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Multigram Solution-Phase Synthesis of Three Diastereomeric Tripeptidic Second-Generation Dendrons Based on (2*S*,4*S*)-, (2*S*,4*R*)-, and (2*R*,4*S*)-4-Aminoprolines

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Abstract: Three diastereomeric second-generation (G2) dendrons were prepared by using (2*S*,4*S*)-, (2*S*,4*R*)-, and (2*R*,4*S*)-4-aminoprolines on the multigram scale with highly optimized and fully reproducible solution-phase methods. The peripheral 4-aminoproline branching units of all the dendrons have the 2*S*,4*S* configuration throughout, whereas those units at the focal point have the 2*S*,4*S*, 2*S*,4*R*, and 2*R*,4*S* configurations. These latter configurations led to the dendrons being named (2*S*,4*S*)-**1**, (2*S*,4*R*)-**1**, and (2*R*,4*S*)-**1**, respectively. The 4-aminoproline derivatives used in this study are new, although many closely related compounds exist. Their syntheses were optimized. The dendron assembly involved amide coupling, the efficiency of which was also optimized by employing the following well-known reagents: EDC/HOBt, DCC/HOSu, TBTA/HOBt, TBTU/HOBt, BOP/HOBt, pentafluorophenol, and PyBOP/HOBt. It was found that the use of PyBOP is by far the best for dendrons (2*S*,4*S*)-**1** and (2*R*,4*S*)-**1**, and pentafluorophenol active ester is best for (2*S*,4*R*)-**1**. Because of their multigram scale, all couplings were done in solution instead of by solid-phase procedures. Purifications were, nevertheless, easy. The optical purities of the key intermediates as well as the three G2 dendrons were analyzed by chiral HPLC analysis. These novel, diastereomeric second-generation dendrons have a rather compact and conformationally highly rigid structure that makes them interesting candidates for applications, for example, in the field of dendronized polymers and in organocatalysis.

Keywords: amide coupling · aminoprolines · dendrimers · peptides · synthetic methods

Introduction

Proline and its derivatives are among the more important amino acids. They have an impact on geometry control and *cis*/*trans* isomerization in proteins[1] and, thus, play a vital role in the tertiary structure and function of these biological macromolecules.[2] Prolines are also efficient in preventing protein aggregation and misfolding.[3] Furthermore, oligoprolines can adopt two distinct helical conformations, known as PPI and PPII, with rather different end-to-end distances.[4] As these conformations depend on solvent polarity, oligoprolines can potentially be used as switches in actuator applications.[2a,5] From the organic chemistry point of view, proline derivatives play a key role in the rapid development of organocatalysis in that they are powerful catalysts in, for example, Michael addition, Mannich reaction, and aldol condensation.[6]

4-Substituted prolines exhibit strong conformational biases that depend on their stereochemistry and the electronic characteristics of the substituent. *Trans* and *cis* 4-substituted prolines strongly favor *trans*- and *cis*-configurated amide bonds in the corresponding peptides.[7] 4-aminoproline stereoisomers have remarkable functions in biologically active compounds[8] and photochemical-energy transformation,[9] and were also discovered as useful building blocks for dendrimers. Recently, Giralt, Albericio, and co-workers described the solid-phase synthesis of proline- and 4-aminoproline-based dendrimers.[10] Several tens of milligrams of second-generation (G2) dendrimers were obtained. Owing

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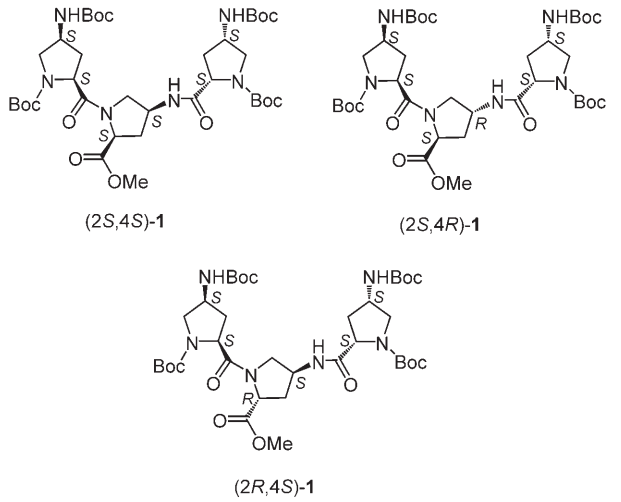
to a proposed detrimental steric hindrance during construction, however, a linear oligoproline spacer was introduced between the 4-substituted proline units, the latter of which served as the branching points. For many, if not all, of the above applications, the reduced conformational space of prolines is of essential importance. This is due to the cyclic nature of proline and the especially low mobility of its somewhat-strained five-membered ring.

We describe herein a facile synthetic access to three diastereomeric *tert*-butyloxycarbonyl (Boc)-protected (2*S*,4*S*)-, (2*S*,4*R*)-, and (2*R*,4*S*)-4-aminoprolines. Their availability on the multigram scale together with an optimized amide-coupling procedure allowed the synthesis of the corresponding G2 dendrons (2*S*,4*S*)-**1**, (2*S*,4*R*)-**1**, and (2*R*,4*S*)-**1**,[11] respectively (Scheme 1). These dendrons are important and versatile building blocks used in a long-term research project aimed at ultrarigid dendronized polymers with a programmed and fixed secondary structure for applications in supramolecular chemistry as well as organocatalysis.



Scheme 1. The three diastereomeric proline-based G2 dendrons **1** synthesized herein (for simplicity, only the absolute configuration of the branching unit at the focal point is considered in naming these compounds).

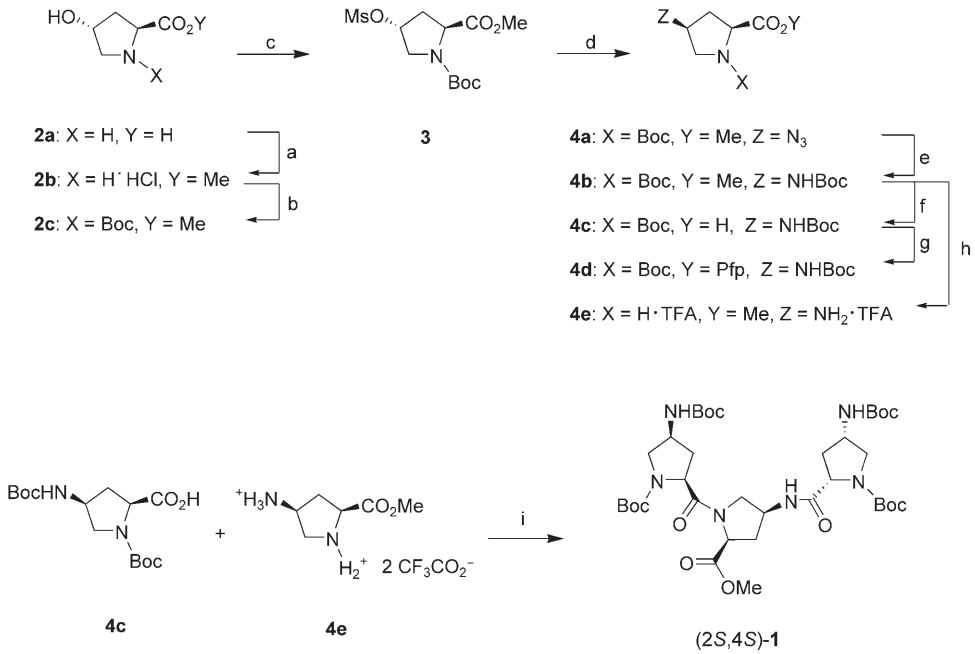
Abstract in Chinese:

本文报道由两种不同构型的4-羟基脯氨酸合成了三种不同构型的4-氨基脯氨酸，在此基础上，采用溶液多肽合成路径有效合成了三种高刚性、紧密堆积、具有不同构型的二代树枝化多肽基元。这些二代树枝化多肽基元的外围均有相同构型的(2*S*,4*S*)-4-氨基脯氨酸构成，而树枝化核点则分别由(2*S*,4*S*)-, (2*S*,4*R*)-及(2*R*,4*S*)-4-氨基脯氨酸组成。对不同构型的二代树枝化多肽基元的合成方法进行了有效的探索，发现PyBOP/HOBt 缩合体系对合成(2*S*,4*S*)及(2*R*,4*S*)构型的二代树枝化基元有效，而五氟酚活性酯路线则对合成(2*S*,4*R*)构型的二代树枝化基元有效。三种二代树枝化多肽基元的总体合成收率均可达45%以上。这类高刚性、紧密堆积的手性多肽树枝化基元在制备螺旋形树枝化聚合物及有机不对称催化合成领域有很高的研究价值。

Results and Discussion

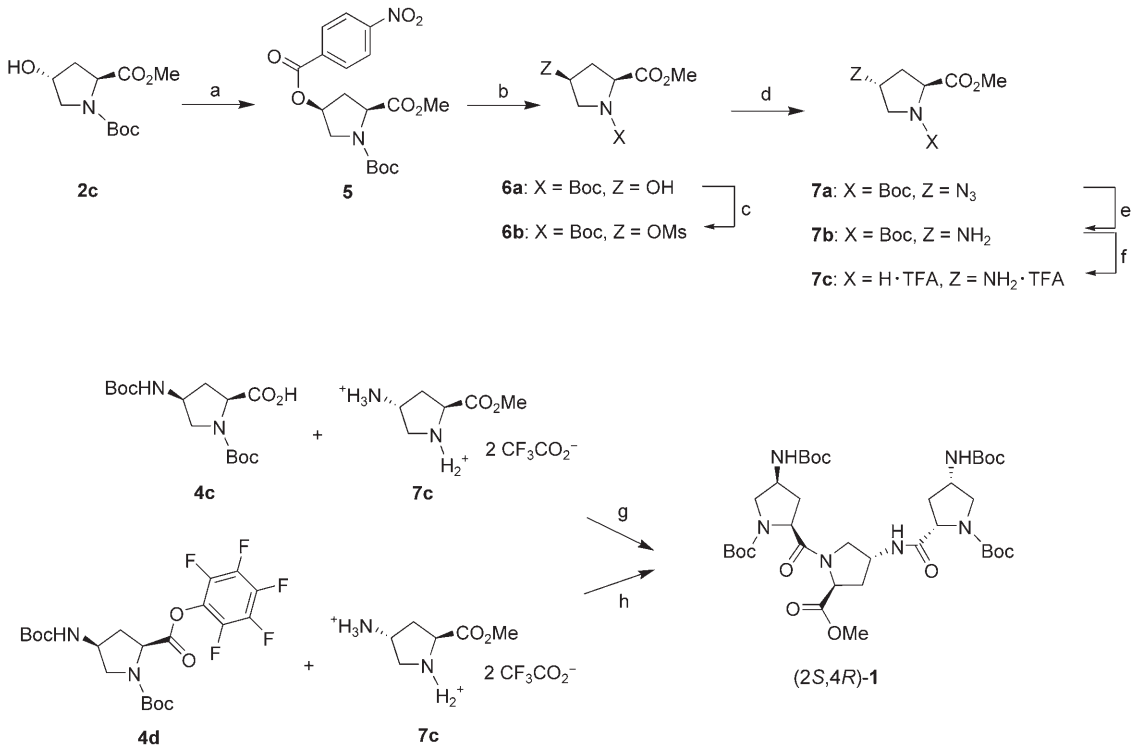
Many 4-aminoprolines are known. Most of them carry benzyloxycarbonyl (Cbz) and 9-fluorenylmethyloxycarbonyl (Fmoc) groups as protecting groups,[8a,12] although Boc has also been used.[13] In the present work, the diastereomeric 4-aminoprolines were used exclusively in their Boc-protected forms (**4b**, **7b**, and **10b**) with one or two Boc groups per compound. For some of our potential polymer applications, both Cbz and Fmoc are less attractive. The former, in particular, can sometimes be difficult to remove completely when present in a macromolecule in the hundreds or thousands, while Fmoc protecting groups often render the corresponding polymers poorly soluble.[14] The few synthetic steps that lead to these three protected 4-aminoprolines are described in Schemes 2–4 together with the synthesis of the corresponding G2 dendrons (2*S*,4*S*)-**1**, (2*S*,4*R*)-**1**, and (2*R*,4*S*)-**1**. In all the dendrons **1**, the 4-aminoproline derivative **4b** was selected for incorporation as a peripheral branching unit, whereas the focal branching unit was systematically varied in its absolute configuration by using **4b**, **7b**, and **10b**, respectively. There are two reasons for this. First, it was expected that a change in the stereochemistry of the focal branching units would possibly affect the overall shape of the dendrons the most, and thus the largest impact on all shape-related matters for future applications would be generated.[15] Second, building block **4b** is the most easily accessible, which is attractive because there are twice as many peripheral branching units than those positioned at the focal point.

The synthetic sequences to the G2 dendron (2*S*,4*S*)-**1** are delineated in Scheme 1. All steps were optimized and carried out on at least the several-gram scale. The synthesis started from the commercially available 4-hydroxyproline **2a**, which was subjected to conventional steps involving esterification, amine protection with Boc anhydride, and mesylation to give the known intermediate **3**, which was then converted into the azide **4a**. The latter compound was also obtained from the hydroxy compound **2c** by the Mitsunobu reaction.[16] This alternative route proceeded in fewer steps, but gave a complex mixture of products. As this mixture would have required too much effort to purify, this route was not followed further. Because azide **4a** plays an important role in the present dendron synthesis, it was prepared on the 50-g scale. Its reduction followed by protection of the amine formed in situ (to give **4b**) required careful optimization. Two methods were tried: hydrogenation and the Staudinger reduction.[17] Although direct hydrogenation with Pd/C in the presence of Boc_2O is an established method and provides the Boc-protected amine **4b** in high yield (86–92%), the formation of some impurities due to dimerization could not be prevented.[18] These impurities made the purification more difficult; therefore, this method was not considered further, as the present work has a strong focus on developing highly efficient and large-scale routes to 4-aminoproline-based dendrons. Fortunately, the Staudinger protocol turned out to be the method of choice specifically be-



Scheme 2. Synthetic procedure for G2 dendron (2*S*,4*S*)-**1**. Reagents and conditions: a) $SOCl_2$, MeOH, −10 °C, 4 h (100 %); b) Boc_2O, $NaHCO_3$, THF/H_2O, 0–5 °C, overnight (95 %); c) MsCl, pyridine, 0–5 °C, 10 h (91 %); d) NaN_3, DMF, 45–55 °C, overnight (89 %); e) PPh_3, Boc_2O, THF/H_2O, 50 °C, 19 h (96 %); f) LiOH, $MeOH/H_2O$, −5 °C, 6 h (87 %); g) DCC, pentafluorophenol, DCM, −15 °C, overnight (92 %); h) TFA, DCM, 0–5 °C, 10 h (100 %); i) HOBt, PyBOP, DIPEA, DMF/DCM, −10 °C, 16 h (86 %). DCM = dichloromethane, DIPEA = diisopropylethylamine, Ms = methanesulfonyl, Pfp = pentafluorophenol, TFA = trifluoroacetic acid,

cause it did not lead to any observable by-products. Its additional advantage was that it could be carried out in the presence of Boc_2O to furnish **4b** directly. Compound **4b** is the key branching unit and was therefore prepared on the 30-g scale as a completely colorless, analytically pure, crystalline material. In the next step, a part of **4b** was hydrolyzed to **4c**, and the other part was deprotected with TFA to **4e** in virtually quantitative steps. Both products were used for the assembly of the G2 dendron (2*S*,4*S*)-**1** by amide coupling despite reports that (2*S*,4*S*)-4-aminoproline couplings of this sort require spacers between the individual branching units to proceed in reasonable yields.[10b] This step was investigated in great detail because it represents the key growth reactions. Several reagent combinations for amide



Scheme 3. Synthetic procedure for G2 dendron (2*S*,4*R*)-**1**. Reagents and conditions: a) DEAD, *p*-nitrobenzoic acid, PPh_3, THF, 0–5 °C, 12 h (92 %); b) NaN_3, MeOH, 40 °C, 12 h (92 %); c) MsCl, pyridine, 0–5 °C, 10 h (95 %); d) NaN_3, DMF, 45–55 °C, overnight (92 %); e) PPh_3, THF/H_2O, 50 °C, 19 h (100 %); f) TFA, DCM, 0–5 °C, 10 h (100 %); g) DIPEA, PyBOP, HOBt, DCM/DMF, −12 °C, 12 h (≈0 %); h) TEA, acetonitrile, −8 °C, 12 h (85 %). DEAD = diethyl azodicarboxylate, TEA = triethylamine.

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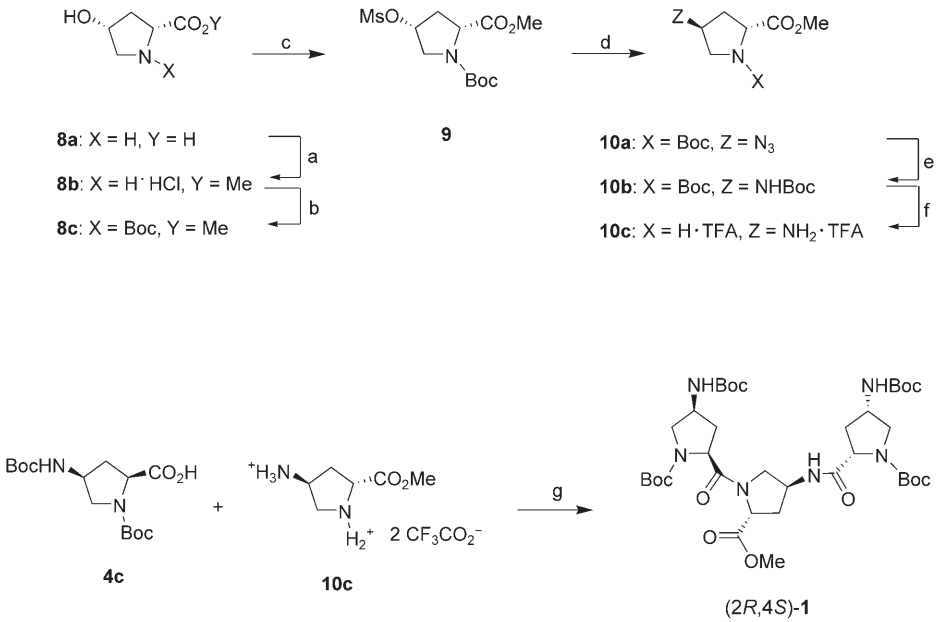

Scheme 4. Synthetic procedure for G2 dendron (2*R*,4*S*)-**1**. Reagents and conditions: a) $SOCl_2$, MeOH, −10 °C, 4 h (100 %); b) Boc_2O, $NaHCO_3$, THF/H_2O, 0–5 °C, overnight (92 %); c) MsCl, pyridine, 0–5 °C, 10 h (93 %); d) NaN_3, DMF, 45–55 °C, overnight (90 %); e) PPh_3, Boc_2O, THF/H_2O, 50 °C, 19 h (92 %); f) TFA, DCM, 0–5 °C, 10 h (100 %); g) HOBt, PyBOP, TEA, DMF/DCM, −10 °C, 16 h (75 %).

formation[19] were tested: EDC/HOBt, DCC/HOSu, HATU/HOBt, HBTU/HOBt, BOP/HOBt, and PyBOP/HOBt.[20] Without going into too much detail, it was by and large observed that the first three methods could not be used. They either gave very poor coupling yields (below 15 %) or led to product mixtures that could not be separated with reasonable effort. Both HBTU/HOBt and BOP/HOBt worked reasonably well in that they gave the desired G2 dendron (2*S*,4*S*)-**1** in yields of 50–60 % and on a relatively large scale (5 g). However, the purification was too tedious because some of the impurities happened to have similar retention times to the product on the column. The final method, PyBOP/HOBt, was by far the best, both with regard to purification effort and yield (up to 86 %). In this way, (2*S*,4*S*)-**1** was reproducibly obtained on the 10-g scale as analytically pure material with a rather impressive overall yield of 45–50 % from **2a**. It is a fortunate coincidence that this reagent is among the cheapest for amide couplings. Dendron (2*S*,4*S*)-**1** and all the other new compounds were fully characterized.

The synthesis of G2 dendron (2*S*,4*R*)-**1** started from **2c** by inversion of the configuration of its 4-hydroxy group under Mitsunobu conditions.[21] *p*-Nitrobenzoic acid was selected for this reaction because of the high reactivity of the resulting benzoate derivative. This provided the opportunity to hydrolyze selectively the benzoate ester with sodium azide in the presence of methyl ester to afford the inverted compound **6a** in an excellent yield of 92 %. By similar procedures as for the synthesis of (2*S*,4*S*)-**1**, mesylation of **6a** followed by azide substitution and Staudinger reduction afforded **7b** in an overall yield of 70 % from **2c**. Compound **7b** was then deprotected to **7c** by TFA. The synthesis of the corresponding G2 dendron (2*S*,4*R*)-**1** was first tried from **4c** and **7c** with the same protocol as for (2*S*,4*S*)-**1**. This, however, did not lead to any product in the reaction mixture (checked by TLC); the reason is still unknown. Therefore, the pentafluorophenol active ester **4d** was treated with the deprotected amine **7c**, which afforded (2*S*,4*R*)-**1** in satisfactory yield (85 %).

The synthesis of G2 dendron (2*R*,4*S*)-**1** started from commercially available compound **8a** and followed closely the procedures described for (2*S*,4*S*)-**1**. (2*R*,4*S*)-**1** was obtained in seven steps in an overall yield of 50 %.

The chemical purities of all the new compounds reported herein were analyzed by ^1H and ^{13}C NMR spectroscopy as well as high-resolution mass spectrometry. The optical purities of the key intermediates **4b**, **7b**, and **10b** as well as the three G2 dendrons (2*S*,4*S*)-**1**, (2*S*,4*R*)-**1**, and (2*R*,4*S*)-**1** were determined by chiral HPLC analysis. For this purpose, HPLC equipped with a Daicel CHIRALCEL®OJ column was applied, and different elution conditions (hexane/isopropanol/ethanol = 90:5:5–9:1:1) were tested in analogy to those reported by Zhao and Pritts.[22] Reasonable elution times of 20–40 min were observed for the solvent ratio 9:1:1. Except for **7b**, 0.1 % TFA was added to the eluent for better separation. All elution curves were strictly monomodal and symmetric (see Supporting Information), which confirms an optical purity higher than the resolution of the method.

Conclusions

This work describes the highly efficient, reliable, and easy synthesis of three diastereomeric G2 dendrons based on 4-aminoprolines. Their optical purities were proved with chiral HPLC analysis to be higher than 98 %. Only single diastereomers were seen. These chiral dendrons are promising as versatile building blocks for numerous applications in organic, polymer, and biomedical chemistry as well as in materials science. An immediate project to be pursued is the synthesis of ultrarigid dendronized polymers with a predetermined screw sense.[23,24] The compactness and rigidity of pendant dendrons not only has a strong effect on backbone flexibility, but may also force the backbone into a helical conformation, as was impressively shown for less-crowded

linear polymers by Okamoto and co-workers[25] and others.[26] It is hoped that the fixed stereochemical configuration of the dendrons synthesized herein, when attached to a linear polymer, will result in a rigid-rod-type polymer with an unprecedented stable biased screw sense.

Experimental Section

Materials

All chemicals were purchased from Fluka or Acros Chemicals Co. at reagent grade and used without further purification unless otherwise specified. (2*S*,4*R*)-4-Hydroxy-l-proline and PyBOP were purchased from Novabiochem, and (2*R*,4*R*)-4-hydroxy-l-proline was purchased from Bachem. Compounds **3**[27] and **4a**[28] were synthesized by modified literature methods at much larger scales. THF was heated at reflux over lithium aluminum hydride, and DCM was distilled from CaH_2 for drying. All reactions were run under nitrogen atmosphere. Macherey–Nagel precoated TLC plates (silica gel 60 G/UV_{254}, 0.25 mm) were used for TLC analysis, and separation on the plates was visualized by treatment with ethanolic ninhydrin solution (1%) and subsequent heating at around 200°C. Silica gel 60 M (Macherey–Nagel, 0.04–0.063 mm, 230–400 mesh) was used as the stationary phase for column chromatography. All samples were dried thoroughly under vacuum prior to analytical measurements to remove strongly adhering solvent molecules.

Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 (^{1}H: 300 MHz; ^{13}C: 75 MHz) or AV 500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) spectrometer at room temperature with $CDCl_3$ or CD_3OD as solvent, and chemical shifts are reported as δ values (ppm) relative to internal Me_4Si. Given the knowledge of isomerization in proline peptides, herein we give only the chemical shifts of the compounds in a relatively simple style. For ^{13}C NMR spectroscopy of G2 dendrons, the number of scans acquired was usually more than 30000 owing to their low signal intensities. High-resolution MALDI-TOF and ESI MS was performed by the MS service of the Laboratorium für Organische Chemie, ETH Zürich, on IonSpec Ultra instruments. Elemental analysis was performed for the three key compounds **4b**, **7b**, **10b**, as well as the three diastereomeric G2 dendrons **1** by the Mikrolabor of the Laboratorium für Organische Chemie, ETH Zürich. The values for the latter three compounds are somewhat off the calculated ones because of contamination with strongly adhering solvent molecules. As with all the other compounds, the composition of the G2 dendrons was unequivocally established by high-resolution mass spectrometry. Melting points were measured on a Büchi B-540 melting-point apparatus and are not corrected. Preparative HPLC separation was performed on a recycling preparation HPLC instrument (LC-9101) from Japan Analytical Industry Co., Ltd (chloroform as eluent (3.5 mL min^{-1}), UV (230 nm) and refractive-index detector, columns: JAIGEL-2H+JAIGEL-2.5H). Optical rotations for **4b**, **7b**, **10b**, and **1** were measured in chloroform on a Perkin–Elmer 241 polarimeter with a 1-dm cuvette. Chiral HPLC analysis was carried out on a recycling preparation HPLC instrument (LC-9101) from Japan Analytical Industry Co., Ltd with a Daicel CHIRALCEL®OJ column (2×25 cm). A mixed solvent of hexane, isopropanol, and ethanol (volume ratio = 9:1:1) was used as eluent at a flow rate of 2 mL min^{-1}.

Syntheses

General procedure for methyl esterification of 4-hydroxyprolines (A): Neat $SOCl_2$ (4.8 mol) was slowly added dropwise into dry methanol (1.3 L) at −10°C. After the mixture was stirred for 1 h, 4-hydroxyproline (1.10 mol) was added in small portions at 0°C. After the mixture was warmed to room temperature, it was stirred to clearness and then heated at reflux for another 3 h. Evaporation of the solvent in vacuo afforded the colorless corresponding methyl ester as the hydrochloride salt in nearly quantitative yield.

General procedure for Boc protection (B): Sodium bicarbonate (0.92 mol) was added to a solution of ammonium chloride (0.47 mol) in THF (300 mL) and water (300 mL) in an ice bath, and then di-*tert*-butyl bicarbonate (0.55 mol) was added in one portion at around 5°C. The mixture was warmed to room temperature and stirred overnight. It was then concentrated to dryness in vacuo, and ethyl acetate (400 mL) was added to extract the residue. The organic phase was washed successively with HCl (0.1 N), saturated sodium bicarbonate, and brine. The mixture was then dried over $MgSO_4$, filtered, and the solvent removed. Purification of the residue by column chromatography or recrystallization with ethyl acetate/hexane (2:1 *v*/*v*) yielded the Boc-protected product as colorless needles.

General procedure for mesylation (C): The respective hydroxy compound (171 mmol) was dissolved in dry pyridine (200 mL) and cooled in an ice bath. MsCl (210 mmol) was added in one portion, and the reaction mixture was stirred for 4 h in the ice bath and then for 6 h at room temperature. The reaction was then quenched by the addition of methanol (50 mL). Evaporation of the solvent gave a residue, which was dissolved in ethyl acetate (300 mL). The organic phase was washed successively with $NaHCO_3$ (1 M), citric acid (1 M), and brine. The mixture was dried over $MgSO_4$, filtered, and the solvent removed. Purification of the residue by column chromatography or recrystallization with ethyl acetate/hexane (6:1 *v*/*v*) afforded the mesylated compound as light-yellow or colorless needles.

General procedure for azide substitution from the mesylated compound (D): The mesylated compound (171 mmol) and NaN_3 (462 mmol) were stirred in dry *N*,*N*-dimethylformamide (DMF; 200 mL) at 45–55°C overnight. The solvent was evaporated, and the residue was taken up with ethyl acetate (250 mL) and H_2O (200 mL). After separation of the layers, the organic phase was washed with H_2O until it was neutral and then successively with HCl (0.1 N) and brine. The mixture was dried over $MgSO_4$, filtered, and the solvent removed. Purification of the residue by column chromatography with ethyl acetate/hexane (1/5 *v*/*v*) afforded the azide as a colorless oil.

General procedure for azide substitution from the hydroxy compound under Mitsunobu conditions (E): DEAD (40% in toluene, 50.4 mmol) was added dropwise to an ice-cooled, stirred solution of the Boc-protected hydroxy compound (38.7 mmol) and Ph_3P (48.4 mmol) in dry THF (150 mL) under nitrogen. After the mixture was stirred for 30 min, diphenylphosphoryl azide (48.4 mmol) was added dropwise, and the temperature was allowed to rise to room temperature. After 12 h of continuous stirring, the solvent was evaporated in vacuo. Purification by column chromatography with ethyl acetate/hexane (1:5 *v*/*v*) afforded the azide as a thick yellowish oil.

General procedure for azide reduction by hydrogenation (F): The azide compound (53.3 mmol) and Boc_2O (66.5 mmol) were dissolved in ethyl acetate (250 mL), and the mixture was added to a glass hydrogenation vessel containing 10% palladium/carbon catalyst. The vessel was shaken at room temperature in a hydrogenator with H_2 (3 bar) for 16 h. The reaction mixture was then filtered through a pad of Al_2O_3 and concentrated by rotary evaporator. The residue was dissolved in ethyl acetate, which was washed successively with $NaHCO_3$ and brine. The aqueous phases were extracted with ethyl acetate three times, and the combined organic phase was dried over $MgSO_4$. After filtration, the solvent was evaporated in vacuo. Purification by column chromatography with ethyl acetate/hexane (1:6 *v*/*v*) followed by recrystallization from ethyl acetate/hexane gave the Boc-protected product as colorless crystals.

General procedure for azide reduction under Staudinger conditions (G): A mixture of the azide compound (125.8 mmol) and PPh_3 (188.7 mmol) in THF (300 mL) and water (13.6 mL) was stirred at 50°C for 16 h. Purification with column chromatography (DCM/MeOH = 20:1 *v*/*v*) afforded the intermediate, which was dissolved in THF (200 mL) and mixed with $NaHCO_3$ (0.63 mol), water (50 mL), and Boc_2O (188.7 mmol). The mixture was stirred in an ice bath for 3 h and then for 6 h at room temperature. Evaporation of the solvent gave a residue, which was dissolved in ethyl acetate and washed successively with $NaHCO_3$ and brine. The aqueous phases were extracted with ethyl acetate three times, and the combined organic phase was dried over $MgSO_4$. After filtration, the sol-

vent was evaporated in vacuo. Purification of the residue by column chromatography with ethyl acetate/hexane (1:6 *v*/*v*) afforded the Boc-protected product as colorless crystals.

General procedure for saponification of methyl ester by LiOH (H): $LiOH{\cdot}H_2O$ (85.7 mmol) was added to a solution of methyl ester (43.6 mmol) in methanol (180 mL) and water (60 mL) at −5 °C with stirring. The reaction temperature was then allowed to rise to room temperature. After the mixture was stirred for 6 h, the solvents were evaporated in vacuo at room temperature, and the residue was dissolved in DCM. The pH of the solution was adjusted carefully to pH 5–6 with 10% $KHSO_4$. The organic phase was washed with brine. All the aqueous phases were extracted with DCM three times. The combined organic phase was dried over $MgSO_4$. After filtration, the solvent was evaporated in vacuo. Purification by column chromatography with ethyl acetate/hexane (1:1 *v*/*v*) afforded the corresponding acid as colorless crystals.

General procedure for Boc removal with TFA (I): TFA (55.0 mmol) was added to a solution of the Boc-protected compound (11.0 mmol) in DCM (30 mL) at 0 °C, and the mixture was stirred for 6 h, after which an excess of methanol was added to quench the reaction. Evaporation of the solvents in vacuo yielded the deprotected product as colorless needlelike crystals.

General procedure for amide coupling with PyBOP (J): The deprotected compound (4.46 mmol) in dry DMF (15 mL) was added to a solution of the acid (9.88 mmol) in dry DCM (26 mL) at room temperature. After the solution was cooled to −10 °C, HOBt (11.5 mmol), DIPEA (44.6 mmol), and PyBOP (9.88 mmol) were added successively under N_2 atmosphere. The mixture was kept at that temperature for 2 h, then for 16 h at room temperature. It was washed successively with $NaHCO_3$ and brine, and all the aqueous phases were extracted with DCM three times. The combined organic phases were dried over $MgSO_4$. After filtration, the solvent was evaporated in vacuo. Purification by column chromatography with ethyl acetate/hexane (1:1 then 2:1 *v*/*v*) yielded the G2 ester as a colorless foam.

3: (2*S*,4*R*)-1-*tert*-Butoxycarbonyl-4-(methylsulfonyloxy)proline methyl ester (**3**) was synthesized in three steps. Step 1: According to general procedure A from $SOCl_2$ (290 mL, 4.8 mol) and **2a** (146 g, 1.10 mol). Compound **2b** was afforded as a colorless product in nearly quantitative yield (199.5 g, 99%). Step 2: According to general procedure B from sodium bicarbonate (77.3 g, 0.92 mol) and **2b** (84.7 g, 0.47 mol) with di-*tert*-butyl bicarbonate (120 g, 0.55 mol) in THF (300 mL) and water (300 mL). Compound **2c** was obtained as colorless needles (110 g, 95%). Step 3: According to general procedure C from **2c** (42 g, 171 mmol) and MsCl (24 g, 210 mmol). Compound **3** was afforded as light-yellow to colorless needles (50.2 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 and 1.43 (2 s, 9H, H-Boc), 2.18–2.28 (m, 1H, CH_2^a), 2.51–2.66 (m, 1H, CH_2^b), 3.03 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.77–3.82 (m, 2H, CH_2), 4.34–4.46 (m, 1H, CH), 5.15–5.25 ppm (m, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.1, 28.3, 36.2, 37.4, 38.6, 38.7, 52.1, 52.2, 52.4, 57.0, 57.4, 80.9, 154.2, 172.7 ppm; MS (ESI): m/z = 346.0935 $[M+Na]^+$.

4a: Route 1: According to general procedure D from **3** (55.4 g, 171 mmol) and NaN_3 (30.0 g, 462 mmol) in dry DMF (200 mL). (2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-azidoproline methyl ester (**4a**) was given as a colorless oil (41.0 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40 and 1.46 (2 s, 9H, H-Boc), 2.13–2.17 (m, 1H, CH_2^a), 2.40–2.50 (m, 1H, CH_2^b), 3.43–3.50 (m, 1H, CH_2^a), 3.66–3.71 (m, 1H, CH_2^b), 3.74 (s, 3H, CH_3), 4.11–4.16 (m, 1H, CH), 4.33 and 4.42 ppm (2 dd, J = 8.8, 4.3, 3.7 Hz, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.4, 28.5, 35.2, 36.2, 50.9, 51.4, 52.4, 52.6, 57.5, 57.9, 58.4, 59.4, 80.7, 80.8, 153.6, 154.1, 172.1, 172.4 ppm; MS (ESI): m/z = 293.1219 $[M+H]^+$. Route 2: According to general procedure E from **2c** (9.5 g, 38.7 mmol), DEAD (40% in toluene, 21.9 mL, 50.4 mmol), Ph_3P (12.7 g, 48.4 mmol), and diphenylphosphoryl azide (13.3 g, 48.4 mmol) in dry THF (150 mL). Chromatography separation gave **4a** as a viscous yellowish oil (7.6 g, 73%). Identical spectral data to those by route 1 were obtained.

4b: Route 1: According to general procedure F from **4a** (14.4 g, 53.3 mmol) and Boc_2O (14.5 g, 66.5 mmol) in ethyl acetate (250 mL) containing 10% palladium/carbon catalyst (1.5 g). (2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-(*tert*-butoxycarbonylamino)proline methyl ester (**4b**) was given as a colorless foam (17.2 g, 94%). M.p.: 116.1 °C; $[\alpha]_D^{25}$ = −17.0 (c = 1.44 M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 and 1.43 (2 s, 18H, H-Boc), 1.84–1.96 (m, 1H, CH_2^a), 2.36–2.50 (m, 1H, CH_2^b), 3.35 and 3.44 (2 d, J = 10.1, 9.8 Hz, 1H, CH_2^a), 3.57–3.66 (m, 1H, CH_2^b), 3.72 (s, 3H, CH_3), 4.22–4.34 (m, 2H, CH), 5.39 ppm (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.2, 28.3, 35.9, 36.8, 49.1, 50.0, 52.2, 52.5, 52.9, 53.4, 57.6, 57.8, 79.6, 80.4, 153.4, 154.2, 155.1, 174.3 ppm; MS (ESI): m/z = 367.1840 $[M+Na]^+$; elemental analysis: calcd (%) for $C_{16}H_{28}N_2O_6$ (344.19): C 55.80, H 8.19, N 8.13; found: C 55.60, H 8.22, N 8.16. Route 2: According to general procedure G from **4a** (34 g, 125.8 mmol), Boc_2O (41.2 g, 188.7 mmol), and PPh_3 (49.5 g, 188.7 mmol) in THF (300 mL) and water (13.6 mL). Compound **4b** was given as colorless crystals (41.6 g, 96%). Identical spectral data to those by route 1 were obtained.

4c: According to general procedure H from $LiOH{\cdot}H_2O$ (3.6 g, 85.7 mmol) and **4b** (15.0 g, 43.6 mmol) in methanol (180 mL) and water (60 mL). (2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-(*tert*-butoxycarbonylamino)proline (**4c**) was given as a colorless foam (12.5 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 and 1.51 (2 s, 9H, H-Boc), 2.04–2.52 (m, 2H, CH_2), 3.32–3.47 (m, 1H, CH_2^a), 3.60–3.74 (m, 1H, CH_2^b), 4.10–4.36 (m, 1H, CH), 4.36–4.49 (m, 1H, CH), 5.40 (br s, 1H, NH), 9.75 ppm (br s, 1H, CO_2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.5, 28.5, 32.9, 50.2, 54.5, 59.0, 80.0, 83.0, 155.6, 157.7, 173.5 ppm; MS (ESI): m/z = 329.1720 $[M-H]^-$.

4d: DCC (1.56 g, 7.56 mmol) was added to a solution of **4c** (2.00 g, 6.05 mmol) and pentafluorophenol (1.34 g, 7.26 mmol) in DCM (25 mL) at −15 °C under N_2 atmosphere, and the mixture was stirred at room temperature overnight. After the solid was filtered off, the solution was washed successively with $NaHCO_3$ and brine and dried over $MgSO_4$. Purification by column chromatography with ethyl acetate/hexane (1:6 and 1:3 *v*/*v*) afforded (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(*tert*-butoxycarbonylamino)proline pentafluorophenol ester (**4d**) as a colorless foam (2.8 g, 92%). $[\alpha]_D^{25}$ = +4.2 (c = 1.02 M, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ = 1.41, 1.43, 1.45, and 1.46 (4 s, 18H, CH_3), 2.18–2.28 (m, 1H, CH_2^a), 2.60–2.70 (m, 1H, CH_2^b), 3.38 and 3.47 (2 dd, J = 11.1, 10.7 Hz, 1H, CH_2^a), 3.73–3.79 (m, 1H, CH_2^b), 4.62 and 4.68 (2 dd, J = 8.4, 8.2 Hz, 1H, CH), 4.93 and 5.00 ppm (2 dd, J = 5.8 Hz, 1H, CH); ^{13}C NMR (125 MHz, CD_3OD): δ = 27.9, 28.2, 28.4, 36.6, 37.8, 49.4, 50.2, 52.4, 52.8, 57.3, 57.4, 80.1, 81.3, 81.6, 137.1, 139.1, 140.3, 142.2, 153.5, 154.1, 155.3, 169.7, 170.0 ppm; MS (MALDI): m/z = 519.1530 $[M+Na]^+$, 535.1264 $[M+K]^+$.

4e: According to general procedure I from TFA (6.3 g, 55.0 mmol) and **4b** (3.8 g, 11.0 mmol) in DCM (30 mL). (2*S*,4*S*)-4-aminoproline methyl ester TFA adduct (**4e**) was given as colorless needles (4.1 g, 100%). ^{1}H NMR (300 MHz, CD_3OD): δ = 2.25–2.36 (m, 1H, CH_2^a), 2.92–3.02 (m, 1H, CH_2^b), 3.53–3.59 (m, 1H, CH_2^a), 3.80–3.87 (m, 1H, CH_2^b), 3.90 (s, 3H, CH_3), 4.13–4.24 (m, 1H, CH), 4.62–4.68 ppm (m, 1H, CH); ^{13}C NMR (75 MHz, CD_3OD): δ = 34.0, 50.0, 50.5, 55.1, 60.9, 169.8 ppm; MS (ESI): m/z = 145.2 $[M+H]^+$.

(2*S*,4*S*)-**1**: According to general procedure J from **4e** (4.98 g, 13.38 mmol), **4c** (9.78 g, 29.64 mmol), HOBt (4.68 g, 34.50 mmol), PyBOP (15.42 g, 29.64 mmol), and DIPEA (17.31 g, 133.80 mmol). (2*S*,4*S*)-1-[(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)-pyrrolidine-2-carbonyl]-4-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)pyrrolidine-2-carbonylamino]proline methyl ester ((2*S*,4*S*)-**1**) was given as a colorless solid foam (8.84 g, 86%). The product (222 mg) was further purified by preparative HPLC to yield 220 mg. M.p.: 134.1 °C; $[\alpha]_D^{25}$ = −60.2 (c = 1.15 M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.35, 1.38, 1.39, and 1.40 (4 s, 36H, CH_3), 1.85–2.00 (m, 2H, CH_2), 2.23 (br s, 2H, CH_2), 2.37–2.55 (m, 2H, CH_2), 3.36–3.56 (m, 4H, CH_2), 3.64–3.79 (m, 1H, CH_2), 3.79 (s, 3H, CH_3), 3.97–4.15 (m, 1H, CH_2), 4.17–4.26 (m, 2H, CH), 4.37–4.47 (m, 2H, CH), 4.51–4.68 (m, 2H, CH), 5.92 and 6.04 (2 br, 2H, NH), 7.72 and 7.84 ppm (2 br, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.3, 28.3, 28.4, 33.1, 34.1, 35.1, 49.2, 49.3, 50.0, 52.7, 53.0, 54.6, 54.9, 56.8, 58.1, 59.6, 79.0, 79.5, 80.1, 80.2, 80.8, 153.1, 154.2, 155.4, 155.7, 172.0, 172.3 ppm; MS (MALDI): m/z = 791.4168 $[M+Na]^+$; elemental analysis: calcd (%) for $C_{36}H_{60}N_6O_{12}$ (768.43): C 56.23, H 7.87, N 10.93; found: C 55.12, H 8.09, N 10.78.

5: DEAD (19.5 mL, 44.79 mmol, 40% in toluene) was added dropwise to a solution of **2c** (5 g, 20.38 mmol), *p*-nitrobenzoic acid (6.8 g, 40.69 mmol), and PPh_3 (11.8 g, 45.00 mmol) in dry THF (100 mL) at 0 °C with stirring. The reaction was then stirred at room temperature for 12 h. After evaporation of the solvent to dryness, the residue was taken with DCM and then washed successively with $NaHCO_3$ and brine. After the mixture was dried over $MgSO_4$, purification by column chromatography with ethyl acetate/hexane (1:3 *v*/*v*) yielded the ester as yellowish crystals, which were recrystallized from hexane/ethyl acetate (9:1 *v*/*v*) to afford pure (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-nitrobenzoyloxy)proline methyl ester (**5**; 7.40 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.43 and 1.47 (2 s, 9H, CH_3), 2.45 (d, J = 14.5 Hz, 1H, CH_2^a), 2.50–2.62 (m, 1H, CH_2^b), 3.53 (s, 3H, CH_3), 3.68–3.84 (m, 2H, CH_2), 4.54 (dd, J = 9.5, 8.5 Hz, 1H, CH), 5.56 (br s, 1H, CH), 8.15 (d, J = 8.5 Hz, 2H, CH), 8.28 ppm (d, J = 8.5 Hz, 2H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.4, 28.5, 35.8, 36.7, 52.3, 52.4, 52.5, 52.7, 57.6, 57.9, 73.6, 74.7, 80.7, 80.8, 123.7, 131.0, 135.2, 150.9, 153.8, 154.2, 164.0, 164.2, 172.3, 172.5 ppm; MS (MALDI): m/z = 417.1266 $[M+Na]^+$.

6a: A mixture of **5** (8.00 g, 20.30 mmol) and NaN_3 (5.27 g, 81.2 mmol) in MeOH (150 mL) was stirred at 40 °C for 12 h. After evaporation of the solvent, the residue was taken up in DCM, and the organic phase was washed successively with $NaHCO_3$ and brine. Purification by column chromatography with ethyl acetate/hexane (1:3 *v*/*v*) gave (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-hydroxyproline methyl ester (**6a**) as colorless crystals (4.60 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.40 and 1.44 (2 s, 9H, CH_3), 2.04–2.09 (m, 1H, CH_2), 2.26–2.36 (m, 1H, CH_2), 3.30 and 3.48 (2 s, 1H, OH), 3.48–3.59 (m, 1H, CH_2), 3.59 and 3.68 (2 d, J = 11.5 Hz, 1H, CH_2), 3.76 and 3.78 (2 s, 3H, CH_3), 4.26–4.36 ppm (m, 2H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.4, 28.5, 37.9, 38.7, 52.7, 53.0, 55.5, 56.1, 57.8, 58.0, 70.4, 71.5, 80.6, 153.8, 154.6, 175.6, 175.9 ppm; MS (ESI): m/z = 168.0633 $[M+Na-Boc]^+$, 268.1164 $[M+Na]^+$.

6b: According to general procedure C from **6a** (3.50 g, 14.27 mmol) and MsCl (2.45 g, 21.40 mmol) to give (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-methylsulfonyloxyproline methyl ester (**6b**) as a colorless foam (4.4 g, 95%); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.42 and 1.47 (2 s, 9H, CH_3), 2.44–2.54 (m, 2H, CH_2), 3.00 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.76–3.81 (m, 2H, CH_2), 4.38–4.52 (m, 1H, CH), 5.22–5.24 ppm (m, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.4, 28.5, 36.4, 37.3, 39.1, 52.1, 52.5, 52.6, 57.2, 57.5, 78.5, 80.9, 81.0, 153.6, 154.0, 171.9, 172.1 ppm; MS (ESI): m/z = 246.0424 $[M+Na-Boc]^+$, 346.0923 $[M+Na]^+$.

7a: According to general procedure D from **6b** (4.4 g, 13.61 mmol) and NaN_3 (5 g, 76.91 mmol) to give (2*S*,4*R*)-1-*tert*-butoxycarbonyl-4-azidoproline methyl ester (**7a**) as a colorless oil (3.4 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.40 and 1.45 (2 s, 9H, CH_3), 2.16–2.19 (m, 1H, CH_2), 2.26–2.36 (m, 1H, CH_2), 3.44 and 3.59 (2 dd, J = 11.4, 3.5, 1.7 Hz, 1H, CH_2), 3.70 (dd, J = 11.6, 5.4 Hz, 1H, CH_2), 3.73 and 3.74 (2 s, 3H, CH_3), 4.16–4.20 (m, 1H, CH), 4.30–4.42 ppm (m, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.7, 28.4, 28.5, 35.5, 36.4, 51.4, 51.5, 52.4, 52.6, 57.9, 58.9, 59.4, 80.8, 153.5, 154.1, 173.1 ppm; MS (ESI): m/z = 193.0696 $[M+Na-Boc]^+$, 293.1216 $[M+Na]^+$.

7b: According to general procedure G from **7a** (3.3 g, 12.21 mmol) and PPh_3 (5.00 g, 19.06 mmol) to give (2*S*,4*R*)-1-*tert*-butoxycarbonyl-4-aminoproline methyl ester (**7b**) as a colorless oil (3.0 g, 100%). $[\alpha]_D^{25}$ = −47.4 (c = 1.22 M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.36 and 1.41 (2 s, 9H, CH_3), 1.94–2.02 (m, 1H, CH_2), 2.06–2.12 (m, 1H, CH_2), 3.06 and 3.16 (2 dd, J = 10.0, 5.1, 4.0 Hz, 1H, CH_2), 3.61–3.66 (m, 2H, CH_2), 3.68 and 3.69 (2 s, 3H, CH_3), 4.30 and 4.39 ppm (2 dd, J = 8.6, 8.2, 5.8, 4.6 Hz, 1H, CH); ^{13}C NMR(125 MHz, $CDCl_3$): δ = 28.4, 28.5, 39.1, 39.7, 49.7, 50.5, 52.1, 52.3, 54.6, 54.9, 58.0, 58.3, 80.2, 80.2, 153.9, 154.6, 173.4, 173.7 ppm; MS (ESI): m/z = 267.1317 $[M+Na]^+$; elemental analysis: calcd (%) for $C_{11}H_{20}N_2O_4$ (244.14): C 54.08, H 8.25, N 11.47; found: C 53.80, H 8.30, N 11.62.

7c: According to general procedure I from **7b** (0.80 g, 3.27 mmol) and TFA (1.87 g, 16.35 mmol) to give (2*S*,4*R*)-4-aminoproline methyl ester TFA adduct (**7c**) as colorless crystals (1.21 g, 100%). ^{1}H NMR (500 MHz, D_2O): δ = 2.44–2.50 (m, 1H, CH_2), 2.58–2.64 (m, 1H, CH_2), 3.39 and 3.43 (2 d, J = 13.2, 5.8 Hz, 1H, CH_2), 3.62–3.66 (m, 1H, CH_2), 3.70 (s, 3H, CH_3), 3.82–3.86 (m, 1H, CH), 4.07–4.09 ppm (m, 1H, CH); ^{13}C NMR (125 MHz, D_2O): δ = 30.3, 32.3, 48.7, 48.8, 54.8, 59.2, 169.1 ppm; MS (ESI): m/z = 145.0977 $[M+H]^+$.

(2*S*,4*R*)-**1**: A solution of **4d** (7.32 g, 14.74 mmol) in acetonitrile (15 mL) was added to a solution of **7c** (2.50 g, 6.70 mmol) and TEA (6.80 g, 67.00 mmol) in dry acetonitrile (30 mL) at −8 °C. After the mixture was stirred at room temperature for 12 h, it was washed successively with $NaHCO_3$ and brine, and all the aqueous phases were extracted with DCM three times. The combined organic phases were dried over $MgSO_4$. After filtration, the solvent was evaporated in vacuo. Purification by column chromatography with ethyl acetate/hexane (1:1 then 2:1 *v*/*v*) gave (2*S*,4*R*)-1-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)-pyrrolidine-2-carbonyl]-4-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)pyrrolidine-2-carbonylamino]proline methyl ester ((2*S*,4*R*)-**1**) as a colorless solid foam (4.30 g, 85%). M.p.: 128.4 °C; $[\alpha]_D^{25}$ = −82.7 (c = 1.09 M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.37, 1.40, 1.41, and 1.44 (4 s, 36H, CH_3), 2.17–2.28 (m, 6H, CH_2), 3.23–3.34 (m, 3H, CH_2), 3.50–3.54 (m, 1H, CH_2), 3.71 and 3.72 (2 s, 3H, CH_3), 3.76–3.82 (m, 2H, CH_2), 4.20–4.27 (m, 2H, CH), 4.30–4.39 (m, 3H, CH), 4.46–4.52 ppm (m, 1H, CH); ^{13}C NMR(125 MHz, $CDCl_3$): δ = 28.2, 28.2, 28.4, 29.7, 31.7, 35.7, 36.6, 47.9, 48.6, 50.0, 51.2, 51.5, 52.2, 52.4, 52.9, 55.3, 57.4, 57.9, 59.3, 79.8, 80.7, 80.9, 81.5, 81.6, 131.8, 131.9, 132.0, 133.7, 135.7, 136.8, 136.9, 136.9, 137.0, 137.2, 137.3, 138.9, 139.1, 139.2, 153.7, 154.3, 155.9, 156.4, 172.2, 172.3, 172.7, 172.9 ppm; MS (ESI): m/z = 791.4163 $[M+Na]^+$; elemental analysis: calcd (%) for $C_{36}H_{60}N_6O_{12}$ (768.43): C 56.23, H 7.87, N 10.93; found: C 55.37, H 7.77, N 10.52.

9: (2*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-methylsulfonyloxyproline methyl ester (**9**) was synthesized in three steps. Step 1: According to general procedure A from $SOCl_2$ (20 g, 168.1 mmol), MeOH (50 mL), and **8a** (5.00 g, 38.13 mmol) to give **8b** as a colorless product in nearly quantitative yield (6.90 g, 100%). Step 2: According to general procedure B from sodium bicarbonate (16.2 g, 152.83 mmol) and **8b** (6.90 g, 38.13 mmol) with di-*tert*-butyl bicarbonate (12.50 g, 57.18 mmol) in THF (50 mL) and water (30 mL) to give **8c** as colorless needles (8.30 g, 92%). Step 3: According to general procedure C from **8c** (7.00 g, 28.54 mmol) and MsCl (3.92 g, 34.22 mmol) to afford **9** as light-yellow to colorless needles (8.60 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 and 1.46 (2 s, 9H, CH_3), 2.47–2.53 (m, 2H, CH_2), 3.00 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.79–3.81 (m, 2H, CH_2), 4.38 and 4.52 (2 d, J = 6.5 Hz, 1H, CH), 5.20–5.23 ppm (m, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.4, 28.5, 31.7, 36.4, 37.3, 39.1, 52.1, 52.5, 52.6, 57.2, 57.5, 78.5, 80.9, 80.9, 153.6, 154.0, 171.9, 172.1 ppm; MS (MALDI): m/z = 324.1116 $[M+H]^+$, 346.0928 $[M+Na]^+$.

10a: According to general procedure D from **9** (8.4 g, 25.98 mmol) and NaN_3 (7.8 g, 120.00 mmol) in DMF (120 mL) to give (2*R*,4*S*)-1-*tert*-butoxycarbonyl-4-azidoproline methyl ester (**10a**) as a colorless oil (6.3 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.39 and 1.44 (2 s, 9H, CH_3), 2.12–2.17 (m, 1H, CH_2), 2.27–2.32 (m, 1H, CH_2), 3.44 and 3.57 (2 d, J = 10.5, 8.0 Hz, 1H, CH_2), 3.66–3.70 (m, 1H, CH_2), 3.71 and 3.72 (2 s, 3H, CH_3), 4.29–4.40 ppm (m, 1H, CH); ^{13}C NMR(125 MHz, $CDCl_3$): δ = 28.3, 28.5, 35.5, 36.4, 51.4, 51.5, 52.4, 52.6, 57.5, 57.9, 58.9, 59.4, 80.8, 153.5, 154.1, 172.9, 173.1 ppm; MS (ESI): m/z = 193.0705 $[M+Na-Boc]^+$, 293.1216 $[M+Na]^+$.

10b: According to general procedure G from **10a** (6.0 g, 22.20 mmol) and PPh_3 (8.7 g, 33.17 mmol), and then Boc_2O (7.27 g, 33.31 mmol) with $NaHCO_3$ (9.30 g, 110.70 mmol) in THF (200 mL) to give (2*R*,4*S*)-1-*tert*-butoxycarbonyl-4-*tert*-butoxycarbonylaminoproline methyl ester (**10b**) as colorless crystals (7.03 g, 92%). M.p.: 119.0 °C; $[\alpha]_D^{25}$ = +39.6 (c = 1.35 M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.38, 1.41, and 1.42 (3 s, 18H, CH_3), 2.12–2.18 (m, 2H, CH_2), 3.20 and 3.33 (2 dd, J = 11.0, 5.0, 3.5 Hz, 1H, CH_2), 3.69 and 3.70 (2 s, 3H, CH_3), 4.25–4.36 (m, 2H, CH), 4.72 ppm (d, J = 9.0 Hz, 1H, NH); ^{13}C NMR(125 MHz, $CDCl_3$): δ = 28.4, 28.5, 36.1, 37.2, 52.3, 52.4, 57.5, 57.9, 80.1, 80.6, 153.7, 154.4, 155.2, 173.2 ppm; MS (ESI): m/z = 367.1839 $[M+Na]^+$; elemental analysis: calcd (%) for $C_{16}H_{28}N_2O_6$ (344.19): C 55.80, H 8.19, N 8.13; found: C 56.05, H 8.07, N 8.11.

10c: According to general procedure I from **10b** (2.05 g, 5.95 mmol) and TFA (15 g, 131.56 mmol) to give (2*R*,4*S*)-4-aminoproline methyl ester TFA adduct (**10c**) as a colorless oil (2.20 g, 100%); ^{1}H NMR (500 MHz,

D_2O): $\delta = 2.57$–2.77 (m, 2H, CH_2), 3.54 and 3.60 (2 d, $J = 9.0$ Hz, 1H, CH_2), 3.87 (s, 3H, CH_3), 3.91–3.98 (m, 1H, CH_2), 4.17–4.25 (m, 1H, CH), 4.78–4.84 ppm (m, 1H, CH); ^{13}C NMR(125 MHz, D_2O): $\delta = 32.1$, 47.3, 47.4, 47.6, 47.8, 48.0, 48.1, 48.3, 48.6, 52.9, 58.5, 167.9 ppm; MS (ESI): $m/z = 145.0975$ $[M+H]^+$.

(2*R*,4*S*)-**1**: According to general procedure J from **4c** (3.46 g, 10.47 mmol), **10c** (1.66 g, 4.46 mmol), TEA (4.13 g, 40.84 mmol), HOBt (14.8 mL in NMP, 14.80 mmol), and PyBOP (6.8 g, 13.07 mmol) to give (2*R*,4*S*)-1-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)-pyrrolidine-2-carbonyl]-4-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-(*tert*-butoxycarbonylamino)pyrrolidine-2-carbonylamino]proline methyl ester ((2*R*,4*S*)-**1**) as a colorless solid foam (2.57 g, 75%). M.p.: 137.0 °C; $[\alpha]_D^{25} = -3.5$ ($c = 1.00$ M, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.41$, 1.43, 1.44, 1.45, and 1.47 (5 s, 36H, CH_3), 1.77 (s, 2H, CH_2), 1.81–1.87 (m, 1H, CH_2), 2.08–2.42 (m, 4H, CH_2), 3.34–3.71 (m, 5H, CH_2), 3.75 (s, 3H, CH_3), 4.11–4.70 ppm (m, 6H, CH); ^{13}C NMR(125 MHz, $CDCl_3$): $\delta = 28.2$, 28.4, 28.5, 28.5, 28.6, 31.7, 33.9, 34.5, 35.3, 35.4, 36.6, 36.8, 37.4, 38.1, 46.5, 47.4, 48.0, 49.2, 49.3, 49.4, 50.0, 50.1, 51.4, 51.9, 52.1, 52.5, 52.6, 53.0, 53.2, 54.5, 54.9, 55.2, 55.4, 55.5, 56.0, 56.8, 56.9, 57.9, 58.2, 58.4, 58.6, 59.3, 59.5, 67.2, 77.0, 77.3, 77.5, 77.5, 79.4, 79.5, 80.2, 80.4, 80.5, 80.8, 81.3, 81.7, 96.2, 153.2, 153.5, 154.1, 154.7, 155.3, 155.7, 156.6, 171.6, 172.2, 172.6, 172.7, 172.9, 173.2 ppm; MS (ESI): $m/z = 791.4167$ $[M+Na]^+$; elemental analysis: calcd (%) for $C_{36}H_{60}N_6O_{12}$ (768.43): C 56.23, H 7.87, N 10.93; found: C 54.62, H 7.85, N 10.76.

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[20] Abbreviations: BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, DCC = 1,3-dicyclohexylcarbodiimide, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HATU = *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = *N*-hydroxybenzotriazole, HOSu = *N*-hydroxysuccinimide, PyBOP = benzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate.

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