Enantioselective Synthesis of (2*S*,3*R*)-3-Hydroxy-3-methylproline, A Novel Amino Acid Found in Polyoxypeptins

Dong-Guang Qin, Hui-Yan Zha, and Zhu-Jun Yao*

State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, 354 Fenglin Road, Shanghai 200032, China

yaoz@pub.sioc.ac.cn

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Abstract: The enantioselective synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline (**3**) was achieved by the Sharpless AD, regioselective opening of cyclic sulfate by NaN_3 and intramolecular ring-closing reaction. The reported route has the advantage of a high overall yield and good enantiomeric purity, as well as starting from readily available chemical substrates and inexpensive reagents.

Induction of programmed cell death (apoptosis) would be favorable for anticancer agents.[1] Recently two new cyclic hexadepsipeptides, polyoxypeptins A (**1** in Figure 1) and B (**2** in Figure 1), were isolated from *Streptomyces* culture broth.[2,3] Both were believed to be potent inducers of apoptosis in human pancreatic carcinoma adenocarcinoma AsPC-1 cells,[4] an apoptosis-resistant cell line. The depsipeptins show two unusual structural characteristics: one is the high percentage of oxygen atoms in the molecules, and the other is that the components are all "unnatural" amino acids, including a complex acyl side chain. Polyoxypeptin A (**1**) consists of six unusual amino acids, 3-hydroxyleucine (3-OH Leu), *N*-hydroxyvaline (N-OH Val), *N*-hydroxyalanine (N-OH Ala), piperazic acid (Pip), 5-hydroxyhexahydropiperazine-3-carboxylic acid (5-OH Pip), and (2*S*,3*R*)-3-hydroxy-3-methylproline (3-OH MePro) (**3**). Among them, 3-OH MePro was found for the first time. Very recently, Kobayashi et al. reported[5] the first synthesis of the 3-OH MePro using a Pd-catalyzed intramolecular N-allylation of alkenyloxirane to the pyrrolidine ring starting from geraniol. As part of our effort to elaborate the total synthesis of polyoxypeptins, herein we report a highly efficient synthesis of 3-OH MePro.

The stereochemical control in the synthesis was achieved by the Sharpless AD[7] reaction on a *Z*-olefin, a regioselective opening of cyclic sulfate by NaN_3, and an intramolecular ring-closing reaction. The detailed synthetic route of 3-OH MePro **3** is outlined in Scheme 1.

First of all, the commercially available 3-butyn-1-ol **4** was protected by a benzyl group in 94% yield. Treatment of butyn ether **5** with *n*-BuLi followed by methyl chloroformate at −78 °C gave the acetylene **6** in 85% yield. Addition of acetylene **6** to $(CH_3)_2CuLi$ in THF[6] at −78 °C afforded the (2*Z*)-α,*β*-unsaturated ester **7** (89% yield). Sharpless dihydroxylation[7] of trisubstituted olefin **7** using AD-mix-α gave the diol **8** in 90% yield and 95.5% enantiomeric ratio (er) (measured by HPLC).

A two-step synthesis of the cyclic sulfate[8] **9** involved cyclic sulfite formation using Et_3N and $SOCl_2$ in CH_2Cl_2 and a ruthenium (III) chloride catalyzed oxidation in $CCl_4-CH_3CN-H_2O$ gave the cyclic sulfate **9** in 90% yield (two steps). Reaction of the cyclic sulfate **9** with sodium azide in DMF at 80 °C, followed by hydrolysis of the sulfate salt, afforded the α-azido-*β*-hydroxy ester as the only product in 86% yield. Hydrogenolysis (1 atm H_2) of the azido group[9] in the presence of a catalytic amount of Pd−C (10%) and Boc_2O in EtOAc followed by removal of the benzyl group with $Pd(OH)_2$−C (20)% in MeOH gave the diol **11** (72%). The byproduct lactone **12** (23%) could be converted to the diol **11** quantitatively by alkaline hydrolysis using 1 N NaOH and esterification with CH_2N_2.

Treatment of diol **11** with MsCl and Et_3N in CH_2Cl_2 at −78 °C for 1 h gave the desired pyrrolidine **13** in 91% yield. The protecting groups of **13** were removed by a two-step procedure with LiOH and TFA (97% yield). The resultant product was treated with Dowex 50 × 2, eluting with 2 N $NH_3 \cdot H_2O$, to give **3** as a crystalline solid. The physical data of our synthetic sample ($[\alpha]_D^{25} = -38.6$ (*c* 0.40, H_2O), lit.[2] = −41.0 (*c* 0.40, H_2O)) are identical to those[2] of the natural one.

In summary, the enantioselective synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline **3** is reported. It provides a highly pratical route to obtain the unusual amino acid in high yield (overall 40%) and good enantiomeric purity, as well as starting from readily available chemical substrates and inexpensive reagents.

Experimental Section

General Methods. All reactions were carried out under argon or nitrogen in oven-dried glassware using standard gastight syringes, cannulas, and septa. Solvents and reagents were purified and dried by standard methods prior to use. Optical rotations were measured at room temperature. IR spectra were recorded on an FT-IR instrument. ^1H NMR spectra were recorded at 300 MHz and are reported in parts per million (*δ*) downfield relative to TMS as internal standard, and ^{13}C NMR spectra were recorded at 75 MHz and assigned in parts per million (*δ*). Flash column chromatography was performed on silica gel (10−40 *μ*m) using a mixture of petroleum ether and ethyl acetate as the eluent.

1-Benzyloxy-3-butyne (5). To a suspension of NaH (60%, 18 g, 0.45 mol) in DMF (350 mL) was added 3-butyn-1-ol **4** (22 g, 0.32 mol) dropwise over 20 min at 0 °C. After the mixture was stirred at 0 °C for 2 h, BnBr (57 mL, 0.48 mol) in DMF (50

* To whom correspondence should be addressed. Fax: +8621 6416 6128.

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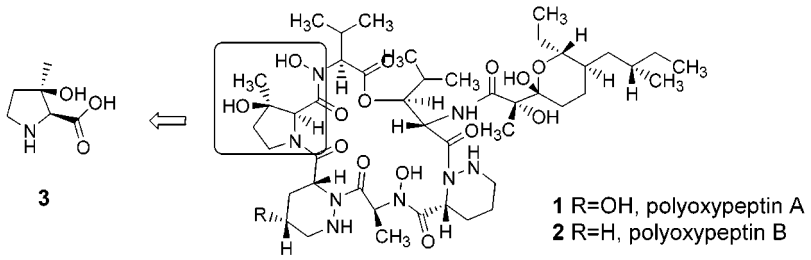
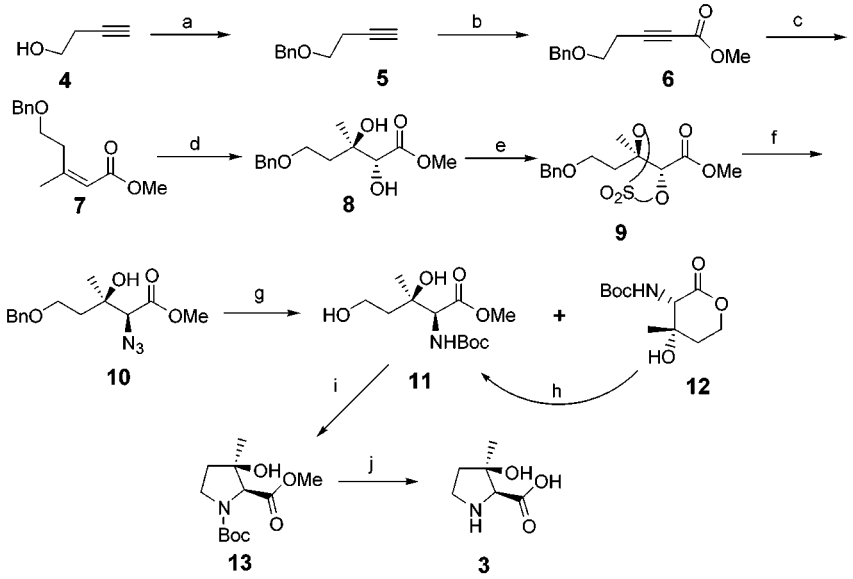


Figure 1. Chemical structures of polyoxypeptins A and B.

Scheme 1



Reagents and conditions: (a) NaH, BnBr, DMF, 94%. (b) *n*-BuLi, $ClCO_2Me$, THF, −78 to 0 °C, 85%. (c) Me_2CuLi, THF, −78 °C, 89%. (d) AD-mix-a, $CH_3SO_2NH_2$, *t*-BuOH−H_2O, 0 °C; 90% yield, 95.5% er (HPLC). (e) (*i*) Et_3N, $SOCl_2$, CH_2Cl_2, 0 °C to room temperature; (*ii*) $NaIO_4$, cat. $RuCl_3 \cdot H_2O$, $CCl_4/CH_3CN/H_2O$, 0 °C to room temperature, 90%. (f) NaN_3, DMF, 80 °C, 86%. (g) (*i*) Pd−C (10%), Boc_2O, EtOAc; (*ii*) 20% $Pd(OH)_2$/C, MeOH, 95% (72% for **11**, and 23% for **12**). (h) (*i*) 1 N NaOH; (*ii*) CH_2N_2, Et_2O, 100%. (i) MsCl, Et_3N, CH_2Cl_2, 96%. (j) (*i*) $LiOH \cdot H_2O$, THF/MeOH/H_2O; (*ii*) CF_3CO_2H, CH_2Cl_2, then Dowex 50 × 2, 97%.

mL) was added. The ice bath was removed, and the mixture was stirred for 3 h at room temperature. The reaction was quenched by aqueous NH_4Cl (100 mL), and the aqueous layer was extracted with EtOAc (200 mL × 3). The combined organic layers were washed with brine and dried ($MgSO_4$). The solvent was removed, and the residue was distilled to give 48 g (94% yield) of butyn ether **5** as a colorless oil (80 °C, 2 mmHg). ^1H NMR (300 MHz, $CDCl_3$): δ 7.25−7.42 (5H, m), 4.58 (2H, s), 3.62 (2H, t, J = 7.5 Hz), 2.52 (2H, dt, J = 5.3, 7.5 Hz), 2.01 (1H, t, J = 2.6 Hz).

5-Benzyloxy-pent-2-ynoic Acid Methyl Ester (6). To a stirred solution of **5** (45 g, 0.28 mol) in THF (500 mL) was added *n*-BuLi (2 M, 170 mL, 0.34 mol) dropwise at −78 °C. The red-black resultant solution was stirred at −78 °C for 2 h, and then methyl chloroformate (45 mL, 0.56 mol) was added. The reaction mixture was stirred at −78 °C for 2 h and then warmed to room temperature and stirred overnight. The reaction was quenched by aqueous NH_4Cl (100 mL), and the aqueous layer was extracted with EtOAc (200 mL × 3). The combined organic layers were washed with H_2O and brine and dried ($MgSO_4$). The solvent was removed, and the residue was distilled to give 52 g (85% yield) of acetylene **6** as a colorless oil (138 °C, 3 mmHg). ^1H NMR (300 MHz, $CDCl_3$): δ 7.32−7.37 (5H, m), 4.57 (2H, s), 3.77 (3H, s), 3.65 (2H, t, J = 5.2 Hz), 2.65 (2H, t, J = 5.2 Hz).

5-Benzyloxy-3-methyl-pent-2-(*Z*)-enoic Acid Methyl Ester (7). A suspension of CuI (21 g, 0.11 mol) in THF (400 mL) was cooled to 0 °C, and 190 mL of CH_3Li (1.2 M) in ether was added dropwise. The resultant black solution was cooled to −78 °C and treated with acetylene **6** (22 g, 0.10 mol) in 80 mL of THF. After the mixture was stirred at −78 °C for 3 h, MeOH (40 mL) was added. The mixture was then warmed to room temperature, and 100 mL of aqueous NH_4Cl was added. The organic layer was separated, and the aqueous layer was extracted with EtOAc (200 mL × 3). The combined organic layers were washed with H_2O and brine and dried ($MgSO_4$). Concentration and flash chromatography gave the (2*Z*)-α,*β*-unsaturated ester **7** as a colorless oil (21 g, 89% yield). ^1H NMR (300 MHz, $CDCl_3$): δ 7.23−7.28 (5H, m), 5.96 (1H, s), 4.46 (2H, s), 3.61 (3H, s), 3.60 (2H, m), 2.92 (2H, t, J = 6.6 Hz), 1.90 (3H, s). MS (EI, *m*/*z*): 235 ($M^+ + 1$). IR (neat, cm^{-1}): 2950, 2861, 1718, 1648, 1199, 1151, 737, 698. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.75; H, 7.82.

(2*R*,3*R*)-Methyl 5-Benzyloxy-2,3-dihydroxy-3-methylpentanoate (8). A 1L round-bottom flask was charged with t-BuOH (300 mL), water (300 mL) $K_3Fe(CN)_6 \cdot 6H_2O$ (75 g, 172 mmol), K_2CO_3 (31 g, 225 mmol), $(DHQ)_2PHAL$ (1.2 g, 1.54 mmol), and $K_2OsO_2(OH)_4$ (120 mg, 0.33 mmol). After the mixture was stirred at room temperature for 5 min, $CH_3SO_2NH_2$ (7.3 g, 77 mmol) was added. The mixture was cooled to 0 °C; α,*β*-unsaturated ester **7** (18.0 g, 77 mmol) was added at once, and the heterogeneous slurry was stirred vigorously for 20 h at 0 °C. Na_2SO_3 (60 g) was added, and the mixture was allowed to warm to room temperature and stirred for 1 h. EtOAc (100 mL) was added, and the aqueous layer was extracted with EtOAc (150 mL × 3). The combined organic layers were dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography, affording diol **8** as a colorless liquid (18.5 g, yield 90%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.27−7.38 (5H, m), 4.53 (2H, s), 4.13 (1H, s), 3.81 (3H, s), 3.72 (2H, m), 3.42 (2H, br), 2.03 (1H, m), 1.74 (1H, m), 1.23 (3H, s). MS (EI, *m*/*z*): 268 (M^+). IR (neat, cm^{-1}): 3464, 2953, 2873, 1737, 1273, 1213, 1096, 746, 696. $[\alpha]_D^{25} = -22.2$ (*c* 1.00, $CHCl_3$). HPLC:

95.5% er (column AS; UV-detector: 214 nm; 20% *i*-PrOH in hexane; flow rate 0.7 mL/min). Anal. Calcd for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found: C, 62.69; H, 7.56.

(4*R*,5*R*)-5-(2-Benzyloxy-ethyl)-2, 2-dioxo-2λ^6-[1, 3, 2]dioxathiolane-4-carboxylic Acid Methyl Ester (9). To a solution of diol **8** (15.391 g, 57.4 mmol) in CH_2Cl_2 (250 mL) were added Et_3N (17.6 mL, 126.3 mmol) and $SOCl_2$ (6.3 mL, 86.4 mmol) at 0 °C. After stirring at room temperature for 1.5 h, the mixture was diluted with CH_2Cl_2 (200 mL), washed with water and brine, and dried over $MgSO_4$. After removal of solvent, the residue was dissolved in CCl_4(100 mL), CH_3CN(100 mL), and H_2O (120 mL). With vigorous stirring, $NaIO_4$ (16.003 g, 74.8 mmol) and $RuCl_3 \cdot H_2O$ (120 mg) were added at 0 °C. The mixture was stirred at room temperature for 1 h, and then EtOAc (200 mL) was added. The mixture was washed with aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. After removal of solvent, the residue was purified by flash chromatography to give the cyclic sulfate **9** as a colorless liquid (17.1 g, yield 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29−7.42 (5H, m), 5.07 (1H, s), 4.52 (2H, s), 3.80 (3H, s), 3.67 (2H, m), 2.39(1H, dt, J = 14.7, 5.7 Hz), 1.95 (1H, m), 1.86 (3H, s). MS (EI, *m*/*z*): 330 (M^+). IR (neat, cm^{-1}): 2960, 2877, 1772, 1749, 1388, 1214, 1062, 875, 834, 749, 700. $[\alpha]_D^{25}$ = 42.8 (*c* 1.14, $CHCl_3$).

(2*S*,3*R*)-Methyl 2-Azido-5-benzyloxy-3-hydroxy-3-methylpentanoate (10). A mixture of cyclic sulfate **9** (15.812 g, 47.9 mmol) and NaN_3 (6.323 g, 97.3 mmol) in 300 mL of DMF was heated to 80 °C with vigorous stirring. After 6 h, the mixture was cooled to room temperature and the solvent removed in vacuo. The residue was dissolved in 400 mL of THF and then H_2SO_4 (concentrated 3 mL) and water (1.8 mL) were added at 0 °C. After the mixture was stirred at room temperature for 1 h, K_2CO_3 (20 g) was added and the stirring was continued for 10 min. After removal of the yellow suspension by filtration, the filtrate was dried over Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography to give the product **10** as a colorless liquid (12.0 g, yield 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31−7.37 (5H, m), 4.53 (2H, s), 3.92 (1H, s), 3.81 (3H, s), 3.77 (2H, m), 2.04 (1H, m), 1.91 (1H, m), 1.29 (3H, s). MS (EI, *m*/*z*): 294 (M^+ + 1). IR (neat, cm^{-1}): 3471, 2955, 2872, 2114, 1743, 1455, 1205, 740, 699. $[\alpha]_D^{25}$ = −23.1 (*c* 1.12, $CHCl_3$). Anal. Calcd for $C_{14}H_{19}O_4N_3$: C, 57.33; H, 6.53; N, 14.32. Found: C, 57.58; H, 6.63; N, 14.67.

(2*S*,3*R*)-2-*tert*-Butoxycarbonylamino-3,5-dihydroxy-3-methyl-pentanoic Acid Methyl Ester (11) and (3*S*,4*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-4-methyl-2-pyrone (12). A mixture of α-azido-β-hydroxy ester **10** (4.830 g, 16.5 mmol), Boc_2O (4.4 g, 20.2 mmol), and Pd−C (10%, 500 mg) in 100 mL of EtOAc was hydrogenolyzed (1 atm) for 20 h. The solid was removed by filtration, and the solution was concentrated in vacuo. The residue was dissolved in MeOH (100 mL), and 20% $Pd(OH)_2$ on C (300 mg) was added. The suspension was stirred vigorously under H_2 (1 atm) until the uptake of hydrogen stopped. The mixture was filtered, and the filtrate was concentrated. Flash chromatography gave the diol **11** (3.26 g, 72%) and the lactone **12** (0.96 g, yield 23%). Data for **11**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.47 (1H, d, J = 7.1 Hz), 4.31(1H, d, J = 7.4 Hz), 3.95 (1H, m), 3.84(1H, m), 3.77 (3H, s), 3.43 (1H, s), 2.71 (1H, br), 1.98 (1H, m), 1.66 (1H, m), 1.44 (9H, s), 1.27 (3H, s). MS (EI, *m*/*z*): 278 (M^+ + 1). IR (neat, cm^{-1}): 3420, 2980, 1716, 1507, 1369, 1165. $[\alpha]_D^{25}$ = 12.7 (*c* 1.28, $CHCl_3$). Data for **12**. ^{1}HNMR (300 MHz, $CDCl_3$): δ 5.86 (1H, br), 5.37 (1H, s), 4.55 (1H, dt, J = 11.5, 2.2 Hz), 4.38 (1H, d, J = 3.8 Hz), 4.33 (1H, dt, J = 11.6, 3.2 Hz), 2.21 (1H, dd, J = 12.9, 2.2 Hz), 1.91 (1H, m), 1.46 (9H, s), 1.16 (3H, s). MS (EI, *m*/*z*): 246 (M^+ + 1). IR (neat, cm^{-1}): 3391, 2934, 1742, 1682, 1520, 1401, 1168, 899. $[\alpha]_D^{25}$ = 126.7 (*c* 1.20, $CHCl_3$). Anal. Calcd for $C_{11}H_{19}O_5N$: C, 53.87; H, 7.81; N, 5.71. Found: C, 53.81; H, 7.53; N, 5.58.

(2*S*,3*R*)-3-Hydroxy-3-methyl-1,2-pyrrolidinedicarboxylic Acid 1-(1,1-Dimethylethyl) 2-Methyl Ester (13). To a stirred solution of diol **11** (1.159 g, 4.18 mmol) in CH_2Cl_2 (30 mL) was added MsCl (0.37 mL, 4.80 mmol) at −78 °C, and then Et_3N (0.67 mL, 4.80 mmol) was added slowly over 10 min. After the mixture was stirred at −78 °C for 1 h, aqueous NH_4Cl (5 mL) was added. The mixture was warmed to room temperature and diluted with CH_2Cl_2 (100 mL), washed by brine, and dried over Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography to give the product **13** as a colorless liquid (1.04 g, yield 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.05 (1H, s), 3.78 (3H, s), 3.61 (2H, m), 2.40 (1H, br), 2.08 (1H, m), 1.91 (1H, m), 1.50 (3H, s), 1.44 (9H, s). MS (EI, *m*/*z*): 260 (M^+ + 1). IR (neat, cm^{-1}): 3447, 2978, 1749, 1705, 1456, 1404, 1368, 1164. $[\alpha]_D^{25}$ = −19.1 (*c* 0.90, $CHCl_3$). Anal. Calcd for $C_{12}H_{21}O_5N$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.51; H, 7.93; N, 5.20.

(2*S*,3*R*)-3-Hydroxy-3-methylproline (3). To a stirred solution of **13** (200 mg, 0.77 mmol) in THF (6 mL), MeOH (2 mL), and water (2 mL) was added $LiOH \cdot H_2O$ (100 mg, 2.31 mmol) at room temperature. After stirring for 6 h, the mixture was acidified by addition of aqueous $NaHSO_4$ (10%) to pH 3 and then extracted with EtOAc (50 mL × 3). The combined organic layer was dried over Na_2SO_4. After removal of solvent, the residue was dissolved in CH_2Cl_2 (4 mL) and TFA (4 mL) at 0 °C. After the mixture was stirred at room temperature for 1.5 h, the solvent was removed in vacuo. The residue was taken up in water (1 mL), and the resulting aqueous solution passed though a column (15 × 1 cm) of a strongly acidic ion-exchange resin (Dowex 50 × 2, 100∼200 mesh), eluting first with water (100 mL) and then with aqueous ammonium hydroxide (2 M, 100 mL). The ammonia fraction was concentrated in vacuo, and the residue solid was crystallized from EtOAc and EtOH (5:1), giving a colorless solid **3** (108 mg, 97% yield). Mp: 196∼199 °C. ^{1}H NMR (300 MHz, D_2O): δ 3.86 (1H, s), 3.54 (1H, m), 3.46 (1H, m), 2.15 (2H, m), 1.60 (3H, s). ^{13}C NMR (75 MHz, D_2O): δ 171.2, 78.8, 70.1, 43.7, 39.9, 24.3. MS (EI, *m*/*z*): 146 (M^+ + 1). IR (film, cm^{-1}): 3375, 2966, 1632, 1401, 1263, 1105, 1025, 880. $[\alpha]_D^{25}$ = −38.6 (*c* 0.40, H_2O).

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