Convergent Synthesis of Dolastatin 15 by Solid Phase Coupling of an *N*-Methylamino Acid

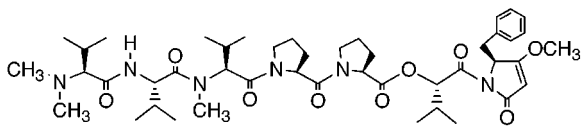
Kenichi Akaji,*,† Yuzo Hayashi,† Yoshiaki Kiso,† and Naohiro Kuriyama‡

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan, and YMC Company, Ltd, Kumiyama-cho, Kyoto 613-0024, Japan

Received June 2, 1998

Convergent synthesis of dolastatin 15 (**1**), a cytostatic depsipeptide isolated from the Indian Ocean sea hare, has been described. For construction of the backbone, a single-step condensation of peptide fragment **2** and pyrrolidone fragment **3** was successfully performed using 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP) developed by us as an efficient coupling reagent. Coupling of an *N*-methylamino acid on solid support was first achieved using CIP for the efficient synthesis of peptide fragment **2**. The effectiveness of CIP for the coupling of *N*-methylamino acids in solution and on solid support were clarified by the syntheses of model di- and tripeptides.

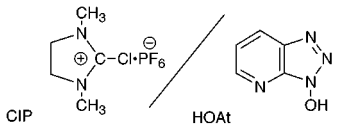
Dolastatin 15 is a cytostatic depsipeptide isolated from the marine mollusk *Dolabella auricularia* by Pettit et al. in 1989.[1] Among the dolastatins, an unprecedented series of linear and cyclic antineoplastic and/or cytostatic peptide isolated from the Indian Ocean sea hare,[2] dolastatin 15 as well as dolastatin 10 represent the two most important members because of the strong and selective activities. Dolastatin 15 inhibits growth of the P388 lymphocytic leukemia cell line with an ED_{50} value of 2.4 ng/mL.



1 Dolastatin 15

Structurally, dolastatin 15 is a lipophilic pentapeptide esterified by an *N*-acylpyrrolidone and contains seven chiral carbons all having *S*-stereochemistry. In the peptide part, two *N*-methylated amino acids, *N*-methylvaline and *N*,*N*-dimethylvaline,[3] are included. The first synthesis of dolastatin 15 was communicated in 1991 by Pettit and co-workers.[4] In 1992, Poncet et al.[5] reported total synthesis of the proposed structure and described that the synthetic compound exhibited physical properties different from those reported by Pettit and co-workers for the natural dolastatin 15 and their synthesis product. The absolute configuration and physical properties of dalastatin 15 was confirmed by the synthesis by Pettit and co-workers in 1994,[6] in which they employed a segment synthetic strategy and obtained a final compound exhibiting essentially the same physical properties as those obtained by Poncet et al. In those syntheses, various coupling procedures including mixed anhydride, chlorocarbonate, carbodiimide, and diethyl cyanophosphonate (DEPC),[7] were selected case by case at each activation step. For the construction of the backbone of dolastatin 15, however, a two-step procedure involving separate incorporation of *N*,*N*-dimethylvaline or bismethylation of valine was necessary because of the low efficiency of these activating reagents in a single-step condensation.[6]

Recently, we have developed a new coupling reagent, 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP) and found that CIP in the presence of the additive 1-hydroxy-7-azabenzotriazole (HOAt)[8] effectively couples sterically hindered α,α-dialkylamino acids.[9] In these couplings, the initially formed oxazolone intermediate is transformed to a highly active ester by the catalytic additive to produce the desired condensation product without detectable racemization. Here, we describe a method of convergent synthesis of dolastatin 15 by single-step fragment condensation, in which all couplings including those of *N*-methylamino acids on solid support are conducted by CIP-mediated activation.



CIP HOAt

Results and Discussion

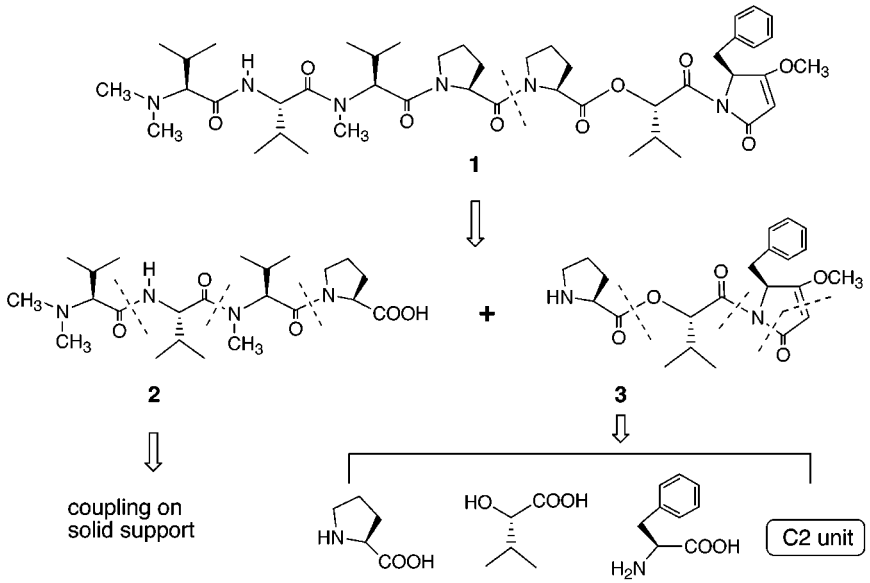
Scheme 1 shows our retrosynthetic route of dolastatin 15. For completing the synthesis of dolastatin 15, single-step segment condensation between peptide fragment **2** and nonpeptide fragment **3** by the CIP/HOAt method was used, since activation at the Pro residue is resistant to isomerization. In contrast to the previous syntheses, an

† Kyoto Pharmaceutical University.
‡ YMC Co.

(1) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005.
(2) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J. C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151.
(3) Bowman, R. E.; Stroud, H. H. *J. Chem. Soc.* **1950**, 1342.
(4) Pettit, G. R.; Herald, D. L.; Singh, S. B.; Thornton, T. J.; Mullaney, J. T. *J. Am. Chem. Soc.* **1991**, *113*, 6692.
(5) Patino, N.; Frérot, E.; Galeotti, N.; Poncet, J.; Coste, J.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1992**, *48*, 4115.
(6) Pettit, G. R.; Thornton, T. J.; Mullaney, J. T.; Boyd, M. R.; Herald, D. L.; Singh, S- B.; Flahiva, E. J. *Tetrahedron* **1994**, *50*, 12097.
(7) Shioiri, T.; Yokoyama, Y.; Kasai, Y.; Yamada, S. *Tetrahedron* **1976**, *32*, 2211.
(8) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(9) (a) Akaji, K.; Kuriyama, N.; Kimura, T.; Fujiwara, Y.; Kiso, Y. *Tetrahedron Lett.* **1992**, *33*, 3177. (b) Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.

10.1021/jo981055a CCC: $18.00
Published on Web 01/05/1999

Scheme 1



N,*N*-dimethylamino acid was incorporated in peptide fragment **2** since CIP-mediated activation is expected to be efficient enough for hindered couplings in solution, as shown from our previous syntheses of mirabazoles.[10] Pro, 2-hydroxyisovaleric acid (Hiva), and Phe were employed for the preparation of fragment **3**. The pyrrolidone ring of **3** was constructed by the CIP-mediated coupling of Phe and Meldrum's ester employed as C2 unit.[11] For the preparation of peptide fragment **2**, CIP-mediated activation for the coupling of an *N*-methylamino acid on solid support was selected as a preferable scheme to facilitate the practical synthesis of dolastatin 15. Thus, prior to the synthesis of dolastatin 15 according to the scheme, we evaluated CIP-mediated activation for its efficiency in preparing a peptide sequence containing *N*-methylamino acids on solid support.

Coupling of *N*-Methylamino Acid on Solid Support. *N*-Methylamino acid is difficult to incorporate by standard peptide coupling methods even in solution phase reactions because of its steric hindrance and marked tendency to racemize.[12] To overcome these difficulties, several halogenophosphonium reagents have been evaluated for the coupling of *N*-methylamino acid.[13] In the practical synthesis of destruxin B containing two consecutive *N*-methylamino acid residues, however, prolonged reaction times (more than 18 h) were necessary to obtain moderate to good isolation yields even with newly developed coupling reagents including BOP-Cl and PyBroP.[14] For the coupling of an *N*-methylamino acid on solid support, few syntheses employing HATU as an effective coupling reagent have been reported[15] and no practical solid-phase syntheses of natural products have been achieved. These reports have prompted us to examine the application of CIP-mediated activation for coupling in solution followed by coupling on solid support.

Table 1. Model Peptides by Solution Phase Synthesis

	time (h)	yield (%)[a]	D-isomer content (%)
Z-MeLeu-Val-OMe **4**	1	95	<0.5[b]
Z-MeVal-Val-OMe **5**	1	91	<0.5[b]
Z-Val-MeVal-OMe **6**	3	92	<1.0[b]
Z-MeLeu-MeLeu-OMe **7**	3	97	<0.5[c]
Z-MeLeu-MeVal-OMe **8**	3	91	<1.5[d]

[a] Isolation yield after silica gel column chromatography. [b] Determined by reverse phase HPLC. [c] Determined by reverse phase HPLC and ^{1}H NMR. [d] Determined by ^{1}H NMR.

To evaluate the coupling efficiency in solution, dipeptides **4** to **8** containing an *N*-methylamino acid were synthesized using CIP−HOAt (Table 1). Each coupling reaction was conducted at 25 °C in CH_2Cl_2, and the desired product was isolated by silica gel column chromatography. Coupling of *N*-methylamino acids proceeded within 1 h to give dipeptides **4** and **5** with quantitative yields. The more difficult coupling to *N*-methylamino acids gave dipeptides **6** to **8** quantitatively after a 3 h reaction using CIP−HOAt.

Epimerization during the CIP-mediated activation was measured by the determination of D-isomer content in the crude isolated products. For dipeptides **4** to **7**, reverse phase HPLC was employed to analyze the D-isomer contents. For dipeptide **8**, however, comparison of peaks of methy ester by ^{1}H NMR was employed since baseline separation of the D-isomer by HPLC was difficult. In the comparison, signals could be found with chemical shifts that differ in the isomers.[16] In each analysis, the D-isomer dipeptide was identified by comparison with standard dipeptide separately synthesized starting from the corresponding D-amino acid.

Table 1 shows that most couplings resulted in a D-isomer content less than 0.5%. Even in the most sterically hindered dipeptide **8**, the D-isomer content was less than 1.5%. These results indicate that CIP-mediated

(10) (a) Akaji, K.; Kuriyama, N.; Kiso, Y. *J. Org. Chem.* **1996**, *61*, 3350. (b) Kuriyama, N.; Akaji, K.; Kiso, Y. *Tetrahedron* **1997**, *53*, 8323.

(11) Jouin, P.; Castro, B.; Nisato, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177.

(12) (a) Frérot, E.; Coste, J.; Poncet, J.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1992**, *33*, 2815. (b) Calmes, M.; Cavelier-Frontin, F.; Jacquier, R.; Mercadier, J.-L.; Sabil, S.; Verducci, J.; Quiot, J.-M.; Vey, A. *Int. J. Pept. Protein Res.* **1993**, *41*, 528.

(13) Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437.

(14) Ward, D. E.; Lazny, R.; Pedras, S. C. *Tetrahedron Lett.* **1997**, *38*, 339.

(15) (a) Angell, Y. M.; García-Echeverría, C.; Rich, D. H. *Tetrahedron Lett.* **1994**, *33*, 5981. (b) Carpino, L. A.; El-Faham, C.; Minor, A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

(16) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669.

Table 2. Model Peptides by Solid Phase Synthesis

	time	yield (%)	D-isomer content (%)[a]
Z-MeVal-Val-OMe **9**	1 h × 1	≈100[b]	<0.5
Z-Val-MeVal-Gly-OMe	2 h × 2	59[c]	<1.0
10	12 h × 1	74[c]	<2.0
Fmoc-MeLeu-MeVal-Gly-OMe	3 h × 1	53[d]	<2.0
11	12 h × 1	56[d]	<2.0

[a] Determined by reverse phase HPLC. [b] Determined by Kaiser ninhydrin test. [c] Determined by amino acid analysis of peptide-resin. [d] Determined by UV analysis of the Fmoc group.

activation is an efficient method for coupling in solution, with practically no racemization within 1 to 3 h at 25 °C. In contrast, it had been reported in solution synthesis of destruxin B that DCC/DMAP activation, a highly efficient conventional peptide coupling procedure, resulted in a 72% isolation yield with 37% isomerization after a 24 h reaction.[14]

Coupling efficiency on solid support was then examined using three model peptides: Z-MeVal-Val-OMe **9**, Z-Val-MeVal-Gly-OMe **10**, and Fmoc-MeLeu-MeVal-Gly-OMe **11**. As a solid support, 2-chlorotrityl chloride (ClTrt) polystyrene resin,[17] from which the desired model peptides could be cleaved by treatment with weak acid such as AcOH, was selected. Incorporation of the *N*-methylamino acid was carried out by use of a double coupling (2 h × 2) or a 12 h single coupling employing 5 equiv of the *N*-methylamino acid. The extent of the reaction was monitored by the Kaiser ninhydrin test for dipeptide **9**, by the amino acid ratio of Val/Gly in acid hydrolysate of the product resin for tripeptide **10**, and by UV analysis of the Fmoc deprotection step[18] for tripeptide **11**. Each product obtained by cleavage from the resin followed by methylation with (trimethysilyl)diazomethane[19] was characterized by the same methods described by solution phase reaction. D-Isomer content of each crude product was analyzed using HPLC or methyl ester-derived signal comparison by ^{1}H NMR. Each corresponding D-isomer was identified by separate syntheses using D-amino acid.

Table 2 summarizes the coupling yields and D-isomer contents in model peptide syntheses on solid support. Quantitative to moderate coupling yields were obtained by single coupling reactions, although somewhat longer reaction times than that in solution phase synthesis were necessary. Slightly lower yields were obtained by a double coupling protocol for each model peptide. As a reaction solvent, DMF gave better results than CH_2Cl_2. D-Isomer contents were less than 2%, indicating practically no racemization occurred during the CIP-mediated activation despite prolonged reaction times. The efficiency of CIP-mediated coupling might be mediated by the formation of the HOAt active ester intermediate, which is believed to have enhanced reactivity because of its neighboring nitrogen effect, as described in our previous synthesis of mirabazole C.[10]

Synthesis of Dolastatin 15. The model studies described above suggest that the coupling of an *N*-methylamino acid is feasible using CIP−HOAt. We then

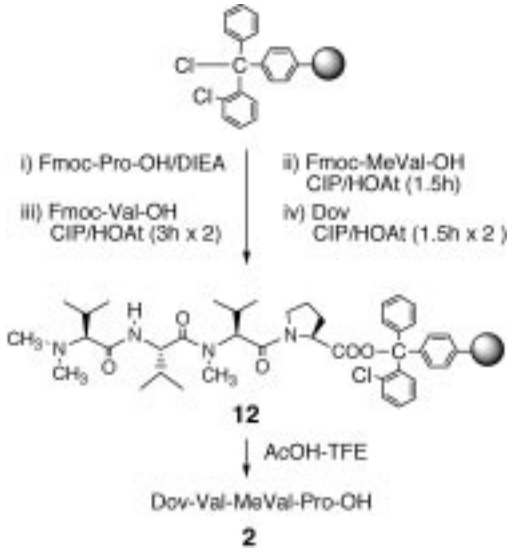


Figure 1. Synthetic route for peptide fragment **2**.

synthesized dolastatin 15 by a convergent route shown in Scheme 1 employing CIP-mediated coupling of *N*-methylamino acids on solid support.

Peptide fragment **2** was synthesized by chain elongation on solid support according to the route shown in Figure 1. As a solid support, 2-chlorotrityl chloride (ClTrt) resin was selected as in the above model experiment. It is particularly useful in the synthesis of prolyl peptide as the bulk of the trityl linker helps prevent diketopiperazine formation.[17] The initial Fmoc-Pro-O-ClTrt resin was prepared by the reaction of ClTrt resin with Fmoc-Pro-OH in the presence of DIEA. After cleavage of the Fmoc group of the resin by piperidine treatment, Fmoc-MeVal-OH was coupled by a 1.5 h reaction using CIP−HOAt. Fmoc deprotection by piperidine and CIP-mediated double coupling reactions were repeated for the incorporation of Fmoc-Val-OH and DOV (dolavaline, *N*,*N*′-dimethylvaline) to give tetrapeptide resin **12**.

The peptide chain was then cleaved from the resin by treatment with AcOH/TFE/CH_2Cl_2 (1:1:8).[20] The crude product obtained was purified by flash chromatography to give the desired peptide fragment **2** with a 50% isolation yield calculated from the starting Fmoc-Pro-O-ClTrt resin. The purified fragment **2** showed a single peak on an analytical HPLC and was shown to be a monomer by FAB-MS spectrometry.

The nonpeptide fragment **20** was synthesized according to the route shown in Figure 2. L-Hydroxyisovalelic acid (Hiva) **13** and Phe-OBzl **14** were condensed by a 1 h reaction using CIP−HOBt to give **15** without difficulty. The hydroxy group of **15** was protected using *tert*-butyldimethylsilyl chloride in the presence of imidazole,[21] and then the Bzl group was cleaved by hydrogenolysis to yield **17** quantitatively. Condensation of **17** and Meldrum's ester was then carried out by a 4 h reaction at 25 °C using CIP in the presence of DMAP.[22] In this particular coupling step, addition of DMAP gave better results than HOAt probably because of its basicity. The

(17) (a) Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943. (b) Barlos, K.; Gatos, D.; Kapolos, S.; Papaphotiu, G.; Schäfer, W.; Wenqing, Y. *Tetrahedron Lett.* **1989**, *30*, 3947.

(18) Meienhofer, J.; Waki, M.; Heimer, E.; Lambros, T. J.; Makofske, R.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35.

(19) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.

(20) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513.

(21) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

(22) Hassner, A.; Krepski, R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069.

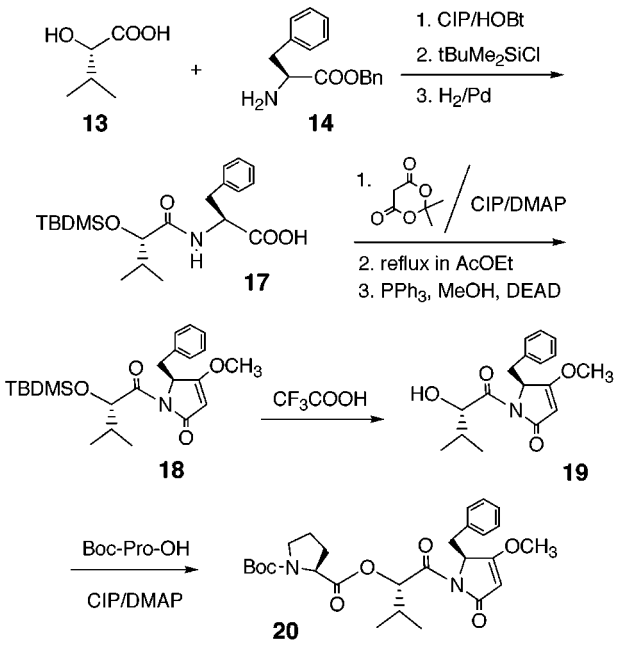


Figure 2. Synthetic route for nonpeptide fragment **20**.

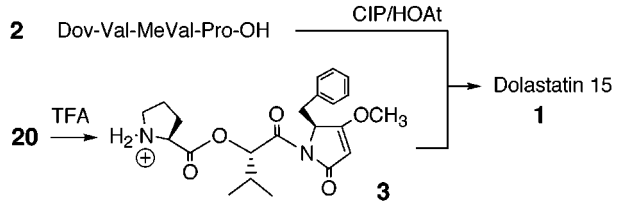


Figure 3. Synthetic route for dolastatin 15.

resulting Meldrum's ester adduct, without purification, was heated in refluxing AcOEt to afford cyclized product. Methylation of the enolic function of the cyclized product was conducted under Mitsunobu conditions at 25 °C for 2 h to give methyl vinyl ether.[23] The product was a mixture of diastereoisomers due to partial epimerization caused by the activation of an *N*-acylamino acid. After purification by flash chromatography, the desired TBDMS-(*S*)-Hiva-(*S*)Dpy **18** was easily separated and obtained at a 46% overall yield calculated from **17** accompanied with 9% formation of (*S*,*R*) isomer. The silyl group of **18** was quantitatively cleaved by TFA treatment to afford the hydroxy product **19**. Esterification of **19** with Boc-Pro-OH was performed by a 10 h reaction at 25 °C using CIP−DMAP. After purification by flash column chromatography, the desired nonpeptide fragment **20** was obtained at a 92% yield.

The final coupling for dolastatin 15 was carried out as shown in Figure 3. The Boc group of **20** was cleaved by a 30 min treatment with TFA at 25 °C, and the product, without purification, was condensed with peptide fragment **2** by a 2 h reaction using CIP−HOAt. The crude product was purified by flash chromatography to yield dolastatin 15 with an 89% isolation yield. The synthetic material had the same spectroscopic and physical properties as those reported for natural and synthetic dolastatin 15.[5,6] In addition, the ^{1}H NMR spectrum of our synthetic dolastatin 15 was identical to the synthetic dolastatin 15 obtained by Poncet and co-workers.

(23) Bajwa, J. S.; Anderson, R. C. *Tetrahedron Lett.* **1990**, *48*, 6973.

Conclusion

We have achieved an efficient synthesis of dolastatin 15, a cytostatic depsipeptide containing a pyrrolidone amino acid and *N*-methylamino acids, in which all condensation reactions were carried out by CIP-mediated activation. In our synthesis, a convergent route utilizing single-step condensation of peptide fragment **2** and pyrrolidone fragment **3** using CIP−HOAt as an efficient coupling reagent was employed. The CIP-mediated reaction was also successfully applied for coupling of *N*-methylamino acid on solid support, demonstrating the first practical application of the solid-phase procedure for natural product synthesis.

Experimental Section

General. Solvents were reagent grade and dried prior to use. All flash chromatographic separations were carried out on Wakogel FC-40 obtained from WAKO Pure Chemical Ind. Ltd. Fmoc amino acid derivatives and 2-chlorotrityl chloride resin were obtained from Calbiochem Novabiochem and used without further purification. For quantification of Fmoc amino acid on the resin, absorbance at 301 nm after cleavage of the Fmoc group with piperidine was measured according to the procedure described by Meienhofer et al.[18]

Melting points were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a 270 MHz spectrometer with TMS as an internal standard. Optical rotations were measured at ambient temperature using a 1 mL cell. Analytical HPLC was carried out on a reverse phase column (4.6 × 150 mm), which was eluted with CH_3CN in 0.1% aqueous TFA: Rt_1, YMC Pack C8 AM202, detected at OD 214 nm; Rt_2, YMC Pack ODS AM302, flow rate 0.9 mL/min, detected at OD230 nm.

Syntheses of Model Peptides 4−8 in Solution (Table 1). Z-Val-MeVal-OMe **6**: To a solution of Z-MeVal-OMe (0.5 mmol) in $CHCl_3$ (0.5 mL) was added 25% HBr/AcOH (1.5 mL) at an ice-bath temperature, and the mixture was stirred for 60 min at 25 °C. The solvent and AcOH were removed by azeotroping with heptane (3 times), yielding a light brown oil.

To the solution of Z-Val-OH (2 equiv) in CH_2Cl_2 (3 mL) were added DIEA (6 equiv) and HOAt (1 equiv). CIP (2 equiv) and the above HBr·H-MeVal-OMe in CH_2Cl_2 (2 mL) were added to the mixture. The reaction mixture was stirred for 3 h at 25 °C. The solvent was removed in vacuo, and the residue was extracted with AcOEt. The organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O and dried ($MgSO_4$). The crude product was isolated by silica gel column chromatography using hexane/AcOEt (7:3) to give the desired dipeptide as an oil. Model peptides **4**, **5**, **7**, and **8** were similarly synthesized, and the isolation yield and reaction time are shown in Table 1. Z-MeLeu-Val-OMe **4**: $[\alpha]^{25}_D$ −41.63 (c = 1.10, MeOH), single peak on HPLC Rt_1 23.06 min (CH_3CN 45%, 1.0 mL/min), FAB-MS, m/z 393.2403 for $[M + H]^+$ (calcd 393.2389 for $C_{21}H_{33}N_2O_5$). Z-MeVal-Val-OMe **5**: $[\alpha]^{25}_D$ −88.22 (c = 0.37, MeOH), single peak on HPLC Rt_1 15.06 min (CH_3CN 45%, 1.0 mL/min), FAB-MS, m/z 379.2217 for $[M + H]^+$ (calcd 379.2233 for $C_{20}H_{31}N_2O_5$). Z-Val-MeVal-OMe **6**: $[\alpha]^{25}_D$ −102.22 (c = 0.32, MeOH), single peak on HPLC Rt_1 23.42 min (CH_3CN 35%, 2.0 mL/min), FAB-MS, m/z 379.2243 for $[M + H]^+$ (calcd 379.2233 for $C_{20}H_{31}N_2O_5$). Z-MeLeu-MeLeu-OMe **7**: $[\alpha]^{26}_D$ −121.08 (c = 0.37, MeOH), single peak on HPLC Rt_1 24.38 min (CH_3CN 45%, 2.0 mL/min), FAB-MS, m/z 421.2714 for $[M + H]^+$ (calcd 421.2702 for $C_{23}H_{37}N_2O_5$). Z-MeLeu-MeVal-OMe **8**: $[\alpha]^{25}_D$ −135.49 (c = 0.36, MeOH), single peak on HPLC Rt_1 26.59 min (CH_3CN 42%, 2.0 mL/min), FAB-MS, m/z 407.2556 for $[M + H]^+$ (calcd 407.2546 for $C_{22}H_{35}N_2O_5$).

Corresponding dipeptides containing D-amino acid were synthesized by the same procedure. Z-(D)-MeVal-Val-OMe: $[\alpha]^{25}_D$ 78.13 (c = 0.32, MeOH), single peak on HPLC Rt_1 16.92 min (CH_3CN 45%, 1.0 mL/min), FAB-MS, m/z 379.2225 for $[M + H]^+$ (calcd 379.2233 for $C_{20}H_{31}N_2O_5$). Z-(D)-Val-MeVal-OMe: $[\alpha]^{21}_D$ −47.73 (c = 0.44, MeOH), single peak on HPLC

Rt_1 25.16 min (CH_3CN 35%, 2.0 mL/min), FAB-MS, *m/z* 379.2229 for $[M + H]^+$ (calcd 379.2233 for $C_{20}H_{31}N_2O_5$). Z-(D)-MeLeu-MeLeu-OMe: $[\alpha]^{24}_D$ 37.38 (*c* = 0.31, MeOH), single peak on HPLC Rt_1 27.03 min (CH_3CN 45%, 2.0 mL/min), FAB-MS, *m/z* 421.2704 for $[M + H]^+$ (calcd 421.2702 for $C_{23}H_{37}N_2O_5$). Z-(D)-MeLeu-MeVal-OMe: $[\alpha]^{26}_D$ −0.548 (*c* = 0.37, MeOH), single peak on HPLC Rt_1 27.54 min (CH_3CN 42%, 2.0 mL/min), FAB-MS, *m/z* 407.2542 for $[M + H]^+$ (calcd 407.2546 for $C_{22}H_{35}N_2O_5$).

Syntheses of Model Peptides 9-11 on Solid Support (Table 2). Z-MeVal-Val-OMe **9**: To 1 g (1.05 mmol) of 2-chlorotrityl chloride (ClTrt) resin were added Fmoc-Val-OH (1.07 g, 3.15 mmol) in CH_2Cl_2 (12 mL) and DIEA (0.82 mL, 4.73 mmol), and the mixture was agitated for 1 h at 25 °C. MeOH/DIEA (9:1) (4 mL) was added, and the mixture was further agitated for 30 min. The resin was filtered, washed with DMF and MeOH, and then dried in vacuo to yield 1.34 g of Fmoc-Val-O-ClTrt resin with substitution 0.687 mmol/g.

To the solution of Z-MeVal-OH (0.77 g, 2.76 mmol) in DMF (12 mL) were added HOAt (188 mg, 1.38 mmol), DIEA (1.1 mL, 6.44 mmol), and CIP (0.77 g, 2.76 mmol). The mixture was then added to the above resin swelled with DMF, and the resin solution was agitated at 25 °C. After 60 min, the resin was ninhydrin test negative. The mixture was filtered, and the resin was washed with MeOH and dried to yield 1.14 g of desired dipeptide resin.

To the Z-MeVal-Val-ClTrt resin (400 mg) obtained above was added TFA/anisole (3.8 mL/0.2 mL), and the mixture was stirred at 0 °C for 1 h. After filtration, the filtrate was concentrated in vacuo, and the residue was extracted with AcOEt. The organic layer was washed with 10% citric acid and H_2O and dried ($MgSO_4$). To the oily product (85 mg, 0.233 mmol) in MeOH (2 mL) was added 10% (trimethylsilyl)-diazomethane in *n*-hexane (2.2 mL, 1.4 mmol), and the mixture was stirred at 25 °C for 1 h under Ar atomosphere. AcOH was added to the mixture, and the solvent was removed in vacuo. The residue was extracted with AcOEt, and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O and dried ($MgSO_4$). The crude product was purified by silica gel column chromatography using hexane/AcOEt (7:3) to yield 82 mg (67%) of the dipeptide **9** as an oil: $[\alpha]^{18}_D$ −84.44 (*c* = 0.36, MeOH), single peak on HPLC Rt_1 14.70 min (CH_3CN 45%, 1.0 mL/min), FAB-MS, *m/z* 379.2280 for $[M + H]^+$ (calcd 379.2233 for $C_{20}H_{31}N_2O_5$).

Model Peptides **10** and **11** were similarly synthesized. For the estimation of coupling yield, amino acid analysis of the corresponding tripeptide resin was used for model peptide **10**, and UV absorption of the Fmoc group for model peptide **11**. Z-Val-MeVal-Gly-OMe **10**: $[\alpha]^{25}_D$ −143.78 (*c* = 0.37, MeOH), single peak on HPLC Rt_1 10.22 min (CH_3CN 35%, 2.0 mL/min), FAB-MS, *m/z* 458.2292 for $[M + Na]^+$ (calcd 458.2267 for $C_{22}H_{33}N_3O_6Na$). Fmoc-MeLeu-MeVal-Gly-OMe **11**: $[\alpha]^{20}_D$ −96.00 (*c* = 0.30, MeOH), single peak on HPLC Rt_1 23.48 min (CH_3CN 44%, 2.0 mL/min), FAB-MS, *m/z* 552.3085 for $[M + H]^+$ (calcd 552.3074 for $C_{31}H_{42}N_3O_6$).

Corresponding tripeptides containing a D-amino acid were synthesized by the same procedure. Z-(D)-Val-MeVal-Gly-OMe: $[\alpha]^{19}_D$ −63.93 (*c* = 0.31, MeOH), single peak on HPLC Rt_1 12.62 min (CH_3CN 44%, 2.0 mL/min), FAB-MS, *m/z* 458.2313 for $[M + Na]^+$ (calcd 458.2267 for $C_{22}H_{33}N_3O_6Na$). Fmoc-(D)-MeLeu-MeVal-Gly-OMe: $[\alpha]^{20}_D$ −19.41 (*c* = 0.34, MeOH), single peak on HPLC Rt_1 27.96 min (CH_3CN 44%, 2.0 mL/min), FAB-MS, *m/z* 552.3066 for $[M + H]^+$ (calcd 552.3074 for $C_{31}H_{42}N_3O_6$).

Estimation of D-Isomer Content. HPLC separation of D-isomer was carried out using YMC-Pack C8 AM-202 column (4.6 × 150 mm), which was eluted with a isocratic condition of CH_3CN in 0.1% aqueous TFA. The eluant was monitored by measuring UV absorption at 214 nm. The elution conditions for model peptides were as follows. CH_3CN (35%) with a flow rate of 1 mL/min; model peptide **5** and **9**: 2 mL of 35% CH_3CN/min; model peptide **6** and **10**: 2 mL of 44% CH_3CN/min; model peptide **11**: 2 mL of 45% CH_3CN/min; model peptide **7**. ^1H NMR signals derived form methyl ester: Z-MeLeu-MeLeu-OMe **7**; 3.49, 3.68, and 3.69 ppm. Z-(D)-MeLeu-MeLeu-OMe; 3.62, 3.67, and 3.74 ppm. Z-MeLeu-MeVal-OMe **8**; 3.43, 3.65, 3.67, and 3.69 ppm. Z-(D)-MeLeu-MeVal-OMe; 3.61, 3.67, 3.70, and 3.71 ppm.

DOV-Val-MeVal-Pro-OH (2). Fmoc-Pro-O-ClTrt resin was prepared from ClTrt resin (3.0 g, 3.90 mmol) and Fmoc-Pro-OH (0.34 g, 1.0 mmol) in CH_2Cl_2 (20 mL) in the presence of DIEA (2.7 mL, 15.6 mmol) according to the procedure described for the synthesis of model peptide on solid support to yield 3.10 g of desired starting resin with substitution 0.297 mmol/g. Piperidine (20%) in DMF was added to the resin swelled with DMF, and the mixture was agitated for 20 min at 25 °C. The N^{α}-deprotected resin was filtered and washed with DMF.

To the solution of Fmoc-MeVal-OH (0.97 g, 2.75 mmol) in DMF (20 mL) were added HOAt (0.188 g, 1.38 mmol), DIEA (1.44 mL, 8.26 mmol), and CIP (0.767 g, 2.75 mmol). The mixture was then added to the above resin, and the resin-mixture was agitated for 1.5 h. After washing the resin with DMF, the N^{α}-Fmoc group was removed by treatment with 20% piperidine/DMF as described above. Fmoc-Val-OH was then introduced by a double coupling procedure (3 h × 2) using Fmoc-Val-OH (1.56 g, 4.6 mmol), HOAt (0.31 g, 2.3 mmol), DIEA (2.24 mL, 12.85 mmol), and CIP (1.28 g, 4.59 mmol) in DMF (20 mL). The Fmoc group of the tripeptide resin was removed with 20% piperidine, and DOV was introduced by a double coupling procedure (1.5 h × 2) using DOV (0.40 g, 2.75 mmol), HOAt (0.28 g, 2.06 mmol), DIEA (1.76 mL, 10.1 mmol), and CIP (1.15 g, 4.13 mmol) in DMF (20 mL). The resin obtained was filtered, washed with DMF and MeOH, and dried to yield 3.10 g of desired tetrapeptide-O-ClTrt resin **12**.

AcOH/TFE/CH_2Cl_2 (1:1:8, 45 mL) was added to the DOV-Val-MeVal-Pro-O-ClTrt resin **12** (2.97 g), and the resin-mixture was stirred at 25 °C for 1.5 h. The mixture was filtered and the solvent of the filtrate was removed in vacuo. The resulting oily residue was purified by flash chromatography using $CHCl_3$/MeOH (5:1) followed by recrystallization with $CHCl_3$/hexane to yield 0.2 g (50%, calculated from the starting Fmoc-Pro-O-ClTrt resin) of the desired tetrapeptide **2** as a solid: $[\alpha]^{25}_D$ −160.36 (*c* = 0.28, MeOH), mp 155−157 °C, Rt_2 12.34 min [CH_3CN (10−60%/30 min)], ^1H NMR (270 MHz, $CDCl_3$) δ 0.76 (d, *J* = 6.6 Hz, 3H), 0.85−0.99 (m, 12H), 1.19 (d, *J* = 6.3 Hz, 3H), 1.76−1.89 (m) 1.93−2.25 (overlapping m) 2.23−2.40 (overlapping m) total 8H, 2.45 (br s, 6H), 3.08 (s, 3H), 3.62−3.81 (m, 2H), 4.37−4.44 (m, 1H), 4.77−4.83 (m, 1H), 5.17 (d, *J* = 10.9 Hz, 1H), 7.34 (br d, *J* = 8.6 Hz, 1H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 17.92, 18.26, 18.40, 19.16, 19.68, 24.83, 26.99, 27.82, 29.16, 30.64, 31.02, 41.76, 43.31, 47.26, 53.89, 59.59, 60.07, 74.23, 74.37, 168.89, 170.55, 172.58, 176.26. FAB-MS, *m/z* 455.3236 for $[M + H]^+$ (Calcd 455.3233 for $C_{23}H_{43}N_4O_5$).

Hiva-Phe-OBzl 15. To the solution of Hiva (0.36 g, 3.04 mmol) in THF (15 mL) were added Phe-OBzl tosylate (1.04 g, 2.43 mmol), DIEA (2.11 mL, 12.1 mmol), HOBt (2.10 g, 13.7 mmol), and CIP (1.27 g, 4.56 mmol), and the mixture was stirred at 25 °C for 3 h. The solvent was removed in vacuo and the residue was extracted with AcOEt. The organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O, dried ($MgSO_4$), and rotary evaporated. The crude product was purified by silica gel column chromatography using hexane/AcOEt (2:1) followed by precipitation with AcOEt/hexane to yield 0.75 g (87%) of **15** as a solid: $[\alpha]^{26}_D$ −38.5 (*c* = 0.5, MeOH), mp 85−86 °C, Rt_2 11.78 min [CH_3CN (40−80%/30min)], IR (KBr) 3265, 1739, 1704, 1649, 1529, 1261, 1176 cm^{-1} ^1H NMR (270 MHz, $CDCl_3$) δ 0.72 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H), 2.02−2.12 (m, 1H), 3.05 (d, *J* = 5.3 Hz, 1H), 3.11 (dd, *J* = 6.9 Hz, *J* = 6.9 Hz, 2H), 3.92 (dd, *J* = 5.3 Hz, *J* = 3.3 Hz, 1H), 4.96 (dd, *J* = 6.9 Hz, *J* = 8.2 Hz, 1H), 5.09 (d, *J* = 12.2 Hz, 1H), 5.15 (d, *J* = 12.2 Hz, 1H), 6.96 (br d, *J* = 8.2 Hz, 1H), 7.03−7.07 (m, 2H), 7.10−7.38 (m, 8H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.49, 19.03, 31.79, 38.08, 52.70, 67.35, 76.24, 127.11, 128.53, 128.62, 129.20, 135.06, 135.65, 171.61, 173.20. Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.03; H, 7.26; N. 4.13. FAB-MS, *m/z* 356.1868 for $[M + H]^+$ (Calcd 356.1862 for $C_{21}H_{26}NO_4$).

TBDMS-Hiva-Phe-OBzl 16. To the stirred solution of **15** (1.3 g, 3.66 mmol) in DMF (8 mL) were added *tert*-butyldimethylsilyl chloride (1.1 g, 7.32 mmol) and imidazole (1.0 g, 14.63 mmol), and the mixture was stirred at 25 °C for 1 h. AcOEt/H_2O (1:1, 40 mL) was added to the mixture, and the organic layer was washed with H_2O and dried ($MgSO_4$). The solvent was removed in vacuo, and the product was purified by silica gel column chromatography using hexane/AcOEt (4:1) to yield 1.76 g (96%) of **16** as an oil: $[\alpha]^{26}_D$ −46.9 (*c* = 0.74, MeOH), Rt_2 22.10 min [CH_3CN (60−80%/30min)], IR (KBr) 1743, 1681, 1506, 1253, 1178, 1051 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ −0.05 (s, 3H), 0.03 (s, 3H), 0.75 (d, *J* = 6.9 Hz, 3H), 0.85 (s, 9H), 0.89 (d, *J* = 6.9 Hz, 3H), 1.98−2.12 (m, 1H), 3.04 (dd, *J* = 5.9 Hz, *J* = 13.9 Hz, 1H), 3.14 (dd, *J* = 5.9 Hz, *J* = 13.9 Hz, 1H), 3.93 (d, *J* = 3.3 Hz, 1H), 4.99 (ddd, *J* = 5.9 Hz, *J* = 5.9 Hz, *J* = 8.9 Hz, 1H), 5.09 (d, *J* = 12.2 Hz, 1H), 5.15 (d, *J* = 12.2 Hz, 1H), 6.94 (br d, *J* = 8.9 Hz, 1H), 7.04−7.10 (m, 2H), 7.18−7.38 (m, 8H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.25, −5.03, 16.12, 17.90, 19.26, 25.73, 32.72, 38.53, 52.29, 67.10, 76.58, 127.11, 128.46, 128.59, 128.66, 129.22, 135.16, 135.63, 171.12, 172.97. FAB-MS, *m*/*z* 470.2740 for $[M + H]^+$ (Calcd 470.2727 for $C_{27}H_{40}NO_4Si$).

TBDMS-Hiva-Phe-OH 17. To the stirred solution of **16** (1.346 g, 2.87 mmol) in MeOH (15 mL) was added catalytic amount of 10% Pd−C, and the mixture was stirred at 25 °C for 1 h under H_2 atomosphere. Pd−C (10%) was removed by filtration, and the filtrate was rotary evaporated. The product was recrystallized from hexane to yield 1.0 g (91%) of **17** as a solid: $[\alpha]^{25}_D$ −21.0 (*c* = 0.38, MeOH), mp 60−61 °C, Rt_2 21.70 min [CH_3CN (40−80%/30 min)], IR (KBr) 1730, 1646, 1625, 1541, 1253, 1049 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ −0.02 (s, 3H), 0.04 (s, 3H), 0.71 (d, *J* = 6.9 Hz, 3H), 0.84 (s, 9H), 0.86 (d, *J* = 6.9 Hz, 3H), 1.95−2.08 (m, 1H), 3.16 (d, *J* = 6.3 Hz, 2H), 3.98 (d, *J* = 3.3 Hz, 1H), 4.96 (dt, *J* = 6.3 Hz, *J* = 8.6 Hz, 1H), 6.97 (d, *J* = 8.6 Hz, 1H), 7.15−7.32 (m, 5H), 9.10 (br s, 1H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.32, −5.00, 16.01, 17.89, 19.25, 25.71, 32.65, 37.93, 52.22, 76.57, 127.26, 128.77, 129.25, 135.54, 174.01, 175.07. Anal. Calcd for $C_{20}H_{33}NO_4Si$: C, 63.29; H, 8.76; N, 3.69. Found: C, 63.17; H, 8.71; N. 3.91. FAB-MS, *m*/*z* 380.2266 for $[M + H]^+$ (Calcd 380.2257 for $C_{20}H_{34}NO_4Si$).

TBDMS-(*S*)-Hiva-(*S*)-Dpy 18. To the stirred solution of **17** (0.50 g, 1.31 mmol) in THF (5 mL) in an ice bath was added DIEA (0.91 mL, 5.22 mmol), Meldrum's ester (0.21 g, 1.44 mmol), CIP (0.54 g, 1.94 mmol), and DMAP (0.08 g, 0.65 mmol). The mixture was stirred at 25 °C for 4 h, and the solvent was removed in vacuo. The residue was extracted with AcOEt, and the organic layer was washed with 10% citric acid, H_2O, dried ($MgSO_4$), and rotary evaporated. The product was dissolved in AcOEt (80 mL), and the mixture was refluxed for 1 h. The solvent was removed in vacuo, and the residue was dissolved in THF (5 mL). To this solution in an ice bath were added MeOH (0.08 mL, 2.00 mmol), Ph_3P (0.52 g, 2.00 mmol), and diethyl diazocarboxylate (0.31 mL, 2.00 mmol). The mixture was stirred at 25 °C for 2 h, and the solvent was removed in vacuo. The product was purified by flash chromatography using $CHCl_3$ to yield 0.25 g (46%) of **18** as a solid: $[\alpha]^{24}_D$ 189.5 (*c* = 0.51, MeOH), mp 104−106 °C, Rt_1 26.54 min [CH_3CN 55%, 2.0 mL/min], IR (KBr) 1720, 1689, 1631, 1460, 1384, 1251, 1147, 1070 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.14 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H), 0.98 (s, 9H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.88−2.05 (m, 1H), 3.18 (dd, *J* = 3.0 Hz, *J* = 13.8 Hz, 1H), 3.62 (dd, *J* = 5.3 Hz, *J* = 13.8 Hz, 1H), 3.80 (s, 3H), 4.76 (dd, *J* = 3.0 Hz, *J* = 5.3 Hz, 1H), 4.80 (s, 1H), 5.27 (d, *J* = 3.0 Hz, 1H), 6.99−7.07 (m, 2H), 7.18−7.23 (m, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −4.91, −4.44, 15.62, 18.40, 20.05, 25.91, 32.02, 35.58, 58.31, 60.41, 75.97, 94.88, 127.02, 128.16, 129.67, 134.55, 169.93, 173.85, 178.29. Anal. Calcd for $C_{23}H_{35}NO_4Si$: C, 66.15; H, 8.45; N, 3.35. Found: C, 65.85; H, 8.42; N. 3.67. FAB-MS, *m*/*z* 418.2421 for $[M + H]^+$ (Calcd 418.2414 for $C_{23}H_{36}NO_4Si$).

A small amount of diastereoisomer, TBDMS-(S)-Hiva-(R)-Dpy, was recovered from the flash chromatography: yield 50 mg (9%), $[\alpha]^{22}_D$ −265.5 (*c* = 0.60, MeOH), mp 99−101 °C, Rt_1 27.86 min [CH_3CN 55%, 2.0 mL/min], IR (KBr) 1720, 1691, 1633, 1460, 1384, 1245, 1145, 1072 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.01 (s, 3H), 0.03 (s, 3H), 0.94 (s, 9H), 0.98 (d, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H), 1.92−2.18 (m, 1H), 3.17 (dd, *J* = 3.0 Hz, *J* = 14.0 Hz, 1H), 3.45 (dd, *J* = 5.3 Hz, *J* = 14.2 Hz, 1H), 3.82 (s, 3H), 4.82 (s, 1H), 4.91 (dd, *J* = 3.0 Hz, *J* = 5.3 Hz, 1H), 5.14 (d, *J* = 2.0 Hz, 1H), 6.96−7.01 (m, 2H), 7.17−7.22 (m, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.32, −4.83, 15.47, 18.42, 20.56, 25.86, 32.02, 34.86, 58.35, 59.69, 76.35, 94.66, 127.06, 128.07, 129.59, 134.34, 169.43, 173.84, 178.18. Anal. Calcd for $C_{23}H_{35}NO_4Si$: C, 66.15; H, 8.45; N, 3.35. Found: C, 65.91; H, 8.29; N. 3.52. FAB-MS, *m*/*z* 418.2408 for $[M + H]^+$ (Calcd 418.2414 for $C_{23}H_{36}NO_4Si$).

(*S*)-Hiva-(*S*)-Dpy 19. To the stirred solution of **18** (145 mg, 0.347 mmol) in CH_2Cl_2 (4 mL) in an ice bath was added TFA (0.4 mL). The mixture was stirred at 25 °C for 3 h, and the solvent was removed in vacuo. The oily residue was purified by flash chromatography to yield 102 mg (97%) of **19** as an oil: $[\alpha]^{26}_D$ 259.0 (*c* = 0.40, MeOH), Rt_2 7.26 min [CH_3CN (40−80%/30 min)], IR (KBr) 3500, 1730, 1635, 1305 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.87 (d, *J* = 6.9 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 2.06−2.18 (m, 1H), 3.12 (dd, *J* = 3.0 Hz, *J* = 13.9 Hz, 1H), 3.60 (d, *J* = 8.3 Hz, 1H), 3.65 (dd, *J* = 5.0 Hz, *J* = 13.9 Hz, 1H), 3.84 (s, 3H), 4.77 (dd, *J* = 3.3 Hz, *J* = 8.3 Hz, 1H), 4.82 (s, 1H), 4.83 (dd, *J* = 3.0 Hz, *J* = 5.0 Hz, 1H), 6.92−7.00 (m, 2H), 7.18−7.25 (m, 3H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.35, 19.95, 30.44, 34.66, 58.53, 60.05, 75.61, 94.82, 127.24, 128.26, 129.61, 133.71, 169.81, 174.25, 178.45. FAB-MS, *m*/*z* 304.1553 for $[M + H]^+$ (Calcd 304.1549 for $C_{17}H_{22}NO_4$).

Boc-Pro-Hiva-Dpy 20. To the stirred solution of Boc-Pro-OH (83 mg, 0.386 mmol) in CH_2Cl_2 (6 mL) in an ice bath were added DIEA (0.224 mL, 1.29 mmol), CIP (0.215 g, 0.771 mmol), **19** (78 mg, 0.257 mmol), and DMAP (16 mg, 0.129 mmol). The mixture was stirred at 25 °C for 10 h, and the solvent was removed in vacuo. The residue was extracted with AcOEt, and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O, dried ($MgSO_4$), and rotary evaporated. The product was purified by flash chromatography using $CHCl_3$/AcOEt (10:1) to yield 0.12 g (92%) of **20** as a solid: $[\alpha]^{27}_D$ 117.8 (*c* = 0.51, MeOH), mp 152−154 °C, Rt_2 21.26 min [CH_3CN (40−80%/30 min)], IR (KBr) 1751, 1732, 1705, 1681, 1631, 1456, 1394, 1305 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) two conformers in the ratio of 3:1, δ 0.95 (d, *J* = 6.9 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 1.46, 1.48 (s, 9H), 1.83−2.05 (m, 2H), 2.18−2.35 (overlapping m, 3H), 3.06 (dd, *J* = 3.3 Hz, *J* = 13.9 Hz, 1H), 3.25−3.65 (overlapping m, 3H), 3.75, 3.78 (s, 3H), 4.40 (t, *J* = 6.1 Hz) 4.53 (dd, *J* = 2.6 Hz, *J* = 8.3 Hz, total 1H), 4.73−4.83 (overlapping m, 2H), 5.87, 5.93 (each d, *J* = 2.6 Hz, 1H), 7.09−7.29 (m, 5H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.83, 19.83, 23.47, 28.32, 28.50, 30.75, 34.93, 46.34, 58.35, 58.72, 59.98, 79.60, 79.89, 94.73, 126.97, 128.25, 129.92, 133.98, 154.03, 169.14, 169.58, 172.67, 178.42. Anal. Calcd for $C_{27}H_{36}N_2O_7$: C, 64.78; H, 7.25; N, 5.60. Found: C, 64.60; H, 7.29; N. 5.92. FAB-MS, *m*/*z* 501.2616 for $[M + H]^+$ (Calcd 501.2601 for $C_{27}H_{37}N_2O_7$).

Dolastatin 15 (1). To the stirred solution of **20** (85 mg, 0.170 mmol) in CH_2Cl_2 (4 mL) in an ice bath was added TFA/anisole (0.85 mL-85 μL), and the mixture was stirred at 25 °C for 1 h. The TFA was removed by azeotroping with hexane (three times) to yield **3** as a light yellow oil.

To the stirred solution of **2** (116 mg, 0.255 mmol) in CH_2Cl_2 (2.5 mL) in an ice bath were added DIEA (0.136 mL, 0.78 mmol), HOAt (26 mg, 0.19 mmol), CIP (53 mg, 0.19 mmol), and the above obtained H-Pro-Hiva-Dpy **3** in CH_2Cl_2/DIEA (2.5 mL/72 μL). The mixture was stirred at 25 °C for 2 h, and the solvent was removed in vacuo. The residue was extracted with AcOEt, and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O, dried ($MgSO_4$), and rotary evaporated. The product was purified by flash chromatography using hexane/acetone (3:2) followed by reprecipitation with AcOEt/hexane to yield 119 mg (84%) of **1** as a solid: $[\alpha]^{26}_D$ −80.7 (*c* = 0.56, MeOH), mp 177−179 °C, Rt_2 9.08 min [CH_3CN (40−70%/30 min)], IR (KBr) 3423, 1735, 1629, 1448, 1309, 1191 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.78 (d, *J* = 6.6 Hz, 3H), 0.92−1.09 (overlapping m, 21H), 1.80−2.43 (overlapping m, 12H),

2.26 (s, 6H), 2.47 (d, J = 6.3 Hz, 1H), 3.05 (dd, J = 3.3 Hz, 13.8 Hz, 1H), 3.18 (s, 3H), 3.54 (dd, J = 4.3 Hz, J = 13.8 Hz, 1H), 3.57−3.65 (m, 1H), 3.75 (s, 3H), 3.72−3.94 (m, 3H), 4.62−4.67 (m, 1H), 4.73 (s, 1H), 4.76−4.87 (m, 3H), 5.14 (d, J = 10.8 Hz, 1H), 5.90 (d, J = 2.3 Hz, 1H), 6.90 (br d, J = 9.2 Hz, 1H), 7.13−7.22 (m, 5H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 15.81, 17.70, 18.15, 18.54, 19.16, 19.57, 19.86, 20.14, 24.64, 24.73, 27.31, 27.69, 28.39, 28.57, 28.88, 30.73, 31.09, 34.88, 42.87, 46.41, 47.85, 53.71, 58.09, 58.29, 58.35, 59.23, 59.89, 76.37, 77.86, 94.75, 127.01, 128.16, 130.01, 134.16, 169.14, 169.34, 169.49, 170.26, 171.50, 171.68, 173.01, 178.22. Anal. Calcd for $C_{45}H_{69}N_6O_9$: C, 64.57; H, 8.19; N, 10.04. Found: C, 64.47; H, 7.98; N. 9.89. FAB-MS, m/z 837.5132 for $[M + H]^+$ (Calcd 837.5126 for $C_{45}H_{69}N_6O_9$).

Acknowledgment. We thank Professor Joël Poncet for sending the ^{1}H NMR spectrum of his synthetic dolastatin **15**.

Supporting Information Available: HPLC and ^{1}H NMR analysis for the estimation of D-isomer content and ^{1}H and ^{13}C NMR spectra for compound **1**, **2**, and **15**−**20** (21 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.

JO981055A