40−1.50 (1H, m), 1.66−1.71 (2H, m), 1.77−1.84 (1H, m), 2.35−2.55 (2H, m), 2.82 (3H, bs), 4.01−4.11 (2H, m), 4.50−4.62 (4H, m), 4.70−4.75 (1H, m), 6.33 (1H, d, J = 9.6), 6.99 (1H, d, J = 7.8), 7.28−7.32 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.29, −4.91, 11.88, 13.01, 15.74, 17.86, 21.48, 23.26, 24.94, 25.70, 27.35, 28.28, 29.68, 30.76, 33.84, 36.75, 37.08, 40.59, 56.31, 56.92, 69.19, 71.68, 73.81, 80.86, 127.58, 127.83, 128.08, 128.39, 137.76, 156.98, 169.23, 169.82, 171.92, 172.45, 173.32, 173.86; *m/z* (FAB) 708.4 [$(M + H)^+$, 21], 608.4 (100).

Boc-(*R*)-N(Me)-Leu-Thr(Bzl)-Ist(TBDMS)-OTce (26). DCC (70 mg, 0.34 mmol) was added over 5 min to a solution of **25** (200 mg, 0.28 mmol), 2,2,2-trichloroethanol (32 μL, 0.34 mmol), and DMAP (7 mg, 0.056 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was allowed to attain rt and, after being stirred for 24 h, was filtered. The solvent was removed and the residue taken up in AcOEt (100 mL) and washed with 10% citric acid solution (2 × 20 mL), 5% aqueous $NaHCO_3$ solution (2 × 20 mL), and saturated aqueous NaCl solution (2 × 20 mL). Drying and filtration followed by solvent removal gave an oil that was purified by chromatography (silica gel, AcOEt−hexanes, 1:6), giving **26** as a clear, viscous oil (189 mg, 80%): RP-HPLC t_R 27.42 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$+ 21.5 (*c* 1.3, $CHCl_3$); IR (film) ν 3855, 3345, 2959, 2859, 1753, 1694, 1597, 1472, 1387, 1368, 1321, 1258, 1225, 1157 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.64 (3H, d, J = 6.6), 0.83 (9H, s), 0.84 (3H, t, J = 7.5), 0.93 (3H, d, J = 6.3), 0.95 (3H, d, J = 6.2), 1.11 (3H, d, J = 6.4), 1.10−1.36 (2H, m), 1.38−1.54 (1H, m), 1.64−1.82 (3H, m), 1.47 (9H, s), 2.51 (1H, dd, J_1 = 16.3, J_2 = 5.2), 2.64 (1H, dd, J_1 = 16.4, J_2 = 5.1), 2.79 (3H, bs), 4.00−4.20 (3H, m), 4.47 (1H, dd, J_1 = 6.1, J_2 = 3.7), 4.58−4.76 (5H, m), 6.37 (1H, bd, J = 9.1), 7.09 (1H, m), 7.34 (5H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.85, −4.52, 11.78, 13.40, 14.54, 14.73, 21.56, 24.61, 24.70, 24.87, 25.69, 27.40, 28.29, 33.76, 36.59, 37.00, 40.03, 55.78, 55.87, 56.20, 69.56, 71.70, 73.79, 73.99, 80.05, 128.00, 128.12, 128.49, 156.00, 168.92, 170.08, 171.57, 171.64, 171.83; *m/z* (FAB) 840.5 [$(M' + H)^+$, 32], 838.5 [$(M + H)^+$, 30], 740.4 (100); *m/z* (FABHRMS) 838.377 458, $C_{39}H_{66}Cl_3N_3O_8Si$ requires $(M + H)^+$ 838.376 098.

Boc-(*R*)-N(Me)-Leu-Thr-Ist(TBDMS)-OTce (27). Compound **26** (180 mg, 0.22 mmol) in dry THF (1.5 mL) was hydrogenolyzed for 90 min in the presence of 10% Pd−C (72 mg) at rt and atmospheric pressure. The mixture was filtered, and solvent removal gave **27** as an oil (150 mg, 90%): RP-HPLC t_R 24.35 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +11.7 (*c* 1.0, CH_2Cl_2); IR (film) ν 3360, 2960, 2920, 2860, 1750, 1700, 1680, 1660, 1525, 1510, 1500, 1460, 1385, 1365, 1320, 1250, 1150, 1095 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 (3H, s), 0.09 (3H, s), 0.86 (9H, s), 0.89−0.98 (12H, m), 1.14 (3H, d, J = 6.3), 1.20−1.50 (2H, m), 1.60−1.90 (4H, m), 2.55 (1H, dd, J_1 = 16.0, J_2 = 5.5), 2.68 (1H, dd, J_1 = 12.1, J_2 = 4.1), 2.79 (3H, bs), 3.25 (1H, bs), 4.04−4.25 (3H, m), 4.34−4.46 (1H, m), 4.64-4.70 (1H, m), 4.69 (1H, d, J = 12.1), 4.79 (1H, d, J = 12.0), 4.65 (1H, m), 7.00 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.81, −4.47, 11.62, 13.90, 18.31, 18.38, 23.25, 24.87, 24.93, 25.72, 27.16, 28.32, 29.60, 30.60, 34.37, 36.72, 39.68, 56.38, 57.32, 57.48, 66.37, 69.24, 74.27, 170.80, 170.99, 172, 90, 173.08; *m/z* (FAB) 751.9 [$(M'' + H)^+$, 12], 750.4 [$(M' + H)^+$, 22], 748.6 [$(M' + H)^+$, 19] 650.5 (100); *m/z* (FABHRMS) 748.329 538, $C_{32}H_{60}Cl_3N_3O_8Si$ requires $(M + H)^+$ 748.329 353.

Boc-(*R*)-N(Me)-Leu-Thr[Z-N(Me)-O(Me)-Tyr]-Ist(TB-

DMS)-OTce (28). Z-N(Me)-O(Me)-Tyr-OH (157 mg, 0.46 mmol) in CH_2Cl_2 (1 mL), DMAP (8 mg, 0.066 mmol) in CH_2Cl_2 (100 μL), and DCC (104 mg, 0.50 mmol) in CH_2Cl_2 (0.3 mL) were added to a solution of **27** (154 mg, 0.21 mmol) in CH_2Cl_2 (0.8 mL) at 0 °C. The mixture was allowed to attain rt and stirred for 24 h. After filtration and solvent removal, the residue was taken up in AcOEt (100 mL) and washed with 10% citric acid (2 × 20 mL), 5% aqueous $NaHCO_3$ solution (2 × 20 mL), and saturated aqueous NaCl solution (2 × 20 mL). Drying and filtration followed by solvent removal gave an oil that was purified by chromatography (silica gel, AcOEt−hexanes, 1:4), giving **28** as a clear, colorless oil (174 mg, 79%): RP-HPLC t_R 20.75 min, C_4 Nucleosil column, linear gradient from 10% B to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN + 0.036% TFA), at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +16.9 (*c* 0.9, $CHCl_3$); IR (film) ν 3347, 2957, 2930, 2900, 1748, 1692, 1514, 1458, 1389, 1321, 1250, 1153, 1036 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (3H, s), 0.09 (3H, s), 0.84 (9H, s), 0.88−1.00 (12H, m), 1.19 (3H, d, J = 6.6), 1.13−1.27 (2H, m), 1.46 (9H, s), 1.40−1.50 (1H, m), 1.58−1.70 (2H, m), 1.72-1.82 (1H, m), 2.57−2.74 (2H, m), 2.75 (3H, bs), 2.95 (3H, bs), 2.92−3.00 (1H, m), 3.11−3.20 (1H, m), 3.77 (3H, s), 4.06−4.15 (1H, m), 4.28−4.41 (2H, m), 4.64 (1H, d, J = 12.0), 4.71 (1H, d, J = 12.0), 4.76−4.84 (2H, m), 5.06 (1H, d, J = 12.1), 5.16 (1H, d, J = 12.0), 5.18−5.22 (1H, m), 6.74 (1H, bd, J = 7.5), 6.75 (2H, d, J = 8.4), 6.99 (1H, bd, J = 8.9), 7.07 (2H, d, J = 9.1), 7.26−7.32 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.70, −7.52, 11.40, 14.50, 17.94, 21.5, 23.24, 24.90, 25.76, 27.04, 28.32, 29.60, 30.60, 34.11, 34.46, 36.50, 39.30, 55.21, 56.50, 57.00, 60.01, 67.50, 68.97, 74.11, 77.20, 80.05, 113.97, 127.67, 127.97, 128.44, 129.80, 136.30, 157.00, 158.00, 168.00, 170.00, 172.00; *m*/*z* (FAB) 1076.0 [(M′ + H)$^+$, 5], 1074.0 [(M + H)$^+$, 5], 976.0 (100).

Boc-(*R*)-N(Me)-Leu-Thr[Z-N(Me)-O(Me)-Tyr]-Ist(TB-DMS)-OH (29). Zinc dust (118 mg, 1.80 mmol) was added portionwise to a solution of **28** (59 mg, 0.05 mmol) in THF (1.6 mL), followed by 1 M aqueous NH_4OAc solution (0.28 mL), and the mixture was stirred vigorously under nitrogen for 24 h. After filtration and solvent removal, the residue was taken up in AcOEt (50 mL) and washed with 5% aqueous $KHSO_4$ solution (2 × 10 mL) and saturated aqueous NaCl solution (2 × 10 mL). Drying and filtration followed by solvent removal gave an oil that was purified by chromatography (silica gel, CH_2Cl_2/MeOH, 99:1 → CH_2Cl_2/MeOH, 98:2, gradient elution), giving **29** as a colorless semisolid (34 mg, 66%): RP-HPLC t_R 19.12 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ +16.3 (*c* 0.6, $CHCl_3$); IR (film) 3428, 3328, 2960, 2943, 1743, 1707, 1655, 1515, 1457, 1389, 1322, 1300, 1250, 1152, 1093 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.07 (3H, s), 0.09 (3H, s), 0.82 (3H, d, J = 6.6), 0.87 (9H, s), 0.88 (3H, t, J = 6.5), 0.94 (3H, d, J = 6.9), 0.96 (3H, d, J = 6.9), 1.14−1.30 (3H, m), 1.18 (3H, d, J = 6.3), 1.46 (9H, s), 1.41−1.90 (3H, m), 1.80−1.90 (1H, m), 2.40−2.60 (2H, m), 2.75−3.00 (7H, m), 3.20−3.35 (1H, m), 3.78 (3H, s), 4.03−4.18 (2H, m), 4.50−4.58 (1H, m), 4.80−4.90 (1H, m), 5.00−5.16 (1H, m), 5.01 (1H, d, J = 12.0), 5.13 (1H, d, J = 12.0), 6.74−6.83 (2H, m), 6.98−7.26 (4H, m), 7.27−7.35 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.94, −4.31, 11.93, 13.31, 17.82, 21.26, 21.45, 23.16, 25.03, 25.66, 27.27, 28.30, 30.36, 30.97, 34.16, 34.30, 37.58, 41.16, 55.16, 55.92, 56.36, 57.67, 59.63, 67.43, 68.60, 69.50, 70.08, 81, 113.91, 127.45, 127.87, 128.38, 129.76, 135.41, 157.17, 158.37, 168.7, 169.89, 172.6, 173.6, 173.8; *m*/*z* (FAB) 966.2 [(M + Na)$^+$, 65], 940.1 (100).

Boc-(*R*)-N(Me)-Leu-Thr[Z-N(Me)-O(Me)-Tyr]-Ist(TB-DMS)-Hip-Leu-Pro-OTce (4a). Acid **29** (23 mg, 0.024 mmol) and alcohol **21** (15 mg, 0.03 mmol) in $CHCl_3$ (500 μL) were added to a solution of DMAP·CF_3COOH (6 mg, 0.025 mmol), DMAP (4 mg, 0.036 mmol), and DIPCDI (3.75 μL, 0.01 mmol) in $CHCl_3$ (100 μL) under nitrogen and the mixture heated to reflux. After 8 h, DMAP·CF_3COOH (6 mg, 0.25 mmol), DMAP (4 mg, 0.036 mmol), and DIPCDI (3.75 μL, 0.01 mmol) in $CHCl_3$ (100 μL) were added and stirring continued for a further 16 h at rt. The solvent was removed, and the crude residue was purified by chromatography (silica gel, AcOEt−hexanes, 1:4), giving the two diastereomers of **4a** (approximate proportions 1:1) as a semisolid, (21 mg, 60%): RP-HPLC t_R 22.91 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B, over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA), at a flow rate of 1 mL min^{-1}; IR (film) ν 3317, 2960, 2933, 2900, 2861, 1746, 1700, 1661, 1636, 1538, 1514, 1455, 1387, 1361, 1322, 1250, 1156, 1100 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.04 (3H, s), 0.10 (3H, s), 0.78 (3H, d, J = 6.5), 0.81 (9H, s), 0.85−0.89 (21H, m), 1.12 (3H, d, J = 6.5), 1.95−1.26 (2H, m), 1.26 (3H, d, J = 6.1), 1.29 (3H, d, J = 7.0), 1.43 (9H, s), 1.40−1.48 (2H, m), 1.50−1.68 (4H, m), 1.94 (1H, q, J = 7.0), 1.99−2.15 (2H, m), 2.32−2.43 (4H, m), 2.74−2.82 (7H, m), 2.90−2.98 (1H, m), 3.14-3.34 (1H, m), 3.63−3.68 (1H, m), 3.75 (3H, s), 3.91−3.95 (2H, m), 4.11−4.26 (2H, m), 4.51 (1H, d, J = 12.0), 4.52 (1H, d, J = 12.0), 4.76−4.82 (4H, m), 4.82−5.14 (5H, m), 5.24−5.30 (1H, m), 6.72 (2H, d, J = 8.5), 6.76 (2H, d, J = 8.5), 6.98 (2H, d, J = 8.1), 7.06 (2H, d, J = 8.1), 7.14−7.24 (2H, m), 7.25−7.31 (5H, m), 7.42 (1H, d, J = 10.0); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.86, −3.80, 12.13, 13.21, 14.30, 14.89, 16.44, 17.49, 18.11, 19.28, 21.42, 22.23, 22.91, 23.39, 24.64, 25.93, 27.61, 28.24, 29.22, 29.69, 30.01, 33.63, 34.07, 34.36, 39.73, 41.07, 47.62, 48.23, 48.63, 55.16, 55.99, 57.60, 58.82, 60.40, 67.04, 67.33, 67.80, 70.85, 73.87, 80.6, 80.95, 113.83, 127.40, 127.76, 127.88, 128.34, 129.77, 156.16, 158.00, 169.20, 169.93, 171.58, 171.80, 205.23; *m*/*z* (FAB) 1464.8 [(M′ + Na)$^+$, 6], 1462.8 [(M + Na)$^+$, 7], 1442.9 [M′ + H)$^+$, 8], 1440.9 [M + H)$^+$, 8] 1342.8 (100). Anal. Calcd for $C_{70}H_{109}Cl_3N_6O_{17}Si$: C, 58.41; H, 7.58; N, 5.84. Found: C, 58.23; H, 7.37; N, 5.91.

Boc-(*R*)-N(Me)-Leu-Thr[Z-N(Me)-O(Me)-Tyr]-Ist(TB-DMS)-Hip-Leu-Pro-OH (4b). Zinc dust (32 mg, 0.48 mmol) was added portionwise to a solution of **4a** (21 mg, 0.015 mmol) in THF (600 μL), followed by 1 M NH_4OAc solution (115 μL), and the mixture stirred vigorously under Ar for 24 h. AcOEt (10 mL) was added, the mixture filtered, and the filtrate washed with saturated aqueous NaCl solution (2 × 10 mL) and dried. Filtration and solvent removal gave **4b** as an oil (16 mg, 81%): RP-HPLC t_R 22.27 min, C_4 Nucleosil column, linear gradient from 10% B to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film) ν 3309, 2964, 2931, 2890, 280, 1746, 1704, 1659, 1632, 1547, 1515, 1452, 1385, 1368, 1321, 1262, 1100, 1021cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.07 (6H, s), 0.82 (9H, s), 0.83−0.91 (24H, m), 1.14 (3H, d, J = 6.3), 1.18−1.28 (2H, m), 1.28 (3H, d, J = 7.2), 1.32 (3H, d, J = 7.2), 1.48 (9H, s), 1.40−1.50 (2H, m), 1.52−1.68 (4H, m), 1.90−2.50 (8H, m), 2.78 (3H, bs,), 2.80 (3H, bs), 2.80−2.92 (1H, m), 3.20−3.30 (1H, m), 3.56−3.68 (2H, m), 3.77 (3H, s), 3.92−4.20 (3H, m), 4.49−4.94 (3H, m), 4.97−5.17 (4H, m), 5.20−5.25 (1H, m), 6.72−6.80 (2H, m), 7.00−7.20 (4H, m), 7.26−7.32 (5H, m), 7.40−7.45 (1H, m); *m*/*z* (ES) 1332.03 [(M + Na)$^+$, 33], 1310.14 [(M + H)$^+$, 100].

Boc-(*R*)-N(Me)-Leu-Thr[H-N(Me)-O(Me)-Tyr]-Ist(TB-DMS)-Hip-Leu-Pro-OH (4c). Acid **4b** (15 mg, 0.01 mmol) in THF (300 μL) was hydrogenolyzed in the presence of 10% Pd−C (17 mg) for 4 h at rt and atmospheric pressure. Filtration followed by solvent removal gave **4c** as a white solid (13 mg, 95%): mp 75−77 °C; RP-HPLC t_R 18.96 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film) ν 3313, 2963, 2931, 2858, 1731, 1661, 1632, 1515, 1455, 1387, 1368, 1322, 1260, 1162, 1096, 1022 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.81−0.94 (33H, m), 1.08 (3H, d, J = 6.6), 1.13 (3H, d, J = 6.6), 1.18−1.34 (2H, m), 1.26 (3H, d, J = 7.0), 1.29 (3H, d, J = 7.0), 1.44−1.50 (2H, m), 1.48 (9H, s), 1.60−1.80 (4H, m), 1.90−2.10 (4H, m), 2.24−2.43 (4H, m), 2.73−2.77 (6H, m), 2.80−3.07 (2H, m), 3.53−3.64 (2H, m), 3.75 (3H, s), 3.91−4.16 (3H, m), 4.62−4.84 (4H, m), 5.04−5.11 (2H, m), 6.77−6.82 (3H, m), 7.00−7.07 (4H, m), 7.33 (1H, d, J = 10.1); *m*/*z* (ES) 1175.08 [(M + H)$^+$, 100].

Cyclo-[N(Me)-O(Me)-Tyr-O-[Boc-(*R*)-N(Me)-Leu-]-Thr-Ist(TBDMS)-Hip-Leu-Pro-] (1a). HATU (12 mg, 0.031

mmol), HOAt (4 mg, 0.031 mmol), and DIEA (8.65 μL, 0.05 mmol) were added to **4c** (30 mg, 0.025 mmol) in THF (23 mL) at 0 °C, and the mixture was stirred for 3 h under Ar. The solvent was removed and the residue taken up in AcOEt (75 mL) and washed with 5% aqueous $KHSO_4$ solution (2 × 10 mL), 5% aqueous $NaHCO_3$ solution (2 × 10 mL), and saturated aqueous NaCl solution (2 × 10 mL). Drying and filtration, followed by solvent removal, gave an oil that was purified by chromatography (silica gel, CH_2Cl_2−AcOEt, 8:1), giving **1a** as an oil (8 mg, 28%): RP-HPLC t_R 21.16 min, C_4 Nucleosil, linear gradient from 10% B in A to 100% B, over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film) ν 3338, 2960, 2933, 2890, 2860, 1736, 1665, 1642, 1536, 1515, 1451, 1387, 1368, 1321, 1250, 1167 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.06 (3H, s), 0.04 (3H, s), 0.82 (9H, s), 0.80−0.92 (24H, m), 1.08−1.15 (1H, m), 1.24 (9H, s), 1.27 (3H, d, J = 7.0), 1.32 (3H, d, J = 7.0), 1.49 (9H, s), 1.45−1.50 (2H, m), 1.56−1.78 (5H, m), 1.94 (1H, q, J = 6.5), 1.98−2.29 (2H, m), 2.40−2.48 (1H, m), 2.52 (3H, s), 2.75 (3H, s), 2.77 (3H, s), 3.13−3.18 (2H, m), 3.36 (1H, dd, J_1 = 14.5, J_2 = 4.5), 3.52 (1H, dd, J_1 = 10.5, J_2 = 4.1), 3.53−3.64 (2H, m), 3.68−3.72 (1H, m), 3.78 (3H, s), 4.03 (1H, td, J_1 = 10.1, J_2 = 2.0), 4.15 (1H, dd, J_1 = 9.0, J_2 = 9.0), 4.58 (1H, dd, J_1 = 7.9, J_2 = 6.0), 4.70−4.84 (3H, m), 4.94−4.98 (1H, m), 5.01 (1H, d, J = 3.5), 6.82−6.84 (1H, m), 6.83 (2H, d, J = 8.5), 7.08 (2H, d, J = 8.3), 7.34−7.40 (1H, m), 7.87−7.93 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.78, −3.63, 12.03, 14.21, 15.26, 16.01, 16.89, 19.08, 20.95, 22.68, 23.12, 23.79, 24.82, 25.12, 25.94, 27.73, 27.87, 28.34, 29.68, 30.37, 33.42, 34.11, 38.59, 39.79, 41.38, 47.06, 47.72, 49.35, 55.20, 55.27, 57.24, 66.01, 68.33, 70.79, 77.19, 81.48, 114.12, 128.83, 129.90, 130.38, 130.92, 158.63, 168.57, 168.96, 169.35, 170.58, 170.97, 171.81, 172.50, 205.26; m/z (FAB) 1180.6 [$(M + Na)^+$, 65], 1158.6 [$(M + H)^+$, 100]. Anal. Calcd for $C_{60}H_{100}N_6O_{14}Si$: C, 62.28; H, 8.65; N, 7.27. Found: C, 62.42; H, 8.63; N, 6.72.

Didemnin A (1c). Macrocycle **1a** (10.2 mg, 8.65 × 10^{-3} mmol) was dissolved in 5 M HCl in dioxane (1 mL), and after 60 min, the solvent was removed and chased with Et_2O, giving didemnin A hydrochloride (8.4 mg, 98%): mp 141−147 °C (lit.[12] mp 146−148 °C); $[\alpha]_D$ −148.5 (*c* 0.4, $CHCl_3$) [lit.[12] $[\alpha]_D$ −148.7 (*c* 0.4, $CHCl_3$)]; RP-HPLC R_t 19.82 min, C_4 Nucleosil column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA), at a flow rate of 1 mL min^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.82−0.92 (24H, m), 1.11−1.19 (1H, m), 1.22 (3H, d, J = 6.9), 1.32 (3H, d, J = 6.8), 1.30−1.35 (1H, m), 1.35−1.63 (6H, m), 1.71−1.81 (2H, m), 1.93−2.07 (1H, m), 2.07−2.18 (2H, m), 2.28−2.34 (1H, m), 2.49−2.52 (1H, dd, J_1 = 11, J_2 = 10.5), 2.54 (3H, s), 2.72 (3H, bs), 2.79 (3H, bs), 2.86−2.94 (1H, bs), 2.72−2.79 (1H, bd, J = 10.5), 3.15−3.18 (1H, dd, J_1 = 14.5, J_2 = 10.5), 3.33−3.36 (1H, dd, J_1 = 14.5, J_2 = 4.5), 3.54−3.57 (1H, dd, J_1 = 10.5, J_2 = 4.5), 3.56−3.61 (1H, m), 3.78 (3H, s), 3.96−4.00 (1H, m), 4.03−4.08 (1H, m), 4.11−4.80 (1H, bs), 4.56−4.62 (1H, m), 4.68−4.81 (3H, m), 4.99−5.01 (1H, q, J = 3.5), 5.16 (1H, bs), 6.83 (2H, d, J = 8.5), 6.95 (1H, bs), 7.07 (2H, d, J = 8.5), 7.21−7.25 (1H, bs), 7.95 (1H, bs); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.55, 14.95, 15.26, 16.82, 18.56, 20.89, 22.00, 23.08, 23.76, 24.58, 24.85, 25.10, 27.12, 29.35, 29.35, 29.65, 29.69, 31.36, 33.96, 34.14, 38.51, 38.64, 40.14, 55.38, 55.56, 57.31, 66.17, 67.85, 70.58, 80.96, 80.98, 81.57, 114.12, 130.33, 158.63, 168.41, 169.33, 169.70, 170.38, 171.24, 172.28, 172.28, 172.93, 204.83; m/z 944.2 [$(M + H)^+$, 100].

Boc-Leu-Pro-OBzl. Boc-Leu-OH (3.01 g, 12 mmol), DCC (3.22 g, 15.6 mol), and HOBt (1.82 g, 13.2 mmol) were added to a stirred solution of Pro-OBzl·HCl (7.31 g, 30 mmol) and NMM (4.63 mL, 62 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The mixture was allowed to attain rt and was maintained at this temperature for 18 h. After cooling and filtration, the solvent was removed and the residue taken up in AcOEt (80 mL) and washed with 10% aqueous $KHSO_4$ solution, 10% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (9.65 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:6), giving Boc-Leu-Pro-OBzl as white rosettes (3.64 g, 72%): mp 185−186 °C; $[\alpha]_D$ −64.7 (*c* 2.0, CH_2Cl_2); IR (film, CH_2Cl_2) ν 3485, 2957, 2871, 1746, 1709, 1651, 1501, 1429, 1391, 1366, 1250, 1169, 1045, 1022 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.90 (3H, d, J = 6.6), 0.96 (3H, d, J = 6.5), 1.42 (9H, s), 1.75 (1H, m), 1.92−2.28 (4H, m), 3.50−3.80 (2H, m), 4.32−4.54 (1H, m), 4.54−4.75 (1H, m), 5.05 (2H, d, J = 12.0), 5.23 (2H, d, J = 12.0), 7.34 (5H, bs); ^{13}C NMR (50 MHZ, $CDCl_3$) δ 22.19, 23.89, 24.99, 25.38, 28.83, 29.45, 42.46, 47.18, 50.72, 59.31, 67.39, 79.99, 128.66−129.04, 136.1, 156.2, 172.32; m/z (FAB) 419.4 [$(M + H)^+$, 55], 319.4 (100); m/z (FABHRMS) 419.255 191, $C_{23}H_{34}N_2O_5$ requires $(M + H)^+$, 419.254 598. Anal. Calcd for $C_{23}H_{34}N_2O_5$: C, 66.03; H, 8.13; N, 6.70. Found: C, 66.11; H, 8.12; N, 6.78.

TBDMS-Hip-Leu-Pro-OBzl (31). Ester **16** (1.39 g, 3.696 mmol) in THF (15 mL) was hydrogenolyzed in the presence of 10% Pd−C (235 mg) for 120 min at room rt and atmospheric pressure. The system was cooled to −20 °C and the mixture filtered through Celite, washing with THF (20 mL), directly onto a solution of HBTU (1.467 g, 3.88 mmol) and HOBt (509 mg, 3.70 mmol) at −10 °C. DIEA (628 μL, 3.70 mmol) was added, and after 3 min, a solution of **30** (2.27 g, 5.39 mmol) [formed by treating Boc-Leu-Pro-OBzl (2.25 g, 5.39 mmol) with 60% TFA in CH_2Cl_2 for 2 h at rt, followed by removal of the solvent and chasing with Et_2O], in THF (7 mL) and DIEA (1.49 mL, 8.69 mmol), was added. The temperature was maintained at −5 °C for 3 h, allowed to attain rt, and stirred for 16 h. The solvent was removed and the residue taken up in AcOEt (50 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration, followed by solvent removal, gave an oil (2.52 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 3:7), giving the two diastereomers of **31** (approximate proportions, 1:1) as a colorless caramel (1.79 g, 83%): RP-HPLC t_R 22.7 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution at 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film, CH_2Cl_2) 3295, 3060 and 3040, 2957, 2934, 2880, 2858, 1736, 1634, 1528, 1454, 1387, 1252, 1171, 1070 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.01 (3H, s), 0.03 (3H, s), 0.85−0.97 (12H, m), 0.92 (9H, s), 0.93 (9H, s), 1.33 (3H, d, J = 7.0), 1.37 (3H, d, J = 7.0), 2.40−2.65 (3H, m), 1.92−2.28 (4H, m), 3.64−3.76 (1H, m), 4.69−4.82 (1H, m), 5.05 (1H, d, J = 11.8), 5.20 (2H, d, J = 11.8), 6.73 (1H, d, J = 8.9), 6.98 (1H, d, J = 9.0), 7.34 (5H, bs); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.24, −4.81, 15.70, 17.43, 17.57, 18.84, 21.48, 21.61, 18.05, 23.28, 24.43, 24.55, 24.76, 25.68, 28.87, 31.36, 31.77, 41.27, 41.67, 48.68, 48.55, 48.89, 58.71, 66.84, 83.84, 83.29, 128.09, 128.47, 135.40, 169.24, 170.67, 170.89, 171.16, 171.20, 209.11, 211.62; m/z (FAB) 611.5 [$(M + Na)^+$, 15], 589.5 [$(M + H)^+$, 100]; m/z (FABHRMS) 589.369 045, $C_{32}H_{52}N_2O_6Si$ requires $(M + H)^+$, 589.367 291.

H-Hip-Leu-Pro-OBzl (32). The ester **31** (908 mg, 1.54 mmol) and TBAF (1.03 g, 3.24 mmol) were dissolved in dry THF (17 mL) at rt, and after 30 min, H_2O (30 mL) was added and the solution extracted with AcOEt (4 × 40 mL). The combined organic phases were washed with saturated aqueous NaCl solution and dried. Filtration and solvent removal gave an off-white solid that was purified by chromatography (AcOEt−hexanes, 2:3) giving the two diastereomers of **32** (approximate proportions, 1:1) as a white solid (708 mg, 97%): mp 180−186 °C, (lit.[12] mp 128−130 °C); RP-HPLC t_R 17.5 and 16.5 min, Nucleosil C18 column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film, CH_2Cl_2) ν 3450−3293, 3060 and 3040, 2961, 2946, 2883, 2852, 1746, 1632, 1533, 1454, 1357, 1387, 1265, 1173, 1095, 1045, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.71 (3H, d, J = 6.8), 0.81 (3H, d, J = 6.6), 0.88 (3H, d, J = 6.5), 0.91 (3H, d, J = 6.5), 0.94 (3H, d, J = 6.5), 0.99 (3H, d, J = 7.1), 1.07 (3H, d, J = 6.5), 1.36 (3H, d, 6.5), 1.43−1.52 (2H, m), 1.60−1.66 (1H, m), 1.93−1.20 (3H, m), 2.12−2.23 (2H, m), 3.53−3.58 (1H, m), 3.65 (1H, q, J = 7.1), 3.67−3.73 (1H, m), 3.89 (1H, q, J = 7.1), 3.96 (1H, d, J = 4.2), 4.22 (1H, d, J = 4.1), 4.54−4.56 (1H, m), 4.58−4.62 (1H, m), 4.69−4.73 (1H, m), 5.1 (1H, d, J = 12.1),

5.18 (1H, d, J = 12.1), 6.57 (1H, d, J = 8.5), 6.63 (1H, d, J = 8.5), 7.28−7.38 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.06, 14.26, 15.85, 16.48, 20.07, 20.53, 22.02, 22.25, 25.37, 25.46, 29.45, 29.53), 31.59, 32.09, 41.13, 42.29, 49.93, 50.91, 51.02, 59.52, 67.60, 81.02, 128.78, 1.29.2, 169.48, 171.58, 172.17, 209.76; m/z (FAB) 497.4 [$(M + Na)^+$, 12], 475.5 [$(M + H)^+$, 100]; m/z (FABHRMS) 497.263 162, $C_{26}H_{38}N_2O_6$ requires $(M + Na)^+$ 497.262 757. Anal. Calcd for $C_{26}H_{38}N_2O_6$: C, 65.82; H, 8.02; N, 5.91. Found: C, 65.97; H, 8.18; N, 5.76.

Boc-Ist(TBDMS)-Hip-Leu-Pro-OBzl (33). DCC (95 mg, 0.46 mmol) in CH_2Cl_2 (154 μL) was added dropwise, over 15 min, to a stirred solution of **32** (195 mg, 0.41 mmol), **15** (149 mg, 0.38 mmol), and DMAP (16 mg, 0.13 mmol) in CH_2Cl_2 (746 μL) under Ar at between −5 and −10 °C. After 3 h, the temperature was allowed to rise to 4 °C (refrigerator) and was then maintained for 12 h. The mixture was filtered, the solvent removed, and the residue taken up in AcOEt (40 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration, followed by solvent removal, gave an oil (412 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 3:7), giving the two diastereomers of **33** (approximate proprotions, 1:1) as a clear, colorless oil, (292 mg, 84%): RP-HPLC t_R 26 and 25 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film, CH_2Cl_2) ν 3365−3200, 3069, 3038, 2959, 2930, 2882, 2857, 1746, 1688, 1640, 1533, 1456, 1389, 1258, 1171, 1086 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.01 (3H, s), 0.03 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.77−1.03 (18H, m), 0.84 (9H, s), 0.85 (9H, s), 1.33 (3H, d, J = 7.4), 1.32−1.36 (2H, m), 1.49 (3H, d, J = 7.5), 1.38−1.62 (3H, m), 1.42 (9H, s), 1.44 (9H, s), 1.51−1.77 (1H, m), 1.88−2.37 (3H, m), 2.17−2.33 (2H, m), 2.47−2.74 (2H, m), 3.34−3.72 (1H, m), 3.72−3.82 (1H, m), 3.99−4.40 (1H, m), 4.03−4.16 (1H, m), 4.49 (1H, d, J = 10.3), 4.54−4.59 (1H, m), 4.63−4.70 (2H, m), 4.75 (1H, d, J = 4.5), 4.77−4.81 (1H, m), 4.95−5.19 (2H, m), 5.22 (1H, d, J = 5.2), 5.32 (1H, d, J = 10.5), 6.38 (1H, d, J = 10.9), 6.71 (1H, d, J = 7.4), 6.76 (1H, d, J = 8.4), 8.60 (1H, d, J = 9.5); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.05, −4.49, 11.83, 12.03, 13.01, 13.51, 13.83, 14.08, 16.92, 17.10, 17.85, 19.14, 19.65, 21.57, 22.09, 22.96, 23.28, 24.36, 24.60, 24.85, 25.73, 26.97, 27.33, 28.35, 28.46, 28.93, 29.09, 29.65, 34.12, 34.16, 40.45, 40.85, 41.18, 42.20, 46.74, 46.16, 47.99, 48.34, 48.90, 49.42, 57.62, 58.81, 58.96, 60.46, 66.62, 66.88, 68.18, 69.69 , 78.98, 79.24, 79.84, 82.95, 128.08−128.49, 135.48 135.61, 155.85, 158.27, 157.44, 168.40, 169.07, 170.65, 170.86, 171.42 171.79, 203.09 205.97; m/z (FAB) 846.6 [$(M + H)^+$, 15], 746.6 (100); m/z (FABHRMS) 868.516 630, $C_{45}H_{75}N_3O_{10}Si$ requires $(M + Na)^+$ 868.511 930.

Boc-Thr(Bzl)-OSEM (35). SEM-Cl (2.6 mL, 14.56 mmol) was added to a suspension of Li_2CO_3 (1.08 g, 14.56 mmol) and Boc-Thr(Bzl)-OH (3.07 g, 9.93 mmol) in DMF (10 mL), containing activated molecular sieves (1 g, 4 Å pore size), and this mixture was stirred under Ar at rt for 18 h. The mixture was filtered through Celite, and H_2O (20 mL) was added to the filtrate. Extraction with AcOEt (5 × 20 mL), followed by washing the combined organic phases with saturated NaCl solution, drying, filtration, and solvent removal, gave an oil (3.5 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:9), giving **35** as a clear, colorless oil [2.42 g, 78%, based on recovered Boc-Thr(Bzl)-OH (0.95 g)]: RP-HPLC t_R 22.3 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution at 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −1.35 (c 0.7, CH_2Cl_2); IR (film, CH_2Cl_2) ν 3500−3275, 2979−2875, 1721, 1501, 1368, 1250, 1167, 1070 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.02 (9H, s), 0.90−0.99 (2H, m), 1.31 (3H, d, J = 6.3), 1.49 (9H, s), 3.65−3.72 (2H, m), 4.12−4.25 (1H, m), 4.32−4.41 (2H, m), 4.39−4.63 (1H, m), 5.28−5.34 (3H, m), 7.29−7.33 (5H, bs); ^{13}C NMR (50 MHz, $CDCl_3$) δ −1.51, 16.23, 17.89, 28.26, 58.28, 68.04, 70.86, 74.56, 79.81, 89.83, 127.15, 127.31, 137.83, 156.09, 170.79; m/z (FAB) 462.4 [$(M + Na)^+$, 5], 440.4 [$(M + H)^+$, 28]; m/z (FABHRMS) 440.244 850, $C_{22}H_{37}NO_6Si$ requires $(M + H)^+$ 440.246 842.

Boc-Thr-OSEM (36). Benzyl ether **35** (950 mg, 2.16 mmol) in MeOH (10 mL) was hydrogenolyzed in the presence of 10% Pd−C (146 mg) for 60 min at rt and atmospheric pressure. The mixture was filtered through Celite and the solvent removed, giving **36** as an oil (730 mg, 97%): RP-HPLC t_R 19.5 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −8.4 (c 1.03, CH_2Cl_2) [lit.[14] $[\alpha]_D$ −6.8 (c 2.1, $CHCl_3$)]; IR (film, CH_2Cl_2) ν 3550−3350, 2979, 2880, 1719, 1508, 1368, 1250, 1165, 1113 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.02 (9H, s), 0.92−1.01 (2H, m), 1.27 (3H, d, J = 6.3), 1.45 (9H, s), 3.69−3.77 (2H, m), 4.22−4.41 (2H, m), 5.36−5.38 (3H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ −1.46, 17.98, 19.94, 28.27, 58.47, 68.13, 68.18, 80.01, 89.89, 157.06, 171.14; m/z (CI) 372.2 [$(M + Na)^+$, 55], 350.2 [$(M + H)^+$, 10], 236.0 (100).

Boc-Thr[Z-N(Me)-O(Me)-Tyr]-OSEM (37). DCC (1 g, 0.72 mmol) in CH_2Cl_2 (800 μL) was added to Z-N(Me)-O(Me)-Tyr-OH (1.53 g, 4.67 mmol), **36** (1.25 g, 3.59 mmol), and DMAP (86 mg, 0.72 mmol) in CH_2Cl_2 (4 mL) at −10 °C and the mixture allowed to attain rt and stirred for 15 h. After the mixture was cooled to 0 °C, CH_2Cl_2 (15 mL) was added and the solution filtered and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (2.62 g) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:5), giving **37** as an oil (2.08 g, 88%): RP-HPLC t_R 21.4 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −10.3 (c 1.2, CH_2Cl_2) [lit.[14] $[\alpha]_D$ −15.2 (c 1.1, $CHCl_3$]; IR (film, CH_2Cl_2) ν 3200, 2957−2838, 1742, 1705, 1613, 1586, 1514, 1454, 1402, 1308, 1248, 1180, 1142, 1109, 1034 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.03 (9H, s), 0.91−1.00 (2H, m), 1.31 (3H, d, J = 6.3), 1.46 (9H, s), 2.73 (3H, s), 2.81 (3H, s), 2.85−3.04 (1H, m), 3.10−3.30 (1H, m), 3.65−3.79 (2H, m), 3.78 (3H, s), 4.43−4.51 (1H, m), 4.69−4.88 (1H, m), 5.06−5.40 (5H, m), 5.40−5.52 (1H, m), 6.75−6.81 (2H, m), 6.99−7.71 (2H, m), 7.31−7.32 (5H, bs.); ^{13}C NMR (50 MHz, $CDCl_3$) δ −1.46, 16.74, 17.01, 17.95, 28.24, 31.64, 32.19, 33.86, 34.17, 55.14, 56.88, 57.05, 67.28, 68.24, 71.52, 71.74, 80.21, 90.40, 113.88, 127.62, 128.79, 129.40, 129.80, 138.67, 137.84, 138.53, 158.31, 169.59, 169.72; m/z (FAB) 697.6 [$(M + Na)^+$, 100]; m/z (FABHRMS) 675.330 007, $C_{34}H_{50}N_2O_{10}$ requires $(M + H)^+$ 675.331 300.

Boc-Thr[Z-N(Me)-O(Me)-Tyr]-OH (38). Aqueous HF in MeCN [1.383 mL of a solution of 15% HF (1 mL) in H_2O (1 mL)], previously cooled to −25 °C, was added dropwise to a solution of **37** (358 mg, 0.519 mmol) in MeCN (2.767 mL) at −25 °C, and the temperature of the system was maintained between −25 and −15 °C for 7 h. Cold (0 °C) solutions of 10% aqueous $KHSO_4$ (18 mL) and cold (0 °C) AcOEt (15 mL) were added, and the pH of the aqueous phase was adjusted to 4 by addition of solid $NaHCO_3$. The aqueous phase was then extracted with AcOEt (4 × 20 mL), dried, and filtered. Solvent removal gave an oil (313 mg) that was purified by chromatography (silica gel, MeOH−CH_2Cl_2, 1:32), giving **38** as a white semisolid (204 mg, 72%): RP-HPLC t_R 17.9, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution at 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −20.5 (c 2, CH_2Cl_2); IR (film, CH_2Cl_2) ν 3400, 3050, 2900, 1715, 1613, 1514, 1456, 1402, 1368, 1248, 1165, 1061, 1036 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 (3H, d, J = 6.5), 1.45 (9H, s), 2.74 (3H, s), 2.75 (3H, s), 2.76−3.31 (2H, m), 3.77 (3H, s), 4.42−4.52 (1H, m), 4.66−4.83 (1H, m), 5.01−5.16 (2H, m), 5.30−5.53 (2H, m), 6.72−6.81 (2H, m), 6.95−7.09 (2H, m), 7.35 (5H, bs); ^{13}C NMR (75 MHZ, $CDCl_3$) δ 16.44, 16.82, 28.23, 31.71, 31.97, 33.82, 33.68, 55.14, 56.87, 56.75, 60.58, 60.39, 67.51, 67.76, 71.83, 72.47, 80.40, 113.91, 127.59, 128.69, 129.77, 236.42, 156.00, 156.19, 156.71, 158.31, 159.47, 169.78; m/z (FAB) 567.1 [$(M + Na)^+$, 46], 545.1 [$(M + H)^+$, 7], 445.1 (100); m/z (FABHRMS) 567.233 280, $C_{28}H_{35}N_2O_9$ requires $(M + Na)^+$ 567.231 851.

Boc-Thr[Z-N(Me)-O(Me)-Tyr]-Ist-Hip-Leu-Pro-OBzl (5a). A 5.7 M solution of HCl in dioxane (3 mL) was added to a solution of **33** (141 mg, 0.17 mmol) in dioxane (1 mL) at rt, and after 3 h the solvent was removed and chased with Et_2O. The white solid formed (**34**, 105 mg, 0.167 mmol) in THF (2 mL) was then added to a solution of **38** (119 mg, 0.22 mmol), HBTU (83 mg, 0.22 mmol), HOBt (30 mg, 0.22 mmol), and DIEA (37 μL, 0.22 mmol) in dry THF (1.2 mL) under nitrogen at −15 °C. After being stirred for 5 min, DIEA (57 μL, 0.33 mmol) was added dropwise over 15 min, and the temperature was maintained between −10 and −5 °C for 4 h. The solvent was removed and the residue taken up in AcOEt (30 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (329 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:1) giving the two diastereomers of **5a** (approximate proportions, 1:1) as a glassy solid (159 mg, 82%): mp 89−93 °C; RP-HPLC t_R 21.0 and 22.1 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution at 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; IR (film, CH_2Cl_2) ν 3350, 2961, 2927, 2893, 1744, 1688, 1638, 1514, 1454, 1368, 1304, 1248, 1171, 1067, 1036 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.74−0.92 (18H, m), 1.05−1.15 (2H, m), 1.18−1.20 (2H, m), 1.23 (3H, d, J = 6.8), 1.25 (3H, d, J = 6.8), 1.29 (3H, d, J = 6.9), 1.42 (9H, s), 1.45 (9H, s), 1.50−1.66 (3H, m), 1.89−2.02 (4H, m), 2.17−2.25 (2H, m), 2.37−2.42 (1H, m), 2.81 (3H, s), 2.88 (3H, s), 2.91 (3H, s), 2.95 (3H, s), 2.84−2.93 (2H, m), 3.17−3.25 (1H, m), 3.53−3.59 (1H, m), 3.75 (3H, s), 3.88−3.98 (4H, m), 4.49 (1H, d, J = 3.1), 4.51 (1H, d, J = 3.1), 4.53−4.57 (1H, m), 4.68−4.72 (1H, m), 4.96−4.99 (1H, m), 5.02−5.33 (4H, m), 5.02 (1H, d, J = 3.2), 5.23 (1H, d, J = 3.1), 5.26−5.33 (1H, m), 5.47 (1H, d, J = 9.5), 6.74 (2H, d, J = 7.8), 6.77 (2H, d, J = 7.7), 7.08 (2H, d, J = 7.7), 7.17 (1H, d, J = 7.5), 7.21 (1H, d, J = 9.5), 7.23−7.36 (10H, m,), 7.75 (1H, d, J = 7.9), 7.79 (1H, d, J = 8.2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.95, 13.27, 15.16, 16.47, 17.33, 18.79, 21.28, 23.65, 24.65, 24.72, 27.09, 28.08, 28.93, 31.20, 31.32, 33.62, 33.98, 38.38, 41.01, 47.12, 49.38, 54.96, 55.17, 57.89, 58.83, 60.01, 60.16, 67.18, 71.05, 71.32, 80.34, 81.24, 113.89, 127.51, 128.59, 129.69, 129.77, 135.52, 136.77, 156.93, 158.27, 169.87, 170.62, 171.15, 171.85, 172.39, 204.88; m/z (FAB) 1181.2 [$(M + Na)^+$, 20], 1159.2 [$(M + H)^+$, 80], 1059.2 (100). Anal. Calcd for $C_{62}H_{87}N_5O_{16}$: C, 64.30; H, 7.52; N, 6.05. Found: C, 64.14; H, 7.66; N, 5.95.

Boc-Thr[H-N(Me)-O(Me)-Tyr]-Ist-Hip-Leu-Pro-OH (5b). Depsipeptide **5a** (45 mg, 39.12×10^{-3} mmol) in THF (1.7 mL) was hydrogenolyzed in the presence of 10% Pd−C (39 mg) at rt and atmospheric pressure for 3 h. The mixture was filtered through Celite and the solvent removed, giving the two diastereomers of **5b** (approximate proportions, 1:1) as an oil (37 mg, 99%): RP-HPLC t_R 14.7 and 15.2 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA), at a flow rate of 1 mL min^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.79−1.08 (18H, m), 1.80−1.38 (3H, m), 1.26 (3H, bs), 1.29 (3H, d, J = 7.1), 1.47 (9H, s), 1.50−1.66 (3H, m), 1.84−1.94 (1H, m), 1.90−2.28 (4H, m), 2.35−2.50 (4H, m), 2.30−2.35 (1H, m), 2.44−3.18 (4H, m), 2.60 (3H, m), 3.53−3.61 (1H, m), 3.77 (3H, s), 3.88−4.07 (4H, m), 4.12−4.72 (4H, m), 5.18−5.24 (1H, m), 5.24 (1H, bs), 6.84 (2H, d, J = 7.9), 7.08 (2H, d, J = 8.0), 7.13 (1H, d, J = 8.2), 7.18 (1H, d, J = 8.2), 7.62−7.68 (1H, bs); m/z (FAB) 972.7 [$(M+K)^+$, 33], 934.9 (M^+, 100].

Cyclo-N(Me)-O(Me)-Tyr-O-[Boc-Thr-]-Ist-Hip-Leu-Pro (3a). HATU (18 mg, 46.94×10^{-3} mmol), HOAt (7 mg, 47.73×10^{-3} mmol), and DIEA (13.3 μL, 78.24×10^{-3} mmol) were added to a stirred solution of **5b** (36 mg, 39.12×10^{-3} mmol) in THF (34 μL) at 0 °C. The mixture was allowed to attain rt, and after 17 h the solvent was removed and the residue taken up in AcOEt (10 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (49 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:1), giving macrocycle **3a** as a clear viscous oil (27 mg, 76%): mp 164−168 °C; RP-HPLC t_R 20.4 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −209.4 (c 0.3, $CHCl_3$); IR (film, CH_2Cl_2) ν 3343, 2961, 2927, 2893, 1734, 1640, 1514, 1454, 1368, 1302, 1248, 1167, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.78 (3H, d, J = 7.1), 0.85 (3H, d, J = 7.0), 0.87 (3H, d, J = 7.0), 0.89−0.93 (9H, m), 1.10−1.20 (1H, m), 1.20 (3H, d, J = 6.4), 1.30 (3H, d, J = 6.9), 1.36 (2H, m), 1.40 (2H, m), 1.44 (9H, s), 1.48−1.72 (2H, m), 1.72−1.78 (1H, m), 1.83−1.88 (1H, m), 2.01−2.17 (3H, 2m), 2.27−2.29 (1H, m), 2.47−2.53 (1H, m), 2.53 (3H, s), 2.93 (1H, bs), 3.14−3.19 (2H, m), 3.34−3.37 (1H, dd, J_1 = 14.8, J_2 = 4.1), 3.54−3.56 (1H, dd, J_1 = 10.5, J_2 = 4.1), 3.58−3.63 (1H, m), 3.68−3.72 (1H, m), 3.78 (3H, s), 3.94−3.98 (1H, m), 3.98 (1H, q, J = 7.5), 4.07−4.11 (1H, 3d, J = 3.8), 4.57−4.61 (2H, m), 4.77−4.81 (1H, m), 4.97−4.98 (1H, q, J = 3.5), 5.02 (1H, d, J = 10.5), 5.18 (1H, d, J = 4.2), 6.81 (2H, d, J = 8.5), 7.05 (2H, d, J = 8.5), 7.19 (1H, d, J = 10.2), 7.64 (1H, d, J = 10.1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.56, 14.68, 14.97, 15.27, 16.61, 18.45, 20.64, 23.50, 24.71, 24.78, 26.92, 27.73, 27.94, 31.55, 33.94, 33.94, 38.27, 38.52, 40.64, 46.86, 49.54, 49.65, 55.16, 55.19, 55.84, 57.12, 65.96, 67.30, 71.00, 80.27, 81.41, 114.02, 130.22, 158.53, 168.30, 169.31, 170.12, 170.29, 171.20, 172.38, 204.51; m/z (FAB) 938.9 [$(M + Na)^+$, 55], 916.9 [$(M + H)^+$, 100]; m/z (FABHRMS) 916.532 120, $C_{47}H_{73}N_5O_{13}$ requires $(M + H)^+$ 916.528 300.

Boc-didemnin A (1b). A 5.7 M solution of HCl in dioxane (2.8 mL) was added to a solution of **3a** (36 mg, 39.34×10^{-3} mmol) in dioxane (200 μL) at rt, and after 1 h, the solvent was removed and chased with Et_2O. The white solid formed (**3b**) was dissolved in CH_2Cl_2 (400 μL) and added to a solution of Boc-(*R*)-N(Me)-Leu-OH (24 mg, 99.28×10^{-3} mmol), BOP (48 mg, 109.24×10^{-3} mmol), HOBt (16 mg, 109.24×10^{-3} mmol), and DIEA (16.8 μL, 99.28×10^{-3} mmol) in CH_2Cl_2 (250 μL) at 0 °C. The mixture was allowed to attain rt, and after 40 min, the solvent was removed and the residue taken up in AcOEt (10 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (19 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 1:1) giving **1b** (38 mg, 92%) as a white solid: mp 121−124 °C; RP-HPLC t_R 21.2 min, Nucleosil C_{18} column, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; $[\alpha]_D$ −84.19 (c 0.37, $CHCl_3$); IR (film, CH_2Cl_2) ν 3338, 2959, 2930, 2875, 1734, 1667, 1640, 1539, 1514, 1454, 1389, 1368, 1321, 1248, 1157, 1076 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.82−0.92 (24H, m), 1.11−1.19 (1H, m), 1.22 (3H, d, J = 6.3), 1.32 (3H, d, J = 6.5), 1.30−1.35 (1H, m), 1.40 (s, 9H), 1.35−1.63 (6H, m), 1.71−1.81 (2H, m), 1.93−2.07 (1H, m), 2.07−2.18 (2H, m), 2.28−2.34 (1H, m), 2.49 (1H, d, J = 10.8), 2.52 (1H, d, J = 10.5), 2.54 (3H, s), 2.72 (3H, bs), 2.79 (3H, bs), 2.86−2.94 (1H, bs), 3.15−3.18 (1H, dd, J_1 = 14.5, J_2 = 4.5), 3.33−3.36 (1H, dd, J_1 = 14.5, J_2 = 4.5), 3.54−3.57 (1H, dd, J_1 = 10.5, J_2 = 4.5), 3.56−3.61 (1H, m), 3.78 (3H, s), 3.96−4.00 (1H, m), 4.03−4.08 (1H, m), 4.11−4.80 (1H, bs), 4.56−4.62 (1H, m), 4.68−4.81 (3H, m), 4.99−5.01 (1H, q, J = 3.5), 5.16 (1H, bs), 6.83 (2H, d, J = 8.5), 6.95 (1H, bs), 7.07 (2H, d, J = 8.5), 7.21−7.25 (1H, bs), 7.95 (1H, bs); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.55, 14.95, 15.26, 16.82, 18.56, 20.89, 22.00, 23.08, 23.76, 24.58, 24.85, 25.10, 27.12, 29.35, 29.35, 29.65, 29.69, 31.36, 33.96, 34.14, 38.51, 38.64, 40.14, 55.38, 55.56, 57.31, 66.17, 67.85, 70.58, 80.96, 80.98, 81.57, 114.12, 130.33, 158.63, 168.41, 169.33, 169.70, 170.38, 171.24, 172.28, 172.28, 172.93, 204.83; m/z (FAB) 1066.3 [$(M + Na)^+$, 31%], 1044.3 [$(M + H)^+$, 100]. Anal. Calcd for $C_{49}H_{77}N_6O_{13}$: C, 56.43; H, 7.39; N, 8.06. Found: C, 56.57; H, 7.37; N, 8.07.

Didemnin A (1c). A 6 M solution of HCl in dioxane (2.8 mL) was added to a solution of **1b** (32 mg, 30.65×10^{-3} mmol) in dioxane (100 μL) and the solution stirred for 30 min. The solvent was removed and chased with Et_2O, giving **1c** hydrochloride as a white solid (31 mg, 99%). The physical and spectroscopic data obtained for this compound were identical

to those reported for the didemnin A obtained in the first synthesis described above.

Pyr-Pro-OBzl (39). Pyruvic acid (1.58 mL, 22 mmol), DCC (5.35 g, 26 mmol), HOBt (3.27 g, 24 mmol), and DIEA (3.67 mL, 22 mmol) were added to a solution of H-Pro-OBzl·HCl (6.26 g, 26 mmol) in CH_2Cl_2 (14 mL) at 0 °C, and after 5 min, DIEA (4.4 mL, 26 mmol) was added and stirring was continued for 32 h at rt. The mixture was filtered, the solvent removed, and the residue taken up in AcOEt (50 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration, followed by solvent removal, gave an oil that was purifed by chromatography (silica gel, AcOEt−hexanes, 3:7), giving **39** as an off-white solid (2.04 g, 36%): mp 42−47 °C; $[\alpha]_D$ −66.8 (*c* 0.1, $CHCl_3$); IR (film, CH_2Cl_2) ν 3035, 2956, 2884, 1744, 1717, 1645, 1499, 1443, 1383, 1352, 1273, 1175, 1092 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.75−2.40 (4H, m), 2.37 (3H, s), 2.44 (3H, s), 3.45−3.82 (2H, m), 4.52−4.61 (1H, m), 4.88−4.97 (1H, m), 5.14−5.15 (2H, m), 5.17−5.20 (2H, m), 7.34 (5H, bs); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.11, 25.22, 26.5, 27.10, 28.53, 31.48, 47.53, 44.81, 59.76, 67.02, 67.31, 128.11, 128.64, 135.24, 170.1, 170.2, 198 (CO); *m/z* (CI) 293 [$(M+NH_4)^+$, 100]. Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.08. Found: C, 65.04; H, 6.01; N, 5.11.

Pyr-Pro-OH (40). Ester **39** (174 mg, 0.63 mmol) in THF (4 mL) was hydrogenolyzed in the presence of 10% Pd−C (containing 50% H_2O) (105 mg) at rt and atmospheric pressure for 3 h. The mixture was filtered through Celite, and solvent removal gave an oil (120 mg) that was purified by chromatography (silica gel, AcOEt−hexanes, 3:2) giving **40** as a white solid (85 mg, 72%): mp 67−69 °C; $[\alpha]_D$ −112 (*c* 0.12, $CHCl_3$); IR (film, CH_2Cl_2) ν 3450−3000, 2961−2870, 1719, 1643, 1615, 1452, 1354, 1205, 1175, 1094, 1018 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.85−2.45 (4H, m), 2.43 and 2.47 (3H, s), 3.42−3.85 (2H, m), 4.52−4.61 (1H, m), 4.88−4.97 (1H, m), 7.21−7.40 (1H, bs); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.03, 25.23, 26.48, 27.00, 28.23, 31.44, 47.57, 48.37, 59.61, 59.395, 162.47, 162.52, 175.04, 176.29, 197.18; *m/z* (CI) 220 [$(M + N_2H_7)^+$, 15], 203 [$(M + NH_4)^+$, 100], 186 [$(M + H)^+$, 16]. Anal. Calcd for $C_8H_{11}NO_4$: C, 51.88; H, 5.99; N, 7.56. Found: C, 52.13; H, 5.85; N, 7.65.

Dehydrodidemnin B (1e). Acid **40** (60 mg, 0.35 mmol) in CH_2Cl_2 (350 μL), PyBroP (160 mg, 0.35 mmol) in CH_2Cl_2 (400 μL), and DIEA (87 μL, 0.51 mmol) were added to **1c** (80 mg, 0.09 mmol), and the mixture was cooled to −5 °C and stirred at this temperature for 30 min. The mixture was allowed to attain rt, and after 4 h the solvent was removed and the residue taken up in AcOEt (10 mL) and washed with 5% aqueous $KHSO_4$ solution, 5% aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution. Drying and filtration followed by solvent removal gave an oil (200 mg) that was purified by chromatography (silica gel, AcOEt−CH_2Cl_2, 1:1), giving **1e** as a white solid (60 mg, 62%): mp 152−160 °C; RP-HPLC t_R 19.0 and 19.8 min, linear gradient from 10% B in A to 100% B over 20 min, followed by isocratic elution with 100% B for 10 min, where A is H_2O/0.045% TFA and B is MeCN/0.036% TFA, at a flow rate of 1 mL min^{-1}; R_f 0.40 and 0.28 (CH_2Cl_2−AcOEt, 2:3), 0.52 and 0.45 ($CHCl_3$−MeOH, 9.5:0.5); $[\alpha]_D$ −95.9 (*c* 1.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.84−0.93 (24H, m), 1.16−1.70 (9H, m), 1.72−1.81 (1H, m), 1.81−1.90 (1H, m), 1.90−2.24 (6H, m), 2.30−2.39 (1H, m), 2.49 (3H, s) 2.51 (3H, s), 2.55 (3H, s), 2.52−2.64 (1H, m), 2.85 (1H, bs), 2.94 (1H, bs), 3.09 (3H, s), 3.13 (3H, s), 3.15−3.18 (1H, m), 3.21−3.26 (1H, dd, J = 15.8, 6.1), 3.32−3.36 (1H, dd, J_1 = 14.5, J_2 = 4.1), 3.54−3.60 (1H, m), 3.66−3.72 (1H, m), 3.78 (3H, s), 3.80−3.87 (1H, m), 3.96−3.99 (1H, m), 4.03−4.11 (2H, m), 4.15−4.23 (1H, 2q, J = 7.5), 4.55−4.57 (1H, 2d, J = 5.5, 2.2), 4.59−4.62 (1H, t), 4.56−4.64 (1H, dd, J_1 = 6.5, J_2 = 2.5), 4.68−4.71 (1H, t), 4.76−4.81 (1H, t), 5.10−5.18 (1H, m), 5.17 (1H, d, J = 3.5), 5.18 (1H, d, J = 3.5), 5.27−5.31 (2H, m), 6.82 (2H, d, J = 8.5), 6.83 (2H, d, J = 8.5), 7.05 (2H, d, J = 8.5), 7.06 (2H, d, J = 8.5), 7.02 (1H, d, J = 7.1), 7.16 (1H, d, J = 9.5), 7.17 (1H, d, J = 9.5), 7.59 (1H, d, J = 5.5), 7.77 (1H, d, J = 9.5), 7.83 (1H, d, J = 9.4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.63, 11.68, 14.11, 14.70, 15.26, 15.30, 16.00, 16.20, 16.88, 16.93, 18.62, 18.85), 20.89, 20.94, 21.62, 21.36, 23.44, 23.57, 23.84, 23.93, 24.66, 24.77, 24.85, 25.02, 26.22, 26.34, 27.09, 27.6, 27.06, 27.30, 27.95, 27.99, 29.33, 29.69, 31.31−31.37, 33.97, 34.06, 36.02, 36.45, 38.68, 38.76, 41.01, 41.15, 47.00, 48.42, 48.48, 48.86, 49.20, 49.51, 54.65, 54.75, 55.26, 55.58, 55.61, 57.14, 57.27), 57.47, 57.79, 66.24, 33.39, 67.80, 67.99, 70.34, 70.67, 81.0, 81.52, 114.10, 130.31, 156.0, 158.65, 161.1, 161.60, 168.20, 169.53, 169.59, 170.452, 171.25, 171.80, 171.95, 172.26, 172.33, 197.5, 204.80, 204.85; *m/z* (FAB) 1132.6 [$(M + Na)^+$, 42], 1110.8 [$(M + H)^+$, 100].

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Supporting Information Available: ^1H NMR spectra for all synthetic intermediates described in the experimental section, including synthetic and natural didemnins, **1c** and **1e**. $^1H-^1H$ COSY spectra for compound **3a** and for natural **1e**. $^1H-^1H$ TOCSY spectra for compounds **21**, **5a**, **4a**, and **1a**−**e** and for natural **1c** (44 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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