Synthesis of Alkyl Bridged-Tris-α-Amino Acids as C₃-Symmetric and Linear Linkers

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Abstract: Although bis- α -amino acids have been used to synthesize dimer models of aggregative peptides involved in neurodegenerative diseases, tris- α -amino acids are employed to a lesser extent for trimer models. The reported tris- α -amino acids substituted on the 1,3,5-positions of an aromatic ring are not suitable for mimicking trimers due to their low flexibility and high planarity. Here, we design and synthesize two new alkyl bridged-tris- α -amino acids with Fmoc protecting groups as new flexible linkers for trimer models.

Introduction

Accumulated evidence suggests that misfolded protein aggregates contribute to the pathogenesis of intractable diseases such as Alzheimer's (AD), Parkinson's, and prion diseases.^[11] Elucidation of the toxicity mechanism of the corresponding misfolded protein aggregates, including oligomers or fibrils, should improve the understanding of such intractable diseases at the molecular level. Currently, the structures of these oligomers or fibrils remain elusive due to their labile or quasi-stable properties and the presence of various strains.^[2] Thus, the development of dimer and trimer models as the minimum units of these oligomers and fibrils should reveal the important structure for biological activity.

Regarding amyloid β (A β) peptides involved in AD,^[3] dimer models have extensively been developed to examine their contribution to the onset of AD.^[4] Among them, bis- α -amino acids, L,L-2,6-diaminopimelic acid (DAP) and L,L-2,8-diaminoazelaic acid (DAZ), have been used to synthesize chemically and metabolically stable full-length A β dimers to compare intermolecular disulfide bond formation.^[5,6] Some of these dimers exhibit potent neurotoxicity in vitro. Although trimers may also contribute to the pathogenesis of AD, the lack of flexible linkers similar to DAP and DAZ has limited the synthesis of the trimer models of full-length A β .^[7]

Tris- α -amino acids would be useful to tether trimers. Aromatic C₃-symmetric tris- α -amino acid derivatives have been synthesized using the Horner-Wadsworth-Emmons (HWE) reaction followed by asymmetric hydrogenation,^[8] cross-coupling

reactions,^[9] or alkylation of a glycine equivalent (Fig. 1).^[10] These derivatives have been utilized as building blocks of synthetic peptides. For example, Kotha and Todeti reported the synthesis of C₃-symmetric dipeptide trimers.^[9e] Frejd and Ritzén prepared chiral peptide dendrimers, which contain 1,3,5-phenyltris-L-alanine (PtA)^[8] as the central core unit.^[11] However, the application of such amino acids to biologically important peptides has yet to be reported. Recently, Irie and colleagues incorporated PtA into the C-terminal region of Aβ40 proteins to synthesize quasi-stable trimer models.^[12] However, the neurotoxicity of these trimer models did not exceed that of the corresponding monomer or the dimer model,^[6b] suggesting that the low flexibility, high planarity, or hydrophobicity of the aromatic ring may inhibit the optimal conformation that shows neurotoxicity.



Figure 1. Tris- α -amino acid derivatives previously reported and those synthesized in this study. Boc = *tert*-butoxycarbonyl, Fmoc = 9-fluorenylmethyloxycarbonyl.

The above issue may be addressed by replacement of a benzene ring that binds three α -amino acid moieties with a flexible alkyl chain. In this paper, we report the synthesis of two new types

of tris- α -amino acid derivatives connected with alkyl spacers. One is C₃-symmetric *tert*-butyltris-L-alanine (*t*ButA), and the other is linear tris- α -amino acid, which was named α , α -di-Lhomonorleucyl-L-glycine (di-hNor-Gly), along with their 9fluorenylmethyloxycarbonyl (Fmoc)-protected derivatives (**1** and **2**, respectively, Fig. 1). These linkers should lead to various types of trimer models of pathogenic aggregative peptides such as C₃symmetric trimers and those forming intermolecular parallel β sheets.

Results and Discussion

Synthesis of Fmoc-(*S*,*S*,*S*)-*tert*-butyltris-L-alanine (Fmoc*t*ButA, 1)

Two paths were envisioned to synthesize **1**, whose amino acid moieties would be installed via the HWE reaction and subsequent asymmetric hydrogenation (Scheme 1).^[13,14] In a three-directional approach, **1** would be obtained by asymmetric hydrogenation of tris-dehydroamino acid **3** (route A). Although a trialdehyde may be a precursor of **3**, it would be labile and unsuitable for the synthesis. Thus, a stepwise introduction was planned to access compound **3** by the HWE reaction from dialdehyde **5** or **6** through compound **4**. Compound **5** or **6** could be derived from carboxylic acid **7**.

FmocHN

NHFmod

asymmetric

hydrogenation

asymmetric hydrogenation

MeO₂C

HO

Route A

.CO₂Me

CO₂Me

CO₂M

| NHCbz

HWE reaction

OTBS

NHCbz

HWE reaction

oxidation

ΟН

CbzHN

| NHCbz

NHCbz

онс

MeO₂C

MeO₂C

.CO₂H

CO₂H

Route B

. NHCbz

. ŇHCbz

CbzHN

.CO₂Me

CO₂Me

CO₂M

NHCbz

NHCbz

HWE reaction

oxidation

ОН

NHFmoc

MeO₂C

to synthesize psuedo C₂-symmetric trimers. Additionally, route B could be used to synthesize *t*ButA stereoisomers, where each stereocenter has a different configuration, namely, (S,S,R)- and (S,R,R)-*t*ButA. However, both routes A and B would be suitable to synthesize (S,S,S)- and (R,R,R)-*t*ButA.

acid moieties would produce orthogonally protected amino acids

The synthesis began with ozonolysis of the double bond of silyl ether **10**, which was readily prepared from carboxylic acid **7** by a known procedure (Scheme 2).^[15] The resultant dialdehyde was immediately converted to protected bis-dehydroamino acid **12** by the HWE reaction using commercially available glycine phosphonate **11** in 31% yield from **10**. The *tert*-butyldimethylsilyl (TBS) group of **12** was removed under acidic conditions to give alcohol **13** in 85% yield. Oxidation of the primary alcohol of **13** using 2-azaadamantane-2-ol (AZADOL)^[16] was attempted to produce hemiaminal **14**. However, the reaction gave a complex mixture, including a bicyclic compound, which did not react under the Wittig reaction conditions for a one-carbon elongation.



Scheme 2. Attempted synthesis of Fmoc-(S,S,S)-*tert*-butyltris-L-alanine via bisdehydroamino acid **13**. DBN = 1,5-diazabicyclo[4.3.0]non-5-ene, CSA = (±)-10camphorsulfonic acid, AZADOL = 2-azaadamantane-2-ol.

To avoid the production of stable six-membered bicyclic compounds, the bis-dehydroamino acid unit was introduced after the one carbon elongation (Scheme 3). Carboxylic acid **7** was converted to known alcohol **16** in four steps, including the Arndt-Eistert synthesis and a LiAlH₄ reduction.^[17] After silylation of compound **16**, the olefin of **17** was cleaved by oxidation with ozone. Reductive treatment with triphenylphosphine produced better results than the use of dimethyl sulfide and yielded the dialdehyde upon stirring at room temperature for 20 minutes. The dialdehyde was immediately subjected to the HWE reaction to give protected bis-dehydroamino acid **18** in 61% yield from compound **17**.

Next, the third dehydroamino acid unit was introduced (route A). Removal of the TBS group of **18** and subsequent oxidation of resultant primary alcohol **4** using AZADOL gave aldehyde **15**. The HWE reaction of **15** gave protected tris-dehydroamino acid **3** in 73% yield. Asymmetric hydrogenation of **3** with (*S*,*S*)-2,3-bis(*tert*-butylmethylphosphino)-quinoxaline-Rh ((*S*,*S*)-QuinoxP*-Rh)^[18]



On the other hand, a stepwise asymmetric hydrogenation would realize compound 1 (route B). Compound 1 would be synthesized from compound 8, which would be produced from alcohol 9. Compound 9 would be derived via an asymmetric hydrogenation of compound 4. A stepwise introduction of amino

catalyst gave protected *t*ButA **20** in 84% yield. The enantiomeric excess and diastereomeric ratio of **20** were >99%ee and >97%de, respectively.



Scheme 3. Synthesis of Fmoc-(*S*,*S*,*S*)-*tert*-butyltris-L-alanine (1). (*S*,*S*)-QuinoxP*-Rh = (*S*,*S*)-2,3-Bis(*tert*-butylmethylphosphino)quinoxaline(1,5cyclooctadiene)rhodium(I)hexafluoroantimonate or (*S*,*S*)-2,3-Bis(*tert*butylmethylphosphino)quinoxaline(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate.

We also investigated stepwise route B. The two alkene functionalities of **18** were hydrogenated asymmetrically under the same conditions as route A using a (*S*,*S*)-QuinoxP*-Rh catalyst to produce protected bis- α -amino acid **9** in 85% yield (>99%ee and >98%de). The TBS group of **18** was removed during hydrogenation, which may be because methanol, which served as the solvent, also acted as a nucleophile. After AZADO oxidation of the primary alcohol of **9**, the HWE reaction of **19** gave compound **8** in 64% overall yield. Asymmetric hydrogenation of **8** using (*S*,*S*)-QuinoxP*-Rh catalyst gave protected *t*ButA **20** in 91% yield (>98%de).

With protected tButA (20) in hand, the protecting group manipulation was performed to access Fmoc-protected *t*ButA (1), which can be used in solid-phase peptide synthesis. Hydrolysis of 20 under basic conditions and subsequent removal of the benzyloxycarbonyl (Cbz) groups, gave tButA 21. Although the hydrophilic property of the three free amino acid moieties prevented extraction of 21 from water, the ¹H and ¹³C NMR spectra (D₂O) confirmed its structure. Fmoc groups were introduced to the primary amino groups of 21 to produce 1 in 54% yield from 20. Since both ¹H and ¹³C NMR analyses showed that the corresponding signals of each homoalanine (- $CH_2CH_2CHNHR^2(CO_2R^1))$ moiety of **20** and **21** were equivalent, the C₃-symmetric structure of the product was confirmed.

Synthesis of Fmoc-(S,S)- α , α -di-L-homonorleucyl-L-glycine (Fmoc-di-hNor-Gly, 2)

We then focused on the synthesis of Fmoc-di-hNor-Gly (2), which is a linear tris- α -amino acid for trimer peptide models. Scheme 4 depicts our envisioned retrosynthesis. The terminal amino acid moieties of Fmoc-di-hNor-Gly (2) would be introduced via the HWE reaction of dialdehyde 23 and subsequent asymmetric hydrogenation of 22. Compound 23 would be derived from bisolefin 24, which would be prepared from isocyanide 25.



Scheme 4. Retrosynthetic analysis of Fmoc-(S,S)- α , α -di-L-homonorleucyl-L-glycine (2).

Compound **25** was dialkylated by following Schöllkopf and Hoppe's procedure^[19] to give isocyanide **26** in 84% yield (Scheme 5). Hydrolysis of the isocyanide group of **26** and subsequent treatment of CbzCl and Na₂CO₃ in toluene at 80 °C produced Cbz-protected amino acid **24** in 73% yield. The terminal olefins of **24** were cleaved by ozonolysis and then treated with triphenylphosphine. The resultant dialdehyde was immediately converted to compound **22** by the HWE reaction. Asymmetric hydrogenation of **22** using the (*S*,*S*)-QuinoxP*-Rh catalyst gave protected di-hNor-Gly (**27**) in 98% yield. The enantiomeric excess and diastereomeric ratio of **27** were >99%ee and >96%de, respectively.



Scheme 5. Synthesis of Fmoc-(S,S)- α , α -di-L-homonorleucyl-L-glycine (2). DMSO = dimethyl sulfoxide, TFA = trifluoroacetic acid.

Acidic hydrolysis of the *tert*-butyl ester, alkaline hydrolysis of the methyl ester, and subsequent hydrogenolysis of the Cbz groups gave di-hNor-Gly (28). Finally, the Fmoc groups were

introduced to the primary amino groups of **28** to produce **2** in 55% yield from **27**. Although the tetrasubstituted carbons of **27**, **28**, and **2** have the same two side chains, these compounds are chiral because the side chains contain a chiral center (namely, pseudo σ -symmetry). Hence, the two chains are not equivalent. As expected, the signals derived from each carbon in ¹³C NMR spectra were observed independently.

Conclusion

Here, we established an enantioselective synthetic route toward two new C₃-symmetric and linear tris- α -amino acids containing alkyl spacers, (*S*,*S*)-*t*ButA (**21**) and (*S*,*S*)-di-hNor-Gly (**28**). Both compounds were converted into the corresponding Fmocprotected amino acids. These synthetic routes should allow the syntheses of various derivatives with modified protecting groups and configurations. They should realize powerful tools to synthesize various cross-linked trimer models of pathogenic aggregative peptides using a solid-phase Fmoc strategy, as exemplified in the synthesis of the trimer models of A β 40.^[12] Currently we are trying to synthesize these trimer models, including C₃-symmetric and β -sheet peptides. These results, which consider the structure–activity relationships, will be reported in due course.

Experimental Section

General: All non-aqueous reactions, except hydrogenation reactions, were carried out under a nitrogen or argon atmosphere in dried glassware. Analytical thin-layer chromatography was performed with TLC Silica gel 60 F254 (Merck, Darmstadt, Germany). Silica gel column chromatography was performed with Wakogel C-200 (Wako Pure Chemical Industries, Osaka, Japan) or Chromatorex BW-300 (Fuji Silica Chemical, Aichi, Japan). Flash column chromatography was performed with a Model 800E with a Model prep UV-10V UV detector (Yamazen, Osaka, Japan), and YMC*GEL ODS-A (YMC, Kyoto, Japan) was used as the carrier. HPLC was performed with Model 600E with a Model 2487 UV detector (Waters, Milford, MA, USA). Proton nuclear magnetic resonance (¹H NMR) spectra were recorded on an AVANCE III 400 (Bruker, Germany) at 400 MHz or an AVANCE III 500 (Bruker, Germany) at 500 MHz. Chemical shifts were reported relative to Me₄Si (δ 0.0) in CDCl₃, and residual solvent of CD₃OD (δ 3.31) and D₂O (δ 4.79). Multiplicity is indicated by one or more of the following: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); br (broad). Carbon nuclear magnetic resonance (¹³C NMR) spectra were recorded on an AVANCE III 400 (Bruker, Germany) at 101 MHz or an AVANCE III 500 (Bruker, Germany) at 126 MHz. Chemical shifts were reported relative to CDCl₃ (δ 77.0) and CD₃OD (δ 49.0). Infrared spectra were recorded on a FT/IR-470 Plus Fourier-transform infrared spectrometer (Jasco, Tokyo, Japan). Specific rotations were recorded on a P-2200 digital polarimeter (Jasco, Tokyo, Japan). Low- and highresolution mass spectra were recorded on a JMS700 mass spectrometer (JEOL, Tokyo, Japan) for FAB-MS (matrix, m-nitrobenzyl-alcohol) or timsTOF (Bruker, Germany) for ESI-MS and APCI-MS.

Compound 12: A solution of **10** (605 mg, 2.85 mmol) in CH₂Cl₂ (30 mL) was cooled to -78 °C. The solution was bubbled with O₃ until it turned blue. Excess O₃ was removed from the reaction mixture by purging with O₂. To the mixture was added Me₂S (3 mL) at -78 °C, and the reaction mixture was stirred for 25 minutes. Then the reaction mixture was washed with water, and the aqueous layer was extracted with CH₂Cl₂. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄. Filtration and concentration afforded a crude dialdehyde. To a solution of (*Z*)- α -phosphonoglycine trimethyl ester (1.98 g, 5.99 mmol) in CH₂Cl₂ (10 mL) was added DBN (0.750 g, 6.04 mmol). The mixture was

stirred at 0 °C for 15 minutes. The dialdehyde in CH₂Cl₂ (5 mL) was added slowly at 0 °C. The reaction mixture was gradually warmed to room temperature while stirring overnight. The reaction mixture was washed with 1 M aqueous HCl solution. The aqueous layer was further extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 3/7) to afford 12 (577 mg, 31% for 2 steps) as colorless oil. $R_f = 0.62$ (hexane/ethyl acetate = 1/1); ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.28 (m, 10H), 6.55 (t, J = 7.6 Hz, 2H), 6.50 (br s, 2H), 5.12 (s, 4H), 3.74 (s, 6H), 3.48 (d, J = 5.4 Hz, 2H), 2.31-2.18 (m, 4H), 1.95-1.85 (m, 1H), 0.86 (s, 9H), 0.03 ppm (s, 6H); ¹³C NMR (101 MHz, CDCl₃): δ 164.8 (2C), 154.1 (2C), 136.0 (2C), 134.2 (2C), 128.5 (4C), 128.24 (2C), 128.21 (4C), 127.0 (2C), 67.4 (2C), 65.2, 52.4 (2C), 40.0, 29.8 (2C), 25.9 (3C), 18.3, -5.4 ppm (2C); IR (neat on KBr plate): ũ 3316, 3033, 2952, 2856, 1725, 1659, 1500, 1226, 1049, 837, 777, 698 cm⁻¹; HRMS (FAB): *m*/z calcd for C₃₄H₄₇N₂O₉Si: 655.3051 [M+H]+; found: 655.3054.

Compound 13: To a solution of **12** (44.7 mg, 68.3 μmol) in MeOH/CH₂Cl₂ (1.5 mL/1.5 mL) was added (±)-10-camphorsulfonic Acid (CSA, 3.2 mg, 13.8 μmol) at room temperature. The reaction mixture was stirred overnight at room temperature and then was added triethylamine (9.10 mL). The mixture was evaporated and purified by silica gel column chromatography (hexane/ethyl acetate, 1/1 to 1/4) to afford **13** (31.4 g, 85%) as colorless oil. R_f = 0.30 (hexane/ethyl acetate = 3/7); ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.29 (m, 10H), 6.71 (br s, 2H), 6.56 (t, *J* = 7.4 Hz, 2H), 5.14 (s, 4H), 3.74 (s, 6H), 3.50-3.48 (m, 2H), 3.05 (br s, 1H), 2.42-2.34 (m, 2H), 2.22-2.15 (m, 2H), 1.94-1.85 ppm (m, 1H); ¹³C NMR (101 MHz, CDCl₃): δ 164.9 (2C), 154.4 (2C), 135.9 (2C), 134.7 (2C), 128.5 (4C), 128.3 (2C), 128.2 (4C), 127.0 (2C), 67.5 (2C), 63.3, 52.4 (2C), 39.8, 30.1 ppm (2C); IR (neat on KBr plate): 0 3307, 3032, 2952, 1717, 1655, 1508, 1238, 1054, 753, 698 cm⁻¹; HRMS (FAB): *m/z* calcd for C₂₈H₃₃N₂O₉: 541.2186 [M+H]⁺; found: 541.2191.

Compound 17: A solution of 16 (722 mg, 6.43 mmol) in dry CH₂Cl₂ (20 mL) was cooled to 0 °C under a nitrogen atmosphere. To this solution were added TBSCI (1.13 g, 7.51 mmol) and imidazole (861 mg, 12.6 mmol). The reaction mixture was warmed to room temperature and stirred overnight. Water (10 mL) was then added, and the two layers were separated. The aqueous layer was further extracted with CH₂Cl₂ 3 times, and the organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 9/1) to afford 17 (1.21 g, 83%) as colorless oil. Rf = 0.21 (hexane/ethyl acetate = 1/0); ¹H NMR (500 MHz, CDCl₃): δ 5.69-5.64 (m, 2H), 3.63 (t, J = 6.7 Hz, 2H), 2.52-2.44 (m, 2H), 2.37-2.28 (m, 1H), 2.02-1.95 (m, 2H), 1.64 (dd, J = 13.9, 6.9 Hz, 2H), 0.90 (s, 9H), 0.05 ppm (s, 6H); ¹³C NMR (126 MHz, CDCl₃): δ 129.9 (2C), 62.3, 39.6, 38.9 (2C), 34.2, 26.0 (3C), 18.3, -5.3 ppm (2C); IR (neat on KBr plate): ũ 3055, 2928, 2857, 1472, 1255, 1102, 835, 775 cm⁻¹; HRMS (APCI): *m/z* calcd for C₁₃H₂₇OSi: 227.1826 [M+H]⁺; found: 227.1824.

Compound 18: A solution of 17 (688 mg, 3.04 mmol) in CH₂Cl₂ (30 mL) was cooled to -78 °C. The solution was bubbled with O3 until the color of it turned blue (6 minutes). Excess O3 was removed from the reaction mixture by purging with O_2 for 3 minutes. To the mixture was added Ph_3P (4.08 g, 15.6 mmol) at -78 °C, and the reaction mixture was stirred at -78 °C for 10 minutes and warmed to room temperature with stirring for an additional 20 minutes. Then the reaction mixture was washed with water, and the aqueous layer was extracted with ethyl acetate twice. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 3/7) to afford the dialdehyde as colorless oil. To a solution of (Z)- α phosphonoglycine trimethyl ester (2.18 g, 6.59 mmol) in dry CH₂Cl₂ (20 mL) was added DBN (0.800 g, 6.44 mmol) under a nitrogen atmosphere. The mixture was stirred at 0 °C and allowed to gradually warm to room temperature with stirring for 2.5 h. The dialdehyde in dry CH₂Cl₂ (4 mL) was added slowly by cannula at 0 °C. The reaction mixture was gradually warmed to room temperature while stirring overnight. The reaction mixture was washed with 1 M aqueous HCl solution. The aqueous layer was further extracted with ethyl acetate twice. The organic layers were washed

with water, saturated aqueous NaHCO₃ solution, and brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford **18** (1.25 g, 61% for 2 steps) as colorless oil. R_f = 0.26 (hexane/ethyl acetate = 7/3); ¹H NMR (500 MHz, CDCl₃): δ 7.38-7.30 (m, 10H), 6.63 (t, *J* = 7.3 Hz, 2H), 6.32 (br s, 2H), 5.14 (s, 4H), 3.74 (s, 6H), 3.64 (t, *J* = 6.3 Hz, 2H), 2.29-2.18 (m, 4H), 2.01-1.94 (m, 1H), 1.55-1.51 (m, 2H), 0.89 (s, 9H), 0.04 ppm (s, 6H); ¹³C NMR (126 MHz, CDCl₃): δ 164.8 (2C), 154.1 (2C), 136.0 (2C), 135.9 (2C), 128.5 (4C), 128.2 (2C), 128.1 (4C), 126.5 (2C), 67.4 (2C), 60.7, 52.3 (2C), 37.0, 34.2, 32.8 (2C), 25.9 (3C), 18.3, -5.4 ppm (2C); IR (neat on KBr plate): \bar{u} 3321, 3033, 2952, 2856, 1726, 1658, 1502, 1256, 1051, 837, 776, 698 cm⁻¹; HRMS (FAB): *m/z* calcd for C₃₅H₄₉N₂O₉Si: 669.3207 [M+H]⁺; found: 669.3217.

Compound 4: To a solution of **18** (51.8 mg, 77.4 μmol) in MeOH/CH₂Cl₂ (0.5 mL/0.5 mL) was added CSA (2.0 mg, 8.6 μmol) at room temperature. The reaction mixture was stirred at room temperature for 70 minutes and then was added triethylamine (1.50 mL). The mixture was evaporated and purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 3/7) to afford **4** (29.7 g, 70%) as colorless oil. R_f = 0.25 (hexane/ethyl acetate = 3/7); ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.28 (m, 10H), 6.61 (t, *J* = 7.4 Hz, 2H), 6.54 (s, 2H), 5.13 (s, 4H), 3.73 (s, 6H), 3.64-3.61 (m, 2H), 2.32-2.16 (m, 4H), 2.05 (br s, 1H), 2.00-1,94 (m, 1H), 1.55-1.51 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 164.9 (2C), 154.3 (2C), 135.9 (2C), 135.4 (2C), 128.5 (4C), 128.2 (2C), 128.1 (4C), 126.6 (2C), 67.4 (2C), 60.2, 52.4 (2C), 36.3, 34.3, 32.6 ppm (2C); IR (neat on KBr plate): ü 3310, 3033, 2952, 1714, 1658, 1505, 1240, 1053, 754, 699 cm⁻¹; HRMS (ESI): *m/z* calcd for C₂₉H₃₃N₂O₉: 553.2192 [M-H]⁻; found: 553.2191.

Compound 15: To a solution of 4 (63.8 mg, 115 µmol) in CH₂Cl₂ (5 mL) were added PhI(OAc)₂ (52.9 mg, 184 µmol) and AZADOL (2.6 mg, 17 µmol). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate, washed with water, extracted with ethyl acetate (3 times), and washed with brine. The combined organic layers were dried over Na2SO4, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford 15 (38.7 mg, 61%) as colorless oil. R_f = 0.30 (hexane/ethyl acetate = 2/3); ¹H NMR (400 MHz, CDCl₃): δ 9.69 (s, 1H), 7.37-7.28 (m, 10H), 6.56 (t, J = 7.4 Hz, 2H), 6.43 (br s, 2H), 5.13 (s, 4H), 3.74 (s, 6H), 2.45-2.34 (m, 3H), 2.27-2.24 ppm (m, 4H); ¹³C NMR (101 MHz, CDCl₃): δ 201.4, 164.6 (2C), 154.1 (2C), 135.8 (2C), 134.1 (2C), 128.5 (4C), 128.3 (2C), 128.1 (4C), 127.0 (2C), 67.4 (2C), 52.5 (2C), 47.9, 32.7 (2C), 32.1 ppm; IR (neat on KBr plate): ũ 3312, 3033, 2952, 2732, 1721, 1659, 1500, 1233, 1049, 754, 699 cm⁻¹; HRMS (ESI): *m*/z calcd for C₂₉H₃₂N₂O₉Na: 575.2000 [M+Na]⁺; found: 575 1990

Compound 3: To a solution of (Z)-a-phosphonoglycine trimethyl ester (25.5 mg, 77.0 $\mu mol)$ in dry CH_2Cl_2 (1 mL) was added DBN (10.6 mg, 85.4 µmol) under an argon atmosphere. The mixture was stirred at 0 °C for 15 minutes. A solution of 15 (33.8 mg, 61.2 µmol) in dry CH₂Cl₂ (4 mL) was then added slowly by cannula at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring overnight. The reaction mixture was washed with 1 M aqueous HCI solution. The aqueous layer was further extracted with ethyl acetate twice. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford 3 (33.7 mg, 73%) as colorless oil. Rf = 0.34 (hexane/ethyl acetate = 2/3); ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.27 (m, 15H), 6.58 (t, J = 7.3 Hz, 3H), 6.40 (br s, 3H), 5.12 (s, 6H), 3.71 (s, 9H), 2.24-2.21 (m, 6H), 2.05-2.00 ppm (m, 1H); ¹³C NMR (101 MHz, CDCl₃): δ 164.7 (3C), 154.2 (3C), 135.9 (3C), 135.2 (3C), 128.6 (6C), 128.3 (3C), 128.2 (6C), 126.6 (3C), 67.5 (3C), 52.4 (3C), 37.4, 33.1 ppm (3C); IR (neat on KBr plate): ũ 3311, 3032, 2952, 1724, 1659, 1504, 1235, 1053, 755, 699 cm⁻¹; HRMS (APCI): *m/z* calcd for C40H44N3O12: 758.2920 [M+H]+; found: 758.2925.

Compound 9: Compound **18** (933 mg, 1.40 mmol) and $[Rh((S,S)-QuinoxP^*)(cod)]SbF_6$ (21.6 mg, 27.6 µmol) were charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, degassed MeOH (3 mL) was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred

vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/4) to afford **9** (665 mg, 85%) as orange oil. Ee and de were determined by HPLC (Daicel CHIRAL CEL OX-RH, λ = 254 nm, CH₃CN/H₂O = 9/11, 0.5 mL/minutes, >99%ee, >98%de). R_f = 0.12 (hexane/ethyl acetate = 3/7); [α]₀²⁷ = +13.4 (c = 1.64 in CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.37-7.29 (m, 10H), 5.41 (d, *J* = 8.1 Hz, 1H), 5.37 (d, *J* = 8.0 Hz, 1H), 5.10 (s, 4H), 4.37-4.33 (m, 2H), 3.74 (s, 3H), 3.73 (s, 3H), 3.60-3.58 (m, 2H), 1.86-1.20 ppm (m, 11H); ¹³C NMR (126 MHz, CDCl₃): δ 172.8 (2C), 156.0, 155.9, 136.29, 136.26, 128.5 (4C), 128.2 (2C), 128.1 (4C), 67.1, 67.0, 60.4, 54.0, 53.7, 52.3 (2C), 36.2, 33.0, 29.8, 29.4, 28.7, 28.6 ppm; IR (neat on KBr plate): ũ 3335, 3033, 2952, 1714, 1531, 1455, 1215, 1051, 742, 698 cm⁻¹; HRMS (FAB): *m/z* calcd for C₂₉H₃₉N₂O₉: 559.2656 [M+H]⁺; found: 559.2662.

Compound 19: To a solution of 9 (515 mg, 922 µmol) in CH₂Cl₂ (10 mL) were added PhI(OAc)₂ (444 mg, 1.38 mmol) and AZADOL (14.2 mg, 92.7 µmol). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford 19 (342 mg, 67%) as pale yellow oil. $R_f = 0.23$ (hexane/ethyl acetate = 1/1); $[\alpha]_D^{27} =$ +17.5 (c = 1.20 in CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 9.71 (s, 1H), 7.37-7.29 (m. 10H), 5.37 (d. J = 7.5 Hz, 1H), 5.33 (d. J = 7.8 Hz, 1H), 5.10 (s. 4H), 4.37-4.33 (m, 2H), 3.74 (s, 6H), 2.34 (d, J = 5.4 Hz, 2H), 2.03-1.96 (m, 1H), 1.85-1.77 (m, 2H), 1.67-1.57 (m, 2H), 1.44-1.24 ppm (m, 4H); ¹³C NMR (126 MHz, CDCI3): 8 201.5, 172.5 (2C), 155.8 (2C), 136.2 (2C), 128.5 (4C), 128.2 (2C), 128.1 (4C), 67.1 (2C), 53.8 (2C), 52.4 (2C), 48.0, 31.8, 29.8, 29.6, 29.2, 29.1 ppm; IR (neat on KBr plate): ũ 3342, 3033, 2953, 1729, 1714, 1531, 1455, 1215, 1047, 753, 699 cm⁻¹; HRMS (FAB): m/z calcd for C₂₉H₃₇N₂O₉: 557.2499 [M+H]+; found: 557.2506.

Compound 8: To a solution of (Z)-a-phosphonoglycine trimethyl ester (86.6 mg, 261 µmol) in dry CH₂Cl₂ (3 mL) was added DBN (36.0 mg, 290 µmol) under a nitrogen atmosphere. The mixture was stirred at 0 °C for 15 minutes. A solution of 19 (126.7 mg, 228 $\mu mol)$ in dry CH_2Cl_2 (3 mL) was then added slowly by cannula at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring for 5.5 h. The reaction mixture was washed with 1 M aqueous HCl solution. The aqueous layer was further extracted with ethyl acetate twice. The organic layers were washed with water, saturated aqueous NaHCO₃ solution, and brine. The combined organic layers were dried over Na2SO4, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 2/3) to afford 8 (166 mg, 96%) as colorless oil. $R_{\rm f}$ = 0.36 (hexane/ethyl acetate = 1/1); [$\alpha]_{\rm D}{}^{27}$ = +15.5 (c = 1.24 in CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.36-7.28 (m, 15H), 6.60 (t, J = 6.7 Hz, 1H), 6.36 (br s, 1H), 5.46-5.42 (m, 2H), 5.13-5.05 (m, 6H), 4.33-4.29 (m, 2H), 3.72 (s, 9H), 2.18-2.15 (m, 2H), 1.83-1.74 (m, 2H), 1.67-1.52 (m, 3H), 1.38-1.21 ppm (m, 4H); ¹³C NMR (126 MHz, CDCl₃): δ 172.6 (2C), 164.7, 155.9 (2C), 154.2, 136.23 (2C), 136.18, 136.0, 128.48 (2C), 128.47 (4C), 128.2 (2C), 128.1 (4C), 128.05 (2C), 127.96, 126.6, 67.4, 67.0 (2C), 53.9 (2C), 52.3 (3C), 36.4, 31.8, 29.51, 29.48, 29.0, 28.8 ppm; IR (neat on KBr plate): ũ 3334, 3033, 2952, 1714, 1519, 1455, 1218, 1052, 753, 699 cm⁻¹; HRMS (FAB): *m*/*z* calcd for C₄₀H₄₈N₃O₁₂: 762.3238 [M+H]⁺; found: 762.3229.

Compound 20: Method A: A solution of $[Rh(cod)_2]BF_4$ (2.4 mg, 5.9 µmol) and (*S*,*S*)-QuinoxP* (2.6 mg, 7.8 µmol) in degassed MeOH (0.5 mL) was stirred vigorously at room temperature under nitrogen atmosphere. After 45 minutes, additional degassed MeOH (0.5 mL) was added to the mixture. Compound **3** (25.8 mg, 34.0 µmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford **20** (21.9 mg, 84%) as pale yellow oil. Ee and de were determined by HPLC (Daicel CHIRAL CEL OX-RH, λ = 254 nm, CH₃CN/H₂O = 3/2, 0.5 mL/minutes, >99%ee, >97%de). Method B: Compound **8** (193 mg, 254 µmol) and [Rh((*S*,*S*)-QuinoxP*)(cod)]SbF₆ (2.0 mg, 2.6 µmol) were charged in a hydrogenation bottle. After the bottle was evacuated and filled

with hydrogen several times, degassed MeOH (1 mL) was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The suspended reaction mixture was dissolved in CHCl3 and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford 20 (177 mg, 91%) as colorless oil. De was determined by HPLC (Daicel CHIRAL CEL OJ-RH, λ = 254 nm, CH₃CN/H₂O = 1/1, 0.5 mL/minutes, >98%de). R_f = 0.24 (hexane/ethyl acetate = 1/1); $[\alpha]_D^{27}$ = +22.3 (c = 1.35 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.37-7.28 (m, 15H), 5.39 (d, J = 6.9 Hz, 3H), 5.13-5.06 (m, 6H), 4.34-4.29 (m, 3H), 3.73 (s, 9H), 1.79-1.68 (m, 3H), 1.64-1.53 (m, 3H), 1.37-1.17 ppm (m, 7H); ¹³C NMR (101 MHz, CDCl₃): δ 172.8 (3C), 155.9 (3C), 136.2 (3C), 128.5 (6C), 128.22 (3C), 128.16 (6C), 67.1 (3C), 53.9 (3C), 52.4 (3C), 36.1, 29.5 (3C), 28.4 ppm (3C); IR (neat on KBr plate): ũ 3340, 3033, 2952, 1714, 1531, 1455, 1215, 1050, 753, 698 cm⁻¹; HRMS (FAB): *m/z* calcd for C₄₀H₅₀N₃O₁₂: 764.3394 [M+H]+; found: 764.3401.

Compound 1: To a solution of **20** (147 mg, 193 µmol) in MeOH (8 mL) was added 2 M aqueous NaOH solution (1.49 mL). The reaction mixture was stirred at room temperature for 75 minutes. The volatile in the reaction mixture was removed under reduced pressure. The residual water layer was adjusted to pH 2 using 1 M aqueous HCI solution and extracted with ethyl acetate 3 times. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄. Filtration and concentration afforded a crude tricarboxylic acid. To a solution of the tricarboxylic acid in deoxygenated MeOH (5 mL) was added Pd/C (10 wt%, 35.6 mg), and the reaction mixture was stirred overnight under a hydrogen atmosphere at room temperature. The reaction mixture was filtered through celite and washed with MeOH/H₂O (1/1 to 0/1) to afford triamino acid **21** as a white solid: ¹H NMR (400 MHz, D₂O): δ 3.75 (t, *J* = 5.9 Hz, 3H), 1.93-1.79 (m, 6H), 1.56-1.30 ppm (m, 7H); ¹³C NMR (101 MHz, D₂O): δ 174.7 (3C), 54.9 (3C), 35.9, 27.32 (3C), 27.28 ppm (3C).

To a suspension of the 21 in H₂O/acetone (3 mL/3 mL) were added Fmoc-OSu (214 mg, 636 µmol) and Na₂CO₃ (137 mg, 1.29 mmol). The reaction mixture was stirred at room temperature for 4 days. The reaction mixture was adjusted to pH 2 using 1 M aqueous HCl solution and extracted with ethyl acetate 9 times. The organic layers were washed with brine. The combined organic layers were dried over Na2SO4, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/4, 1% acetic acid), ODS flash column chromatography (CH₃CN/H₂O = 3/7 to 7/3, 0.1% TFA), and HPLC (YMC Pack ODS-A No.2015000608, λ = 254 nm, CH₃CN/H₂O = 7/3, 0.1% TFA. 8.0 mL/minutes, retention time 19.0 minutes) to afford 1 (103 mg, 54%) as a white solid. $R_f = 0.04$ (hexane/ethyl acetate = 0/1, 1%) AcOH); $[\alpha]_D^{27}$ = +26.6 (c = 1.31 in CHCl₃); ¹H NMR (400 MHz, CDCl₃, 313 K): δ 7.63 (d, J = 7.5 Hz, 6H), 7.58-7.34 (m, 6H), 7.27 (t, J = 7.5 Hz, 6H), 7.18 (t, J = 7.3 Hz, 6H), 6.79 (br s, 1H), 5.81 (br s, 2H), 4.83-3.87 (m, 12 H), 2.00-1.44 (m, 6H), 1.44-0.97 ppm (m, 7H); ¹³C NMR (101 MHz, CDCl₃, 313 K): δ 176.4 (3C), 156.5 (3C), 143.6 (6C), 141.3 (6C), 127.7 (6C), 127.1 (6C), 125.0 (6C), 112.0 (6C), 67.4 (3C), 53.8 (3C), 47.0 (3C), 35.8, 29.1 (3C), 28.2 ppm (3C) (The signals derived from a minor conformation were also observed); IR (neat on KBr plate): ũ 3321, 3066, 2952, 2869, 2603, 1715, 1520, 1450, 1219, 1050, 741 cm⁻¹; HRMS (ESI): m/z calcd for $C_{58}H_{55}N_3O_{12}Na: 1008.3678 [M+Na]^+; found: 1008.3677.$

Compound 26: To a suspension of NaH (60%) (1.30 g, 32.4 mmol) in dry Et₂O/dry DMSO (100 mL/5 mL) was added 25 (1.53 g, 10.8 mmol) dropwise under a nitrogen atmosphere at room temperature. The mixture was stirred for 30 minutes. 6-Bromo-1-hexene (5.14 g, 31.5 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. 10 mL of H₂O was added dropwise to the reaction mixture at room temperature. The two layers were separated, and the aqueous layer was extracted with Et₂O 3 times. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 19/1) to afford 26 (2.78 g, 84%) as colorless oil. R_f = 0.44 (hexane/ethyl acetate = 9/1); ¹H NMR (400 MHz, CDCl₃): δ 5.83-5.73 (m, 2H), 5.04-4.94 (m, 4H), 2.10-2.04 (m, 4H), 1.91-1.83 (m, 2H), 1.76-1.68 (m, 2H), 1.65-1.54 (m, 2H), 1.49 (s, 9H), 1.46-1.37 (m, 4H), 1.34-1.23 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 167.8, 158.2, 138.2 (2C), 114.8 (2C), 83.4, 68.9, 39.1 (2C), 33.3 (2C), 28.4 (2C), 27.8 (3C), 23.4 ppm (2C); IR (neat on KBr plate): $\tilde{\upsilon}$ 3077, 2933, 2863, 2137, 1748, 1641, 1370, 1254, 1153, 911, 844 cm^1; HRMS (APCI): *m/z* calcd for $C_{19}H_{32}NO_2$: 306.2428 [M+H]+; found: 306.2427.

Compound 24: Isocyanide 26 (2.77 g, 9.08 mmol) was dissolved in EtOH (10 mL) containing 12 M aqueous HCl solution (398 μ L) and the resulting mixture was stirred overnight at room temperature. Additional 12 M aqueous HCl solution (390 µL) was added and the mixture was stirred for 40 minutes. The solvent was removed under reduced pressure and the remaining oil was taken up in toluene (100 mL). The mixture was cooled to 0 °C, and CbzCl (1.80 g, 10.6 mmol) and 1 M aqueous Na₂CO₃ solution (18.5 mL) were added. The reaction mixture was stirred at room temperature for 2 h and at 80 °C for 1.5 h. Additional CbzCl (1.52 g, 8.93 mmol) and 1 M aqueous Na₂CO₃ solution (18.2 mL) were added and the mixture was stirred at 80 °C for 35 minutes. The two layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The organic layers were washed with 0.1 M aqueous HCl solution and saturated aqueous NaHCO3 solution. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/Et₂O = 1/0 to 49/1) to afford 24 (2.84 g, 73% for 2 steps) as colorless oil. Rf = 0.30 (hexane/Et₂O = 9/1); ¹H NMR (400 MHz, CDCl₃): δ 7.40-7.28 (m, 5H), 5.88 (br s, 1H), 5.80-5.70 (m, 2H), 5.06 (s, 2H), 5.00-4.90 (m, 4H), 2.34-2.27 (m, 2H), 2.02-1.97 (m, 4H), 1.71-1.63 (m, 2H), 1.45 (s, 9H), 1.41-1.22 (m, 6H), 1.06-0.94 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 172.9, 153.9, 138.7 (2C), 136.9, 128.4 (2C), 127.9, 127.8 (2C), 114.4 (2C), 82.2, 66.0, 64.1, 35.5 (2C), 33.5 (2C), 28.7 (2C), 27.9 (3C), 23.4 ppm (2C); IR (neat on KBr plate): ũ 3418, 3075, 2928, 2859, 1717, 1640, 1496, 1253, 1158, 1067, 911, 697 cm⁻¹; HRMS (FAB): *m*/z calcd for C₂₆H₄₀NO₄: 430.2957 [M+H]⁺; found: 430.2963.

Compound 23: A solution of 24 (685 mg, 1.60 mmol) in CH₂Cl₂ (15 mL) was cooled to -78 °C. The solution was bubbled with O3 until the color of it turned blue (10 minutes). Excess O_3 was removed from the reaction mixture by purging with O2 for 2 minutes. To the mixture was added Ph3P (1.64 g, 6.26 mmol) at -78 °C, and the reaction mixture was stirred at -78 °C for 10 minutes and warmed to room temperature with stirring for an additional 30 minutes. Then the reaction mixture was washed with water, and the aqueous layer was extracted with CH2Cl2 3 times. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 4/1 to 3/2) to afford 23 (672 mg, 97%) as colorless oil. Rf = 0.38 (hexane/ethyl acetate = 3/2); ¹H NMR (400 MHz, CDCl₃): δ 9.71 (t, J = 1.6 Hz, 2H), 7.38-7.28 (m, 5H), 5.88 (br s, 1H), 5.06 (s, 2H), 2.43-2.29 (m, 6H), 1.72-1.65 (m, 2H), 1.62-1.54 (m, 4H), 1.46 (s, 9H), 1.33-1.21 (m, 2H), 1.09-1.98 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 202.2 (2C), 172.5, 153.9, 136.8, 128.5 (2C), 128.0, 127.9 (2C), 82.7, 66.1, 63.8, 43.6 (2C), 35.3 (2C), 27.9 (3C), 23.5 (2C), 21.8 ppm (2C); IR (neat on KBr plate): ũ 3416, 3033, 2938, 2865, 2721, 1718, 1497, 1252, 1157, 1070, 847, 742, 698 cm⁻¹.

Compound 22: To a solution of (Z)- α -phosphonoglycine trimethyl ester (712 mg, 2.15 mmol) in dry CH₂Cl₂ (4 mL) was added DBN (280 mg, 2.25 mmol) under an argon atmosphere. The mixture was stirred at 0 °C for 20 minutes. A solution of 23 (443 mg, 1.02 mmol) in dry CH₂Cl₂ (6 mL) was then added slowly by cannula at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring overnight. The reaction mixture was washed with 1 M aqueous HCl solution. The aqueous layer was further extracted with ethyl acetate twice. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 3/2) to afford 22 (552 mg, 64%) as colorless oil. R_f = 0.35 (hexane/ethyl acetate = 1/1); ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.28 (m, 15H), 6.57 (t, J = 7.3 Hz, 2H), 6.31 (br s, 2H), 5.88 (s, 1H), 5.14 (s, 4H), 5.04 (s, 2H), 3.72 (s, 6H), 2.34-2.27 (m, 2H), 2.23-2.12 (m, 4H), 1.68-1.60 (m, 2H), 1.44-1.39 (m, 13H), 1.31-1.20 (m, 2H), 1.06-0.95 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 172.6, 165.0 (2C), 154.2 (2C), 154.0, 138.1 (2C), 136.7, 136.1 (2C), 128.5 (4C), 128.4 (2C), 128.2 (2C), 128.1 (4C), 128.0, 127.8 (2C), 125.5 (2C), 82.5, 67.3 (2C), 66.2, 64.0, 52.3 (2C), 35.2 (2C), 27.93 (2C), 27.88 (2C), 27.8 (3C), 23.5 ppm (2C); IR (neat on KBr plate): ũ 3410, 3326, 3033, 2951, 2861, 1714, 1659, 1504, 1227, 1063, 754, 698 cm⁻¹; HRMS (FAB): m/z calcd for C₄₆H₅₇N₃O₁₂Na: 866.3840 [M+Na]⁺; found: 866.3838.

Compound 27: A solution of [Rh(cod)₂]BF₄ (3.9 mg, 9.6 µmol) and (S,S)-QuinoxP* (4.1 mg, 12.3 µmol) in degassed MeOH (0.5 mL) was stirred vigorously at room temperature under nitrogen atmosphere. After 50 minutes, additional degassed MeOH (1.5 mL) was added to the mixture. Compound 22 (421 mg, 499 µmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/1) to afford 27 (412 mg, 98%) as colorless oil. Ee and de were determined by HPLC (Daicel CHIRAL CEL OX-RH, λ = 254 nm, CH₃CN/H₂O = 7/3, 0.5 mL/minutes, >99%ee, >96%de). $R_f = 0.39$ (hexane/ethyl acetate = 1/1); [α]_D²⁷ = +8.67 (c = 0.92 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.27 (m, 15H), 5.87 (s, 1H), 5.34 (d, J = 7.9 Hz, 1H), 5.26 (d, J = 8.3 Hz, 1H), 5.12 (s, 2H), 5.10 (s, 2H), 5.06 (s, 2H), 4.37-4.30 (m, 2H), 3.73 (s, 3H), 3.72 (s, 3H), 2.30-2.23 (m, 2H), 1.78-1.72 (m, 2H), 1.67-1.54 (m, 4H), 1.44 (s, 9H), 1.32-1.19 (m, 10H), 1.00-1.91 ppm (m, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 173.0, 172.9, 172.7, 155.9, 155.8, 153.9, 136.8, 136.25, 136.20, 128.5 (4C), 128.4 (2C), 128.14, 128.11, 128.07 (2C), 128.0 (2C), 127.9, 127.8 (2C), 82.3, 66.9 (2C), 66.0, 64.0, 53.81, 53.77, 52.3 (2C), 35.5, 35.3, 32.5, 32.3, 29.0, 28.8, 27.9 (3C), 25.0, 24.8, 23.7, 23.4 ppm. IR (neat on KBr plate): ũ 3412, 3353, 3033, 2950, 2861, 1716, 1499, 1214, 1066, 698 cm⁻¹; HRMS (FAB): *m*/z calcd for C₄₆H₆₁N₃O₁₂Na: 870.4153 [M+Na]⁺; found: 870.4154.

Compound 2: To a solution of 27 (285 mg, 336 µmol) in CH₂Cl₂ (5 mL) was added TFA (5mL) at room temperature. The mixture was stirred for 1.5 h. The volatile in the reaction mixture was removed under reduced pressure. The residue was reconstituted in Et₂O (30 mL) and washed with saturated aqueous NH₄Cl solution 3 times. The organic layer was dried over Na₂SO₄, filtered and concentrated. Residual solvent was removed by azeotropic drying with pentane to afford monocarboxylic acid as colorless oil. To a solution of the monocarboxylic acid in MeOH (7 mL) was added 2 M aqueous NaOH solution (1.65 mL). The reaction mixture was stirred at room temperature for 1 h. The volatile in the reaction mixture was removed under reduced pressure. The residual water layer was adjusted to pH 1 using 1 M aqueous HCl solution and extracted with ethyl acetate 3 times. The organic layers were washed with and brine. The combined organic layers were dried over Na₂SO₄. Filtration and concentration afforded a crude tricarboxylic acid as a white solid. 5 mL of MeOH was added to the tricarboxylic acid and Pd/C (10 wt%, 62.0 mg). The reaction mixture was stirred overnight under a hydrogen atmosphere at room temperature. The reaction mixture was filtered through celite and washed with H₂O to afford triamino acid 28 as a white solid: ¹H NMR (400 MHz, D₂O): δ 3.79-3.76 (m, 2H), 1.91-1.73 (m, 8H), 1.48-1.33 (m, 10H), 1.29-1.19 ppm (m, 2H); ¹³C NMR (101 MHz, D₂O): δ 175.9, 174.5 (2C), 65.1, 54.5 (2C), 35.8, 35.9, 30.1, 30.0, 28.23, 28.18, 23.9, 23.7, 22.6, 22.5 ppm.

To a suspension of the 28 in H₂O/acetone (3 mL/3 mL) were added Fmoc-OSu (349 mg, 1.04 mmol) and Na₂CO₃ (209 mg, 1.97 mmol) at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring 1.5 days. The reaction mixture was adjusted to pH 1 using 1 M aqueous HCl solution and extracted with ethyl acetate 3 times. The organic layers were washed with brine. The combined organic layers were dried over Na₂SO₄, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 0/1, 1% acetic acid) and HPLC (YMC Pack ODS-A No.2015000608, λ = 254 nm, CH₃CN/H₂O = 7/3, 0.1% TFA, 8.0 mL/minutes, retention time 26.9 minutes) to afford 2 (186 mg, 55% for 4 steps) as a white solid. $R_f = 0.37$ (hexane/ethyl acetate = 0/1, 1% acetic acid); $[\alpha]_D^{27}$ = +1.58 (c = 0.92 in MeOH); ¹H NMR (400 MHz, CD₃OD): δ 7.74-7.71 (m, 6H), 7.64-7.58 (m, 6H), 7.36-7.24 (m, 12H), 4.43-4.25 (m, 6H), 4.20-4.10 (m, 5H), 2.17-2.04 (m, 2H), 1.83-1.69 (m, 4H), 1.68-1.56 (m, 2H), 1.47-1.11 (m, 10H), 0.97-0.71 ppm (m, 2H); ¹³C NMR (126 MHz, CD₃OD): δ 176.9, 176.1 (2C), 158.6 (2C), 156.1, 145.33 (2C), 145.27, 145.24, 145.16 (2C), 142.6 (6C), 128.8 (6C), 128.1 (6C), 126.3 (4C), 126.1 (2C), 120.9 (6C), 67.9 (2C), 67.2, 64.4, 55.3 (2C), 48.4 (3C), 36.0, 35.9, 32.6, 32.5, 30.1, 30.0, 26.7, 26.6, 24.8, 24.6 ppm; IR (neat on KBr plate): ũ 3405, 3066, 2940, 2861, 2604, 1693, 1514, 1449, 1207, 740 cm⁻¹; HRMS (ESI): *m*/z calcd for C₆₁H₆₁N₃O₁₂Na: 1050.4147 [M+Na]+; found: 1050.4146.

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FULL PAPER

Entry for the Table of Contents



The lack of suitable trivalent linkers has limited trimer peptide models. This study designed and synthesized two new alkyl bridgedtris-α-amino acids with Fmoc protecting groups as flexible linkers, which are characterized by C₃-symmetric and linear structures, respectively.

Contents

- I. Copy of NMR spectra of new compounds
- II. Experimental procedure of synthesis of stereoisomers of 9, 20, and 27
- III. Copy of HPLC chart of compounds 9, 20, and 27

I. NMR spectra of new compounds







S20

II. Experimental procedure of synthesis of stereoisomers of 9, 20, and 27

A solution of $[Rh(cod)_2]BF_4$ (4.0 mg, 9.9 µmol), (*S*,*S*)-QuinoxP* (2.2 mg, 6.6 µmol) and (*R*,*R*)-QuinoxP* (2.2 mg, 6.6 µmol) in degassed MeOH (0.5 mL) was stirred vigorously at room temperature under nitrogen atmosphere. After 40 minutes, additional degassed MeOH (2.5 mL) was added to the mixture. Compound **18** (97.5 mg, 146 µmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 1/9) to afford a mixture of (2*S*,8*S*)-, (2*S*,5*S*,8*R*)-, (2*R*,8*R*)-**9** (19.5 mg, 24%) and a mixture of (2*S*,5*S*,8*R*)-, (2*S*,5*S*,8*R*)-**9** and (2*S*,5*R*,8*R*)-**9**:(2*R*,8*R*)-**9** = 1.0:2.3:1.3)

A solution of $[Rh(cod)_2]BF_4$ (1.9 mg, 4.7 µmol), (*S*,*S*)-QuinoxP* (1.4 mg, 4.2 µmol) and (*R*,*R*)-QuinoxP* (1.4 mg, 4.2 µmol) in degassed MeOH (0.5 mL) was stirred vigorously at room temperature under nitrogen atmosphere. After 4 h, additional degassed MeOH (1.5 mL) was added to the mixture. Compound **3** (6.5 mg, 8.6 µmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 3/7) to afford a mixture of (2*S*,8*S*,11*S*)-, (2*S*,8*S*,11*R*)-, (2*S*,8*R*,11*R*)-, (2*R*,8*R*,11*R*)-**20** (5.4 mg, 82%) as colorless oil. ((2*S*,8*S*,11*S*)-**20**:(2*S*,8*S*,11*R*)-**20** and (2*S*,8*R*,11*R*)-**20**:(2*R*,8*R*,11*R*)-**20** = 1.0:3.8:0.4)

A solution of $[Rh(cod)_2]SbF_6$ (2.7 mg, 4.9 µmol) and (R,R)-QuinoxP* (2.9 mg, 8.7 µmol) in degassed MeOH (0.7 mL) was stirred vigorously at room temperature under nitrogen atmosphere. After 50 minutes, additional degassed MeOH (1.3 mL) was added to the mixture. Compound **8** (97.5 mg, 146 µmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 2/3) to afford a mixture of starting material **8** and the product. The mixture was hydrogenated by the same procedure as above using $[Rh(cod)_2]SbF_6$ (3.5 mg, 6.3 µmol) and (R,R)-QuinoxP* (3.2 mg, 9.6 µmol). After completion of the reaction, the reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (because = 1/0 to 2/3) to afford a mixture of starting material **8** and the product. The mixture was hydrogenated by the same procedure as above using $[Rh(cod)_2]SbF_6$ (3.5 mg, 6.3 µmol) and (R,R)-QuinoxP* (3.2 mg, 9.6 µmol). After completion of the reaction, the reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 1/0 to 2/3) to afford (2S,8S,11R)-**20** (44.4 mg, 54%) as colorless oil.

III. Copy of HPLC chart of compounds 9, 20, and 27

Ee and de were calculated from the peak area of each stereoisomer in HPLC (Daicel CHIRAL CEL OX-RH, $\lambda = 254$ nm, CH₃CN/H₂O = 9/11, 0.5 mL/minutes) by weighing each peak.

Ee and de were calculated from the peak area of each stereoisomer in HPLC (Daicel CHIRAL CEL OX-RH, $\lambda = 254$ nm, CH₃CN/H₂O = 3/2, 0.5 mL/minutes) by weighing each peak.

peak	ret. time	wight of	wight of
No.	(min)	paper (mg)	paper (%)
1	16.6	13.6	19.3
2, 3	19.3, 22.4	51.3	72.8
4	25.8	5.6	7.9

CbzHN,,, CO₂Me MeO₂C NHCbz NHCbz 20

(>99%ee, >97%de)

Peak	ret. time	wight of	wight of
No.	(min)	paper (mg)	paper (%)
1	16.7	87.9	98.8
2	19.6	1.1	1.2

A diastereomer (peak 3 in the above chart) and enantiomer were not detected.

De was calculated from the peak area of each stereoisomer in HPLC (Daicel CHIRAL CEL OJ-RH, $\lambda = 254$ nm, CH₃CN/H₂O = 1/1, 0.5 mL/minutes) by weighing each peak.

Ee and de were calculated from the peak area of each stereoisomer in HPLC (Daicel CHIRAL CEL OX-RH, $\lambda = 254$ nm, CH₃CN/H₂O = 7/3, 0.5 mL/minutes) by weighing each peak.

MeO ₂ C	NHCbz 8' 8'	2C NHCbz	NHCbz	
(8S,8'S)-, (8S,2S,8'R)-, (8S,2R,8'R)-, (8R,8'R)- 27				
peak	ret. time	wight of	wight of	
No.	(min)	paper (mg)	paper (%)	
1	16.7	21.3	18.6	
2, 3	18.9, 19.6	56.8	49.7	
4	22.6	36.2	31.7	

27 (>99%ee, >96%de)

peak	ret. time	wight of	wight of
No.	(min)	paper (mg)	paper (%)
1	16.6	75.4	98.2
2	19.6	1.4	1.8

Enantiomer was not detected.