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Peptides Containing γ,δ-Dihydroxy-L-leucine

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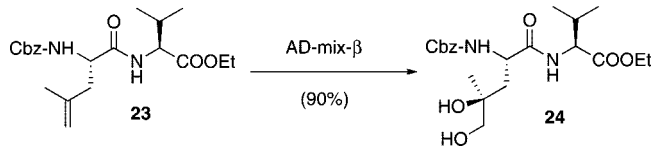

(±)-Dehydroleucine was prepared and resolved by porcine kidney acylase. Under the conditions of the Sharpless asymmetric dihydroxylation (SAD), employing AD-mix-α, *N*α-carbobenzyloxy-(2*S*)-4,5-dehydroleucine methyl ester (**16**) gave rise to a 6.5:1.0 mixture of γ-lactones **17**, favoring the 4*R* configuration. Such carbamate-protected α-amino-γ-hydroxylactones are not recommended as intermediates for peptide synthesis, since model studies showed that lactone **13** was unreactive toward amines. Moreover, the lactone ring could not be opened hydrolytically without epimerization at Cα. *N*α-Carbobenzyloxy-(2*S*)-4,5-dehydroleucine (**22**) was condensed with valine ethyl ester (**19**) to give dipeptide **23**. Treatment of **23** with AD-mix-β, under the SAD conditions, converted the dehydroleucine residue to γ,δ-dihydroxyleucine with 4*S* configuration, as occurs in alloviroidin (**3**), a natural product isolated from *Amanita suballiacea*.

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Introduction

γ,δ-Dihydroxyleucine occurs at position 7 of several members of the cyclic peptide toxins isolated from *Amanita* mushrooms.[1] The most well-studied of these is phalloidin (**1**, Figure 1), the prototypical phallotoxin that binds tightly to F-actin. The (2*S*,4*R*)-γ,δ-dihydroxyamino acid also occurs in phallacidin, secophalloidin and some virotoxins (e.g., viroidin, **2**).[2] The 2*S* stereochemistry was demonstrated by degradation to L-aspartic acid.[3] Alloviroidin (**3**) contains the (2*S*,4*S*) diastereoisomer of 4,5-dihydroxyleucine and has equal affinity for actin.[4]

A single synthesis of γ,δ-dihydroxyleucine has appeared in the literature. In 1957, Wieland and Weiberg reported bromination of compound **4** and subsequent treatment with acid and silver salts that led to a mixture of all four stereoisomers of **7** (Scheme 1).[5] The two racemates were separated by crystallization and then the enantiomers separated by crystallization of diastereoisomeric salts with ditoluoyl tartaric acid.[6]

Considerable effort has been directed toward understanding SARs of the phalloidins and there have been two reports of viroidin analogs.[7] Much of the early work by Wieland and co-workers involved partial syntheses employing the natural product as starting material.[8] Total syntheses have invariably resorted to substitution of the dihydroxyleucine residue.[9]

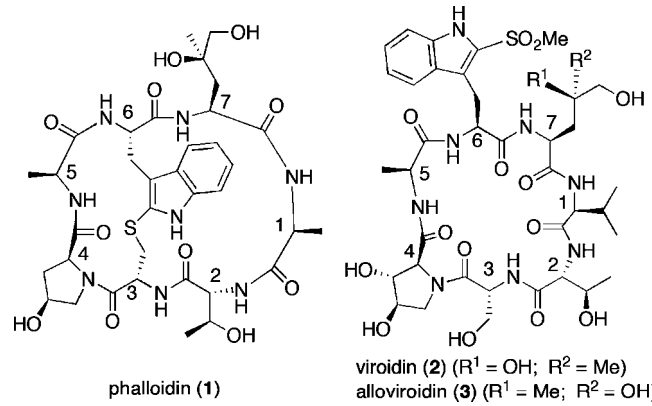

FIGURE 1. Cyclic peptide toxins from *Amanita*.

Our aim was to produce a stereoisomerically pure γ,δ-dihydroxyleucine, for incorporation into virotoxins. We hoped that a diastereoselective dihydroxylation of an (*S*)-dehydroleucine deriva-

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(6) Wieland, T.; Krantz, H. *Chem. Ber.* **1958**, *91*, 2619–2629.
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(8) (a) Munekata, E.; Faulstich, H.; Wieland, T. *J. Am. Chem. Soc.* **1977**, *99*, 6151–6153. (b) Munekata, E.; Faulstich, H.; Wieland, T. *Liebigs Ann. Chem.* **1979**, 1020–1027.

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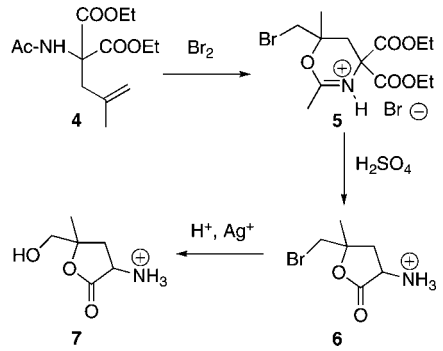
SCHEME 1. Wieland's Synthesis of γ,δ-Dihydroxyleucine



tive would enable us to achieve this goal. The 2*S* configuration of γ,δ-dihydroxyleucine has been shown to be vital for biological activity in the phalloidins.[10] Since both viroidin (**2**) and alloviroidin (**3**) bind actin with equal affinity,[4] either configuration at C4 would be acceptable.

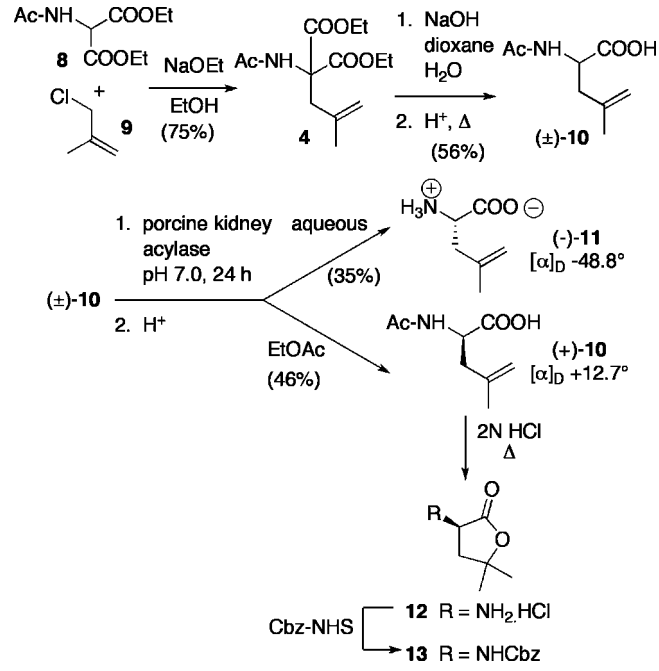
Results and Discussion

Our preparation of dehydroleucine began with the condensation of the anion of ethyl acetamidomalonate (**8**) with methallyl chloride (**9**)[11] (Scheme 2). We then followed the work of Schmidt and Schmidt who utilized (*S*)-dehydroleucine in their synthesis of eponemycin.[12] Hydrolysis of the esters, acidification and decarboxylation resulted in racemic *N*-acetyl-4,5-dehydroleucine. The enzymatic resolution of (±)-**10** was best conducted at room temperature, with strict control of pH in the 7−8 range, for no more than 24 h. The isolation of (*S*)-dehydroleucine in high optical purity followed the recommendations of Chenault et al.[13] Specifically, the acidic layer was applied directly to a column of Dowex-50 (H^+). Attempts to concentrate the solution, even by freezedrying, were accompanied by partial lactonization.[14] Treatment of (+)-**10** with acid led to deacetylation and concomitant lactonization. Hydrochloride salt **12** could be converted to the Cbz-derivative **13** that was used in model studies (*vide infra*).

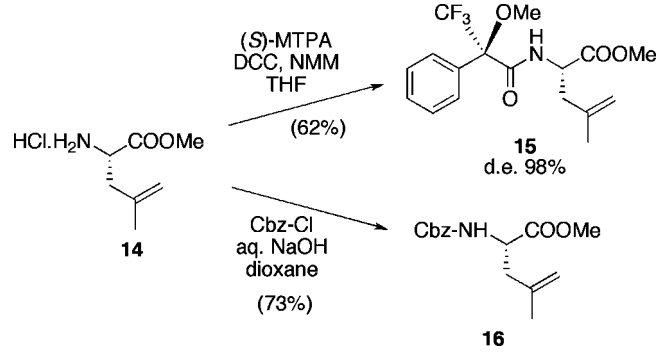
The free L-3,4-dehydroleucine, (−)-**11**, was readily converted to its methyl ester **14**. Formation of the Mosher amide **15** (Scheme 3) and ^{19}F NMR analysis indicated a 99:1 ratio in favor of the *S*-configuration at Cα. The carbobenzyloxy group was introduced to protect *N*α; we hoped that the aromatic ring would provide an "anchor" to bind in the so-called southwest binding pocket of the ligand during the asymmetric dihydroxylation (*vide infra*).

We were reluctant to predict which of the AD-mixes[15] would give the desired diastereomer. There have been several cases reported where the Sharpless mnemonic has failed to predict the

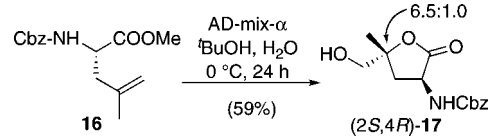
SCHEME 2. Synthesis and Resolution of (±)-Dehydroleucine



SCHEME 3. Functionalization of the (−)-Dehydroleucine



SCHEME 4. Diastereoselective Dihydroxylation



outcome in the dihydroxylation of 1,1-disubstituted olefins.[16] We were also uncertain of the impact the existing Cα stereocenter would have on the stereochemical course of the reaction. It turned out that under the standard conditions of the Sharpless asymmetric dihydroxylation (SAD) reaction with AD-mix-α, an inseparable mixture of diastereomers of **17** was obtained in a 6.5:1.0 ratio (Scheme 4). Considerable effort was directed toward determining the stereochemistry at C4. Standard NMR experiments (NOESY and ROESY) failed to show any crosspeaks that would have established the relative stereochemistry of the substituents on the five-membered ring. This suggests that the γ-lactone is very conformationally mobile. Attempts to separate and characterize the diastereomers were unsuccessful, although the configuration was later demonstrated to be 4*R* as shown. AD-mix-β gave a 1:1 mixture of diastereomers of **17**.

With diastereomerically enriched lactone **17** in-hand, we began to consider how to incorporate such a residue into a peptide

(9) Two recent examples: (a) Anderson, M. O.; Shelat, A. A.; Guy, R. K. *J. Org. Chem.* **2005**, *70*, 4578–4584. (b) Schuresko, L. A.; Lokey, R. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 3547–3549.

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(11) Albertson, N. F.; Archer, S. *J. Am. Chem. Soc.* **1945**, *67*, 308–310.

(12) (a) Schmidt, U.; Schmidt, J. *J. Chem. Soc., Chem. Commun.* **1992**, *52*, 9–530. (b) Schmidt, U.; Schmidt, J. *Synthesis* **1994**, 300–304.

(13) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354–6364.

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(15) AD-mixes are reagent mixtures for the SAD reaction, as defined in: Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, 2483–2547.

(16) (a) Hale, K. J.; Manaviazar, S.; Peak, S. A. *Tetrahedron Lett.* **1994**, *35*, 425–428. (b) Gardiner, J. M.; Bruce, S. E. *Tetrahedron Lett.* **1998**, *39*, 1029–1032.

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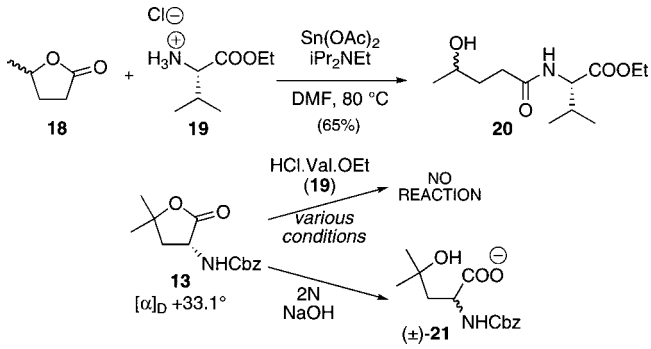
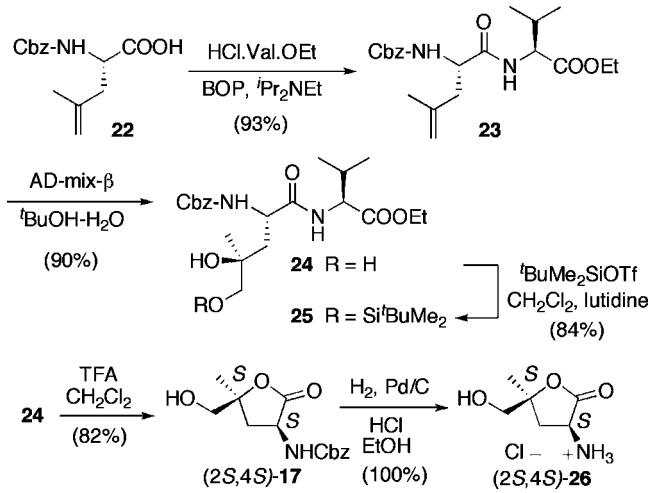
SCHEME 5. Amide Formation with Lactones 18 and 13



SCHEME 6. Dihydroxylation of Dipeptide 23



backbone. There are scattered reports of the direct reaction of lactones with amines under neutral conditions,[17] $Sn(OAc)_2$ catalysis,[18] catalysis by 2-hydroxypyridine[19] and via modification of the Weinreb method.[20] We decided to explore this strategy, to minimize the number of steps and protecting group manipulations associated with the dihydroxyleucine residue. We screened a number of methods using (±)-valerolactone (**18**) and valine ethyl ester (**19**) and found that the conditions of Hansen et al. gave the best results (Scheme 5). Using lactone **13** as a better model system, without the complication of the additional, unassigned stereocenter at C4, we were unable to form any dipeptide. Indeed, none of the examples cited above involve a γ-lactone bearing a carbamate-protected amine at Cα; Cα substituents were invariably small (e.g., H, Me, OMe, CN). We presume that this group prevents nucleophilic attack at the lactone carbonyl for steric reasons. The δ-disubstitution was also unprecedented and undoubtedly contributes to the low reactivity of **13** toward nucleophiles. Our recourse seemed obvious: we would hydrolytically open the lactone ring and subsequently perform a conventional peptide coupling. Unfortunately, Cα was epimerized under mildly basic conditions. An extensive study by Michl showed that this is a general phenomenon for γ-lactones bearing an *N*-acyl or *N*-carbamate substituent.[21]

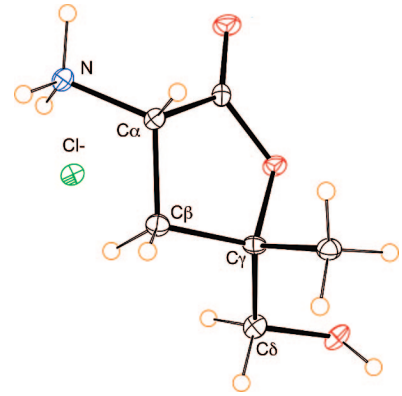


FIGURE 2. ORTEP diagram for (2*S*,4*S*)-**26**.

Dihydroxylation of β,γ-unsaturated ester **16** led to isolation of γ-lactone (2*S*,4*R*)-**17** that was unlikely to be a useful synthetic intermediate for peptide synthesis, based on the precedents in Scheme 5. During dihydroxylation of γ,δ-unsaturated esters, concomitant lactonization is prevented by sterically hindered esters.[22] We reasoned that an amide bond would also be resistant to uncatalyzed lactonization. Thus we prepared dipeptide **23**, bearing the alkene as a masked diol (Scheme 6). Dihydroxylation of **23** with AD-mix-β gave a single peptide, **24**. The diol was protected as a silyl acetal according to Corey and Hopkins,[23] but this functionality was unstable to silica gel. The primary alcohol in **24** was protected as a TBDMS ether to improve solubility. Other attempts to derivatize diol **24**, to produce crystals suitable for X-ray analysis, were unsuccessful. However, cleavage of the peptide bond under acidic conditions gave lactone (2*S*,4*S*)-**17**, the diastereomer of the major product in Scheme 4. Hydrogenolysis, in the presence of hydrochloric acid, gave the hydrochloride salt **26**, a compound that Wieland had previously described as crystalline. The crystal structure of **26** revealed that the $-NH_3^+$ and $-CH_2OH$ substituents were on the same face of the ring (Figure 2). Thus the configuration of **26** is (2*S*,4*S*), as occurs in alloviroidin (**3**). Treatment of dipeptide olefin **23** with AD-mix-α led to a mixture of diastereomers that was not synthetically useful.

Conclusion

In summary, we have found an effective method for the preparation of a dipeptide containing (2*S*,4*S*)-4,5-dihydroxyleucine. We found that compound **17**, the dihydroxyleucine in its γ-lactone form, was not a useful building block, since it cannot be opened directly by an amine and it undergoes Cα-epimerization on hydrolytic opening of the lactone ring. We therefore incorporated dehydroleucine into a dipeptide and introduced the diol stereoselectively via a Sharpless asymmetric dihydroxylation. We were unable to produce the (2*S*,4*R*) diastereomer in an analogous manner. Studies on the incorporation of dipeptide **25** into larger peptides will be reported in due course.

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Experimental Section

Dehydroleucine Methyl Ester Hydrochloride (14).[24] Thionyl chloride (112 μL, 184 mg, 1.5 mmol, 2.0 equiv.) was added dropwise to a solution of dehydroleucine (−)-**11** (100 mg, 0.77 mmol, 1.0 equiv.) in anhydrous methanol (3 mL) at −10 °C under N_2. The solution was warmed to rt and left to stir overnight. The mixture was concentrated to give **14** as a colorless oil (136 mg, 98%). R_f 0.78 (6:4:1 $CHCl_3$/CH_3OH/H_2O); $[\alpha]_D^{24}$ −5.1° (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 1.81 (s, 3H), 2.60 (dd, *J* = 14.2, 8.4 Hz, 1H), 2.70 (dd, *J* = 14.2, 5.6 Hz, 1H), 3.84 (s, 3H), 4.23 (dd, *J* = 8.0, 6.2 Hz, 1H), 4.94 (br, 1H) 5.00 (t, *J* = 1.3 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 19.6, 37.9, 50.1, 51.7, 115.1, 137.9, 168.8; HRMS (+TOF) calcd for $C_7H_{14}NO_2$ $(M + H)^+$ 144.1019; obsd: 144.1008.

Mosher Amide 15. *N*-Methyl morpholine (67 μL, 62 mg, 0.61 mmol, 1.1 equiv) was added to a solution of dehydroleucine methyl ester hydrochloride **14** (100 mg, 0.56 mmol, 1 equiv) in THF (2 mL) at 0 °C under N_2. (*S*)-(−)-Methoxy(trifluoromethyl)phenyl acetic acid (MTPA) (143 mg, 0.61 mmol, 1.1 equiv) and *N*,*N*′-dicyclohexyl carbodiimide (DCC) (138 mg, 0.67 mmol, 1.2 equiv) were added. The mixture was stirred at 0 °C for 3 h and then at rt overnight. The resulting *N*,*N*′-dicyclohexyl urea was removed by filtration and the filtrate concentrated. The residue was dissolved in ethyl acetate (15 mL) and washed successively with 10% citric acid (10 mL), 5% $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 5:1 Hex/EtOAc to give **15** as an oil (123 mg, 62%). R_f 0.53 (2:1 Hexanes/EtOAc); $[\alpha]_D^{28}$ +16.5° (*c* 0.85, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (s, 3H), 2.39 (dq, *J* = 8.8, 5.2 Hz, 1H), 2.56 (dd, *J* = 14.0, 5.1 Hz, 1H), 3.51 (dd, *J* = 3.3, 1.6 Hz, 3H), 3.80 (s, 3H), 4.60 (app d, *J* = 0.8 Hz, 1H), 4.70 (app. t, *J* = 1.5 Hz, 1H), 4.80 (dt, *J* = 8.6, 4.4 Hz, 1H), 7.35−7.58 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 40.4, 50.1, 55.2, 83.7, 84.0, 114.8, 122.1, 125.0, 127.5, 128.4, 129.4, 132.7, 140.0, 166.2, 172.0; ^{19}F NMR (236 MHz, $CDCl_3$) δ −69.35; HRMS (+TOF) calcd for $C_{17}H_{21}NO_4F_3$ $(M + H)^+$: 360.1417; obsd: 360.1419.

Mosher Amide of (±)-11. Compound (±)-**10** (100 mg, 0.58 mmol) was suspended in aqueous NaOH (2.5 N, 3 mL) and heated at reflux for 4 h. The solution was neutralized to pH 7 (monitored with UIP) by the addition of 6 M HCl. The solution was then loaded onto a column (25 mm diameter, 30 mm high) of Dowex-50 (H^+), rinsed with water (∼150 mL), eluted with 1 N aqueous NH_4OH. Fractions were monitored by TLC, staining with nihydrin. Relevant fractions were freezedried to give (±)-**11** as a colorless, amorphous powder in quantitative yield. A portion of this material (50 mg) was derivatized with MTPA, as described above, to give a 1:1 mixture of diastereomers (75 mg, 54%). ^{19}F NMR (236 MHz, $CDCl_3$) δ −69.35, −69.49.

Cbz-dehydroleucine-OMe (16).[25] Thionyl chloride (229 μL, 376 mg, 3.2 mmol, 2 equiv) was gradually added to a suspension of (−)-**11** (204 mg, 1.6 mmol, 1 equiv) in MeOH (4 mL) at −10 °C under N_2. The solution was gradually warmed to rt, stirred for 2 d, and concentrated. The residue was dissolved in a biphasic mixture of CH_2Cl_2 (3 mL) and H_2O (1.5 mL) and cooled to 0 °C at which point $NaHCO_3$ (728 mg, 8.7 mmol, 6.6 equiv) and *N*-(benzyloxycarbonyl-oxy)succinimide (393 mg, 1.6 mmol, 1.2 equiv) were added sequentially. The reaction mixture was gradually warmed to rt overnight and diluted with CH_2Cl_2 and H_2O (20 mL each). The aqueous layer was back-extracted with EtOAc (2 × 15 mL). The organic extracts were combined, filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography eluting with 2:1 Hex/EtOAc to give **16** as a colorless oil (266 mg, 73%). R_f 0.50 (3:1 Hexanes/EtOAc); $[\alpha]_D^{27}$ +7.3 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (s, 3H), 2.38 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.54 (dd, *J* = 14.0, 5.4 Hz, 1H), 3.73 (s, 3H), 4.49 (td, *J* = 8.1, 5.6 Hz, 1H), 4.75 (br, 1H), 4.84 (app. t, *J* = 1.5 Hz, 1H), 5.10 (s, 2H), 5.27 (d, *J* = 7.8 Hz, 1H), 7.27−7.38 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 40.6, 52.1, 52.2, 66.9, 114.6, 127.9, 128.0, 128.4, 136.2, 140.2, 155.7, 172.6; HRMS (+TOF) calcd for $C_{15}H_{20}NO_4$ $(M + H)^+$: 278.1386; obsd: 278.1387.

Lactone (2*S*,4*R*)-17. AD-mix-α (1.833 g) was added to a mixture of ^{t}BuOH (6.5 mL) and H_2O (6.5 mL) at rt. The clear orange solution was cooled to 0 °C and Cbz-dehydroleucine-OMe, (+)-**16**, (363 mg, 1.31 mmol) was added. The reaction mixture was stirred at 0 °C for 24 h, quenched with Na_2SO_3 (1.964 g), stirred for an additional 1 h at rt, and extracted with CH_2Cl_2 (6 × 30 mL). The organic layers were combined, filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 95:5 CH_2Cl_2/MeOH, to give **17** as a 6.5:1.0 mixture of diastereomers (215 mg, 59%). R_f 0.55 (9:1 CH_2Cl_2/CH_3OH); $[\alpha]_D^{26}$ −6.3 (*c* 0.95, $CHCl_3$). NMR data reported are for the major diastereomer: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3H), 2.08 (t, *J* = 11.7 Hz, 1H), 2.35 (br, 1H), 2.77 (t, *J* = 11.2 Hz, 1H), 3.54 (d, *J* = 12.0 Hz, 1H), 3.70 (d, *J* = 12.0 Hz, 1H), 4.69 (dd, *J* = 17.1, 9.6 Hz, 1H), 5.10 (s, 2H), 5.49 (d, *J* = 6.3 Hz, 1H), 7.29−7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.4, 35.8, 38.0, 52.3, 67.2, 68.6, 84.9, 128.1, 128.2, 128.5, 136.0, 156.1, 174.9; HRMS (+TOF) calcd for $C_{15}H_{14}N_5O$ $(M + H)^+$: 280.1179; obsd: 280.1183.

Amide 20. Diisopropylethylamine (182 μL, 142 mg, 1.10 mmol, 1.1 equiv), γ-valerolactone (95 μL, 100 mg, 1.00 mmol, 1 equiv) and $Sn(OAc)_2$ (47 mg, 0.20 mmol, 0.2 equiv) were added sequentially to a solution of L-valine ethyl ester hydrochloride (272 mg, 1.50 mmol, 1.5 equiv) in DMF (3 mL) at 0 °C under N_2. The mixture was warmed to 80 °C and stirred for 44 h, concentrated, and the product isolated by flash chromatography eluting with 95:5 CH_2Cl_2/MeOH to give **20** as a 1:1 mixture of diastereomers (159 mg, 65%). R_f 0.37 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (dd, *J* = 6.9, 0.6 Hz, 3H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.98 (d, *J* = 1.2 Hz, 1.5H), 1.21 (d, *J* = 1.2, Hz, 1.5H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.66−1.75 (m, 1H), 1.80−1.89 (m, 1H), 2.12−2.20 (m, 1H), 2.37−2.48 (m, 1H), 2.42 (t, *J* = 6.8 Hz, 1H), 2.43 (t, *J* = 7.3 Hz, 1H), 3.27 (br, 1H), 3.81−3.87 (m, 1H), 4.14−4.26 (m, 2H), 4.53 (dd, *J* = 8.7, 5.0 Hz, 1H), 6.48 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.7, 18.8, 23.5, 31.1, 33.0, 34.2, 57.0, 61.2, 67.1 and 67.2, 172.1 and 172.2, 173.6; HRMS (+TOF) calcd for $C_{12}H_{24}NO_4$ $(M + H)^+$: 246.1699; obsd: 246.1701.

Cbz-L-dehydroleucine-OH (22). Aqueous NaOH (2M, 10 mL) was added dropwise to a suspension of dehydroleucine (380 mg, 2.94 mmol, 1 equiv) in THF (5 mL) at 0 °C. Cbz-Cl (497 μL, 602 mg, 3.53 mmol, 1.2 equiv) was added dropwise over 30 min, with vigorous stirring. The cloudy reaction mixture was left to stir overnight at rt and concentrated to remove THF. The residue was diluted with H_2O (20 mL), extracted with ether (2 × 10 mL), acidified with 6 M HCl to pH 1 and extracted with EtOAc (3 × 25 mL). The organic layers were combined, washed with brine (25 mL) and concentrated to give **22** as a colorless oil (630 mg, 81%). R_f 0.35 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{27}$ +4.4° (*c* 1.2, CH_3OH), Lit.[26] *ent*-**22** $[\alpha]_D^{24.3}$ −5.8 (*c* 1.18, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.75 (s, 3H), 2.38 (dd, *J* = 14.1, 10.0 Hz, 1H), 2.56 (dd, *J* = 14.1, 4.7 Hz, 1H), 4.36 (dd, *J* = 10.0, 4.8 Hz, 1H), 4.78 (br, 1H), 4.81 (br, 1H), 4.95 (br, 1H), 5.07 (s, 2H), 7.26−7.35 (m, 5 H); ^{13}C NMR (100 MHz, CD_3OD) δ 20.0, 38.9, 51.7, 65.5, 112.3, 126.7, 126.9, 127.4, 136.2, 140.3, 156.5, 173.7; HRMS (+TOF) calcd for $C_{14}H_{18}NO_4$ $(M + H)^+$: 264.1230; obsd: 264.1224.

Cbz-L-Dehydroleucine-Val-OEt (23). Diisopropylethylamine (1.2 mL, 910 mg, 6.9 mmol, 3.0 equiv) was added to a solution of Cbz-protected dehydroleucine **22** (618 mg, 2.3 mmol, 1.0 equiv), valine ethyl ester hydrochloride (426 mg, 2.3 mmol, 1.0 equiv) and BOP reagent (1.1 g, 2.6 mmol, 1.1 equiv) in acetonitrile (15 mL). The mixture was stirred at 0 °C for 1 h and then at rt overnight. The mixture was concentrated and the product isolated by flash chromatography eluting with 2:1 Hex/EtOAc, to give **23** as a colorless solid (851 mg, 93%). R_f 0.53 (2:1 Hexanes/EtOAc); $[\alpha]_D^{30}$ −2.4° (*c* 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.75 (s, 3H), 2.16 (app. pd, *J* = 6.9, 4.8 Hz, 1H), 2.39 (dd, *J* = 14.2, 8.8 Hz, 1H), 2.56 (dd, *J* = 14.2, 5.8 Hz, 1H), 4.14−4.24 (m, 2H), 4.31 (br, 1H), 4.50 (dd, *J* =

(25) Racemic material has been prepared via another route, but no data reported: Kazmaier, U.; Maier, S. *Tetrahedron* **1996**, *52*, 941–954.

(26) Qu, H.; Gu, X.; Liu, Z.; Min, B. J.; Hruby, V. J. *Org. Lett.* **2007**, *9*, 3997–4000.

8.7, 4.8 Hz, 1H), 4.80 (br, 1H), 4.87 (t, $J = 1.4$ Hz, 1H), 5.11 (s, 2H), 5.26 (br, 1H), 6.63 (d, $J = 7.8$ Hz, 1H), 7.29−7.37 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 17.7, 18.8, 21.9, 31.3, 40.4, 53.2, 57.2, 61.2, 67.1, 114.5, 127.9, 128.1, 128.5, 136.2, 140.8, 156.1, 171.3, 171.5; HRMS (+TOF) calcd for $C_{21}H_{31}N_2O_5$ $(M + H)^+$: 391.2227; obsd: 391.2240.

Dipeptide 24. AD-mix-β (563 mg) was dissolved in ^{t}BuOH (2 mL) and H_2O (2 mL) at rt. The clear orange solution was cooled to 0 °C and dipeptide olefin **23** (157 mg, 0.4 mmol) was added. The mixture was stirred for 48 h at 0 °C, quenched with Na_2SO_3 (604 mg), stirred for 1 h at rt, diluted with H_2O (10 mL) and extracted with EtOAc (6 × 15 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography eluting with 20:1 EtOAc/MeOH, to give **24** as a colorless solid (154 mg, 90%). R_f 0.32 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{29}$ −25.8° (*c* 0.95, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 0.93 (d, $J = 2.3$ Hz, 3H), 0.95 (d, $J = 2.3$ Hz, 3H), 1.19 (s, 3H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.77 (dd, $J = 14.8$, 8.7 Hz, 1H), 2.03 (dd, $J = 14.6$, 3.3 Hz, 1H), 2.15 (app. qd, $J = 13.1$, 6.5 Hz, 1H), 3.38 (dd, $J = 15.9$, 11.1 Hz, 2H), 4.10−4.21 (m, 2H), 4.30 (d, $J = 5.5$ Hz, 1H), 4.39 (dd, $J = 8.4$, 3.8 Hz, 1H), 5.09 (s, 2H), 7.25−7.36 (m, 5 H); ^{13}C NMR (100 MHz, CD_3OD) δ 12.5, 16.3, 17.4, 22.3, 29.8, 39.0, 50.8, 57.2, 60.2, 65.7, 68.6, 71.1, 126.8, 127.0, 127.5, 136.2, 156.2, 170.9, 173.5; HRMS (+TOF) calcd for $C_{21}H_{33}N_2O_7$ $(M + H)^+$: 425.2282; obsd: 425.2280.

Dipeptide 25. Triethylamine (273 μL, 199 mg, 1.96 mmol, 2.4 equiv), DMAP (20 mg, 0.16 mmol, 0.2 equiv) and TBDMSOTf (206 μL, 238 mg, 0.90 mmol, 1.1 equiv) were added to a solution of **24** (347 mg, 0.82 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 0 °C under N_2. The mixture was warmed to rt overnight, concentrated, and the product isolated by flash chromatography eluting with 2:1 Hex/EtOAc to give **25** (368 mg, 84%). R_f 0.42 (2:1 Hex/EtOAc); $[\alpha]_D^{26}$ +3.3 (*c* 1.15, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.07 (d, $J = 4.4$ Hz, 6H), 0.88 (d, $J = 6.8$ Hz, 3H), 0.89 (s, 9H), 0.93 (d, $J = 6.8$ Hz, 3H), 1.26 (t, $J = 8.8$ Hz, 3H), 1.29 (s, 3H), 1.76 (dd, $J = 14.8$, 4.2 Hz, 1H), 1.84 (br, 1H), 2.13 (d, $J = 6.6$ Hz, 1H), 2.14−2.23 (m, 1H), 3.29 (br, 1H), 3.43 (app.t, $J = 10.6$ Hz, 2H), 4.08−4.24 (m, 3H), 4.42 (br, 1H), 4.48 (q, $J = 8.8$ Hz, 1H), 5.12 (dd, $J = 10.9$, 6.1 Hz, 2H), 6.12 (d, $J = 10.9$, 5.4 Hz, 1H), 7.28−7.36 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.5, 14.2, 17.5, 18.3, 19.0, 23.6, 25.8, 30.9, 41.1, 51.1, 57.3, 61.1, 66.8, 70.6, 72.2, 128.0, 128.1, 128.4, 136.3, 156.0, 171.8,172.4; HRMS (+TOF) calcd for $C_{27}H_{46}N_2O_7Si$ $(M + H)^+$: 538.3074; obsd: 538.3065.

Lactone (2*S*,4*S*)-17. A mixture of trifluoroacetic acid (100 μL) and CH_2Cl_2 (2 mL) was added to compound **24** (43 mg, 0.10 mmol) at 0 °C under N_2. The mixture was gradually warmed to rt overnight, concentrated, and the product isolated by flash chromatography eluting with 95:5 CH_2Cl_2/MeOH to give (2*S*,4*S*)-**17** (23 mg, 82%). R_f 0.53 (9:1 CH_2Cl_2/CH_3OH); $[\alpha]_D^{25}$ −5.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H), 2.34 (d, $J = 9.5$ Hz, 1H), 2.54 (br, 1H), 3.44 (d, $J = 8.0$ Hz, 1H), 3.73 (d, $J = 12.2$ Hz, 1H), 4.70 (dd, $J = 17.1$, 8.8 Hz, 1H), 5.11 (dd, $J = 18.5$, 12.2 Hz, 2H), 5.79 (d, $J = 7.3$ Hz, 1H), 7.30−7.36 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5, 29.7, 35.8, 51.1, 67.3, 67.4, 84.6, 128.1, 128.2, 128.5, 136.0, 156.1, 174.9; HRMS (+TOF) calcd for $C_{15}H_{14}N_5O$ $(M + H)^+$: 280.1179; obsd: 280.1183.

Hydrochloride Salt of Lactone (2*S*,4*S*)-26. Concentrated HCl (48 μL) and 10% Pd/C (22 mg, 0.206 mmol) were added to a solution of (2*S*,4*S*)-**17** (70 mg, 0.25 mmol) in EtOH (2.4 mL) at rt under N_2. The mixture was hydrogenated for 4 h, then filtered through Celite, and concentrated to give the hydrochloride salt as a colorless solid. Recrystallization from ethanol/ether yielded colorless crystals (46 mg, 100%); R_f 0.46 (3:3:3:1 ^{n}BuOH/EtOH/NH_3/H_2O); mp 210−213 °C, Lit.[6] 205−207 °C. $[\alpha]_D^{23}$ −7.4° (*c* 0.75, 6 N HCl), Lit.[6] $[\alpha]_D^{20}$ −12° (*c* 2, 6 N HCl). ^{1}H NMR (400 MHz, D_2O): δ 1.45 (s, 3H), 2.43 (dd, $J = 13.0$, 10.4 Hz, 1H), 2.60 (dd, $J = 13.2$, 9.6 Hz, 1H), 3.61 (d, $J = 12.7$ Hz, 1H), 3.77 (d, $J = 12.7$ Hz, 1H), 4.64 (t, $J = 9.9$ Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 20.9, 32.8, 49.4, 65.9, 87.5, 173.2; HRMS (+TOF) calcd for $C_6H_{12}NO_3$ $(M - HCl)^+$: 146.0811; obsd: 146.0813.

Hydrochloride Salt of Lactone (2*S*,4*R*)-26. (2*S*,4*R*)-**17** (32 mg) was treated, as for the diastereomer, to cleave the Cbz group to give the hydrochloride salt of aminolactone (2*S*,4*R*)-**26** (18 mg, 86%) as a 6.5:1.0 mixture of diastereomers. mp 197−200 °C, Lit.[6] 199−200 °C. $[\alpha]_D^{23}$ −22° (*c* 0.45, 6 M HCl), Lit.[6] $[\alpha]_D^{20}$ −35.5° (*c* 2, 6N HCl). NMR spectra are reported for the MAJOR (2*S*,4*R*) diastereomer. ^{1}H NMR (400 MHz, D_2O) δ 1.46 (s, 3H), 2.24 (dd, $J = 13.4$, 11.0 Hz, 1H), 2.88 (dd, $J = 13.4$, 9.8 Hz, 1H), 3.65 (d, $J = 12.6$ Hz, 1H), 3.71 (d, $J = 12.6$ Hz, 1H), 4.64 (t, $J = 10.3$ Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 22.1, 35.3, 50.0, 66.9, 87.9, 173.7.

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Supporting Information Available: Procedures for the preparation of compounds **4**, (±)-**10**, (+)-**10** and (−)-**11**, and **13**. ^{1}H and ^{13}C NMR spectra for pertinent compounds and crystallographic data for compound (2*S*,4*S*)-**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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