Synthesis of L-2,3-*trans*-3,4-*cis*-Dihydroxyproline Building Blocks for Peptide Synthesis

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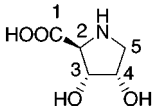
L-2,3-*trans*-3,4-*cis*-*N*-Fluorenylmethoxycarbonyl-3,4-dihydroxy-3,4-*O*-isopropylidineproline (**9**) has been prepared from D-gulonolactone in nine steps and an overall yield of 22%. Compound **9** has been converted to its allyl ester **13**. Compounds **9** and **13** were investigated as building blocks for the incorporation of dihydroxyproline into peptides, with compound **9** serving as a carboxyl component and compound **13** as a precursor to an amino component for peptide coupling reactions. Their utility was demonstrated by the synthesis of dipeptides **11** and **15**.

Introduction

In 1994, L-2,3-*trans*-3,4-*cis*-dihydroxyproline (DHP) (**1**) was identified as the sixth residue in the repeating



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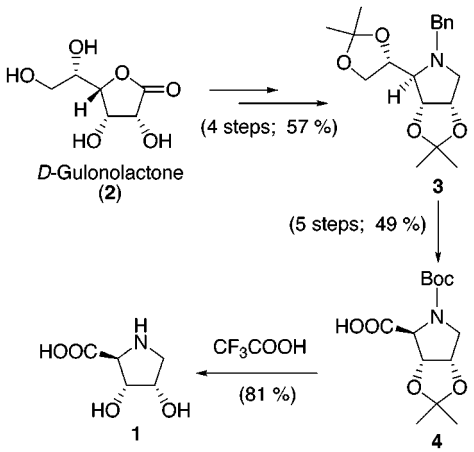
decapeptide sequence of Mefp1, an adhesive peptide produced by the marine mussel *Mytilus edulis*.[1] This was the first example in which the position of a dihydroxyproline was established in the primary sequence of a protein. Two other L-isomers of dihydroxyproline have been isolated from nature.[2]

All eight stereoisomers of dihydroxyproline, or related aza-sugar derivatives, have been synthesized previously.[3] Interest in these compounds has been derived largely from their ability to inhibit glycosidase enzymes.[4,5] Our goal, however, is to investigate the role of dihydroxyproline in peptide structure and function.[6] A prerequisite to this study is an efficient synthesis of suitably protected dihydroxyproline building blocks.

Fleet and Son reported the synthesis of L-2,3-*trans*-3,4-*cis*-dihydroxyproline (**1**) from D-gulonolactone (**2**)

Scheme 1



(Scheme 1) in 1988.[7] Pyrrolidine **3** was obtained efficiently in four steps and elaborated to protected DHP **4**. Although **4** could be used as a building block in peptide synthesis, its utility is limited by the fact that both the Boc and acetonide protecting groups are acid labile.

We report, herein, the synthesis of some potentially useful building blocks of L-2,3-*trans*-3,4-*cis*-DHP, derived from **3**, and demonstrate their utility in the synthesis of dipeptides **11** and **15**.

Results and Discussion

Compound **3** was prepared according to Fleet and Son.[7] As noted by Herdeis et al., *N*-benzyl pyrrolidines (e.g., **3**) are not especially stable.[8] On standing, compound **3** gave off the characteristic odor of benzaldehyde, suggesting that an oxidative cleavage of the benzylamine was taking place. We considered it sensible to replace the benzyl group by an electron-withdrawing protecting group early in the synthesis. We chose the Fmoc group,[9] which is cleaved under mild, basic conditions, to develop

(1) (a) Taylor, S. W.; Waite, J. H.; Ross, M. M.; Shabanowitz, J.; Hunt, D. F. *J. Am. Chem. Soc.* **1994**, *116*, 10803−10804. For earlier reports, see: (b) Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038−1040. (c) Waite, J. H.; Housley, T. J.; Tanzer, M. L. *Biochemistry* **1985**, *24*, 5010−5014.

(2) For a review of naturally occurring proline derivatives, see: Mauger, A. B. *J. Nat. Prod.* **1996**, *59*, 1205−1211.

(3) For some recent examples with extensive references to previous work, see: (a) Huang, Y.; Dalton, D. R.; Carroll, P. J. *J. Org. Chem.* **1997**, *62*, 372−376. (b) Pohlit, A. M.; Correia, C. R. D. *Heterocycles* **1997**, *45*, 2321−2325. (c) Schumacher, K. K.; Jiang, J.; Joullié, M. M. *Tetrahedron: Asymmetry* **1998**, *9*, 47−53.

(4) Sinnot, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202.

(5) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340−347.

(6) Joullié et al. have described the synthesis of various astins containing substituted prolines. See: (a) (3*S*,4*R*)-3,4-dichloro-L-proline, Jiang, J.; Schumacher, K. K.; Hauze, D. B.; Joullié, M. M. 208th National Meeting of the American Chemical Society, 1994, ORGN 30. (b) L-*trans*-4-hydroxyproline and L-2,3-*trans*-3,4-*cis*-dihydroxyproline, Schumacher, K. K.; Hauze, D. B.; Jiang, J.; Zhang, Z.; Reddy, R. E.; Davis, F. A.; Joullié, M. M. 211th National Meeting of the American Chemical Society, 1996, ORGN 168. (c) 3,4-dehydroproline, Hauze, D. B.; Schumacher, K. K.; Jiang, J.; Zhang, Z.; Reddy, R. E.; Davis, F. A.; Joullié, M. M. 211th National Meeting of the American Chemical Society, 1996, ORGN 169.

(7) Fleet, G. W. J.; Son, J. C. *Tetrahedron* **1988**, *44*, 2637−2647.

(8) Herdeis, C.; Aschenbrenner, A.; Kirfel, A.; Schwabenländer, F. *Tetrahedron: Asymmetry* **1997**, *8*, 2421−2432.

(9) (a) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404−3409. (b) Atherton, E.; Sheppard, R. C. In *The Peptides*: *Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, p 1.

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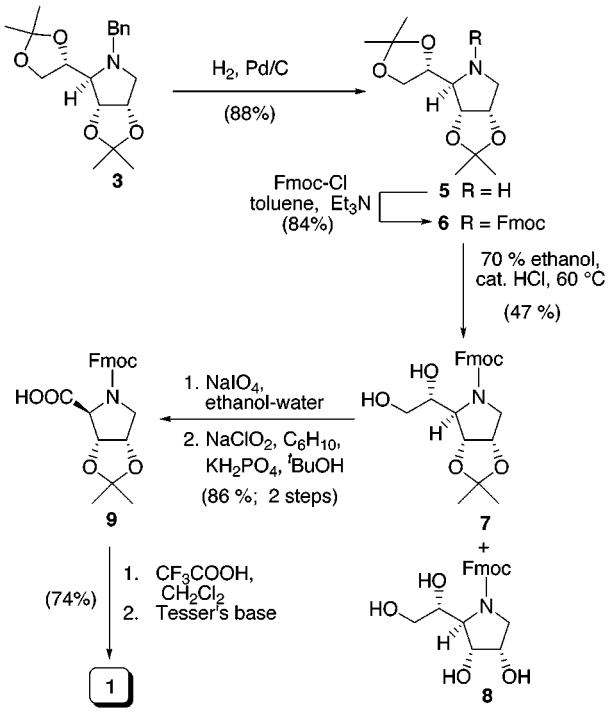
Scheme 2



an orthogonal scheme for protection. Hydrogenolysis of the benzyl group in **3** gave amine **5**, which was readily converted to its Fmoc derivative **6** (Scheme 2).

Attempts to selectively deprotect the side chain isopropylidene in compound **6** proved problematic. Fleet et al. performed a similar selective deprotection on compound **3**, using 80% aqueous acetic acid at 50 °C.[7] These conditions appeared to be too harsh for our substrate; a considerable amount of tetraol **8** was observed in the reaction mixture. Table 1 summarizes the various reaction conditions investigated. Entry 6 represents the best results obtained to date, in which a 47% yield of **7** is accompanied by a 53% recovery of **6**. Although this procedure is not optimal, it represents a quantitative yield based on recovered starting material.

Periodate cleavage of the diol in compound **7** and analysis of the crude reaction mixture by ^{1}H NMR spectroscopy confirmed the presence of an aldehyde (δ 9.62 ppm). The aldehyde was subjected directly to oxidation, using sodium chlorite.[7] This provided building block **9** in 86% yield, over two steps.

To make a direct comparison with the DHP isolated from Mefp1, compound **9** was converted to **1** (Scheme 2). The free DHP (**1**) obtained in this manner has a small, positive optical rotation, in reasonable agreement with the literature.[12]

With building block **9** in hand, we were eager to investigate its incorporation into peptides. We sought to construct dipeptides which were relevant to the Mefp1 sequence.[13] Specifically, we wanted to explore the DHP residue as a carboxyl component and also as an amino component in peptide bond formation.

Reaction of compound **9** with *trans*-4-hydroxyproline derivative **10**, using the popular BOP reagent gave only a 55% yield of dipeptide **11**. The more reactive BroP reagent[14] and longer reaction times gave improved results. The suitability of the acetonide protective group for the DHP diol was demonstrated by treatment of **11** with TFA. This effected simultaneous cleavage of the *tert*-butyl ether and the acetonide to give **12** in an efficient manner (Scheme 3).

Building block **9** could be converted to allyl ester **13** by conventional methods.[15] Removal of the Fmoc group with diethylamine[16] gave an amine which was coupled in situ with Fmoc-Tyr(O^{t}Bu)-OH (**14**) under a variety of reaction conditions. With BOP, only a 17% yield of **15** was obtained; the diastereomer **16** was also observed in the reaction mixture. Better yields were observed with the more reactive coupling reagents, BroP and BOP−Cl, but diastereomer **16** was again present. This presumably occurs because the rate of racemization of an oxazolone, derived from **14**, is competitive with the unusually slow coupling reactions.[17] This underscores the low reactivity of the dihydroxyproline as a nucleophile. Although **15** and **16** were readily separated by flash chromatography, it was desirable to find reaction conditions where the formation of **16** is minimized. Fortunately, using diphenylphosphoryl azide, a respectable yield of **15** was obtained, and HPLC analysis indicated minimal (2.4%) racemization.

The difficulty experienced in the peptide coupling reactions is worthy of some comment.[18] These reactions are slow because the amino component is a secondary amine embedded in a pyrrolidine ring. The amine derived from **13** is a particularly hindered nucleophile because the amine is part of a rigid bicyclic system.

Summary

We have successfully adapted Fleet's DHP synthesis for the production of building block **9**, which has been demonstrated to be a useful intermediate in peptide synthesis. Although peptide couplings involving DHP are slow, good yields of dipeptides **11** and **15** have been achieved using appropriate reaction conditions. This work sets the stage for the synthesis of larger peptides containing DHP and studies to assess the role of DHP in peptide structure and function.

Experimental Section

General. Reactions were carried out under an atmosphere of dry nitrogen. THF and ether were freshly distilled from sodium/benzophenone. Toluene was freshly distilled from sodium. Acetone was dried over anhydrous $CaSO_4$, distilled, and stored over 4 Å molecular sieves. Acetonitrile and dichloromethane were freshly distilled from calcium hydride. Pyri-

(10) Setoi, H.; Kayakiri, H.; Takeno, H.; Hashimoto, M. *Chem. Pharm. Bull.* **1995**, *35*, 3995−3999.

(11) Han, S.-Y.; Liddell, P. A.; Joullié, M. M. *Synth. Commun.* **1988**, *13*, 275−283.

(12) The NMR data in the present study is in good agreement with that reported in ref 1a. The configuration of the DHP isolated from Mefp1 remains unknown as a result of a lack of material. Fleet and Son (ref 7) reported a rotation of $[\alpha]^{20}_D$ +7.5° (*c* 0.16, H_2O).

(13) The revised sequence of the repeating decapeptide unit is -(Ala-Lys-Pro-Ser-Tyr-DHP-Hyp-Thr-DOPA-Lys)- (ref 1a).

(14) (a) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669−672. (b) Castro, B.; Dormoy, J. R. *Bull. Soc. Chim. Fr.* **1973**, 3359−3361.

(15) Kunz, H.; Waldmann, H.; Unverzagt, C. *Int. J. Pept. Protein Res.* **1985**, *26*, 493−497.

(16) For one-pot removal of Fmoc group and coupling of the next amino acid, see: Colucci, W. J.; Tung, R. D.; Petri, J. A.; Rich, D. H. *J. Org. Chem.* **1990**, *55*, 2895−2903.

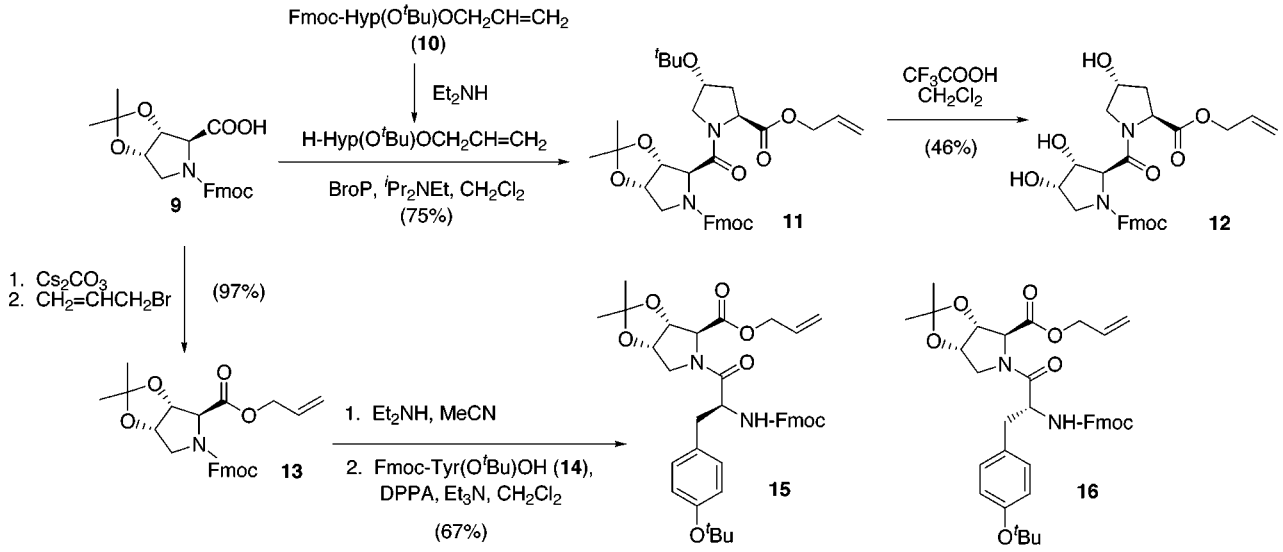
(17) Benoiton, N. L. *Biopolymers* **1996**, *40*, 245−254.

(18) For a recent review on difficult peptide coupling reactions, see: Humphrey, J. M.; Chamberlain, A. R. *Chem. Rev.* **1997**, *97*, 2243−2266.

Table 1.

entry	reaction conditions	recvrd **6** (%)	isoltd **7** (%)	ref
1	80% HOAc, 50 °C, 2 d	0	9	7
2	TsOH, MeOH/H_2O (10:1), 3 d	22	45	10
3	Dowex H^+, MeOH/dioxane/H_2O (1:0.6:1), 0 °C, 3 d	64	20	11
4	Dowex H^+, MeOH/H_2O (10:1), 40 °C, 5 d	17	22	
5	cat. HCl, 70% EtOH, 2 d	70	23	
6	cat. HCl, 70% EtOH, 60 °C, 2.5 h	53	47	

Scheme 3



dine, benzylamine, diethylamine, diisopropylethylamine, and triethylamine were distilled from calcium hydride and stored over potassium hydroxide. Cyclohexene was dried and distilled from calcium hydride and stored over 4 Å molecular sieves. *tert*-Butyl alcohol was dried over $MgSO_4$, distilled from CaH_2, and stored over 4 Å molecular sieves. Methanesulfonyl chloride was dried and distilled from phosphorus pentoxide under vacuum. Allyl bromide was obtained from Riedel-de-Haën, washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and distilled prior to use. D-Gulonolactone, *p*-toluenesulfonic acid, cesium carbonate, and BOP reagent were obtained from Acros. DPPA was obtained from Fluka. BroP was prepared according to ref 14b. Lithium aluminum hydride and trifluoroacetic acid were obtained from Riedel-de-Haën. 2,2-Dimethoxypropane was obtained from Aldrich. Fmoc-Tyr-(O^{t}Bu)-OH was obtained from Sigma. Fmoc-Hyp(O^{t}Bu)-OH was obtained from Bachem. Tesser's base was prepared by mixing together dioxane, methanol, and 4 N NaOH in a 30:9:1 ratio by volume.[19] R_f values refer to TLC on silica gel. Analytical and preparative HPLC was performed on 250 mm C-18 columns. The analytical column was 4.6 mm in diameter, and a flow rate of 0.6 mL min^{-1} was employed. The preparative column was 10 mm in diameter, and a flow rate of 2.8 mL min^{-1} was employed. Disodium 3-trimethylsilyl-1-propanesulfonate (DSS) was used to reference ^{1}H NMR spectra run in D_2O.

1,4-Dideoxy-2,3;5,6-*di*-*O*-isopropylidene-1,4-imino-D-allitol (5). Tertiary amine **3** (775 mg, 2.33 mmol) was dissolved in absolute ethanol (30 mL), and the solution was stirred under an atmosphere of hydrogen in the presence of 10% palladium on charcoal (300 mg) at room temperature overnight. The reaction mixture was filtered through a pad of Celite and rinsed thoroughly with ethanol (10 mL). The extract and washings were concentrated. The residue was purified by flash column chromatography, eluting first with ethyl acetate, and then increasing the polarity to 9:1 ethyl acetate−methanol, to elute 1,4-dideoxy-2,3;5,6-*di*-*O*-isopropylidene-1,4-imino-D-allitol (**5**) as a yellow oil (498 mg, 88%): TLC $R_f = 0.28$ (ethyl acetate); $[\alpha]^{20}_D = +29.1°$ (*c* 1.50, $CHCl_3$) [lit.[7] +34.1° (*c* 0.41, $CHCl_3$)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (s, 6H), 1.28 (s, 3H), 1.31 (s, 3H), 2.74 (dd, $J = 13.6$, 4.0 Hz, 1H), 2.89 (d, $J = 13.6$ Hz, 1H), 2.98 (d, $J = 7.7$ Hz, 1H), 3.5 (s, 1H), 3.67 (dd, $J = 8.4$, 5.8 Hz, 1H), 3.80 (ddd, $J = 7.6$, 4.8, 1.5 Hz, 1H), 3.95 (dd, $J = 8.4$, 6.5 Hz, 1H), 4.57 (dd, $J = 5.6$, 4.0 Hz, 1H), 4.61 (d, $J = 5.7$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.5, 24.6, 25.8, 26.3, 52.1, 67.3, 67.7, 74.3, 81.6, 82.4, 109.2, 110.5; HRMS (EI) calcd for $C_{12}H_{21}NO_4$ $(M)^+$ 243.1470, obsd 243.1456.

***N*-Fluorenylmethoxycarbonyl-1,4-dideoxy-2,3;5,6-*di*-*O*-isopropylidene-1,4-imino-D-allitol (6).** A solution of amine **5** (1.17 g, 4.81 mmol, 1.0 equiv) in toluene (5 mL) was added dropwise to a solution of fluorenylmethylchloroformate (1.37 g, 5.29 mmol, 1.1 equiv) in toluene (7 mL). Triethylamine (738 μL, 5.29 mmol, 1.1 equiv) was added to the solution. Further toluene (4 mL) was added to disperse the triethylamine hydrochloride. The suspension was stirred at room temperature under nitrogen for 15 h. The solid was removed by filtration and rinsed thoroughly with toluene (1 mL). The filtrate and washings were concentrated. The yellow residue was purified by flash column chromatography, eluting with 4:1 hexanes−ethyl acetate. This afforded 2.24 g (84%) of *N*-fluorenylmethoxycarbonyl-1,4-dideoxy-2,3;5,6-*di*-*O*-isopropylidene-1,4-imino-D-allitol (**6**) as a colorless oil: TLC $R_f = 0.52$ (1:1 hexanes−ethyl acetate); $[\alpha]^{20}_D = -49.5°$(*c* 2.65, $CHCl_3$); ^{1}H NMR (200 MHz, DMSO-d_6, 370 K) δ 1.22 (s, 3H), 1.24 (s, 3H), 1.28 (s, 3H), 1.33 (s, 3H), 3.32 (dd, $J = 12.6$, 4.6 Hz, 1H), 3.62−3.87 (m, 5H), 4.25 (t, $J = 5.8$ Hz, 1H), 4.39−4.55 (m, 2H), 4.59−4.70 (m, 2H), 7.25−7.42 (m, 4H), 7.62 (d, $J = 7.5$ Hz, 2H), 7.82 (d, $J = 6.8$ Hz, 2H); ^{13}C NMR (50 MHz, DMSO-d_6, 370 K) δ 24.0, 24.2, 25.6, 26.2, 46.4, 51.5, 64.9, 65.5, 65.7, 73.9, 78.3, 79.9, 108.3, 110.2, 119.2, 124.0, 126.3, 126.9, 140.3, 143.3, 153.5; HRMS (CI) calcd for $C_{27}H_{32}NO_6$ $(M + H)^+$ 466.2229, obsd 466.2235. Anal. Calcd for $C_{27}H_{31}NO_6$: C 69.63; H 6.75; N 3.01. Found: C 69.46; H 6.96; N 2.99.

***N*-Fluorenylmethoxycarbonyl-1,4-dideoxy-2,3-*O*-isopropylidene-1,4-imino-D-allitol (7).** Compound **6** (305 mg, 0.6 mmol, 1 equiv) was suspended in 70% aqueous ethanol (4 mL). The suspension was warmed to 60 °C, resulting in a homogeneous solution. Concentrated hydrochloric acid (10 μL) was added, and the solution was stirred at 60 °C for 2.5 h.

(19) Chang, C.-D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Peptide Protein Res.* **1980**, *15*, 59−66.

The mixture was filtered through a plug of alumina in a Pasteur pipet, washing well with absolute ethanol. The filtrate and washings were concentrated. The residue was purified by flash column chromatography, eluting with ethyl acetate, to recover compound **6** (148 mg, 53%) and afford compound **7** as a colorless oil (130 mg, 47%): TLC $R_f = 0.27$ (ethyl acetate); $[\alpha]^{20}_D = -34.3°$ (*c* 1.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.34 (s, 3H), 3.29−3.41 (br m, 2H), 3.49−3.60 (br m, 2H), 3.90 (d, $J = 12.9$ Hz, 1H), 4.05 (d, $J = 8.6$ Hz, 1H), 4.25 (t, $J = 6.8$ Hz, 1H), 4.36 (dd, $J = 10.6$, 7.2 Hz, 1H), 4.52 (dd, $J = 10.6$, 6.6 Hz, 1H), 4.74 (t, $J = 5.1$ Hz, 1H), 4.86 (d, $J = 5.8$ Hz, 1H), 7.29−7.39 (m, 4H), 7.41−7.59 (m, 2H), 7.75 (d, $J = 7.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 26.8, 47.1, 52.1, 62.7, 65.1, 67.7, 70.5, 79.1, 81.8, 118.8, 119.9, 124.9, 125.0, 127.1, 127.7, 141.2, 141.3, 143.4, 143.7, 155.9; HRMS (CI) calcd for $C_{24}H_{28}NO_6$ $(M + H)^+$ 426.1916, obsd 426.1910. Anal. Calcd for $C_{24}H_{27}NO_6$: C 67.75; H 6.40; N 3.29. Found: C 67.27; H 6.53; N 3.24.

L-2,3-*trans*-3,4-*cis*-*N*-Fluorenylmethoxycarbonyl-3,4-dihydroxy-3,4-*O*-isopropylidene-proline (9). Diol **7** (122 mg, 0.28 mmol, 1 equiv) was dissolved in ethanol−water (5:2 ratio, 4.5 mL). Sodium periodate (166 mg, 0.78 mmol, 2.7 equiv) was added, resulting in a cloudy suspension which was stirred at room temperature for 15 min. The mixture was filtered and concentrated. The crude aldehyde was dissolved in *tert*-butyl alcohol (4.5 mL) and cyclohexene (0.3 mL). A solution of sodium chlorite (313 mg, 2.87 mmol, 10 equiv) and potassium dihydrogen phosphate (390 mg, 2.87 mmol, 10 equiv) in water (4 mL) was added dropwise to the aldehyde mixture. The resulting biphasic reaction mixture was stirred at room temperature under an atmosphere of N_2 for 12 h. The reaction mixture was concentrated, and the residue was partitioned between ethyl acetate (50 mL) and water (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography, eluting with 9:1 CH_2Cl_2−MeOH. This afforded L-2,3-*trans*-3,4-*cis*-*N*-fluorenylmethoxycarbonyl-3,4-dihydroxy-3,4-*O*-isopropylidene-proline (**9**) as a colorless solid (96 mg, 82%): TLC $R_f = 0.21$ (9:1 CH_2Cl_2−methanol); $[\alpha]^{20}_D = -47.7°$ (*c* 0.85, $CHCl_3$); ^{1}H NMR (200 MHz, DMSO-d_6, 343 K) δ 1.27 (s, 3H), 1.37 (s, 3H), 3.45−3.70 (m, 4H), 4.20−4.38 (m, 4H), 4.70−4.85 (m, 2H), 7.26−7.41 (m, 4H), 7.61 (d, $J = 7.2$ Hz, 2H), 7.83 (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.6, 26.7, 46.7, 52.3, 67.5, 68.2, 78.9, 82.1, 111.6, 119.8, 125.1, 127.0, 127.6, 141.1, 143.6, 156.7, 177.2; HRMS (CI) calcd for $C_{23}H_{24}NO_6$ $(M + H)^+$ 410.1603, obsd 410.1608. Anal. Calcd for $C_{23}H_{23}NO_6$: C 67.47; H 5.66; N 3.42. Found: C 67.41; H 5.62; N 3.65.

L-2,3-*trans*-3,4-*cis*-3,4-Dihydroxyproline (1). Trifluoroacetic acid (0.5 mL) was added to a solution of compound **9** (30 mg, 0.07 mmol) in CH_2Cl_2 (0.5 mL). The orange solution was stirred at room temperature under N_2 overnight. The reaction mixture was concentrated, dissolved in Tesser's base (1 mL), and stirred at room temperature for 1 h. The reaction mixture was concentrated, and the residue was purified by ion exchange chromatography (Dowex H^+), eluting with 0.5 M NH_4OH. The fractions which gave a positive ninhydrin test were lyophilized to give **1** as a colorless solid (8 mg, 74%): TLC $R_f = 0.34$ (6:4:1 $CHCl_3$−methanol−H_2O); $[\alpha]^{20}_D = +5.9°$ (*c* 0.35, H_2O) [lit.[7] +7.5° (*c* 0.16, H_2O)]; ^{1}H NMR (400 MHz, D_2O, DSS as internal reference) δ 3.30 (dd, $J = 12.4$, 4.4 Hz, 1H), 3.55 (dd, $J = 12.4$, 4.9 Hz, 1H), 3.97 (d, $J = 4.9$ Hz, 1H), 4.37 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 51.1, 67.2, 72.8, 76.9, 175.3; HRMS (FAB) calcd for $C_5H_{10}NO_4$ $(M + H)^+$ 148.06098, obsd 148.06349.

L-2,3-*trans*-3,4-*cis*-*N*-Fluorenylmethoxycarbonyl-3,4-dihydroxy-3,4-*O*-isopropyl-ideneproline Allyl Ester (13). Cesium carbonate (23 mg, 0.07 mmol, 0.5 equiv) was added to a suspension of acid **9** (57 mg, 0.13 mmol, 1.0 equiv) in dry methanol (1 mL). The reaction mixture was stirred at room temperature under N_2 for 2 h. The solvent was removed to give a colorless residue, which was dissolved in DMF (1 mL) and treated with allyl bromide (14 μL, 0.17 mmol, 1.2 equiv). The mixture was stirred at room temperature under N_2 for 14 h. The suspension was diluted with ethyl acetate (30 mL) and washed with water (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated. The colorless residue was purified by flash column chromatography, eluting with 2:1 hexanes−ethyl acetate, to give compound **10** as a colorless oil (60 mg, 97%): TLC $R_f = 0.49$ (2:1 hexanes−ethyl acetate); $[\alpha]^{20}_D = -22.8°$ (*c* 0.85, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.49 (s, 3H), 3.58−3.74 (m, 1H), 3.98 (dd, $J = 12.4$, 7.1 Hz, 1H), 4.20−4.48 (m, 2H), 4.59−4.61 (m, 2H), 4.64−4.73 (m, 1H), 4.76−4.80 (m, 2H), 5.22−5.38 (m, 2H), 5.78−6.19 (m, 1H), 7.29−7.43 (m, 4H), 7.53−7.62 (m, 2H), 7.62−7.77 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.8, 26.8, 47.0, 52.2, 52.5, 66.1, 67.2, 78.4, 79.4, 82.3, 83.4, 112.6, 118.9, 119.9, 124.9, 125.1, 127.0, 127.6, 141.2, 155.1, 169.6; HRMS (DCI) calcd for $C_{26}H_{28}NO_6$ $(M + H)^+$ 450.19166, obsd 450.19152.

Fmoc-Hyp(O^{t}Bu)-OAll (10). Cesium carbonate (99 mg, 0.3 mmol, 0.5 equiv) was added to a suspension of Fmoc-Hyp(O^t-Bu)-OH (250 mg, 0.6 mmol, 1.0 equiv) in dry methanol (2.5 mL). The resulting homogeneous solution was stirred at room temperature under N_2 for 2 h. The mixture was concentrated. The residue was dissolved in DMF (2.5 mL) and treated with allyl bromide (64 μL, 0.07 mmol, 1.2 equiv). The mixture was stirred at room temperature under N_2 for 15 h. The suspension was partitioned between ethyl acetate (30 mL) and water (30 mL). The organic layer was washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography, eluting with 2:1 hexanes−ethyl acetate, to give Fmoc-Hyp(O^{t}Bu)-OAll (**10**) as a colorless oil (252 mg, 92%): TLC $R_f = 0.33$ (2:1 hexanes−ethyl acetate); $[\alpha]^{20}_D = -27.4°$ (*c* 0.90, $CHCl_3$); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.11 (s, 9H), 1.93−2.19 (m, 2H), 3.12−3.34 (m, 1H), 3.53 (dd, $J = 10.6$, 5.5 Hz, 1H), 4.15−4.36 (m, 5H), 4.51−4.60 (m, 2H), 5.16−5.36 (m 2H), 5.78−5.99 (m, 1H), 7.28−7.45 (m, 4H), 7.57−7.65 (m, 1H), 7.80−7.89 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) (mixture of rotamers) δ 27.9, 37.3 & 38.2, 46.4, 53.4 & 53.8, 57.1 & 57.5, 64.7 & 65.0, 66.6 & 66.7, 68.0, 68.9, 73.6, 117.6 & 117.9, 120.0, 124.9, 127.0, 127.6, 132.2, 140.7, 143.4 & 143.7, 153.5 & 153.9, 171.5; HRMS (CI) calcd for $C_{27}H_{32}NO_5$ $(M + H)^+$ 450.22806, obsd 450.22802.

Dipeptide 11. Diethylamine (1 mL) was added to a solution of Fmoc-Hyp(O^{t}Bu)-OAll (**10**) (58 mg, 0.1 mmol, 1.0 equiv) in acetonitrile (1 mL), and the mixture was stirred at room temperature under N_2 for 30 min. The mixture was concentrated, and the residue was redissolved in acetonitrile (2 mL) and concentrated again. A solution of compound **9** (50 mg, 0.1 mmol. 1.0 equiv) in dry CH_2Cl_2 (1.5 mL) was added to the residue. Diisopropylethylamine (56 μL, 0.3 mmol, 2.5 equiv) was added, followed by BroP reagent (55 mg, 0.1 mmol, 1.0 equiv). The resulting solution was stirred at room temperature under N_2 for 5 d. The mixture was concentrated, and the brown residue was purified by flash column chromatography, eluting with 2:1 hexanes−ethyl acetate, to give dipeptide **11** as a colorless oil (80 mg, 75%): $R_f = 0.26$ (2:1 hexanes−ethyl acetate); HPLC $t_R = 11.34$ min (85% MeCN in H_2O; 0.05% TFA); $[\alpha]^{20}_D = -28.7°$ (*c* 0.60, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ 1.11 (s, 3H), 1.18 (s, 6H), 1.32 (s, 1H), 1.35 (s, 2H), 1.47 (s, 1H), 1.51 (s, 2H), 2.06−2.35 (m, 2H), 2.49 (br, 1H), 3.29 (dd, $J = 10.1$, 5.6 Hz, 0.25H), 3.68 (dd, $J = 10.1$, 5.6 Hz, 0.75H), 3.78−4.00 (m, 3H), 4.22−4.89 (m, 10H), 5.22−5.38 (m, 2H), 5.76−5.97 (m, 1H), 7.25−7.42 (m, 4H), 7.51−7.63 (m, 2H), 7.76 (d, $J = 7.8$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) (mixture of rotamers) δ 24.9, 26.9, 28.2, 36.6 & 36.7, 47.0 & 47.2, 52.9 & 53.1, 53.3, 57.5, 65.3 & 65.4, 65.8, 67.2 & 67.7, 69.4, 74.2, 78.9 & 80.0, 82.3 & 83.3, 112.4 & 112.5, 118.6 & 118.7, 119.9, 124.7, 124.8, 125.2, 127.0, 127.6, 131.6, 141.2, 143.8, 143.9, 144.0, 154.5 & 155.2, 168.6 & 168.9, 171.6 & 171.7; HRMS (FAB^+) calcd for $C_{35}H_{43}N_2O_8$ $(M + H)^+$ 619.30194, obsd 619.30190.

Dipeptide 12. Trifluoroacetic acid (0.5 mL) was added to a solution of dipeptide **11** (13 mg, 0.02 mmol) in dichloromethane (0.5 mL). The orange solution was stirred at room temperature under N_2 for 23 h and then concentrated. The residue was purified by RP-HPLC (70−95% MeCN in H_2O over 30 min) to give, after lyophilization, dipeptide **12** as a colorless solid (5 mg, 46%): R_f 0.47 (9:1 CH_2Cl_2−MeOH); HPLC $t_R = 4.89$ min (70−95% MeCN in H_2O over 30 min); $[\alpha]^{20}_D = -34.9°$ (*c* 0.30, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers)

δ 2.04−2.10 (m, 1H), 2.17−2.36 (m, 1H), 3.45−3.56 (m, 2H), 3.64−3.72 (m, 1.25H), 3.78−3.88 (m, 0.75H), 3.91−3.96 (m, 1H), 4.15−4.23 (m, 2H), 4.24−4.30 (m, 1H), 4.31−4.41 (m, 2H), 4.45−4.52 (m, 2H), 4.57−4.63 (m, 2H), 5.21−5.25 (m, 1H), 5.32−5.37 (m, 1H), 5.88−5.99 (m, 1H), 7.29−7.41 (m, 4H), 7.57−7.65 (m, 2H), 7.78−7.81 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) (mixture of rotamers) δ 38.0 & 38.2, 52.2 & 52.4, 55.9 & 56.2, 59.8 & 59.9, 65.1 & 65.3, 66.9 & 67.1, 68.9 & 69.0, 70.6, 71.0, 71.7, 76.1, 77.0, 118.8 & 118.9, 121.0, 126.1, 126.2, 128.2, 128.3, 128.8, 128.9, 133.3, 142.6, 145.1, 145.3, 145.4, 156.5 & 156.8, 171.7 & 171.8, 173.1 & 173.2; HRMS (FAB$^+$) calcd for $C_{28}H_{31}N_2O_8$ $(M + H)^+$ 523.20804, obsd 523.20774.

Dipeptide 15. Diethylamine (0.5 mL) was added to a solution of compound **13** (10 mg, 0.020 mmol, 1.0 equiv) in acetonitrile (0.5 mL), and the mixture was stirred at room temperature under N_2 for 30 min. The mixture was concentrated, and the residue was redissolved in acetonitrile (2 mL) and concentrated again. A solution of Fmoc-Tyr(O^{t}Bu)-OH (12 mg, 0.025 mmol. 1.1 equiv) in dry CH_2Cl_2 (1.5 mL) was added to the residue. Triethylamine (8 μL, 0.056 mmol, 2.5 equiv) was added, followed by DPPA (5 μL, 0.025 mmol, 1.1 equiv). The resulting solution was stirred at room temperature under N_2 for 10 d. The mixture was concentrated, and the product was isolated by preparative HPLC, eluting with 85% MeCN in H_2O (0.05% TFA), followed by lyophilization, to give dipeptide **15** as a colorless solid (10 mg, 67%): R_f = 0.26 (2:1 hexanes−ethyl acetate); $[\alpha]^{20}_D = -20.9°$ (*c* 0.65, $CHCl_3$); HPLC t_R = 11.02 min (85% MeCN in H_2O, 0.05% TFA); ^{1}H NMR ($CDCl_3$, 200 MHz) (mixture of rotamers) δ 1.29 (s, 3H), 1.30 (s, 6H), 1.40 (s, 4H), 1.42 (s, 2H), 1.43 (br, 1H), 2.89−3.11 (m, 2H), 3.18 (dd, J = 12.3, 4.4 Hz, 0.75H), 3.54 (dd, J = 12.3, 4.4 Hz, 0.25H), 3.86 (d, J = 12.5 Hz, 1H), 4.08−4.41 (m, 3H), 4.56−4.41 (m, 5H), 4.87 (br, 1H), 5.23−5.41 (m, 2H), 5.53 (d, J = 8.6 Hz, 0.25H), 5.67 (d, J = 8.4 Hz, 0.75H), 5.84−5.96 (m, 1H), 6.85−6.90 (m, 2H), 7.11 (dd, J = 12.1, 8.4 Hz, 2H), 7.25−7.43 (m, 4H), 7.56 (d, J = 7.0 Hz, 2H), 7.75 (d, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) (mixture of rotamers) δ 24.3 & 24.9, 26.5 & 26.8, 28.8, 38.4, 47.1, 51.9 & 52.2, 53.5, 65.4, 66.2, 67.0, 78.3, 79.3, 81.3, 83.7, 112.5 & 112.8, 118.9 & 119.1, 119.9, 124.2, 125.1, 127.0, 127.6, 129.9, 130.2, 130.6, 131.3, 141.2, 143.8, 154.3 & 155.4, 168.9, 170.7 & 171.2; HRMS (FAB$^+$) calcd for $C_{39}H_{45}N_2O_8$ $(M + H)^+$ 669.31759, obsd 669.31812.

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Supporting Information Available: Experimental procedures for the conversion of D-gulonolactone (**2**) to compound **3** and spectral data which augments that in ref 7. This material is available free of charge via the Internet at http://pubs.acs.org.

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