Synthesis and application of L-*N*-Boc-*N*-methyl-β-hydroxyvaline in the preparation of a depsipeptide[1]

James E. Dettwiler, Laurent Bélec, and William D. Lubell

Abstract: Enantiopure (>99% ee) L-*N*-*tert*-butyloxycarbonyl-*N*-methyl-β-hydroxyvaline (**2**) was synthesized in six steps and 43% overall yield from D-serine methyl ester (**5**). Methyl (4*S*)-*N*-(9-phenylfluoren-9-yl)-oxazolidine-4-carboxylate (**7**) was prepared in two steps and 73% yield by *N*-phenylfluorenation of **5** followed by cyclization of *N*-(PhF)amino alcohol **6** with formaldehyde and catalytic *p*-toluenesulfonic acid (PhF = 9-phenylfluoren-9-yl). The addition of MeLi to oxazolidine carboxylate **7** produced the tertiary alcohol **8** in 91% yield. Oxazolidines **8** equilibrated with oxazolidine **9** under acidic conditions. Reduction of pure **8** or the mixture of oxazolidines **8** and **9** with $NaCNBH_3$ and hydrochloric acid in anhydrous dioxane afforded *N*-methyl amino diol **11** in 86%–92% yields. Attempts to selectively oxidize *N*-(PhF)amino diol **11** were unsuccessful; however, hydrogenation of **11** in the presence of di-*tert*-butyl dicarbonate gave the corresponding *N*-(Boc)amino diol **12** in 82% yield. Selective oxidation of diol **12** was performed using a cocktail containing TEMPO free radical, $NaClO_2$, and NaOCl to give L-*N*-Boc-*N*-methyl-β-hydroxyvaline (**2**) in 87% yield. Coupling of β-hydroxyvaline **2** and (*S*)-2-hydroxy-3-methylbutanoate (**15**) was accomplished by using the methodology of Mitsunobu to provide depsipeptide Boc-(*S*)-HOMeVal-(*R*)-Hmb (**4**) for use as a building block in the synthesis of the cyclic antifungal depsipeptide aureobasidin B.

Key words: *N*-methylated amino acid, serine, depsipeptide, aureobasidin.

Résumé : La L-*N*-*tert*-butyloxycarbonyl-*N*-méthyl-β-hydroxyvaline (**2**) a été synthétisée sous sa forme énantiopure (>99 % ee) par un protocole comprenant six étapes à partir de l'ester méthylique de la D-sérine (**5**) avec un rendement total de 43 %. Le méthyle (4*S*)-*N*-(9-phénylfluoren-9-yl)-oxazolidine-4-carboxylate (**7**) a été préparé en deux étapes avec un rendement de 73 % par une *N*-phénylfluorénation de **5**, suivie par une cyclisation de l'alcool *N*-(PhF)amino **6** avec du formaldéhyde en présence catalytique d'acide *p*-toluène sulfonique (PhF = 9-phenylfluoren-9-yl). L'addition de MeLi sur l'oxazolidine carboxylate **7** a donné l'alcool tertiaire **8** avec un rendement de 91 %. L'oxazolidine **8** a équilibré avec l'oxazolidine **9** dans des conditions acides. La réaction de **8** pur ou du mélange des oxazolidines **8** et **9** avec du $NaCNBH_3$ et de l'acide chlorhydrique dans du dioxane anhydre a donné l'amino diol *N*-méthylé **11** avec des rendements de 86 % – 92 %. Les essais d'oxydation du *N*-(PhF)amino diol **11** ont échoué, cependant, le *N*-(Boc)amino diol **12** a été obtenu à partir de l'hydrogénation de **11** en présence de di-*tert*-butyle dicarbonate avec un rendement de 82 %. L'oxydation sélective du diol **12** a été effectuée avec un mélange du radical libre TEMPO, $NaClO_2$ et NaOCl pour donner la L-*N*-Boc-*N*-méthyl-β-hydroxyvaline (**2**) avec un rendement de 87 %. Le couplage entre la β-hydroxyvaline **2** et le (*S*)-2-hydroxy-3-méthylbutanoate (**15**) a été effectué avec la méthode de Mitsunobu pour donner le depsipeptide Boc-(*S*)-HOMeVal-(*R*)-Hmb (**4**) qui est un fragment pour la synthèse de l'auréoabsidine B, un depsipeptide antifongique et cyclique.

Mots clés : acide aminé *N*-méthylé, serine, depsipeptide, auréobasidine.

Introduction

L-*N*-Methyl-β-hydroxyvaline (**1**) is an important component of biologically active peptides such as the anti-HIV antibiotic luzopeptin (1) as well as members of the aureobasidin (2) family of antifungal peptides (Fig. 1). Featuring hydroxyl, amino, carboxylate, and *N*-alkyl substituents as well as a quaternary carbon, **1** is a synthetic challenge for which no efficient route presently exists. Methods to prepare L-*N*-methyl-β-hydroxyvaline (**1**) have usually required multiple steps and have often resulted in low enantiomeric purity (3–6). First synthesized in racemic form (3) and resolved, L-*N*-Boc-*N*-methyl-β-hydroxyvaline (**2**) was most recently prepared from D-*N*-Boc-serine methyl ester in eight steps with

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Scheme 1. Synthesis of L-*N*-*tert*-butyloxycarbonyl-*N*-methyl-β-hydroxyvaline (**2**).

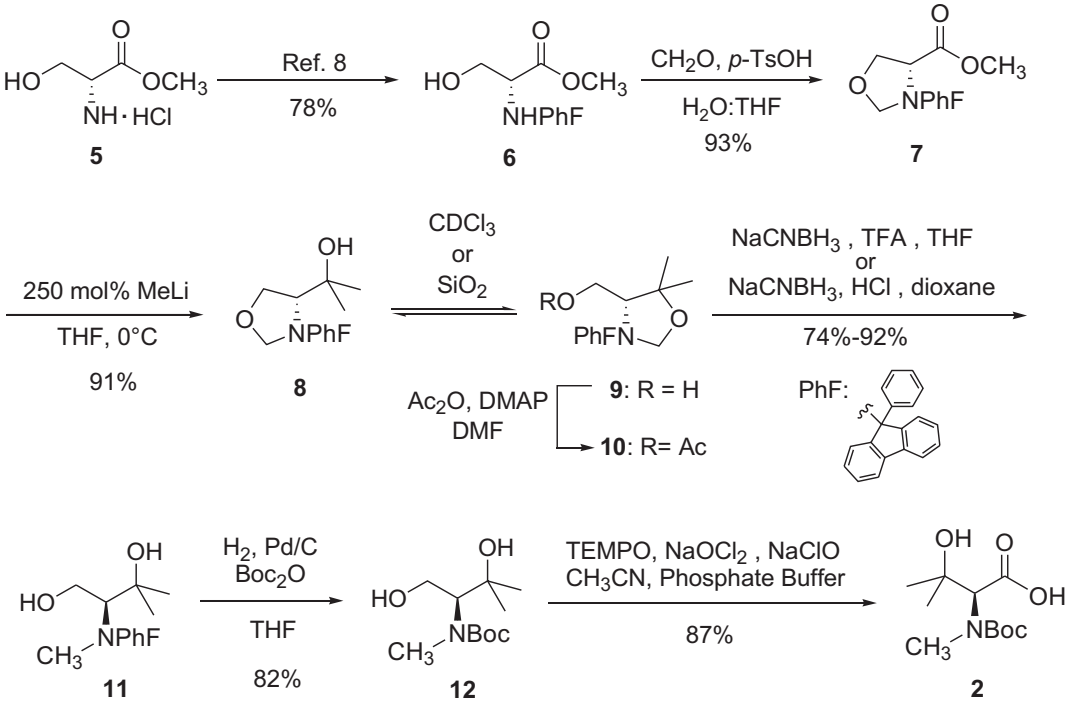
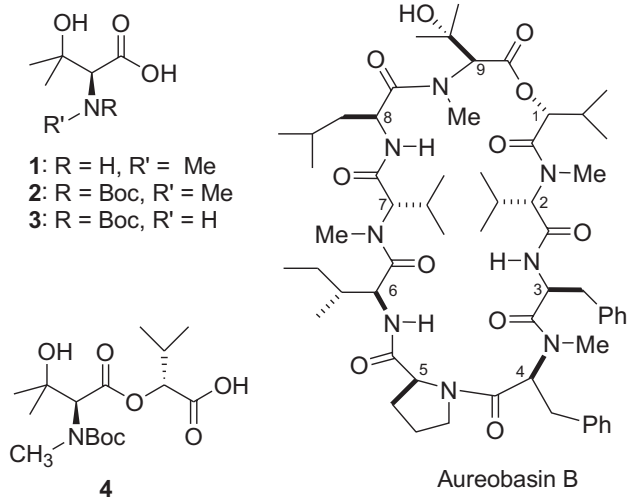


Fig. 1. L-Hydroxymethyl-*N*-methyl-β-hydroxyvaline (**1**), L-*N*-*tert*-butyloxycarbonyl-*N*-methyl-β-hydroxyvaline (**2**), L-*N*-*tert*-butyloxycarbonyl-β-hydroxyvaline (**3**), depsipeptide Boc-(*S*)-HOMeVal-(*R*)-Hmb (**4**), and aureobasidin B.



an overall yield of 66%, albeit with an enantiomeric purity of 90% (4, 5). Racemization during this synthesis was likely due to the employment of a configurationally labile α-amino aldehyde intermediate (7). Considering the requirement of enantiopure building blocks for effective peptide synthesis, alternative methodology is still necessary for synthesizing enantiopure **1** in protected form.

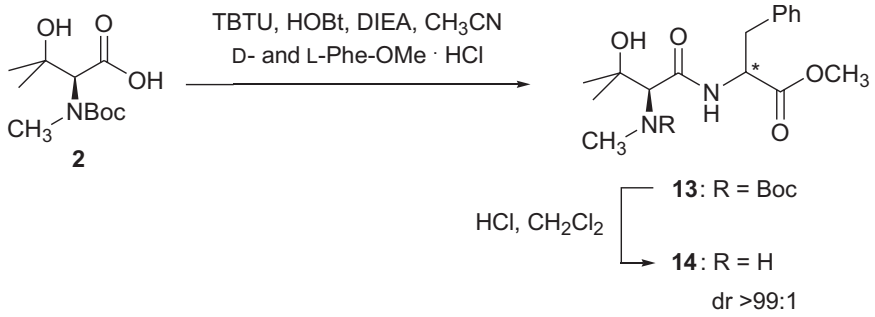
We have recently reported a two-step approach for synthesizing L-*N*-Boc-β-hydroxyvaline (**3**) from D-*N*-Boc-serine methyl ester (8), which involved treatment with MeMgBr followed by selective catalyzed TEMPO oxidation (9, 10) Direct *N*-methylation of L-*N*-Boc-β-hydroxyvaline (**3**) was, however, unsuccessful using various methods. Protocols using CH_3I and $(CH_3)_2SO_4$ as methylating agents in the presence of various bases (NaH, Ag_2O, Ag_2CO_3, or *n*-BuLi) were unsuccessful and either resulted in alcohol methylation or recovered starting material (11). For example, treatment of **3** with CH_3I and NaH in THF gave L-*N*-Boc-β-methoxyvaline in 65% yield. Attempts to convert **3** to an oxazolidine or oxazolidinone were also unsuccessful and resulted predominantly in recovered starting material. Instead of trying a selective *O*-protection, *N*-alkylation, and *O*-deprotection strategy, we decided to develop an alternative route to obtain L-*N*-Boc-*N*-methyl-β-hydroxyvaline (**2**) in enantiopure form (Scheme 1) (12). Our protocol involves a six-step synthesis from D-serine methyl ester (**5**) in 43% overall yield. This route employs *N*-(9-phenylfluoren-9-yl)serine methyl ester (8) as a chiral educt to deliver enantiopure **2** (>99% ee).

Results and discussion

The starting material D-*N*-(PhF)serine methyl ester (**6**) was synthesized according to the literature method by phenylfluorenation of D-serine methyl ester (Scheme 1) (8). Treatment of amino alcohol **6** with formaldehyde and catalytic *p*-toluenesulfonic acid in THF at room temperature afforded oxazolidine carboxylate ester **7** in 93% yield (13). The quaternary carbon was constructed using our best conditions by adding 250 mol% of MeLi to a solution of **7** in THF at 0 °C. Tertiary alcohol **8** could be isolated after filtration through a plug of silica gel; however, on prolonged exposure to silica gel, **8** equilibrated with oxazolidine primary alcohol **9**.

In chloroform, oxazolidine **8** existed in equilibrium with oxazolidine primary alcohol **9**, the latter of which could be selectively acetylated. Treatment of a 1:1 mixture of **8** and **9** with acetic anhydride and catalytic 4-dimethylaminopyridine (DMAP) in chloroform gave **8** and ester **10**. Separation of

Scheme 2. Enantiomeric purity of L-*N*-*tert*-butyloxycarbonyl-*N*-methyl-β-hydroxyvaline (**2**).



the mixture by chromatography on silica gel gave a new equilibrium and the three compounds (**8**–**10**) were isolated in pure form. In $CDCl_3$, pure **8** was found to equilibrate to a 1:1 mix of **8** and **9** after 12 h. Residual acid in chloroform may be responsible for this transformation because spectra of pure **8** and **9** could be obtained in DMSO-d_6. The assignment of the structures of oxazolidine alcohols **8** and **9** was made based on their ^{1}H NMR spectra in DMSO-d_6 in comparison to acetate **10**. Of particular note, large differences in the chemical shifts for the signals of the diastereotopic methylene and methyl protons were indicative of groups held in fixed environments by the oxazolidine ring. In the case of the primary alcohol oxazolidine **9**, the difference in the chemical shifts of the diastereotopic signals for the constrained methyl groups were larger (Δδ = 162.4 Hz) than those observed for tertiary alcohol oxazolidine **8** (Δδ = 94.6 Hz). The chemical shift differences for the diastereotopic methylene protons were greater (Δδ = 71.1 Hz) for the tertiary alcohol oxazolidine **8** relative to those of the primary alcohol oxazolidine **9** (Δδ = 26.6 Hz). Acetate **10** exhibited a similar difference in the chemical shifts of its diastereotopic methyl singlets (Δδ = 143.3 Hz) and methylene protons (Δδ = 28.2 Hz) as was seen for the primary alcohol oxazolidine **9**. Moreover, relative to the methylene signals of **9**, those for **10** were observed to be downfield shifted indicative of acylation of the primary alcohol.

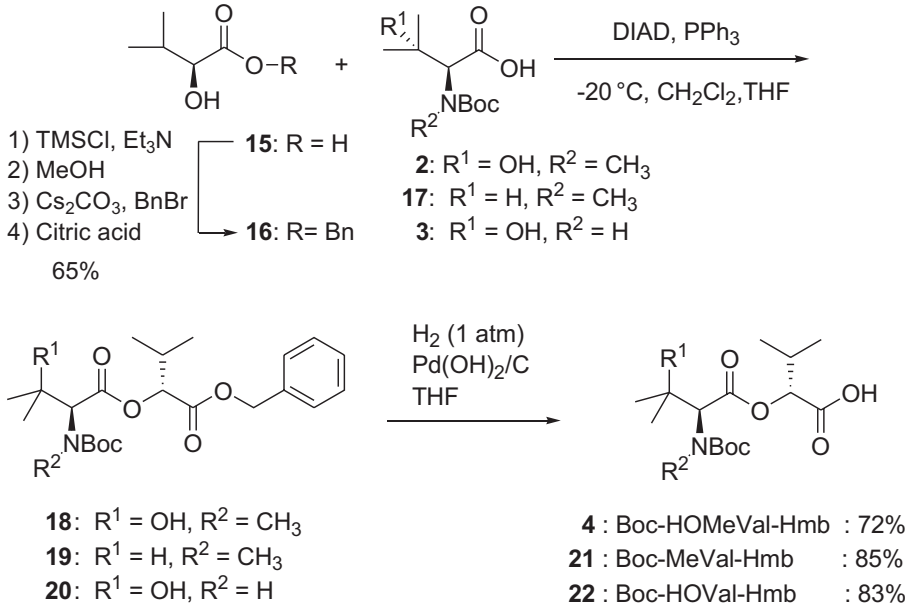
The conversion of oxazolidines **8** and **9** to *N*-methyl diol **11** was accomplished by treatment with $NaCNBH_3$ under acidic conditions (13). Two conditions were tested. In the first condition, oxazolidine **8** at 0 °C was treated with $NaCNBH_3$ in anhydr. THF containing trifluoroacetic acid to give *N*-methylamino diol **11** in 74% yield after purification. In the second condition, oxazolidine **8** in 0.1 mol/L HCl in dioxane was treated with $NaCNBH_3$ to give *N*-methylamino diol **11** in 86% yield after purification. Employing these later conditions to reduce a mixture of oxazolidines **8** and **9** gave amino diol **11** in similar yield. In the ^{1}H NMR spectrum of *N*-PhF-*N*-methylamino diol **11** in $CDCl_3$, a doubling of signals was observed suggesting an equilibrium between two hydrogen-bonded forms. The doubling of signals could be avoided by recording the spectrum of **11** in deuterated methanol containing a trace of trifluoroacetic acid.

Selective oxidation of *N*-PhF-*N*-methylamino diol **11** was unsuccessful using a wide variety of oxidation methods presumably because of the oxidation of the tertiary amine. On the other hand, exchanging *N*-PhF to *N*-Boc protection could be effected by hydrogenation in the presence of di-*tert*-butyl dicarbonate and palladium hydroxide to give the *N*-(Boc)amino diol **12** in 82% yield (14). As in our synthesis of β-hydroxyvaline (10), selective primary alcohol oxidation was accomplished by treating *N*-(Boc)amino diol **12** with TEMPO (2,2,6,6-tetramethyl-1-piperidyloxy) free radical, sodium chlorite, and sodium hypochlorite in a sodium phosphate buffered acetonitrile solution (15). L-*N*-Boc-*N*-Methyl-β-hydroxyvaline (**2**) was then isolated in pure form after aqueous extraction in 87% yield.

To ascertain if any racemization had occurred during the synthesis, the enantiomeric purity of **2** was investigated by coupling to L- and D-Phe-OMe·HCl using benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (16), 1-hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIEA) in acetonitrile to produce dipeptides **13**, which existed as mixtures of carbamate isomers (Scheme 2). The *N*-Boc protecting group of the crude product was removed by bubbling a stream of HCl gas into a solution of **13** in CH_2Cl_2. The 400 MHz ^{1}H NMR spectrum of crude dipeptide (*R*)-**14** was examined during incremental additions of (*S*)-**14** in CD_3OD. Measurement of the signals for the diastereomeric methyl amine singlets at 2.52 and 2.04 ppm demonstrated dipeptide (*S*)-**14** to be of >99% diastereomeric purity. Hence, L-*N*-Boc-*N*-methyl-β-hydroxyvaline (**2**) is considered to be of the same high enantiomeric purity.

The depsipeptide Boc-(*S*)-HOMeVal-(*R*)-Hmb (**4**) was targeted to serve as a building block for the synthesis of analogs of the cyclic antifungal depsipeptide aureobasidin B (17). The preparation of depsipeptides **4**, **21**, and **22** was accomplished by the coupling of the corresponding *N*-Boc protected amino acid (**2**, **3**, and **17**) and (*S*)-2-hydroxy-3-methylbutanoic benzyl ester (**16**) using the Mitsunobu reaction, followed by benzyl ester deprotection (Scheme 3). Benzyl ester **16** was prepared from (*S*)-2-hydroxy-3-methylbutanoic acid (**15**), which was synthesized with retention of configuration via a double inversion from L-valine using the literature procedure in 52% overall yield (18). Because direct alkylation of **15** resulted in mixtures of ether and ester products, a four-step, one-pot procedure was developed to prepare the benzyl ester **16**. First, the hydroxyl and carboxylate groups were both protected with trimethylsilyl (TMS) groups. Then, the carboxylic acid was liberated selectively using MeOH and alkylated with benzyl bromide and Cs_2CO_3 to provide the silylether benzyl ester, which on treatment with citric acid gave α-hydroxy ester **16** in 65% overall yield. The conditions for formation of the depsi bonds were first optimized with Boc-MeVal (**17**) and Boc-

Scheme 3. Synthesis of the depsipeptides **4**, **21**, and **22** (1 atm = 101.325 kPa).



HOVal (**3**). Optimal conditions were then applied to Boc-HOMeVal (**2**). Formation of the depsipeptide bond was performed by an initial reaction of the secondary alcohol of **16** with triphenylphosphine and diisopropyldiazodicarboxylate (DIAD) in dichloromethane, followed by S_N2 displacement of the phosphine intermediate on addition of the *N*-Boc protected amino acid (**2**, **3**, and **17**) to form depsipeptides **18**–**20** and triphenylphosphine oxide (19). Crude depsipeptides **18**–**20** were isolated by chromatography on silica gel to remove the triphenylphosphine oxide. Hydrogenolysis by using $Pd(OH)_2$ on carbon cleaved the benzyl ester affording acids **4**, **21**, and **22**, in 72%, 85%, and 83% overall yields, respectively (14).[3]

Conclusion

Enantiopure (>99%) L-*N*-*tert*-butyloxycarbonyl-*N*-methyl-β-hydroxyvaline (**2**) was synthesized in six steps and 43% overall yield from D-serine methyl ester (**5**). Application of **2**, as well as Boc-MeVal and Boc-HOVal (**3**), in Mitsunobu reactions with (*S*)-benzyl-2-hydroxy-3-methylbutanoate (**16**) has provided three depsipeptides: Boc-(*S*)-HOMeVal-(*R*)-Hmb (**4**), Boc-(*S*)-MeVal-(*R*)-Hmb (**21**), and Boc-(*S*)-HOVal-(*R*)-Hmb (**22**). These building blocks are presently being employed in our program on antimicrobial peptide synthesis (17, 20).

Experimental section

General

For reactions performed under anhydrous conditions, glassware was either oven- or flame-dried and the reaction was run under a positive pressure of argon or nitrogen. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone, CH_2Cl_2 from P_2O_5, dioxane from sodium. Triethylamine (Et_3N) was distilled from ninhydrin then CaH_2. The 0.2 N HCl – dioxane solution was prepared by passing a stream of HCl gas bubbles through dry dioxane and the concentration of the solution was determined by titration of 1 mL aliquots diluted with 1 mL H_2O using a 0.1 N standard NaOH solution and phenolphtalein as the indicator. Chromatography was performed using 230–400 mesh silica gel. Amines were revealed with ninhydrin on TLC plates; Boc groups were removed by dipping the plates in TFA before exposure to ninhydrin. Unless otherwise noted, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD at 300/75 or 400/100 MHz. Chemical shifts (δ) are reported in parts per million (ppm) downfield of internal tetramethylsilane ($(CH_3)_4Si$) or referenced to the residual solvent peak ($CHCl_3$ δ 7.26, 77.16 ppm; methanol δ 3.31, 49.00 ppm). Coupling constants are reported in Hz. Chemical shifts of PhF aromatic carbons are omitted for clarity in the ^{13}C NMR spectra. HR-MS (EI and FAB) were obtained by the Université de Montréal Mass Spectrometry facility. Specific rotations are reported as follows: $[\alpha]_D^{20}$, concentration (*c* in g/100 mL), and solvent. Analytical LC–MS was performed on an inverse phase column (Anal Waters C18, 250 × 4.6 mm) with a flow rate of 0.5 mL/min. A volume of 20 µL of sample with a concentration of 1 mg/mL was injected. Preparative reversed-phase HPLC was performed on an inverse phase column (Prep Prevail C18, 250 × 22 mm) with a flow rate of 15 mL/min: elution condition A, gradient 20% CH_3CN with 0.1% TFA–H_2O with 0.1% TFA to 80% CH_3CN with 0.1% TFA–H_2O with 0.1% TFA over 20 min; condition B, 70% CH_3CN with 0.1% TFA–H_2O with 0.1% TFA.

Methyl (4*S*)-*N*-(9-phenylfluoren-9-yl)-oxazolidine-4-carboxylate (7)

A solution of *N*-(PhF)serine methyl ester (**6**, 11.50 g, 32 mmol, prepared according to ref. 8), *p*-toluenesulfonic

[3] Supplementary data for this article are available on the Web site or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada. DUD 3681. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

acid (6 mol%, 331 mg, 1.92 mmol), and a 37% aqueous formaldehyde solution (1500 mol%, 39 mL, 480 mmol) in THF (320 mL) was stirred for 24 h at room temperature, washed with a solution of satd. $NaHCO_3$ (2 × 200 mL) and brine, dried with $MgSO_4$, filtered and evaporated to a solid residue. Chromatography on silica gel using 10% EtOAc in hexanes as the eluant gave a white solid (11.05 g, 93%). TLC R_f = 0.41 (20% EtOAc in hexanes); mp 106 to 107 °C. $[\alpha]_D^{20}$ –267.3 (*c* 1.0 $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.30 (t, 1H, *J* = 6.8), 3.54 (s, 3H), 3.61 (d, 2H), 4.73 (d, 1H, *J* = 6.4), 4.93 (d, 1H, *J* = 6.4), 7.22–7.70 (m, 13H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 52.0, 60.8, 69.1, 85.1, 119.8, 173.6. HR-MS calcd. for $C_{24}H_{22}NO_3$ ($[M + H]^+$): 372.1600; found: 372.1582.

(4*S*)-4-(1-Hydroxymethylethyl)-3-(9-phenylfluoren-9-yl)-oxazolidine (8)

To a solution of oxazolidine **7** (3.72 g, 10 mmol) in THF (182 mL) at 0 °C, a solution of 1.4 mol/L MeLi in Et_2O (250 mol%, 18 mL, 25 mmol) was added dropwise. The reaction mixture was stirred for 2 h at 0 °C and partitioned between equal volumes of EtOAc and a satd. NaH_2PO_4 solution (100 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine, dried with $MgSO_4$, filtered and evaporated to a solid residue. Chromatography on silica gel using 20% EtOAc in hexanes as the eluant provided **8** as a white solid (3.38 g, 91%). TLC R_f = 0.34 (30% AcOEt in hexanes); mp 156 to 157 °C. $[\alpha]_D^{20}$ –519.3 (*c* 0.3 DMSO). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (s, 3H), 1.05 (s, 3H), 1.95 (s, 1H), 2.49 (dd, 1H, J_1 = 3.1, J_2 = 7.5), 2.96 (t, 1H, *J* = 7.8), 3.49 (dd, 1H, J_1 = 3.1, J_2 = 8.1), 4.79 (d, 1H, *J* = 6.7), 5.10 (d, 1H, *J* = 6.7), 7.15–7.73 (m, 13H). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.78 (s, 3H), 1.01 (s, 3H), 2.32 (dd, 1H, J_1 = 2.4, J_2 = 7.2), 2.32 (t, 1H, *J* = 7.4), 3.63 (dd, 1H, J_1 = 2.5, J_2 = 7.2), 4.13 (s, 1H), 4.71 (d, 1H, *J* = 6.3), 4.89 (d, 1H, *J* = 6.3), 7.10–7.97 (m, 13H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 26.2, 29.0, 65.2, 66.7, 71.1, 77.6, 85.5. HR-MS calcd. for $C_{25}H_{25}NO_2$ (TOF EI): 371.1885; found: 371.1886.

(4*R*)-*O*-Acetoxymethyl-5,5-dimethyl-3-(9-phenylfluoren-9-yl)-oxazolidine (10)

A solution of oxazolidine **8** (50 mg, 0.135 mmol) in $CDCl_3$ (1.4 mL) was stirred for 12 h at room temperature, treated with DMAP (10 mol%, 1.6 mg, 13.5 µmol), and dropwise with acetic anhydride (240 mol%, 42 µL, 0.324 mmol). The reaction mixture was stirred for 24 h and concentrated under reduced pressure to give a residue that was purified by chromatography on silica gel using 15% EtOAc in hexanes as the eluant. First to elute was the acetylated oxazolidine **10** as a white foam (28.0 mg, 50%). TLC R_f = 0.45 (20% AcOEt in hexanes). $[\alpha]_D^{20}$ 229.3 (*c* 0.3 DMSO). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.70 (s, 3H), 1.15 (s, 3H), 1.87 (s, 3H), 2.30 (dd, 1H, J_1 = 4.5, J_2 = 10.3), 3.72 (dd, 1H, J_1 = 4.5, J_2 = 11.1), 3.90 (t, 1H, *J* = 10.7), 4.73 (d, 1H, *J* = 7.3), 4.88 (d, 1H, *J* = 7.3), 7.16–7.60 (m, 13H). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.56 (s, 3H), 1.07 (s, 3H), 1.83 (s, 3H), 2.17 (dd, 1H, J_1 = 4.5, J_2 = 10.3), 3.57 (dd, 1H, J_1 = 4.5, J_2 = 11.1), 3.86 (t, 1H, *J* = 10.7), 4.65 (d, 1H, *J* = 7.2), 4.75 (d, 1H, *J* = 7.1), 7.17–7.94 (m, 13H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.5, 27.9, 63.4, 63.9, 76.9, 79.2, 80.9, 81.2, 169.7. HR-MS calcd. for $C_{27}H_{28}NO_3$ ($[M + H]^+$): 414.2064; found: 414.2065. Second to elute was (4*R*)-4-hydroxymethyl-5,5-dimethyl-(9-phenylfluoren-9-yl)-oxazolidine (**9**) as a white foam (12.5 mg, 25%). TLC R_f = 0.53 (30% AcOEt in hexanes). $[\alpha]_D^{20}$ 326.0 (*c* 0.2 DMSO). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.70 (s, 3H), 1.24 (s, 3H), 2.15 (d, 1H, *J* = 4.9), 2.80 (dd, 1H, J_1 = 6.1, J_2 = 11.1), 3.20 (d, 1H, *J* = 11.0), 4.78 (d, 1H, *J* = 7.1), 4.96 (d, 1H, *J* = 7.0), 7.16–7.76 (m, 13H). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.62 (s, 3H), 1.16 (s, 3H), 1.99 (m, 1H), 3.06 (m, 1H), 3.24 (m, 1H), 4.22 (t, 1H, *J* = 4.7), 4.59 (d, 1H, *J* = 7.0), 4.68 (d, 1H, *J* = 7.0), 7.14–7.96 (m, 13H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.2, 28.5, 61.6, 66.2, 77.1, 80.8, 81.6. HR-MS calcd. for $C_{25}H_{26}NO_2$ ($[M + H]^+$): 372.1958; found: 372.1961. Last to elute was oxazolidine **8** as a white foam (8.9 mg, 18%).

(2*R*)-2-*N*-(9-Phenylfluoren-9-yl)amino-3-methyl-1,3-butanediol (11)

Method 1

A solution of oxazolidine **8** (2.22 g, 6 mmol) in 0.2 mol/L HCl in dioxane (60 mL) was treated with $NaCNBH_3$ (320 mol%, 1.21 g, 19.2 mmol). The reaction mixture was stirred for 1 h at r.t.

Method 2

A solution of oxazolidine **8** (4.5 g, 14.2 mmol) and $NaCNBH_3$ (800 mol%, 4.9 g, 114 mmol) in THF (120 mL) was cooled to 0 °C and treated dropwise with trifluoroacetic acid (300 mol%, 3.3 mL, 42.6 mmol). The reaction mixture was stirred for 30 min at 0 °C. After stirring for the designated time, both reaction mixtures were washed, respectively, with a solution of satd. $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and evaporated to a solid. Chromatography on silica gel using 20% EtOAc in hexanes as eluant gave a white solid (Method 1: 1.93 g, 86%; Method 2: 3.35 g, 74%). TLC R_f = 0.54 (50% AcOEt in hexanes); mp 85 to 86 °C. $[\alpha]_D^{20}$ –449.8 (*c* 1.0 MeOH). ^{1}H NMR (400 MHz, CD_3OD + TFA) δ: 0.99 (s, 3H), 1.36 (s, 3H), 2.82 (dd, 1H, J_1 = 2.0, J_2 = 5.4), 3.12 (dd, 1H, J_1 = 6.6, J_2 = 5.0), 3.15 (s, 3H), 3.42 (dd, 1H, J_1 = 3.5, J_2 = 8.1), 7.22–8.10 (m, 13H). ^{13}C NMR (100 MHz, CD_3OD) showed a mixture of conformers; the chemicals shifts are listed in order of appearance (δ): 26.8, 28.5, 29.6, 33.2, 33.4, 58.2, 60.7, 61.3, 63.0, 75.5, 76.7, 78.5, 79.1, 79.2. HR-MS calcd. for $C_{25}H_{27}NO_2$ (TOF EI): 373.2042; found: 373.2051. Employing a 1:1 mixture of oxazolidines **8** and **9** (155 mg, 0.147 mmol) using method 2 gave diol **11** in 92% yield.

(2*R*)-2-*N*-(Boc)Amino-3-methyl-1,3-butanediol (12)

A solution of diol **11** (900 mg, 2.41 mmol) and di-*tert*-butyl dicarbonate (140 mol%, 737 mg, 3.37 mmol) in THF (100 mL) was treated with palladium hydroxide on carbon (270 mg, 20 wt% in Pd) and stirred under 5 atm of hydrogen (1 atm = 101.325 kPa) for 24 h at room temperature. The catalyst was removed by filtration on Celite® and washed with THF (2 × 20 mL) and MeOH (2 × 20 mL). The combined organic phase was evaporated to a residue that was purified by chromatography on silica gel using 70% EtOAc in hexanes as the eluant. Evaporation of the ninhydrin positive fractions (visualized on TLC after treatment with TFA va-

pors) gave *N*-(Boc)amino diol **12** as a white solid (461 mg, 82%). TLC R_f = 0.49 (80% AcOEt in hexanes); mp 138 to 139 °C. $[\alpha]_D^{20}$ 37.1 (*c* 1.0 MeOH). ^{1}H NMR (400 MHz, CD_3OD) showed a 1:1.3 mixture of carbamate isomers (δ): 1.15 (s, 1.6H), [1.16 (s, 1.2H)], [1.25 (s, 1.5H)], 1.26 (s, 1.6H), 1.46 (s, 4.2H), 1.48 (s, 4.5H), [2.89 (s, 1.1H)], 2.93 (s, 1.7H), 3.83–3.92 (m, 2.5H), [4.04 (dd, 0.5H, J_1 = 4.0, J_2 = 8.0)]. ^{13}C NMR (100 MHz, CD_3OD) showed a mixture of carbamate isomers; the chemical shifts are listed in order of appearance (δ): 26.7, 27.8, 28.0, 28.3, 28.9, 57.7, 58.0, 64.8, 72.6, 72.7, 79.9, 80.1, 157.9, 158.0. HR-MS calcd. for $C_{11}H_{24}NO_4$ ([M + H]$^+$): 234.1705; found: 234.1709. Anal. calcd. for $C_{11}H_{23}NO_4$: C 56.63, H 9.94, N 6.00; found: C 56.50, H 10.28, N 5.92.

(*S*)-β-Hydroxy-*N*-(Boc)-*N*-(methyl)valine (2)

A mixture of diol **12** (278 mg, 1.19 mmol), sodium phosphate buffer (4.6 mL, 0.67 mol/L, pH 6.7), and TEMPO (10 mol%, 24 mg, 0.119 mmol) in acetonitrile (6 mL) was heated to 35 °C and treated dropwise simultaneously over 2 h (Caution! Do not mix bleach and sodium chlorite before adding to reaction mixture) (15) with a solution of sodium chlorite ($NaClO_2$, 200 mol%, 2.38 mmol, 1.2 mL of solution : 5.71 g 80% *w*/*w*, 50.5 mmol in 25 mL of water) and diluted bleach (NaOCl, 2 mol%, 0.04 mmol, 1050 µL of solution : 0.66 mL commercial bleach 10.8% *v*/*v* 25 mL of water). The mixture was stirred at 35 °C overnight, cooled to r.t., acidified with citric acid to pH 3, and extracted with EtOAc (3 × 10 mL). The organic phases were combined and evaporated. The residue was dissolved in a solution of satd. Na_2CO_3 (15 mL) and washed with EtOAc (2 × 10 mL). The aqueous phase was acidified with 1 mol/L H_3PO_4 to pH 3, saturated with NaCl, and extracted with EtOAc (3 × 15 mL). The organic phases were combined, dried with $MgSO_4$, filtered and evaporated to give an oil (256 mg, 87%). TLC R_f = 0.43 ($CHCl_3$–MeOH–AcOH, 89:10:1). $[\alpha]_D^{20}$ –34.4 (*c* 0.5 MeOH). ^{1}H NMR (400 MHz, CD_3OD) showed a 0.7:1 mixture of carbamate isomers (δ): 1.22 (s, 1.9H), [1.26 (1.2H)], [1.38 (s, 1.2H)], 1.42 (s, 1.9H), [1.46 (s, 3.6H)], 1.47 (s, 5.4H), [2.95 (s, 1.2H)], 2.96 (s, 1.8H), [4.46 (s, 0.4H)], 4.59 (s, 0.6H). ^{13}C NMR (100 MHz, CD_3OD) showed a mixture of carbamate isomers; the chemicals shifts are listed in order of appearance (δ): 26.4, 27.6, 27.9, 28.1, 33.2, 33.3, 65.3, 66.2, 72.8, 78.5, 80.5, 81.1, 156.5, 157.4, 172.2, 172.3. HR-MS calcd. for $C_{11}H_{22}NO_5$ ([M + H]$^+$): 248.1498; found: 248.1500. LC–MS: 98% purity (condition A), R_T = 14.5.

Enantiomeric purity of (*S*)-β-hydroxy-*N*-(Boc)-*N*-(methyl)valine (2)

A solution of **2** (30 mg, 0.12 mmol) in acetonitrile (1 mL) at 0 °C was treated with HOBt (100 mol%, 22 mg, 0.12 mmol) and TBTU (150 mol%, 58 mg, 0.18 mmol), stirred for 30 min, and treated with a premixed solution of (*S*)- or (*R*)-phenylalanine methyl ester hydrochloride (300 mol%, 78 mg, 0.36 mmol) and diisopropylethylamine (DIEA, 400 mol%, 0.84 µL, 0.48 mmol) in acetonitrile (1 mL) at 0 °C. The reaction mixture was stirred for 48 h at room temperature until TLC showed the complete disappearance of starting acid **2**. TLC R_f = 0.43 ($CHCl_3$–MeOH–AcOH, 89:10:1). The solvent was evaporated and the solid residue was resuspended in CH_2Cl_2 (3 mL). The organic phase was washed with an aq. satd. solution of $NaHCO_3$ (2 × 3 mL), 1 N NaH_2PO_4 (2 × 3 mL), and brine (3 mL), dried with $MgSO_4$, filtered and evaporated to a solid residue. The Boc group was subsequently removed by treating the residue in dry CH_2Cl_2 at 0 °C with a stream of HCl gas bubbles, stirring for 15 min, and concentrating the reaction mixture under reduced pressure to give a solid that was directly examined by 400 MHz ^{1}H NMR spectroscopy in CD_3OD. Measurement of the signals for the diastereomeric methyl amine singlets at 2.04 and 2.52 ppm during incremental additions of (*S*)-**14** in (*R*)-**14** demonstrated dipeptide (*R*)-**14** to be of >99% diastereomeric purity. Hence, (*S*)-β-hydroxy-*N*-(Boc)-*N*-(methyl)valine (**2**) is considered to be of the same high enantiomeric purity.

(*S*,*S*)-β-Hydroxy-*N*-(Boc)-*N*-(methyl)valinylphenylalanine methyl ester ((*S*)-13)

TLC R_f = 0.30 (30% AcOEt in hexanes). ^{1}H NMR (400 MHz, CD_3OD) showed a 1:2 mixture of carbamate isomers (δ): 1.13 (s, 3H), 1.20 (s, 3H), 1.47 (s, 9H), 2.68 (s, 2.0H), [2.71 (s, 1.1H)], 2.97 (m, 1H), 3.23 (m, 1H), [3.68 (s, 1.5H)], 3.73 (s, 3.8H), [4.44 (s, 0.28H)], 4.54 (s, 0.65H), 4.76 (dd, 1H, J_1 = 4.8, J_2 = 9.5), 7.08–7.35 (m, 5H). MS (TIC) *m*/*z*: 309.2 ([M + H]$^+$).

(*S*,*R*)-β-Hydroxy-*N*-(Boc)-*N*-(methyl)valinylphenylalanine methyl ester ((*R*)-13)

TLC R_f = 0.35 (30% AcOEt in hexanes). ^{1}H NMR (400 MHz, CD_3OD) showed a 1:2 mixture of carbamate isomers (δ): 1.08 (s, 3H), 1.15 (s, 3H), 1.46 (s, 9H), [2.70 (s, 1.1H)], 2.93 (s, 1.9H), 3.00 (m, 1H), 3.24 (m, 1H), [3.66 (s, 1.8H)], 3.72 (s, 3.6H), [4.40 (s, 0.3H)], 4.49 (s, 1H), 4.77 (dd, 0.6H, J_1 = 4.50, J_2 = 8.9), 7.01–7.34 (m, 5H). MS (TIC) *m*/*z*: 309.2 ([M + H]$^+$).

(*S*,*S*)-β-Hydroxy-*N*-(methyl)valinylphenylalanine methyl ester hydrochloride ((*S*)-14)

^{1}H NMR (400 MHz, CD_3OD) δ: 1.07 (s, 3H), 1.29 (s, 3H), 2.04 (s, 3H), 2.84 (dd, 1H, J_1 = 10.9, J_2 = 14.0), 3.17–3.23 (m, 2H), 3.58 (s, 3H), 4.82 (m, 1H), 7.14–7.20 (m, 5H).

(*S*,*R*)-β-Hydroxy-*N*-(methyl)valinylphenylalanine methyl ester hydrochloride ((*R*)-14)

^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (s, 3H), 0.87 (s, 3H), 2.52 (s, 3H), 2.83 (dd, 1H, J_1 = 11.5, J_2 = 14.2), 3.21 (m, 1H), 3.26 (dd, 1H, J_1 = 4.5, J_2 = 14.2), 3.66 (s, 3H), 4.70 (dd, 1H, J_1 = 4.5, J_2 = 11.4), 7.14–7.19 (m, 5H).

(*S*)-*N*-(Boc)-β-methoxyvaline

To a solution of (*S*)-β-hydroxy-*N*-(Boc)valine (**3**, 100 mg, 429 µmol, prepared according to ref. 10) and methyl iodide (800 mol%, 214 µL, 3.43 mmol) in THF (1.5 mL) at 0 °C, sodium hydride (60% dispersion in mineral oil, 300 mol%, 52 mg, 29 mmol) was added cautiously with gentle stirring. The suspension was stirred for 24 h, diluted with EtOAc (2 mL), treated dropwise with water to destroy the excess sodium hydride, and evaporated to an oily residue that was partitioned between ether (5 mL) and water (10 mL). The ether layer was washed with a solution of satd. $NaHCO_3$ (10 mL). The combined aqueous extracts were acidified with citric acid to pH 3 and extracted with EtOAc (3 × 10 mL).

The organic phases were combined, washed with water (2 × 10 mL), 5% aqueous sodium thiosulfate (2 × 10 mL), and water (2 × 10 mL), dried with $MgSO_4$, filtered and evaporated to give an oil that was purified by chromatography on silica gel using a mixture of $CHCl_3$, methanol, and acetic acid (94:4:1) to give an oil (69 mg, 65%). TLC R_f = 0.56 ($CHCl_3$–MeOH–AcOH, 89:10:1). $[\alpha]_D^{20}$ 9.5 (*c* 1.0 MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.26 (s, 3H), 1.28 (s, 3H), 1.45 (s, 9H), 3.23 (s, 3H), 4.2 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 21.7, 21.8, 27.7, 49.0, 60.6, 76.1, 79.7, 156.8, 173.0. HR-MS calcd. for $C_{11}H_{21}NO_5Na$ ($[M + Na]^+$): 270.1312; found: 270.1319.

(*S*)-Benzyl-2-hydroxy-3-methylbutanoate (Hmb-OBn, 16)

To a solution of (*S*)-2-hydroxy-3-methylbutanoic acid (**15**, 2 g, 16.9 mmol, prepared according to ref. 18) and TMSCl (600 mol%, 8.8 mL, 101.4 mmol) in CH_2Cl_2 (170 mL) at 0 °C, Et_3N (600 mol%, 14.2 mL, 101.4 mmol) was added dropwise. The mixture was heated at reflux for 1 h, cooled to 0 °C, and treated with dry MeOH (110 mol%, 732 µL, 18.6 mmol). The reaction was stirred for 1 h at room temperature. The volatiles were removed under reduced pressure on a rotary evaporator. The residue was dissolved in acetonitrile (170 mL) and treated with anhydrous cesium carbonate (150 mol%, 8.3 g, 25.4 mmol) and benzyl bromide (500 mol%, 10 mL, 84.5 mmol), heated at reflux and stirred vigorously for 1 h, cooled to room temperature, treated with solid citric acid (1000 mol%, 32.5 g, 169 mmol), and stirred vigorously for 1 h at room temperature. The precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (150 mL), washed with a solution of satd. $NaHCO_3$ (2 × 50 mL) and brine, dried with $MgSO_4$, filtered and evaporated to a residue. Chromatography on silica gel using a gradient 20%–30% EtOAc in hexanes as the eluant and evaporation of the collected fractions provided an oil (2.3 g, 65%). TLC R_f = 0.37 (50% EtOAc in hexanes). $[\alpha]_D^{20}$ –11.3 (*c* 2.0 $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.82 (d, 3H, *J* = 6.9), 0.98 (d, 3H, *J* = 6.9), 2.00–2.14 (m, 1H), 3.06–3.21 (m, 1H), 4.01–4.11 (m, 1H), 5.14 (d, 1H, *J* = 12.1), 5.21 (d, 1H, *J* = 12.2), 7.25–7.38 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.9, 18.7, 32.1, 67.0, 75.0, 128.3, 128.4, 128.5, 135.2, 174.7. HR-MS calcd. for $C_{12}H_{16}NO_3$ (TOF EI): 208.1099; found: 208.1098.

General procedure for the preparation of depsipeptide benzyl esters

A 0.1 mol/L solution of Hmb-OBn (**16**, 100 mmol%), Ph_3P (250 mol%), and the amino acid (Boc-HOMeVal (**2**, 100 mol%), Boc-HOVal (**3**, 150 mol%), or Boc-MeVal (**17**, 150 mol%)) in CH_2Cl_2–THF (1:1) was cooled to –20 °C, treated with diisopropyl azodicarboxylate (DIAD, 255 mol%) dropwise over 30 min, and stirred at –20 °C for 30 min. The reaction mixture was removed from the ice bath and allowed to stir at room temperature overnight. The volatiles were removed under reduced pressure on a rotary evaporator.

Boc-(*S*)-HOMeVal-(*R*)-Hmb-OBn (**18**, 169 mg) was isolated from the reaction of Hmb-OBn (**16**, 80 mg, 0.384 mmol) and Boc-HOMeVal (**2**, 91 mg, 0.384 mmol) by chromatography on silica gel using 18% EtOAc in hexanes as the eluant and directly employed in the hydrogenation step. An analytically pure sample (LC–MS: 96% purity, condition B, R_T = 23.7) was obtained by purification on RP-HPLC using condition A. TLC R_f = 0.28 (20% AcOEt in hexanes). $[\alpha]_D^{20}$ –22.2 (*c* 0.5 MeOH). ^{1}H NMR (300 MHz, CD_3OD) showed a 1:2 mixture of carbamate isomers (δ): 0.92 (d, 3H, *J* = 6.9), 0.99 (d, 3H, *J* = 6.9), [1.38 (s, 1.08H)], 1.41 (s, 1.98H), 1.22 (s, 2.01H), [1.25 (s, 1.08H)], 1.47 (s, 9H), 2.16–2.31 (m, 1H), [2.93 (s, 1.16H)], 2.94 (s, 1.96H), [4.75 (s, 0.33H)], 4.78 (s, 0.62H), 5.15 (d, 1H, *J* = 12.1), 5.21 (d, 1H, *J* = 12.1), 7.31–7.38 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) showed a mixture of carbamate isomers; the chemical shifts are listed in order of appearance (δ): 16.5, 18.1, 21.3, 26.4, 26.5, 27.6, 28.1, 30.4, 32.3, 33.3, 65.1, 65.6, 67.0, 72.5, 72.6, 77.6, 80.6, 81.0, 128.5, 128.6, 135.9, 156.5, 157.5, 196.6, 170.1. HR-MS calcd. for $C_{23}H_{36}NO_7$ ($[M + H]^+$): 438.2486; found: 438.2478.

Boc-(*S*)-MeVal-(*R*)-Hmb-OBn (**19**, 649 mg) was isolated from the reaction of Hmb-OBn (**16**, 313 mg, 1.50 mmol) and Boc-MeVal (**17**, 521 mg, 2.25 mmol) by chromatography on silica gel using 15% EtOAc in hexanes as the eluant and directly employed in the hydrogenation step. An analytically pure sample (LC–MS: 99% purity, condition A, R_T = 8.0) was obtained by purification on RP-HPLC using condition A. TLC R_f = 0.35 (10% AcOEt in hexanes). $[\alpha]_D^{20}$ –46.6 (*c* 0.5 $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) showed a 1:1 mixture of carbamate isomers (δ): 0.85–0.95 (m, 6H), 0.95–1.06 (m, 6H), 1.46 (s, 9H), 2.22 (m, 2H), 2.78 (s, 1.6H), [2.83 (s, 1.4H)], [4.29 (d, 0.4H, *J* = 10.3)], 4.59 (d, 0.4H, *J* = 10.3), 4.87 (d, 1H, *J* = 4.1), 7.24–7.46 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) showed a mixture of carbamate isomers; the chemical shifts are listed in order of appearance (δ): 17.2, 19.0, 19.3, 20.0, 27.6, 27.8, 28.5, 30.2, 30.7, 63.1, 64.8, 66.9, 80.0, 80.3, 128.5, 128.7, 135.4, 155.8, 156.4, 169.1, 169.3, 170.8, 171.1. HR-MS calcd. for $C_{23}H_{35}NO_7$ (TOF EI): 421.2464; found: 421.2471.

Boc-(*S*)-HOVal-(*R*)-Hmb-OBn (**20**, 1.3 mg) was isolated from the reaction of Hmb-OBn (**16**, 625 mg, 3.0 mmol) and Boc-HOVal (**3**, 1050 mg, 4.5 mmol) by chromatography on silica gel using 15% EtOAc in hexanes as the eluant and directly employed in the hydrogenation step. An analytically pure sample (LC–MS: 99% purity, condition B, R_T = 16.0) was obtained by purification on RP-HPLC using condition A. TLC R_f = 0.38 (25% AcOEt in hexanes). $[\alpha]_D^{20}$ –2.8 (*c* 0.5 MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 0.93 (d, 3H, *J* = 6.8), 0.98 (d, 3H, *J* = 6.8), 1.25 (s, 3H), 1.28 (s, 3H), 1.44 (s, 9H), 2.22 (dsept, 1H, J_1 = 4.6, J_2 = 6.8), 4.17 (s, 1H), 4.86 (d, 1H, *J* = 4.4), 5.16 (d, 1H, *J* = 12.2), 5.21 (d, 1H, *J* = 12.2), 7.28–7.40 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.6, 18.2, 25.8, 26.0, 27.7, 30.4, 63.1, 67.0, 71.3, 77.7, 79.8, 128.4, 128.5, 128.6, 135.9, 156.8, 169.9, 170.9. HR-MS calcd. for $C_{22}H_{33}NO_7$ ($[M + H]^+$): 424.2330; found: 424.2315.

General procedure for benzyl ester removal from depsipeptides

A 0.033 mol/L solution containing the depsipeptide benzyl ester (100 mol%) in THF was treated with palladium hydroxide on carbon (30 wt% of 20 wt% palladium (wet)), stirred under 1 atm of hydrogen for 1 h, filtered onto Celite®, and washed with THF (2×) and MeOH (2×). The filtrate was concentrated under reduce pressure to a residue that was

passed through a pad of silica gel, first using 20% EtOAc in hexanes as the eluant, then followed by 99% EtOAc containing 1% AcOH.

Boc-(S)-HOMeVal-(R)-Hmb (4)

The reaction of ester **18** (89 mg) afforded **4** as a white foam (51 mg, 72%). TLC R_f = 0.40 ($CHCl_3$–MeOH–AcOH, 89:10:1). $[\alpha]_D^{20}$ –28.2 (*c* 0.5 MeOH). ^{1}H NMR (300 MHz, CD_3OD) showed a 1:1.5 mixture of carbamate isomers (δ): 0.99 (d, 3H, *J* = 6.9), 1.03 (d, 3H, *J* = 6.9), 1.26 (d, 3H, *J* = 9.5), 1.41 (d, 3H, *J* = 8.6), 1.48 (s, H), 2.26 (dsept, 1H, J_1 = 4.0, J_2 = 6.7), [3.01 (s, 1.17H)], 3.03 (s, 1.78H), [4.75 (s, 0.37H)], 4.78 (s, 0.64H), 4.88 (d, 1H, *J* = 3.9), 4.95 (br s, 1OH). ^{13}C NMR (75 MHz, CD_3OD) showed a mixture of carbamate isomers; the chemical shifts are listed in order of appearance (δ): 16.4, 18.3, 21.1, 21.3, 26.4, 27.6, 28.1, 30.1, 32.3, 33.3, 65.2, 65.6, 69.4, 72.5, 72.7, 77.5, 78.5, 80.6, 81.0, 156.6, 157.5, 170.1, 171.5. HR-MS calcd. for $C_{16}H_{30}NO_7$ ($[M + H]^+$): 348.2017; found: 348.2016. LC–MS: 99% purity (condition A), R_T = 21.1.

Boc-(S)-MeVal-(R)-Hmb (21)

The reaction of ester **19** (250 mg) afforded **21** as an oil (167 mg, 85%). TLC R_f = 0.58 ($CHCl_3$–MeOH–AcOH, 89:10:1). $[\alpha]_D^{20}$ –75.6 (*c* 0.5 MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ: 0.91 (m, 3H), 0.95–1.07 (m, 6H), 1.25 (d, 3H, *J* = 6.2), 1.45 (s, 9H), 2.13–2.33 (m, 2H), 2.84 (s, 3H), [4.27 (d, 0.43H, *J* = 10.5)], 4.45 (d, 0.45H, *J* = 10.4), 4.8 (d, 1H, *J* = 4.0), 4.84–5.00 (br s, 1OH). ^{13}C NMR (75 MHz, CD_3OD) showed a mixture of carbamate isomers; the chemical shifts are listed in order of appearance (δ): 16.5, 18.2, 18.4, 18.5, 19.3, 21.3, 27.4, 27.6, 27.8, 29.8, 30.1, 30.3, 63.6, 65.1, 69.4, 77.3, 80.5, 80.8, 156.5, 157.1, 170.9. HR-MS calcd. for $C_{16}H_{30}NO_6$ ($[M + H]^+$): 332.2073; found 332.2074. LC–MS: 97% purity (condition A), R_T = 26.0.

Boc-(S)-HOVal-(R)-Hmb (22)

The reaction of ester **20** (330 mg) afforded **22** as a white solid (224 mg, 83%). TLC R_f = 0.38 ($CHCl_3$–MeOH–AcOH, 89:10:1). $[\alpha]_D^{20}$ –29.4 (*c* 0.5 MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.00 (d, *J* = 6.9, 3H), 1.03 (d, *J* = 7.0, 3H), 1.29 (s, 3H), 1.31 (s, 3H), 1.45 (s, 9H), 2.26 (dsept, J_1 = 4.1, J_2 = 6.7, 1H), 4.14 (s, 1H), 4.87 (d, *J* = 3.9, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4, 19.4, 26.9, 27.0, 28.7, 31.2, 64.2, 72.3, 78.5, 80.8, 157.8, 171.7, 173.0. HR-MS calcd. for $C_{15}H_{28}NO_7$ ($[M + H]^+$): 334.1866; found 334.1876. LC–MS: 98% purity (condition A), R_T = 18.5.

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