Probing Host-Selective Phytotoxicity: Synthesis of Destruxin B and Several Natural Analogues

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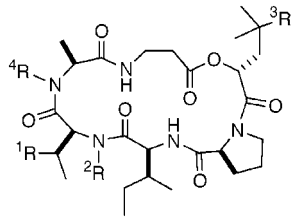
The syntheses of the host-selective phytotoxin destruxin B [cyclo(βAla-Hmp-Pro-Ile-MeVal-MeAla), Hmp = (2*R*)-2-hydroxy-4-methylpentanoic acid], and the closely related natural analogues homodestruxin B (MeVal→MeIle), desmethyldestruxin B (MeVal→Val), hydroxydestruxin B (Hmp→Dhmp, Dhmp = (2*R*)-2,4-dihydroxy-4-methylpentanoic acid), and hydroxyhomodestruxin B (MeVal→MeIle, Hmp→Dhmp) are described. In each case, the MeAla-βAla linkage was formed by cyclization and the precursor linear hexadepsipeptides were formed by condensing two three-residue fragments. Radiolabeled samples of destruxin B, homodestruxin B, and hydroxydestruxin B were prepared by coupling [3-^{14}C]-β-alanine to the appropriate pentadepsipeptide followed by cyclization. A noteworthy feature of the synthesis involves the novel use of a Boc-hydrazide protecting group on dipeptides with a C-terminal *N*-methylalanine residue to inhibit the otherwise facile dioxopiperazine formation during peptide coupling.

Introduction

Although a number of economically important plant diseases appear to be mediated by host-selective toxins, their role in disease remains unknown in the majority of the examples. Host-selective toxins are secondary metabolites produced by plant pathogens, which facilitate colonization of host plants by the producing microorganism, but do not significantly affect nonhost plants. The large array of chemical structures of host-selective toxins would suggest multiple and diverse mechanisms of action; however, one of the major obstacles to investigation of such mechanisms is the availability of these metabolites, which are typically produced in very small amounts. For example, destruxin B (**1**)[1] is a host-selective toxin produced both in vitro[2] and in planta[3,4] by the fungal pathogen *Alternaria brassicae* (Berk.) Sacc., the causative agent of Alternaria blackspot of brassicas, but despite significant work, its production in culture has not been improved to an acceptable level.[5] In addition, related destruxins **2**[2] and **3**[2,6] produced by *A. brassicae* have not been properly evaluated as they are produced in even smaller amounts. Because Alternaria blackspot is the most destructive fungal disease of the economically important oilseeds rapeseed (*Brassica napus* and *B. rapa*) and canola (*B. napus* and *B. rapa*),[7] it was of much interest to probe the host-selective phytotoxicity of the destruxins **1**−**3**. As part of a research program aimed at understanding disease resistance mechanisms, we wished to develop a convergent and efficient synthesis of the host selective phytotoxin destruxin B (**1**) that would be versatile enough to provide access to analogues, radiolabeled congeners, and possible biotransformation products. Herein we report practical and efficient syntheses of **1**−**5**, including ^{14}C-labeled samples of **1**, **2**, and **4**.



1 ^{1}R=Me, ^{2}R=Me, ^{3}R=H, ^{4}R=Me
2 ^{1}R=Et, ^{2}R=Me, ^{3}R=H, ^{4}R=Me
3 ^{1}R=Me, ^{2}R=H, ^{3}R=H, ^{4}R=Me
4 ^{1}R=Me, ^{2}R=Me, ^{3}R=OH, ^{4}R=Me
5 ^{1}R=Et, ^{2}R=Me, ^{3}R=OH, ^{4}R=Me
6 ^{1}R=Me, ^{2}R=H, ^{3}R=H, ^{4}R=H

Destruxin B (**1**) is a cyclic hexadepsipeptide composed of one α-hydroxy acid [(2*R*)-2-hydroxy-4-methylpentanoic acid (Hmp)], one β-amino acid [3-aminopropanoic acid (βAla)], and four α-amino acid residues [L-proline (Pro), L-isoleucine (Ile), *N*-methyl-L-valine (MeVal), and *N*-methyl-L-alanine (MeAla)]. The structures of the two other host-selective phytotoxins of interest are closely related with only the MeVal residue being modified (homodestruxin B (**2**), MeVal→*N*-methyl-L-isoleucine (MeIle); desmethyldestruxin B (**3**), MeVal→valine (Val)). The typical synthetic strategy utilized for preparation of cyclic depsipeptides (and peptides) involves linear or convergent coupling of intact hydroxy acid and amino acid fragments followed by cyclization.[8] This approach focuses the key strategic decisions to the site of cyclization and

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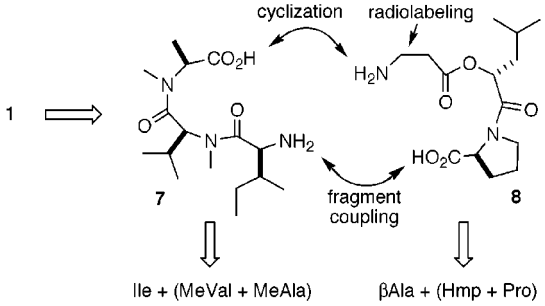
the order of the residue coupling. The choice of cyclization site can be crucial for successful synthesis of a cyclic (depsi)peptide;[8c,9,10] additionally, it has been established that *N*-methylamino acid residues are difficult to incorporate by standard peptide-coupling methods.[10]

Syntheses of compounds **2**−**5** have not been reported previously. A previous synthesis of **1**, reported by Kuyama and Tamura in 1965,[11] involved preparation of the protected hexadepsipeptide AcO-Hmp-Pro-Ile-MeVal-MeAla-*β*Ala-OMe by linear coupling of the six residues starting with *β*Ala-OMe using Cbz-protected amino acid residues with DCC-mediated coupling (16% overall yield). The yield for lactonization of this hexadepsipeptide (i, NaOH; ii, DCC; iii, pyridine) was not reported but is likely to be very low judging from similar examples.[12] Thus, Lee and Izumiya failed to obtain protodestruxin (**6**) by lactonization of the corresponding linear hexadepsipeptide at the *β*Ala-Hmp site using this method; by contrast, cyclizations at both the Hmp-Pro (26%) and Pro-Ile (48%) sites were successful using the *N*-hydroxysuccinimide-activated ester method.[12b] Following this lead, Cavelier et al. successfully prepared a variety of destruxin analogues (i.e., the Hmp residue in **1** was replaced by various α-hydroxy and amino acids, Xxx) based on cyclization of the corresponding hexa(depsi)peptides at the Pro-Ile site.[13] The linear hexa(depsi)peptides were prepared by coupling P-Ile-MeVal-MeAla-*β*Ala with the requisite Xxx-Pro-P′ fragment (P/P′ = Cbz/BnO or Boc/*t*-BuO), and cyclizations (after deprotection) were very efficient (63−78%) under optimized conditions using diphenylphosphoryl azide (DPPA) or benzotriazol-1-yloxytri(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent.

Results and Discussion

At the outset of our work,[14] the published syntheses of **1**[11] and close analogues[12,13] were deemed unsatisfactory for our objectives. Our retrosynthetic analysis of destruxin B (**1**) is presented in Scheme 1. Various guidelines have evolved for selection of the cyclization site for cyclo(depsi)peptide synthesis.[8c,9,10a] Lactamization is more facile than lactonization, and of the five amide bonds in **1**, we selected the MeAla-*β*Ala linkage as the most favorable site for cyclization on the basis of minimal steric hindrance.[8a] The Pro-Ile bond in the requisite linear hexadepsipeptide precursor for cyclization presents a logical disconnection for fragment coupling because C-terminal proline residues in oligopeptide chains are

Scheme 1



resistant to isomerization during chain extension.[8a] This synthetic strategy of coupling the three-residue fragments **7** and **8** not only provides for maximum convergence but, by localizing the difficult *N*-methyl amide bond(s) to a single tripeptide fragment **7**, also should maximize the overall yield in the longest linear sequence. Should cyclization at the MeAla-*β*Ala site prove to be ineffective, an additional advantage of this design is that simply interchanging the sites designated for fragment coupling and cyclization would allow for cyclization at the "proven"[12,13] Pro-Ile site. Finally, we considered the preparation of radiolabeled analogues (vide infra) by incorporation of a radiolabeled residue as late as possible in the synthesis. In the present context, this implies introduction of the labeled N-terminal residue of the linear hexadepsipeptide in the last coupling step prior to cyclization. Cyclization at the MeAla-*β*Ala site would be particularly well suited to this requirement because the precursor hexadepsipeptide would be prepared by coupling *β*Ala and a pentadepsipeptide with formation of the relatively simple *β*Ala-Hmp ester linkage.

A potential disadvantage of the above strategy concerns the synthesis of the fragment **7** by condensation of an N-protected isoleucine (e.g., **14**) with a C-protected MeVal-MeAla dipeptide (e.g., **17**). This is a particularly difficult coupling step because both amino acid residues are hindered, the amine is secondary, and dipeptides with C-terminal *N*-methylamino acids (e.g., MeVal-MeAla) are prone to cyclize to dioxopiperazines (Scheme 2).[10] For example, Tamura et al.[15] found that attempted preparation of **16** from its hydrochloride by treatment with Et_3N instead gave **13a**. We attempted to prepare **17** by deprotection of the corresponding trichloroethylcarbamate (Zn, HOAc); however, ^{1}H NMR ($CDCl_3$) of the product obtained immediately after work up indicated the presence of only a small amount (20−35%) of **17** with the major component being **13a**. Further monitoring of this mixture by ^{1}H NMR suggested that the half-life for the conversion of **17** to **13a** in $CDCl_3$ solution was ca. 15−30 min. In an effort to attenuate the propensity of **17** to cyclize, we considered alternatives to the benzyl ester protecting group. The increased stability of the *tert*-butyl esters in this context has been noted,[16] and several successful acylations of dipeptide *tert*-butyl esters containing a C-terminal *N*-methylamino acid have been reported;[16−19] a competing cyclization to dioxopiperazines was noted in only a few cases.[18] By contrast, examples

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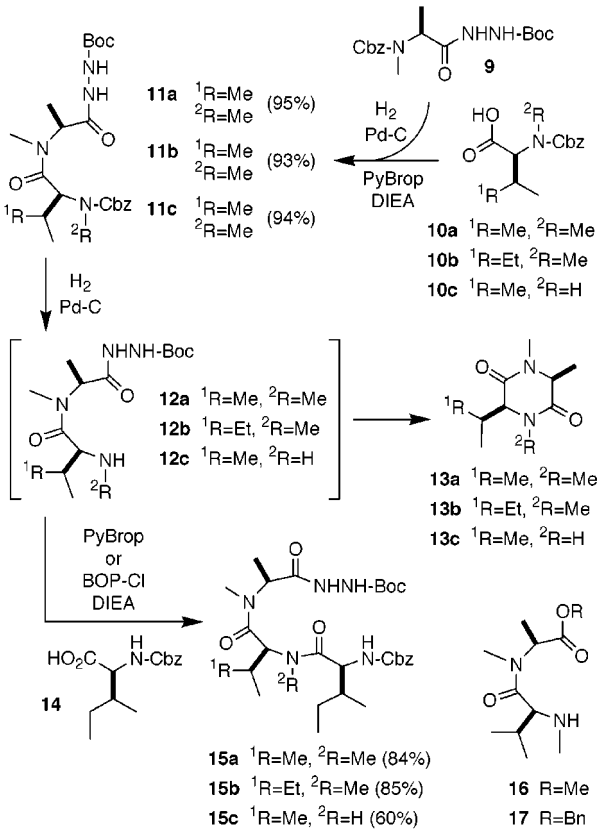
Scheme 2



of N-terminal chain extensions on other dipeptide esters with C-terminal *N*-methylamino acids are rare.[16a,19] Reasoning that a hydrazide protecting group not only should slow the rate of dioxopiperazine formation[20] but also could serve as a latent activating group for the eventual cyclization of the hexadepsipeptide (i.e., via the azide method),[8a] we chose to examine the stability of **12a**.[21]

The dipeptide **11a** was obtained from **9** and **10a** under a variety of conditions (Scheme 2, Table 1).[22] As expected for this type of coupling, the use of DCC with 1-hydroxybenzotriazole (HOBt) was ineffectual and "specialized" coupling reagents such as bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[23] or bromotri(pyrrolidino)phosphonium hexafluorophosphate (PyBrop)[24] were required to obtain good yields of **11a** with negligible isomerization. Hydrogenolysis of **11a** gave **12a**, and in contrast to **17**, cyclization of **12a** to **13a** was slow in $CDCl_3$ solution ($t_{1/2} \approx 16$ h) and even slower in the presence of Et_3N ($t_{1/2} >$ 24 h). The stability of **12a** boded well for successful coupling with **14**, and we examined several reagents and conditions for this reaction (Table 1). The use of PyBrop or BOP-Cl gave acceptable yields of **15a**; yields were markedly improved in the presence of excess *i*-Pr_2EtN (albeit with a concomitant increase in isomerization) presumably due to the increased stability of **12a** under these conditions.

Table 1. Efficacy of Various Coupling Reagents for the Synthesis of 11a and 15a

reactants	reagent	additive	stoichiometry[a]	time (h)[b]	% yield[c] (% isomerization)[d]
9[e] + **10a**[f]	BOP-Cl	*i*-Pr_2EtN	1:1.1:1.2:3	18	87 (<0.5)
	PyBrop	*i*-Pr_2EtN	1:1.1:1.2:3	18	95 (<0.5)
	DCC	HOBt	1:1.1:1.2:1.1	48	55 (<0.5)
	EDC[g]	HOBt	1:1.1:1.2:1.1	72	37 (<0.5)
	DCC	DMAP	1:1.1:1.2:0.1	24	72 (37)
11a[e] + **14**[h]	BOP-Cl	*i*-Pr_2EtN	1:1.1:1.2:3	14	60 (0.7)
			1:1.1:1.2:4	14	75 (1.5)
			1:1.1:1.2:5	14	77 (nd)
			1:2:2:8	14	84 (2.3)
	PyBrop	*i*-Pr_2EtN	1:1.1:1.2:3	14	60 (0.7)
			1:1.5:1.5:4	14	73 (nd)
	DCC	HOBt	1:1.1:1.2:1.1	96	20 (<0.5)
	DCC	DMAP	1:1.1:1.2:0.1	36	21 (23)

[a] Stoichiometry of **9** (or **11a**): **10a** (or **14**): reagent: additive. [b] Reaction in CH_2Cl_2 at 0 °C for 30 min and then ambient temperature for the cited time. [c] Isolated yield. [d] Determined by HPLC: reversed-phase column (5 mm ODS, 4.6 × 200 mm) eluting with 75−100% CH_3CN/H_2O (linear gradient for 35 min) at a flow rate of 1 mL/min. The minor component from the DCC/DMAP coupling was assumed to be the isomerized product. [e] Compound was subjected to hydrogenolysis (to remove the Cbz group) prior to coupling. [f] Product is **11a**. [g] 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide. [h] Product is **15a**.

The required tridepsipeptide fragment **21**[12c] was readily prepared in 90% yield by routine DCC-mediated condensations of **18**, **19**, and **20** as shown in Scheme 3. Efficient coupling of fragments **15a** and **21** (after hydrogenolysis) to give the hexadepsipeptide **23a** resulted with a variety of reagents (DCC/HOBt, PyBrop, or DPPA) (Scheme 4). Cyclization of **23a** was achieved in excellent yield in a three-step one-pot procedure.[8a,25] Thus, the Boc protecting groups in **23a** were removed by reaction with TFA and the resulting amino hydrazide was treated with nitrous acid to produce the acyl azide that, after neutralization to free the amine, cyclized to give destruxin B (**1**; 65% yield) identical in all respects to an authentic sample (Scheme 4).

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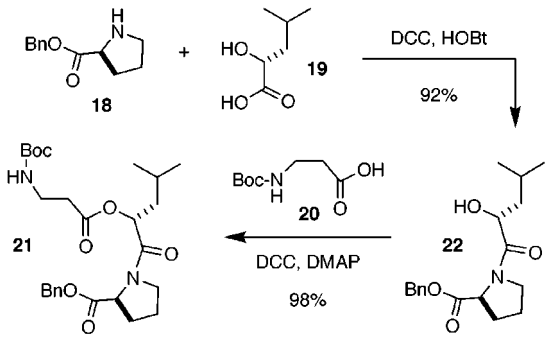
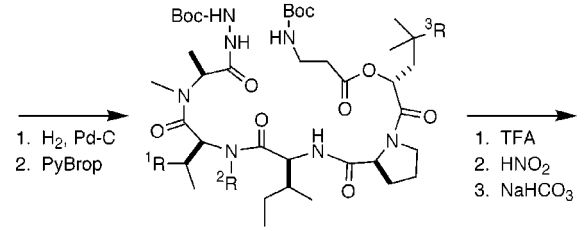
Scheme 3



Scheme 4

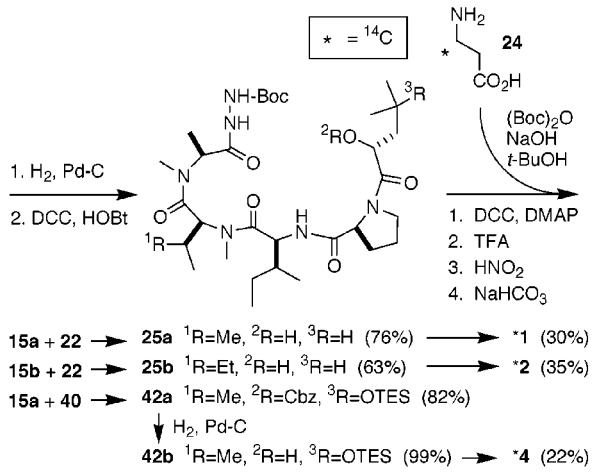


15a + 21 → **23a** ^{1}R=Me, ^{2}R=Me, ^{3}R=H (94%) → **1** (65%)
15b + 21 → **23b** ^{1}R=Et, ^{2}R=Me, ^{3}R=H (92%) → **2** (58%)
15c + 21 → **23c** ^{1}R=Me, ^{2}R=H, ^{3}R=H (85%) → **3** (60%)
15a + 37 → **41a** ^{1}R=Me, ^{2}R=Me, ^{3}R=OTES (80%) → **4** (52%)
15b + 37 → **41b** ^{1}R=Et, ^{2}R=Me, ^{3}R=OTES (80%) → **5** (47%)

The syntheses of homodestruxin B (**2**) and desmethyldestruxin B (**3**) were accomplished with the above strategy, but Cbz-MeIle (**10b**) and Cbz-Val (**10c**), respectively, were incorporated in place of Cbz-MeVal (**10a**) (Scheme 2). The stability of dipeptide **12b** was similar to that of **12a** ($t_{1/2} \approx 14$ h for cyclization to **13b** in $CDCl_3$ solution), and its condensation with **14** to give **15b** proceeded analogously. However, the propensity for cyclization of dipeptide **12c** ($t_{1/2} = 3.5$ h for cyclization to **13c** in CD_3OD solution) was much higher than that for **12a** or **12b**, and this decreased stability was reflected in a lower yield of tripeptide **15c** under otherwise identical coupling conditions. Coupling **21** with the tripeptides **15b** and **15c**, respectively, followed by cyclization gave homodestruxin B (**2**) and desmethyldestruxin B (**3**) with overall yields similar to that for destruxin B (**1**) (Scheme 4).

As a prelude to an investigation of the metabolism of the host-selective toxins by plants resistant and susceptible to Alternaria blackspot, we required radiolabeled samples of destruxin B (**1**) and homodestruxin B (**2**).[26,27] To maximize the flexibility of this investigation, we chose to incorporate ^{14}C into the peptide backbone of the phytotoxins, and to minimize handling of radioactive intermediates, we wished to introduce the radiolabel as late as possible in the synthesis. These objectives were anticipated in our synthesis design (Scheme 1), which provides for the introduction of a commercially available ^{14}C-labeled amino acid residue (i.e., βAla) in the penultimate step. Thus, hydrogenolysis and coupling of **15a** with **22** gave the pentadepsipeptide **25a** in 76% yield[28] and condensation of **25a** with Boc-βAla (**20**) gave the

Scheme 5



15a + 22 → **25a** ^{1}R=Me, ^{2}R=H, ^{3}R=H (76%) → ***1** (30%)
15b + 22 → **25b** ^{1}R=Et, ^{2}R=H, ^{3}R=H (63%) → ***2** (35%)
15a + 40 → **42a** ^{1}R=Me, ^{2}R=Cbz, ^{3}R=OTES (82%)
↓ H_2, Pd-C
42b ^{1}R=Me, ^{2}R=H, ^{3}R=OTES (99%) → ***4** (22%)

hexadepsipeptide **23a** in 88% yield (Scheme 5). For radiolabeling purposes, we wished to keep the scale of the synthesis as small as possible (to maximize the specific activity of the product) while still producing sufficient product to allow isolation and purification by traditional methods. Cognizant of the form of radiolabeled β-alanine available to us,[29] we developed a procedure without purification of the intermediates whereby β-alanine (**24**) was converted into its Boc derivative and coupled with **25a** and the resulting hexadepsipeptide subjected to the three-step one-pot cyclization protocol. The procedure using 5−10 mg of β-alanine was optimized and gave 30−45% overall yields of **1** in several "practice" runs. In the event, radiolabeled **1** was obtained in 30% yield; use of the same approach gave radiolabeled **2** in 35% yield (Scheme 5).

Investigation of the metabolism of radiolabeled destruxin B (***1**) and homodestruxin B (***2**) by plant tissues, as previously described,[26,27] established that hydroxylation of the Hmp residue (to give **4** and **5**, respectively) was the major pathway. Significantly, biotransformations of **1** into **4** and of **2** into **5** were much faster with leaves of plants resistant to *A. brassicae* (*S. alba* cultivar Ochre) than with leaves of susceptible plants (*B. napus* cultivar Westar and *B. juncea* cultivar Cutlass).

The syntheses of **4** and **5** were undertaken to corroborate the proposed structures and provide material to determine their phytotoxicities to plants resistant and susceptible to *A. brassicae*. Thus, we required (2*R*)-2,4-dihydroxy-4-methylpentanoic acid (Dhmp) to replace the Hmp residue in **1** and **2**. To the best of our knowledge, the preparation of Dhmp has not been described. Mori and Takigawa[30] obtained an ammonium salt of Dhmp in very low yield by resolution of the corresponding carboxylate (obtained by treatment of racemic **27** with NaOH). This salt readily lactonized to **27** upon acidification, and the lactone was highly prone to racemization suggesting that protection of the tertiary hydroxy group in Dhmp would be necessary for our purposes. Our

(26) Preliminary communication: Pedras, M. S. C.; Zaharia, I. L.; Gai, Y.; Smith, K. C.; Ward, D. E. *Org. Lett.* **1999**, *1*, 1655−1658.

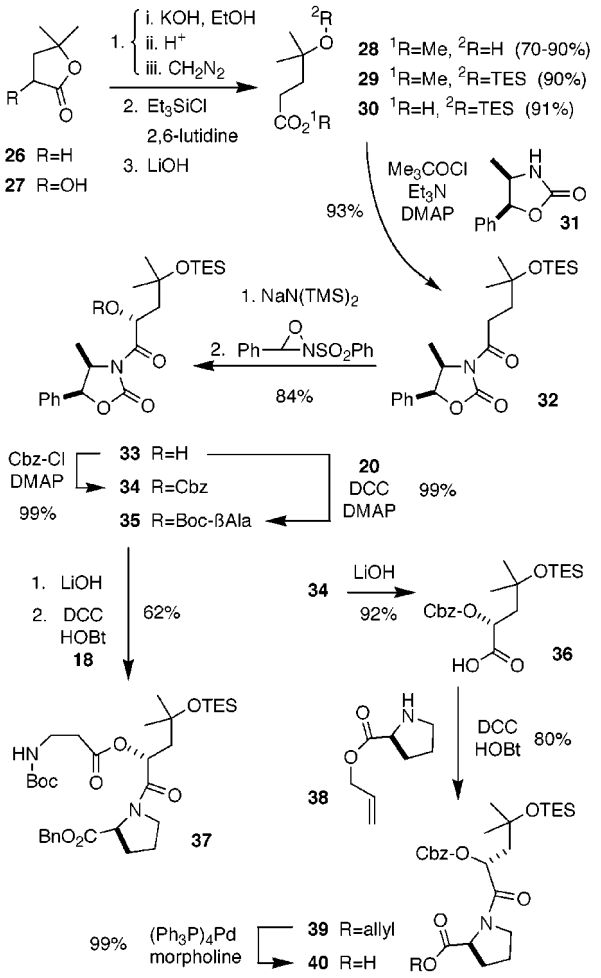
(27) Pedras, M. S. C.; Zaharia, I. L.; Gai, Y.; Zhou, Y.; Ward, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 747−752.

(28) The modest yield is due to competing cyclization of the activated Hmp-Pro fragment. This reaction was not optimized.

(29) [3-^{14}C]-β-alanine (0.250 mCi, 55.5×10^7 dpm, 46.1 mCi/mmol, 0.48 mg, 5.4 μmol) in 2% aqueous EtOH (2.5 mL, sealed ampule) was purchased from Sigma Chemical Company. In each case, this material was diluted with "cold" β-alanine (6 mg). Specific activities: destruxin B (***1**), 1.28×10^7 dpm/mg; homodestruxin B (***2**), 7.94×10^6 dpm/mg; hydroxydestruxin B (***4**), 1.07×10^7 dpm/mg.

(30) Mori, K.; Takigawa, T.; Matsuo, T. *Tetrahedron* **1979**, *35*, 933−940.

Scheme 6



enantioselective synthesis of the doubly protected Dhmp derivative **36** is shown in Scheme 6. Saponification of the readily available lactone **26**[31] followed by careful acidification gave the unstable[32] hydroxy acid, which was immediately treated with diazomethane to give the methyl ester **28** in 70−90% overall yield. The hydroxy group in **28** was protected as its triethylsilyl (TES) ether, and hydrolysis of the resulting ester **29** with LiOH gave the acid **30**. Enantioselective introduction of a (2*R*)-hydroxyl group on **30** was achieved using the method of Evans et al.[33] Thus, acylation of the commercially available oxazolidinone **31** with **30** using the mixed anhydride method[34] gave **32** in excellent yield. Reaction of the sodium enolate of **32** with 2-phenylsulfonyl-3-phenyloxaziridine[35] gave **33** in 84% yield with excellent diastereoselectivity.[36] Despite considerable experimentation, all attempts to remove the oxazolidinone auxiliary in **33** without prior protection of the hydroxy group resulted in extensive concomitant γ-lactonization to give **27**. By contrast, the desired **36** was readily obtained by hydrolysis of the benzyl carbonate **34**. The Boc-βAla ester **35** was also effective in this regard, and hydrolysis of **35** followed by condensation of the resulting acid with **18** gave the desired tridepsipeptide fragment **37** in 62% overall yield. Use of the fragment **37** in place of **21** allowed the synthesis of hydroxydestruxin B (**4**) and hydroxyhomodestruxin B (**5**) as described above for **1** and **2** (Scheme 4). Thus, hexadepsipeptides **41a** and **41b** were prepared by deprotection and coupling of **37** with tripeptides **15a** and **15b**, respectively. Cyclization of **41a** and **41b** gave **4** and **5**, respectively, which were identical (^{1}H and ^{13}C NMR, HRMS, IR, $[\alpha]_D$, and HPLC) to the isolated metabolites.

To facilitate an investigation of the metabolism of **4** by plants resistant and susceptible to Alternaria blackspot, we also prepared a ^{14}C-radiolabeled sample using the (3 + 2) + 1 coupling strategy described above.[27] The required didepsipeptide fragment **40** was obtained by condensation of **36** with proline allyl ester (**38**) followed by Pd-catalyzed deprotection of the allyl ester (Scheme 6). Coupling **15a** with **40** gave the pentadepsipeptide **42b** after hydrogenolysis of the benzyl carbonate (Scheme 5). Without purification of the intermediates, **42b** was subjected to coupling with radiolabeled Boc-βAla and the resulting hexadepsipeptide cyclized to give radiolabeled hydroxydestruxin B (***4**) (Scheme 5).

In summary, the synthesis of destruxin B (**1**) proceeds in six operations from readily available starting materials (i.e., **9**, **10a**, **14**, and **18**−**20**) using a highly convergent strategy. The average yield per operation is >88%, and the overall yields for the longest linear sequences (four steps) are 55% (from **18**) and 49% (from **9**). This route compares very favorably with other reported syntheses[11,12,13] of this type of cyclic depsipeptide in terms of the degree of convergence, the efficiency of the cyclization, and the overall yield. A noteworthy feature of the synthesis is the novel use of a Boc-hydrazide protecting group on an *N*-methylamino acid dipeptide to inhibit the otherwise facile dioxopiperazine formation during peptide coupling. Efficient syntheses of the natural analogues **2**−**5** were achieved using the same convergent strategy by substituting the alternate residue(s) as needed into the appropriate fragment(s). Importantly, the synthetic approach was easily modified to allow for preparation of ^{14}C-radiolabeled compounds ***1**, ***2**, and ***4** by incorporating [3-^{14}C]-β-alanine immediately prior to cyclization.

Ready access to compounds **1**−**5** has been crucial for probing their host-selective phytotoxicity and investigation of the chemical basis of plant disease resistance. Bioassays of **1**−**5** utilizing staining of plant cell suspension cultures of *S. alba* (resistant), *B. juncea* (susceptible), and *B. napus* (susceptible), in planta leaf assays, and leaf uptake of toxin solutions indicated that **2** was the most toxic (EC_{50} 3×10^{-4} M) followed by **1** (EC_{50} 5×10^{-4} M) and **3** ($EC_{50} \gg 5 \times 10^{-4}$ M); metabolites **4** and **5** showed much lower phytotoxicities ($EC_{50} \gg \times 10^{-3}$ M) establishing that hydroxylation is a detoxification step.[37] The rate of hydroxylation of **1** and **2** in cruciferous plants correlated with the resistance of each species to *A. brassica*.[26,27] Interestingly, hydroxydestruxin B (**4**) elicited the biosynthesis of phytoalexins in the resistant plant species (*S. alba*), whereas no phytoalexins were detected in the susceptible species (*B. napus* and *B. juncea*). Although it is well-known that phytoalexins can be biosynthesized

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(33) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346−4348.

(34) Ager, D. J.; Allen, D. R.; Schaad, D. R. *Synthesis* **1996**, 1283−1285.

(35) Vishwakarma, L. C.; Stringer, O. D.; Davies, F. A. *Org. Synth.* **1987**, *66*, 203−210.

(36) The presence of other diastereomers could not be detected by ^{1}H NMR of the crude product.

(37) Pedras, M. S. C.; Biesenthal, C. J.; Zaharia, I. L. *Plant Sci.* **2000**, *156*, 185−192.

in response to a variety of abiotic elicitors,[38] it is remarkable that the detoxification product of **1** (i.e., **4**) could also induce phytoalexin biosynthesis in the resistant species. These results suggest that the blackspot resistance in *S. alba* due to detoxification of destruxin B may be amplified by well-synchronized phytoalexin elicitation.[27]

Experimental Section

General Methods. All solvents were distilled prior to use. Unless otherwise noted, reactions were carried out under an atmosphere of argon and reaction temperatures refer to that of the bath. Concentration refers to removal of volatiles at the water aspirator pressure on a rotary evaporator. Flash column chromatography (FCC) and dry flash column chromatography (DFC) were performed according to the procedures of Still et al.[39] and Harwood,[40] respectively. All mixed solvent eluents are reported as v/v solutions. Unless otherwise noted, NMR spectra were measured in $CDCl_3$ solution at 300 MHz for ^1H and 75 MHz for ^{13}C. Coupling constants (J) are reported to the nearest 0.5 Hz. Optical rotations ($[\alpha]_D$) were determined at ambient temperature using a 1 mL, 10 dm cell; the units are 10^{-1} deg cm^2 g^{-1}, and the concentrations (c) are reported in g/100 mL.

Materials. Cbz-Ala, Cbz-Ile, and Cbz-Val, and compounds **10c**, **14**, **18**, **20**, and **38** were prepared from the corresponding amino acids according to standard procedures.[41] Compounds **9**,[42] **10a**,[42] **10b**,[42] **19**,[43] **26**,[31] and 2-phenylsulfonyl-3-phenyloxaziridine[35] were prepared by published methods. The syntheses of ***4***, **38**−**40**, and **42** have been described.[27] All other reagents were commercially available and, unless otherwise noted, were used as received.

General Method for Hydrogenolysis. A suspension of the protected substrate and 10% Pd on carbon (100 mg/mmol of substrate) in CH_3OH (18 mL/mmol of substrate) was vigorously stirred under an H_2 atmosphere (balloon). After 2 h (reaction complete by TLC), the mixture was filtered with the aid of Celite and the combined filtrate and washings were concentrated. Residual CH_3OH was removed by dissolving the residue in CH_2Cl_2 followed by concentration and drying under high vacuum (0.2 Torr). The resulting product (yield is essentially quantitative) was used without further purification.

Destruxin B (1). The hexadepsipeptide **23a** (250 mg, 0.30 mmol) was dissolved in TFA/CH_2Cl_2 (1:1 v/v, 4 mL), and after being stirred for 30 min at room temperature, the solution was concentrated and reconcentrated from CH_2Cl_2. Trituration of the residue with ether gave a white solid that was dissolved in water (5 mL) and aqueous HCl (1.2 M, 2 mL). The stirred solution was cooled to <5 °C, and a solution of $NaNO_2$ (30 mg, 0.43 mmol) in water (1 mL) was added dropwise. After 15 min, ice-cold CH_2Cl_2 (120 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added and the resulting biphasic mixture was vigorously stirred at room temperature. After 15 h, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (four times). The combined organic layers were dried over Na_2SO_4, concentrated, and fractionated by DFC (15−40% acetone in hexane) to give destruxin B as a white solid (115 mg, 65%): mp 234−235 °C (acetone/hexane) (lit.[2,11,44] 234, 225−227 °C); $[\alpha]_D$ −240 (*c* 0.5, CH_3OH) (lit.[2,11,44] −228, *c* 0.50, CH_3OH; −237, *c* 0.08, CH_3OH; −241, *c* 0.5, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 8.20 (1H, d, J = 8.5 Hz, HN), 7.19 (1H, d, J = 9 Hz, HN), 5.19 (1H, q, J = 7 Hz), 4.95 (1H, d, J = 11 Hz), 4.92−4.80 (2H, m), 4.68 (1H, d, J = 7 Hz), 4.05−3.97 (1H, m), 3.92 (1H, dd, J = 8, 8.5 Hz), 3.43 (1H, ddd, J = 8, 9, 9.5 Hz), 3.23 (3H, s), 3.09 (1H, dd, J = 12, 13.5 Hz), 2.73 (3H, s), 2.70−2.45 (3H, m), 2.35−2.28 (1H, m), 2.13−1.72 (7H, m), 1.46−1.20 (2H, m), 1.31 (3H, d, J = 6.5 Hz), 0.95 (3H, d, J = 6.5 Hz), 0.90 (3H, d, J = 7 Hz), 0.89 (3H, d, J = 7 Hz), 0.85 (3H, d, J = 6.5 Hz), 0.82 (3H, d, J = 7 Hz), 0.81 (3H, t, J = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 173.5, 175.1, 170.9, 169.7 (×2), 71.9, 60.7, 58.1, 55.4, 53.6, 46.4, 38.9, 37.5, 34.4, 33.2, 30.8, 28.8, 28.0, 27.2, 24.4, 24.4, 24.1, 23.3, 21.4, 20.0, 19.6, 15.4, 15.2, 11.3; HRMS *m/z* calcd for $C_{30}H_{51}N_5O_7$ 593.3788, found 593.3788 (EI). Anal. Calcd for $C_{30}H_{51}N_5O_7$: C, 60.67; H, 8.66; N, 11.79. Found: C, 60.62; H, 8.59; N, 11.53.

[3-^{14}C]-*β*Ala Destruxin B (*1). A solution of [3-^{14}C]-*β*-alanine (0.250 mCi, 46.1 mCi/mmol, 0.48 mg, 5.4 μmol) in 2% aqueous EtOH (2.5 mL) was added to a stirred solution of *β*-alanine (**24**, 6 mg, 0.07 mmol) in *t*-BuOH (1 mL). The solution was cooled to 0 °C (ice bath), and NaOH (1 M, 0.074 mL, 0.074 mmol) and a solution of $(Boc)_2O$ (15 mg, 0.081 mmol) in *t*-BuOH (1.5 mL) were added. After being stirred for 22 h at room temperature, the reaction mixture was concentrated to ca. 0.5 mL and diluted with aqueous $KHSO_4$ (0.2 M, 1 mL). The mixture was saturated with NaCl and extracted with ethyl acetate (1 mL × 6). The combined organic layers were dried over Na_2SO_4, concentrated, and dried under high vacuum for 30 min to give ***20*** (9.6 mg) as a white solid. DCC (14 mg, 0.068 mmol) and DMAP (0.6 mg, 0.005 mmol) were added to a stirred solution of ***20*** (9.6 mg) and **25a** (32 mg, 0.049 mmol) in CH_2Cl_2 (0.45 mL) at 0 °C under argon. After 1 h at 0 °C and 21 h at room temperature, the mixture was filtered through Celite and the combined filtrate and washings were concentrated and filtered through a plug of SiO_2 eluting first with 50% ethyl acetate in hexane to remove DCU and then with 80% ethyl acetate in hexane to obtain ***23a*** (36 mg). TFA (0.35 mL) was added to a solution of the ***23a*** (36 mg) in CH_2Cl_2 (0.35 mL), and after 20 min, the mixture was concentrated to dryness. Water (1 mL) was added to the residue, and after the solution was cooled to between 0 and −5 °C (ice−salt bath), HCl (1 M, 0.1 mL, 0.1 mmol) and $NaNO_2$ (6.3 mg, 0.092 mmol) were added sequentially to the stirred solution. After 20 min, ice-cold saturated aqueous $NaHCO_3$ (1 mL) and ice-cold CH_2Cl_2 (7.5 mL) were added and the biphasic mixture was stirred vigorously at ambient temperature for 20 h. The aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (three times), and the combined organic layers were dried over Na_2SO_4, concentrated, and fractionated by FCC (60% acetone in hexane) to give [3-^{14}C]-*β*Ala destruxin B (***1***, 13 mg, 0.075 mCi, specific activity = 1.28 × 10^7 dpm/mg; 30% yield based on **24**).

Cbz-MeAla-NHNH-Boc (9). A solution of DCC (2.680 g, 13 mmol) in CH_2Cl_2 (3 mL) was added in three portions to a stirred mixture of Cbz-*N*-methylalanine (2.844 g, 12 mmol), H_2NNH-Boc (1.585 g, 12 mmol), and HOBt hydrate (1.85 g, 12 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 1 h at 0 °C, the mixture was diluted with ethyl acetate and the precipitated DCU was filtered off. The combined filtrate and washings were washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and fractionated by DFC (10−50% ethyl acetate in hexane) to give **9** as a white hygroscopic solid (3.750 g, 89%): mp 46−48 °C; $[\alpha]_D$ −51 (*c* 2.0, CH_3OH); ^1H NMR δ 7.99 (1H, br s, HN), 7.48−7.25 (5H, m), 6.45 (1H, br s, HN), 5.25−5.05 (2H, m), 4.92−4.88 (1H, m), 2.92 (3H, s), 1.45 (9H, s), 1.39 (3H, d, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 157.0, 155.2, 136.2, 128.5, 128.1, 127.9, 81.6, 67.8, 52.7, 29.0, 28.1, 13.6; HRMS *m/z* calcd for $C_{17}H_{25}N_3O_5$ 352.1872 (M + H), found 352.1880 (FAB). Anal. Calcd for $C_{17}H_{25}N_3O_5$: C, 58.12; H, 7.17; N, 11.96. Found: C, 58.30; H, 7.29; N, 11.89.

Cbz-MeVal-MeAla-NHNH-Boc (11a). Cbz-MeAla-NHNH-Boc (**9**, 400 mg, 1.14 mmol) was deprotected by hydrogenolysis according to general procedure. PyBroP (600 mg, 1.28 mmol) was added portionwise to a stirred solution of the crude hydrogenolysis product, **10a** (300 mg, 1.12 mmol), and DIEA (0.58 mL, 0.43 g, 3.3 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C and then for 18 h at

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room temperature. The mixture was diluted with CH_2Cl_2, washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and fractionated by FCC (45% ethyl acetate in hexane) to give **11a** as a white solid (494 mg, 95%). A similar reaction on a scale 4-times larger than that described gave **11a** in 90% yield: mp 53−55 °C; $[\alpha]_D$ −130 (*c* 2.0, CH_3OH); ^1H NMR δ (a 2.5:1.5:1 mixture of three conformers) 8.92 (d, J = 2.5 Hz), 7.97 (d, J = 2.5 Hz) and 7.95 (d, J = 2.5 Hz) (1H, HN), 7.45−7.25 (5H, m), 5.28−5.00 (3H, m), 4.74 (d, J = 10.5 Hz), 4.66 (d, J = 10.5 Hz) and 4.50 (d, J = 10.5 Hz) (1H), 3.00 (s), 2.99 (s), 2.84 (s), 2.82 (s) and 2.77 (s) (6H), 2.42−2.24 (1H, m), 1.41 (9H, s), 1.37 (d, J = 7 Hz), 1.32 (d, J = 7 Hz) and 1.24 (d, J = 7 Hz) (3H), 1.02−0.85 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 170.5, 168.8, 158.2, 156.8, 154.9, 136.1, 136.5, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8, 127.6, 81.5, 81.4, 68.0, 67.8, 67.5, 60.8, 60.5, 59.3, 53.5, 50.7, 50.4, 30.8, 30.2, 29.6, 29.2, 28.4, 28.1, 27.8, 27.5, 27.4, 19.6, 19.0, 18.4, 18.3, 15.5, 13.3, 13.2; HRMS *m/z* calcd for $C_{23}H_{36}N_4O_6$ 465.2713 (M + H), found 465.2717 (FAB). Anal. Calcd for $C_{23}H_{36}N_4O_6$: C, 59.46; H, 7.81; N, 12.06. Found: C, 59.24; H, 7.60; N, 11.87.

(3*S*,6*S*)-1,4,6-Trimethyl-3-(1-methyethyl)-2,5-piperazinedione (13a). The crude product obtained from hydrogenolysis of **11a** (50 mg, 0.11 mmol) as described above was allowed to stand in $CDCl_3$ solution for 3 days. The mixture was concentrated and fractionated by FCC (60% acetone in hexane) to give **13a** (38 mg, 90%): mp 143−144 °C (lit.[15,45] 148, 140−142 °C); $[\alpha]_D$ +16 (*c* 1.0, CH_3OH) (lit.[15] +13.5, *c* 5, CH_3OH); ^1H NMR δ 3.93 (1H, q, J = 8 Hz), 3.75 (1H, d, J = 6.5 Hz), 3.01 (3H, s), 2.95 (3H, s), 2.29−2.12 (1H, m), 1.58 (3H, d, J = 6.5 Hz), 1.12 (3H, d, J = 7.5 Hz), 1.03 (3H, d, J = 7.5 Hz); ^{13}C NMR δ 167.1, 164.6, 68.2, 57.8, 34.5, 32.2, 31.8, 19.8, 18.8, 18.7; HRMS *m/z* calcd for $C_{10}H_{18}N_2O_2$ 198.1368, found 198.1369 (EI).

Cbz-Ile-MeVal-MeAla-NHNH-Boc (15a). Using BOP-Cl. A mixture of **11a** (330 mg, 0.71 mmol) and 10% Pd/C (200 mg) in methanol (10 mL) was vigorously stirrred at room temperature under H_2 (1 atm) for 0.5 h. The mixture was quickly filtered with the aid of Celite, and the combined filtrate and washings were concentrated and then dried under high vacuum (<0.5 Torr, 5 min). Without delay, the residue (**12a**) was dissolved in a cold mixture of CH_2CI_2 (1.5 mL) and DIEA (0.75 mL, 0.55 g, 4.3 mmol) and the solution was added to a stirred solution of **14** (370 mg, 1.4 mmol), DIEA (0.25 mL, 1.4 mmol), and BOP-Cl (356 mg, 1.4 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C. After 30 min at 0 °C and 14 h at room temperature, the mixture was diluted with CH_2Cl_2, washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and fractionated by FCC (45% ethyl acetate in hexane) to give the titled compound as a white solid (345 mg, 84%). **Using PyBrop.** Compound **11a** (464 mg, 1.0 mmol) was deprotected by hydrogenolysis as above, and without delay, an ice-cold solution of **14** (399 mg, 1.5 mmol) and DIEA (0.070 mL, 4.0 mmol) in CH_2Cl_2 (3.0 mL) was added to the above residue. PyBroP (699 mg, 1.5 mmol) was added, and the mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The mixture was diluted with CH_2Cl_2 and processed as above to give the titled compound as a white solid (421 mg, 73%): mp 79−81 °C; $[\alpha]_D$ −15 (*c* 2.0, CH_3OH); ^1H NMR δ (a 1.5:1 mixture of two conformers) 9.26 (br s), 7.90 (br s) and 6.36 (br s) (2H, br s, HN), 5.46 (d, J = 9 Hz) and 5.41 (d, J = 9.5 Hz) (1H, HN), 5.25−5.05 (4H, m), 4.55−4.45 (1H, m), 3.38 (s), 3.05 (s), 3.02 (s) and 2.82 (s) (6H, H_3CN × 2), 2.44−2.24 (2H, m), 1.75−1.65 (1H, m), 1.42 (s) and 1.41 (s) (9H, $(H_3C)_3C$), 1.36 (d, J = 7 Hz) and 1.28 (d, J = 7 Hz) (3H, H_3CC-3 Ala), 1.15−1.03 (1H, m), 0.95−0.75 (12H, m, H_3C × 4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 171.7, 170.5, 156.4, 154.9, 136.2, 123.5, 128.1, 127.9, 81.6, 66.9, 58.0, 57.7, 55.5, 55.3, 53.5, 50.5, 37.7, 37.6, 31.1, 30.8, 30.5, 28.1, 27.6, 27.0, 24.3, 24.1, 19.6, 19.4, 19.1, 18.1, 15.5, 15.2, 14.9, 13.1, 11.2, 10.9; HRMS *m/z* calcd for $C_{30}H_{51}N_5O_7$ 578.3554 (M + H), found 578.3544 (FAB). Anal. Calcd for $C_{30}H_{51}N_5O_7$: C, 60.29; H, 8.20; N, 12.12. Found: C, 60.17; H, 8.24; N, 11.95.

Boc-β-Ala-Hmp-Pro-OBn (21).[12c] DCC (566 mg, 2.75 mmol) was added to a stirred solution of Hmp-Pro-OBn (**22**) (798 mg, 2.5 mmol), Boc-β-alanine (**20**) (520 mg, 2.75 mmol), and DMAP (34 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 20 h and then diluted with ethyl acetate, and the precipitated DCU was filtered off. The combined filtrate and washings were washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and fractionated by FCC (35% ethyl acetate in hexane) to give the titled compound as an oil (1.20 g, 98%): $[\alpha]_D$ −33 (*c* 1.3, CH_3OH); ^1H NMR (500 MHz) δ (a 2:1* mixture of conformers) 7.40−7.20 (5H, m), 5.24 (dd, J = 3.5, 10 Hz), 4.90* (dd, J = 2, 8.5 Hz), 4.85* (dd, J = 2.5, 11 Hz) and 4.47 (dd, J = 3.5, 8.5 Hz) (2H), 5.16* (d, J = 12 Hz), 5.11 (ap s) and 5.10* (d, J = 12 Hz) (2H), 3.95−3.25 (4H, m), 2.55−2.45 (2H, m), 2.35−1.25 (8H, m), 1.39* and 1.38 (9H, 2 × s), 0.92, 0.90, 0.84* and 0.74* (6H, 4 × d, J = 6.5 Hz); ^{13}C NMR (125 MHz) δ 172.5*, 171.9, 171.7*, 171.3, 169.6*, 169.0, 156.1, 155.9*, 135.7, 135.0*, 128.7, 128.6*, 128.4 (×2), 128.3* (×2), 128.2* (×2), 128.1 (×2), 79.0, 78.9*, 70.9*, 70.6, 67.3*, 66.8, 59.4, 59.2*, 46.8*, 46.5, 39.4, 36.4, 36.3*, 34.7, 34.5*, 31.5*, 28.7, 28.4 (×6), 24.7, 24.5, 24.3*, 23.2*, 23.1, 22.2*, 21.6, 21.1; HRMS *m/z* calcd for $C_{26}H_{38}N_2O_7$ 491.2757 (M + H), found 491.2749 (FAB). Anal. Calcd for $C_{26}H_{38}N_2O_7$: C, 63.65; H, 7.80; N, 5.71. Found: C, 63.49; H, 7.94; N, 5.73.

Hmp-Pro-OBn (22). DCC (2.16 g, 10.5 mmol) was added to a stirred solution of Hmp (**19**) (0.95 g, 7.2 mmol), Pro-OBn (**18**) (freshly prepared by washing the HCl salt (1.47 g, 7.2 mmol) with aqueous $NaHCO_3$), and HOBt hydrate (1.43 g, 9.3 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 20 h and then diluted with ethyl acetate, and the precipitated dicyclohexyl urea (DCU) was filtered off. The combined filtrate and washings were washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and fractionated by FCC (40% ethyl acetate in hexane) to give the titled compound as an oil (2.11 g, 92%): $[\alpha]_D$ −55 (*c* 1.5, CH_3OH); ^1H NMR δ 7.45−7.25 (5H, m), 5.16 (1H, d, J = 12.5 Hz), 5.11 (1H, d, J = 12.5 Hz), 4.53 (1H, m, J = 1.5, 8.5 Hz), 4.27 (1H, m, J = 2.5, 10 Hz), 3.72−3.60 (1H, m), 3.45−3.35 (1H, m), 3.20−3.10 (1H, br s), 2.22−1.90 (5H, m), 1.44 (1H, ddd, J = 4, 10, 14 Hz), 1.30 (1H, ddd, J = 2.5, 9.5, 14 Hz), 0.96 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.5 Hz. ^{13}C NMR (125 MHz) δ 174, 171.6, 135.6, 128.4, 128.3 (×2), 128.1 (×2), 68.0, 66.9, 59.4, 46.3, 43.6, 28.8 (×3), 24.5 (×2), 23.6, 21.3; HRMS *m/z* calcd for $C_{30}H_{51}N_5O_7$ 320.1862 (M + H), found 320.1851 (FAB). Anal. Calcd for $C_{30}H_{51}N_5O_7$: C, 67.69; H, 7.89; N, 4.38. Found: C, 67.45; H, 8.04; N, 4.57.

Boc-β-Ala-Hmp-Pro-Ile-MeVal-MeAla-NHNH-Boc (23a). Using PyBrop. Compound **21** (220 mg, 0.45 mmol) and the tripeptide **15a** (230 mg, 0.40 mmol) were individually deprotected according to the general procedure for hydrogenolysis. PyBroP (280 mg, 0.60 mmol) was added to sitrred solution of the crude deprotected products (180 mg from **21**, 177 mg from **15a**) and DIEA (0.13 mL, 0.74 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 10 min at 0 °C and 17 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, concentrated, and fractionated by DFC (30−65% ethyl acetate in hexane) to give **23a** as a white solid (430 mg, 94%). **Using DCC/HOBt.** DCC (69 mg, 0.33 mmol, 1.1 equiv) was added to a stirred solution of HOBt hydrate (50 mg) and the crude products from hydrogenolysis of **21** (133 mg from 162 mg, 0.33 mmol of **21**) and **15a** (133 mg from 175 mg, 0.30 mmol of **15a**) in CH_2Cl_2 (0.5 mL) at 0 °C. After 1 h at 0 °C and 24 h at room temperature, the reaction mixture was diluted with ethyl acetate (ca. 3 mL) and the precipitated DCU was filtered off. The combined filtrate and washings were processed as above to give **23a** as a white solid (233 mg, 95%). **Using DPPA.** A solution of DPPA (93 mg, 0.39 mmol, 1.3 equiv) in DMF (0.5 mL) and then Et_3N (0.07 mL) were sequentially added to a stirred solution of the crude products

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from hydrogenolysis of **21** (122 mg from 150 mg, 0.30 mmol of **21**) and **15a** (135 mg from 175 mg, 0.30 mmol of **15a**) in DMF (3 mL) at −10 °C. After 1 h at −10 °C, 1 h at 0 °C, and 24 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and processed as above to give **23a** as a white solid (241 mg, 97%). **From 20 and 25a.** DCC (27 mg, 0.13 mmol) was added to a stirred solution of **25a** (75 mg, 0.11 mmol), **20** (25 mg, 0.13 mmol), and DMAP (1.3 mg) in CH_2Cl_2 (0.5 mL) at 0 °C. After 1 h at 0 °C and 24 h at room temperature, the reaction mixture was processed as above to give **23a** (84 mg, 88%): mp 84−86 °C; $[\alpha]_D$ −150 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ (several rotamers, partial data only) 8.93, 7.91, 7.04, 6.47, 6.44, 5.80, and 5.45 (4H, 7 × br s, HN), 3.26, 3.03, 2.97, and 2.80 (6H, 4 × s, H_3CN), 1.29 (18H, s, $(H_3C)_3C$ × 2), 1.32 and 1.26 (3H, 2 × d, J = 7 Hz, H_3C-3 MeAla), 0.96−0.70 (18H, m, H_3CC × 6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 172.3, 171.9, 170.6, 169.9, 169.8, 165.0, 157.9, 154.8, 81.5, 71.3, 71.2, 60.3, 60.2, 58.1, 53.5, 53.4, 46.6, 46.5, 39.0, 36.6, 34.4, 30.4, 28.4, 28.1, 27.1, 24.7, 24.4, 24.3, 23.2, 21.5, 18.0, 15.2, 13.1, 10.8, 10.6; HRMS *m*/*z* calcd for $C_{40}H_{71}N_7O_{11}$ 826.5290 (M + H), found 826.5257 (FAB). Anal. Calcd for $C_{40}H_{71}N_7O_{11}$: C, 58.16; H, 8.66; N, 11.87. Found: C, 57.81; H, 8.93; N, 12.24.

Hmp-Pro-Ile-MeVal-MeAla-NHNH-Boc (25a). The benzyl ester **22** (190 mg, 0.60 mmol) and **15a** (290 mg, 0.50 mmol) were individually deprotected according to the general procedure for hydrogenolysis. DCC (134 mg, 0.65 mmol) was added to a stirred solution of the crude deprotected products (138 mg from **22**, 222 mg from **15a**) and HOBt hydrate (0.113 mg) in CH_2Cl_2 (0.75 mL) at 0 °C. After 10 min at 0 °C and 24 h at room temperature, the reaction mixture was diluted with ethyl acetate (ca. 3 mL) and the precipitated DCU was filtered off. The combined filtrate and washings were washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, concentrated, and fractionated by DFC (30−80% ethyl acetate in hexane) to give **25a** as a white solid (250 mg, 76%): mp 106−108 °C; $[\alpha]_D$ −180 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CDCl3) δ (several rotamers) 9.29 (br s), 7.92 (br s), 7.18 (d, J = 9 Hz), 6.64 (br s) and 6.45 (br s) (3H, HN), 5.25−5.05 (2H, m), 4.80−4.70 (1H, m), 4.57−4.47 (1H, m), 4.35−4.24 (1H, m), 3.71−3.60 (1H, m), 3.46−3.35 (1 H, m), 3.32, 3.09, 3.07, 2.87 (1 H, br s), 3.75−3.60 (6H, 4 × s, H_3CN), 2.40−1.65 (10H, m), 1.39 (9H, s, $(H_3C)_3C$), 1.34 and 1.26 (3H, 2 × d, J = 6.5 Hz, H_3C-3 MeAla), 1.50−1.00 (3H, m), 1.00−0.70 (18H, m, H_3C × 6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 174.3, 172.8, 171.6, 170.9, 170.5, 168.6, 155.0, 81.5, 68.1, 60.3, 58.1, 57.8, 54.0, 53.6, 50.5, 46.5, 43.4, 37.6, 37.2, 30.5, 28.1, 28.0, 27.0, 24.7, 24.5, 24.2, 23.6, 21.3, 19.6, 19.0, 18.1, 15.5, 13.3, 11.2, 10.9; HRMS *m*/*z* calcd for $C_{32}H_{58}N_6O_8$ 661.4476 (M + Li), found 661.4506 (FAB). Anal. Calcd for $C_{32}H_{58}N_6O_8 \cdot H_2O$: C, 57.12; H, 8.99; N, 12.49. Found: C, 57.41; H, 8.93; N, 12.20.

Methyl 4-Hydroxy-4-methylpentanoate (28). A solution of 4,4-dimethyl-butyrolactone **26**[31] (7.90 g, 69 mmol) and NaOH (3.3 g, 83 mmol) in ethanol/water (2:1 v/v, 100 mL) was heated under reflux for 2 h. The reaction mixture was concentated to dryness under high vacuum to give a white solid (11.2 g). A solution of this solid (4.77 g) in water (20 mL) at 0 °C was acidified to pH 4−5 by addition of citric acid (ca. 2.5 g). Excess NaCl was added, and the mixture was extracted with ether (four times). The combined organic layers were dried over Na_2SO_4 and concentrated to give the unstable 4-hydroxy-4-methylpentanoic acid, which was immediately reacted with excess CH_2N_2 in ether solution at 0 °C for 2 h. The resulting mixture was concentrated and fractionated by FCC (30% ethyl acetate in hexane) to give **28** (3.05 g, 71%): ^{1}H NMR δ 3.46 (3H, s), 2.60 (2H, ap t, J = 8 Hz), 2.04 (2H, ap t, J = 8 Hz), 1.44 (6H, s); ^{13}C NMR δ 177.0 (s), 84.9 (s), 51.0 (q), 34.9 (t), 29.6 (t), 27.9 (q × 2); HRMS *m*/*z* calcd for $C_7H_{14}O_3$ 147.1021 (M + H), found 147.1019 (CI). In several smaller-scale experiments, the white solid above was converted into the titled compound in 70−90% yield. The variable yield is attributed to the instability of the intermediate acid and the volatility of **28**.

Methyl 4-Triethylsilyloxy-4-methylpentanoate (29). TESOTf (97%; 1.01 mL, 1.15 g, 4.36 mmol) was added to a stirred solution of **28** (0.530 g, 3.63 mmol) and 2,6-lutidine (0.84 mL, 0.77 g, 7.3 mmol) in CH_2Cl_2 (5 mL) at 0 °C under argon. After 30 min at 0 °C and 14 h at room temperature, the mixture was diluted with ether, washed with water, dried over Na_2SO_4, concentrated, and fractionated by FCC (5% ether in hexane) to give the titled compound (0.850 g, 90%): ^{1}H NMR δ 3.66 (3H, s), 2.41 (2H, ap t, J = 8 Hz), 1.75 (2H, tap, J = 8 Hz), 1.20 (6H, s), 0.93 (9H, t, J = 8 Hz), 0.56 (6H, q, J = 8 Hz); ^{13}C NMR δ 176.1 (s), 73.1 (s), 51.6 (q), 40.0 (t), 30.1 (q × 2), 29.7 (t), 7.3 (q × 3), 6.9 (t × 3); HRMS *m*/*z* calcd for $C_{13}H_{28}O_3Si$ 261.1886 (M + H), found 261.1885 (CI).

4-Triethylsilyloxy-4-methylpentanoic Acid (30). LiOH·H_2O (1.55 g, 37 mmol) was added to a stirred solution of **29** (1.30 g, 5.00 mmol) in THF−CH_3OH−H_2O (2:2:1 v/v, 150 mL) at 0 °C. After 15 min at 0 °C and 3 h at room temperature, the organic solvents were removed on a rotary evaporator and the remaining aqueous soution was acidfied to pH 4−5 by addition of aqueous citric acid (1 M). Excess NaCl was added, and the mixture was extracted with ether (3 × 50 mL). The combined orgainc layers were dried over Na_2SO_4, concentrated, and fractionated by FCC (30% ethyl acetate in hexane) to give **30** (1.12 g, 91%): ^{1}H NMR δ 2.46 (2H, ap t, J = 8 Hz), 1.76 (2H, ap t, J = 8 Hz), 1.22 (6H, s), 0.93 (9H, t, J = 8 Hz), 0.57 (6H, q, J = 8 Hz); ^{13}C NMR δ 181.0 (s), 72.7 (s), 39.5 (t), 29.9 (q × 2), 29.7 (t), 7.2 (q × 3), 6.8 (t × 3); HRMS *m*/*z* calcd for $C_{12}H_{26}O_3Si$ 247.1729 (M + H), found 247.1734 (FAB).

(4*R*,5*S*)-(+)-4-Methyl-5-phenyl-3-[4-triethylsilyloxy-4-methylpentanoyl]-2-oxazolidinone (32). Pivaloyl chloride (0.39 mL, 0.38 g, 3.2 mmol) was slowly added via syringe to a stirred solution of acid **30** (739 mg, 3.0 mmol) and Et_3N (0.46 mL, 334 mg, 3.3 mmol) in THF (3 mL) at −78 °C under argon. The mixture (a thick white paste formed) was allowed to warm to 0 °C, and after 1 h, a solution of **31** (483 mg, 2.73 mmol), DMAP (60 mg, 0.5 mmol), and Et_3N (0.42 mL, 0.30 g, 3 mmol) in THF (2.5 mL) was added. After 14 h at room temperature, the reacton mixture was diluted with ethyl acetate and washed sequentially with aqueous NaOH (1 M) and brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (10−60% ethyl acetate in hexane) to give recovered oxazolidinone **31** (23 mg, 5%) and the titled compound (1.03 g, 93%): $[\alpha]_D$ +20 (*c* 0.8, CH_2Cl_2); ^{1}H NMR δ 1.73 (2H, m), 1.23 (6H, s), 0.93 (9H, t, J = 7 Hz), 0.85 (3H, d, J = 7 Hz), 0.56 (6H, q, J = 7 Hz); ^{13}C NMR δ 173.8 (s), 153.2 (s), 133.7 (s), 128.7 (d × 3), 125.9 (d × 2), 79.0 (d), 72.7 (s), 54.9 (q), 39.1 (t), 31.4 (t), 30.1 (q), 29.9 (q), 14.7 (q), 7.2 (q × 3), 6.9 (t × 3); HRMS *m*/*z* calcd for $C_{22}H_{35}NO_4Si$ 412.2495 (M + Li), found 412.2501 (FAB, LiBr). Anal. Calcd for $C_{22}H_{35}NO_4Si$: C, 65.15; H, 8.70; N, 3.45. Found: C, 64.96; H, 8.70; N, 3.58.

(4*R*,5*S*,2′*R*)-(+)-4-methyl-5-phenyl-3-[2-hydroxy-4-triethylsilyloxy-4-methylpentanoyl]-2-oxazolidinone (33). A solution of **32** (920 mg, 2.27 mmol) in THF (3.4 mL) was added dropwise via syringe to a stirred solution of NaHMDS (1 M solution in THF; 2.72 mL, 2.72 mmol) in THF (3.4 mL) at −78 °C under argon. After 30 min, a precooled (−78 °C) solution of 2-(phenylsulfonyl)-3-phenyloxaziridine[35] (839 mg, 3.18 mmol) in THF (3.4 mL) was added via cannula. After 10 min, the reactiion was quenched by addition of a solution of HOAc (0.69 mL) in THF (13.5 mL). The mixture was diluted with hexane/CH_2Cl_2 (4:1 v/v, 85 mL) and was washed sequentially with 50 mL each of 5% $NaHCO_3$, saturated Na_2SO_3, 1 M $KHSO_4$, 5% $NaHCO_3$, and brine. The organic layer was dried, concentrated, and fractionated by FCC (15% ethyl acetate in hexane) to give the crude product, which was further fractionated by FCC (0.5% CH_3OH in CH_2Cl_2) to give the titled compound (802 mg, 84%) as a viscous oil: $[\alpha]_D$ +24 (*c* 3.5, CH_2Cl_2); ^{1}H NMR δ 7.45−7.15 (5H, m), 5.66 (1H, d, J = 7 Hz), 5.47 (1H, br dd, J = 3, 8.5 Hz), 4.71 (1H, dq, J = 7, 7 Hz), 4.57 (1H, br s), 1.97−1.80 (2H, m), 1.41 (3H, s), 1.32 (3H, s), 0.93 (9H, t, J = 8 Hz), 0.88 (3H, d, J = 7 Hz), 0.59 (6H, q, J = 8 Hz); ^{13}C NMR δ 173.8 (s), 152.8 (s), 133.3 (s), 128.9 (d × 2), 128.8 (d), 125.7 (d × 2), 79.7 (d), 75.0 (s), 69.3 (d), 55.3 (d), 46.6 (t), 31.0 (q), 29.3 (q), 14.4 (q), 7.1 (q × 3), 6.7 (t × 3); HRMS *m*/*z* calcd for $C_{22}H_{35}NO_5Si$ 428.2445 (M + Li), found 428.2442 (FAB, LiBr). Anal. Calcd for $C_{22}H_{35}NO_5Si$: C, 62.67; H, 8.37; N, 3.32. Found: C, 62.40; H, 8.41; N, 3.30.

(2′*R*,4*R*,5*S*)-(+)-4-Methyl-5-phenyl-3-[4-methyl-(2-(phenylmethoxycarbonyloxy)-4-triethylsilyloxypentanoyl)-2-oxazolidinone (34). Benzyl chloroformate (0.15 mL, 1 mmol) and DMAP (122 mg, 1 mmol) were added to a stirred solution of **33** (280 mg, 0.67 mmol) in CH_2Cl_2 (4 mL) at room temperature. After 2 h, additional benzyl chloroformate (0.075 mL, 0.5 mmol) and DMAP (61 mg, 0.50 mmol) were added; after being stirred for 2 h, the reaction mixture was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4, concentrated, and fractionated by FCC (50−75% CH_2Cl_2 in hexane) to give the titled compound as an oil (365 mg, 99%): $[\alpha]_D$ +27 (*c* 1.5, CH_2Cl_2); ^{1}H NMR δ 7.35 (10H, m), 6.22 (1H, dd, J = 5.5, 6 Hz), 5.70 (1H, d, J = 7 Hz), 5.18 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.5 Hz), 4.73 (1H, dq, J = 7, 6.5 Hz), 2.04 (2H, m), 1.36 (3H, s), 1.34 (3H, s), 0.92 (12H, m), 0.58 (6H, q, J = 8 Hz); ^{13}C NMR δ 170.9 (s), 154.8 (s), 152.3 (s), 135.3 (s), 133.3 (s), 129.1 (d), 129.0 (d × 2), 128.8 (d × 2), 128.7 (d), 128.4 (d × 2), 125.9 (d × 2), 79.7 (d), 73.9 (d), 72.7 (s), 70.1 (t), 55.5 (d), 45.1 (t), 31.4 (q), 30.4 (q), 14.2 (q), 7.3 (q × 3), 6.9 (t × 3); HRMS *m*/*z* calcd for $C_{30}H_{41}NO_7Si$ 562.2812 (M + Li), found 562.2814 (FAB, LiBr). Anal. Calcd for $C_{30}H_{41}NO_7Si$: C, 64.84; H, 7.44; N, 2.52. Found: C, 64.65; H, 7.22; N, 2.78.

(4*R*,5*S*,2′*R*)-(+)-4-Methyl-5-phenyl-3-[3-(*t*-butoxycarbonylamino)propanoyloxy-4-triethylsilyloxy-4-methylpentanoyl]-2-oxazolidinone (35). DCC (160 mg, 0.77 mmol) was added to a stirred solution of **33** (270 mg, 0.64 mmol), **20** (133 mg, 0.71 mmol), and DMAP (17 mg, 0.14 mmol) in CH_2Cl_2 (1.8 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 20 h and then diluted with ethyl acetate, and the precipitated DCU was filtered off. The combined filtrate and washings were washed sequentially with aqueous citric acid (0.5 M) and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, concentrated, and fractionated by FCC (20% ethyl acetate in hexane) to give the titled compound (374 mg, 99%), which solidified upon standing in a refrigerator: mp 82−83 °C; $[\alpha]_D$ +29 (*c* 1.2, CH_2Cl_2); ^{1}H NMR δ 7.45−7.25 (5H, m), 6.23 (1H, dd, J = 4, 7.5 Hz), 5.70 (1H, d, J = 6.5 Hz), 5.21 (1H, br s), 4.72 (1H, dq, J = 6.5, 7 Hz), 3.50−3.35 (2H, t), 2.62−2.53 (2H, t), 2.07−1.95 (2H, m), 1.41 (9H, s), 1.35 (3H, s), 1.33 (3H, s), 0.93 (9H, t, J = 8 Hz), 0.88 (3H, d, J = 7 Hz), 0.59 (6H, q, J = 8 Hz); ^{13}C NMR δ 171.8 (s), 171.0 (s), 155.9 (s), 152.2 (s), 133.3 (s), 129.0 (d), 128.8 (d × 2), 125.8 (d × 2), 79.5 (s), 79.0 (s), 72.7 (s), 71.1 (d), 55.3 (d), 44.7 (t), 36.5 (t), 43.7 (t), 31.2 (q), 30.2 (q), 28.4 (q × 3), 14.0 (q), 7.2 (q), 6.8 (t); HRMS *m*/*z* calcd for $C_{30}H_{48}N_2O_8Si$ 599.3340 (M + Li), found 599.3344 (FAB, LiBr). Anal. Calcd for $C_{30}H_{48}N_2O_8Si$: C, 60.78; H, 8.16; N, 4.73. Found: C, 60.85; H, 8.18; N, 4.69.

(2*R*)-4-Triethylsilyloxy-4-methyl-2-(phenylmethoxycarbonyloxy)pentanoic Acid [Cbz-Dhmp(4-OTES)] (36). Hydrogen peroxide (30%; 0.52 mL, 4.59 mmol) and LiOH (1 M in H_2O; 0.9 mL, 0.9 mmol) were added to a stirred solution of **34** (170 mg, 0.31 mmol) in THF (3.2 mL) at 0 °C under argon. After 75 min, 10% aqueous Na_2SO_3 (10 mL) was added and the mixture stirred at room temperature for 20 min. The reaction mixture was diluted with ethyl acetate, washed sequentially with citric acid (0.75 M) and water, dried over Na_2SO_4, concentrated, and fractionated by FCC (eluting first with 45:45:9:1 hexane:CH_2Cl_2:^{i}PrOH:NH_4OH and then with 15% CH_3OH in CH_2Cl_2) to give **31** (50 mg, 92%) and the titled compound (110 mg, 91%): $[\alpha]_D$ +52 (*c* 0.89, CH_2Cl_2); ^{1}H NMR δ 8.45−7.95 (1H, br s), 7.31 (5H, m), 5.20 (1H, d, J = 12 Hz), 5.08 (1H, br d, J = 9 Hz), 5.07 (1H, d, J = 12 Hz), 2.09 (1H, d, J = 14.5 Hz), 1.90 (1H, dd, J = 9, 14.5 Hz), 1.22 (6H, s), 0.88 (9H, t, J = 8 Hz), 0.54 (6H, q, J = 8 Hz); ^{13}C NMR δ 177.4 (s), 155.5 (s), 135.8 (s), 128.7 (d × 2), 128.5 (d × 3), 76.0 (d), 72.7 (s), 69.8 (t), 45.8 (t), 31.2 (q), 30.2 (q), 7.3 (q), 6.8 (t); HRMS *m*/*z* calcd for $C_{20}H_{32}O_6Si$ 403.2128 (M + Li), found 403.2141 (FAB, LiBr). Anal. Calcd for $C_{20}H_{32}O_6Si$: C, 60.58; H, 8.15. Found: C, 60.31; H, 8.15.

Boc-*β*Ala-Dhmp(OTES)-Pro-OBn (Dhmp(OTES) = (2*R*)-2-Hydroxy-4-triethylsiloxy-4-methyl-pentanoic Acid) (37). H_2O_2 (30%; 0.32 mL, 2.8 mmol), LiOH·H_2O (24 mg, 0.57 mmol), and a solution of **35** (282 mg, 0.48 mmol) in THF/H_2O (4:1 v/v, 3 mL) were added sequentially to a stirred mixture of THF/H_2O (4:1 v/v, 4 mL) at 0 °C under argon. After ca. 20 min, the reaction was quenched by addition of aqueous $NaHSO_3$ (10% w/v, 7 mL) and the mixture was diluted with water and extracted with ethyl acetate (three times). The combined organic layers were washed sequentially with aqueous citric acid (1 M) and brine, dried over Na_2SO_4, concentrated, and fractionated by FCC (eluting first with 45:45:9:1 CH_2Cl_2:hexane:^{i}PrOH:NH_4OH and then with 15% CH_3OH in CH_2Cl_2) to give **31** (84 mg, 100%) and desired didepsipeptide Boc-*β*Ala-Dhmp(OTES) (156 mg, 76%): ^{1}H NMR δ 5.38 (1H, dd, J = 5, 5.5 Hz), 5.26 (1H, br s), 3.55−3.35 (2H, m), 2.67−2.52 (2H, m), 2.18 (1H, dd, J = 5, 15 Hz), 1.90 (1H, dd, J = 5.5, 15 Hz), 1.43 (9H, s), 1.34 (3H, s), 1.32 (3H, s), 0.95 (9H, t, J = 8 Hz), 0.62 (6H, q, J = 8 Hz). The aqueous layer from the above extractions was acidified to pH 4 with aqueous citric acid (1 M) and extracted with ethyl acetate; concentration of the extract gave **20** (18 mg, 20%). DCC (82 mg, 0.40 mmol) was added to a stirred solution of the above didepsipeptide (134 mg, 0.31 mmol), **18** (76 mg, 0.37 mmol), and HOBt hydrate (62 mg) in CH_2Cl_2 (1 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 18 h and then diluted with ethyl acetate, and the precipitated DCU was filtered off. The combined filtrate and washings were washed sequentially with 0.5 M aqueous citric acid and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, concentrated, and fractionated by FCC (30% ethyl acetate in hexane) to give the titled compound (156 mg, 81%) (62% overall yield from **35**): $[\alpha]_D$ −22 (*c* 1.4, CH_3OH); ^{1}H NMR δ 7.42−7.25 (5H, m), 5.53−5.44 (2H, m), 5.20 (2H, ap s), 4.47 (1H, dd, J = 4, 8 Hz), 3.89−3.76 (1H, m), 3.72−3.60 (1H, m), 3.45−3.32 (2H, m), 2.57−2.47 (2H, m), 2.22−1.75 (8H, m), 1.41 (9H, s), 1.27 (3H, s), 1.24 (3H, s), 0.93 (9H, t, J = 8 Hz), 0.58 (6H, q, J = 8 Hz); ^{13}C NMR δ 171.6 (s × 2), 169.7 (s), 156.2 (s), 136.0 (s), 128.6 (d × 2, C6H5), 128.3 (d × 3), 79.1 (s), 72.6 (s), 69.5 (d), 66.8 (t), 59.5 (d), 46.6 (d), 45.7 (t), 36.6 (t), 35.0 (t), 31.8 (t), 29.1 (q × 2), 28.6 (q × 3), 24.8 (t), 7.2 (q × 3), 6.7 (t × 3); HRMS *m*/*z* calcd for $C_{32}H_{52}N_2O_8Si$ 627.3653 (M + Li), found 627.3643 (FAB, LiBr). Anal. Calcd for $C_{32}H_{52}N_2O_8Si$: C, 61.91; H, 8.44; N, 4.51. Found: C, 61.95; H, 8.39; N, 4.59.

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Supporting Information Available: Experimental procedures and spectroscopic data for **2**, ***2**, **3**−**5**, **11b**, **11c**, **13b**, **13c**, **15b**, **15c**, **23b**, **23c**, **25b**, **41a**, and **41b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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