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Synthesis of a Carboxylate Functionalized Bis-Amino Acid Monomer

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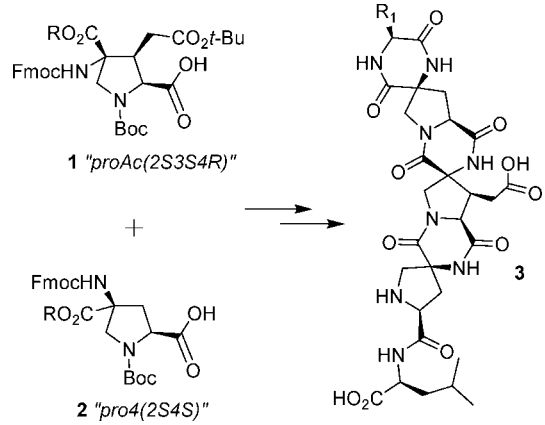

The synthesis of the first functionalized bis-amino acid monomer proAc(2S3S4R) **1** that carries an acetyl side chain is presented. This monomer was incorporated into oligomer **3** and the solution phase structure was determined by using two-dimensional nuclear magnetic resonance. The solution structure confirmed the intended connectivity and stereochemistry of the oligomer. This first functionalized bis-amino acid represents a milestone toward functionalized bis-peptide nanostructures for catalytic, molecular recognition, and nanotechnology applications.

Introduction

Oligomers that adopt well-defined three-dimensional structures have attracted a great deal of interest in the past decade.[1−3] Functionalized oligomers that present proteogenic and nonproteogenic functional groups from a structurally defined backbone have found many applications.[4] Functionalized amphipathic β-peptides have demonstrated antifungal and antimicrobial activities and mixed β-peptide foldamers have been developed that mimic peptide/protein interactions.[5−7] Functionalized peptoids have also demonstrated antimicrobial activity and are being developed as mimics of lung surfactant proteins.[8,9] Phenylene ethynylene foldamers have demonstrated molecular recognition of small molecules.[10] In our own laboratory, we have developed a functionalized oligomer that acts as a molecular switch under the control of metal exchange.[11] An essential requirement toward the development of more sophisticated applications for shape-persistant oligomers is the development of monomers that carry proteogenic and nonproteogenic chemical functionality.

In our laboratory, we have developed a synthetic approach to a unique class of oligomers called bis-peptides.[12] We

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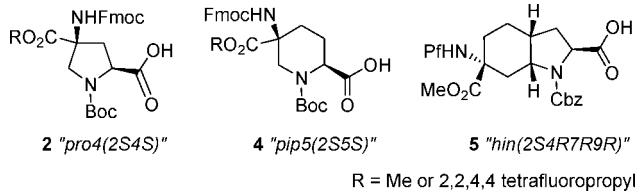


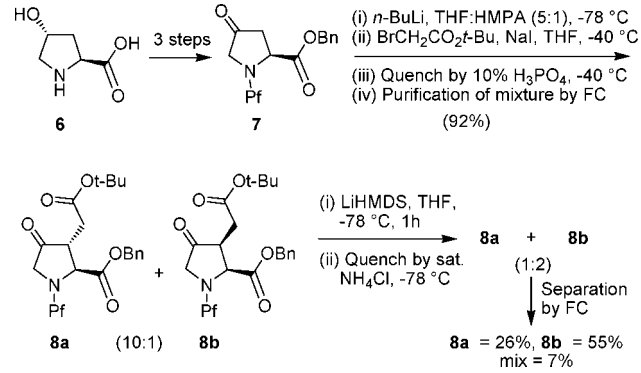
FIGURE 1. Previously synthesized bis-amino acid[16−18] monomers.

synthesize stereochemically pure, cyclic bis-amino acid monomers and couple them through pairs of amide bonds to create spiro-ladder oligomers (bis-peptides) with programmable three-dimensional shapes. Bis-peptides are assembled by using solid phase peptide synthesis and they are trivially functionalized on their two ends through the incorporation of amino acids at the beginning or the end of a bis-peptide synthesis. To enable the development of more highly functionalized bis-peptides we need to develop synthetic approaches to incorporate functionality into the bis-peptide backbone. The approach that we have taken in this report is to create the functionalized bis-amino acid "proAc(2S3S4R)" that carries a protected carboxylic acid functional group on its cyclic core. The carboxylic acid group is a very versatile proteogenic functional group found in aspartic acid and glutamic acid. It plays an essential role in enzyme catalysis such as part of the Asp-His-Ser catalytic triad in proteases and esterases.[13] Miller and co-workers have demonstrated that carboxylic acids on chiral scaffolds can carry out asymmetric epoxidation of alkenes.[14,15] It is a polar group that is solvated well by water and it can be activated and further functionalized through the formation of amides and esters.

Results and Discussion

Synthesis of the proAc(2S3S4R) monomer starts from *trans*-4-hydroxy-L-proline **6**—the same starting material used in the synthesis of of pro4[18,19] and pip5[16] monomers (Figure 1). We built on the work of Rapoport and Lubell,[20−24] who have made extensive use of the phenylfluorenyl (Pf) group as a nitrogen protecting group for regio- and stereoselective alkylation of various amino acid aldehydes and ketone derivatives. The phenylfluorenyl group offered us several advantages over other protecting groups (such as carbamates): it simultaneously masked the secondary amine, protected the stereochemical configuration of the α-carbon, and protected the adjacent benzyl ester from attack by nucleophiles and base hydrolysis. The phenylfluorenyl group was also easily removed by hydrogenolysis and it facilitated NMR analysis because it did not produce amide rotamers. We used the Rapoport and Lubell chemistry

SCHEME 1. Synthesis of Ketone Intermediates 8a and 8b



to convert **6** into ketone **7** through a three-step procedure that included the conversion of **6** to its benzyl ester,[24,25] protection of the secondary amine with a phenylfluorenyl group,[22] and oxidation of the alcohol by using Swern oxidation to produce the ketone **7**.[26,27] We then treated **7** with *n*-BuLi in THF:HMPA at −78 °C and alkylated the resulting enolate using $BrCH_2CO_2t$-Bu at −40 °C. The alkylation occurred exclusively at the 3-position of the ring consistent with previous reports.[28] We obtained a diastereomeric mixture of ketones **8a** and **8b** in a 10:1 ratio as determined by C_{18} reverse-phase (RP) HPLC-MS analysis and 1D proton NMR. After separation of the diastereomers by silica gel chromotography, the stereochemistry of each diastereomer was tentatively assigned by comparing the coupling constants between Hα and Hβ for the respective ketones (J = 6.1 Hz for **8a** and J = 8.2 Hz for **8b**) with those reported previously for similar alkylated ketone derivatives (Scheme 1).[28,29] The alkylation of the enolate with $BrCH_2CO_2t$-Bu at −40 °C represents a bottleneck in the overall process of synthesizing **1**. We found that the combined yield of **8a** and **8b** decreased significantly when we carried the reaction out on scales larger than 100 mmol and in particular in round-bottomed flasks larger than 100 mL. We believe that this is because of poor temperature control in larger volumes and we are exploring the use of continuous flow reactors to increase the throughput of material.

Both diastereomers **8a** and **8b** could lead to useful carboxylate functionalized bis-amino acids with different stereochemistry. In subsequent steps toward the synthesis of these functionalized bis-amino acids we discovered that intermediate **8a** led to mixtures of diastereomers that we could not separate. On the other hand, intermediate **8b** led to the single, well-behaved bis-amino acid **1**. To increase the amounts of intermediate **8b** that we could produce we carried out a controlled epimerization[29] at carbon 3 by treating the 10:1 mixture of **8a** and **8b** with lithium bis(trimethylsilyl)amide (LiHMDS) at −78 °C for 1 h followed by quenching by rapidly adding saturated ammonium chloride to the reaction mixture at −78 °C. The resulting diastereomeric mixture has a more useful ratio of **8a** and **8b** of 1:2. The two diastereomers were then separated by silica gel chromatography (Scheme 1).

Toward the synthesis of carboxyl functionalized bis-amino acid **1** we converted the ketone **8b** to a protected amino ester.

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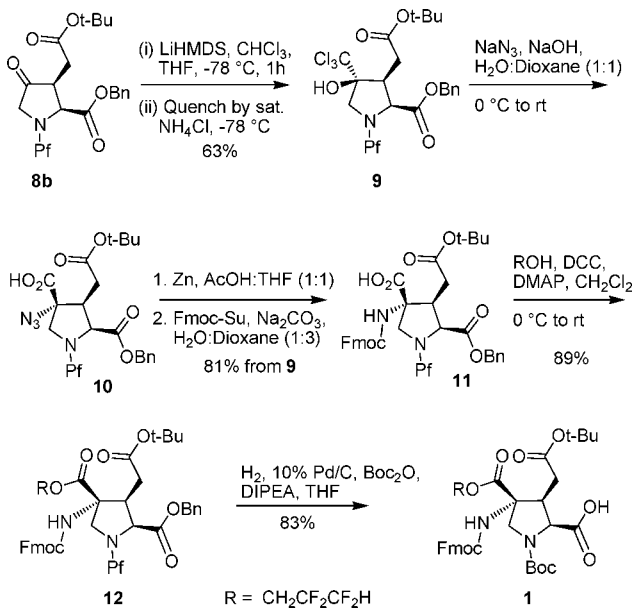
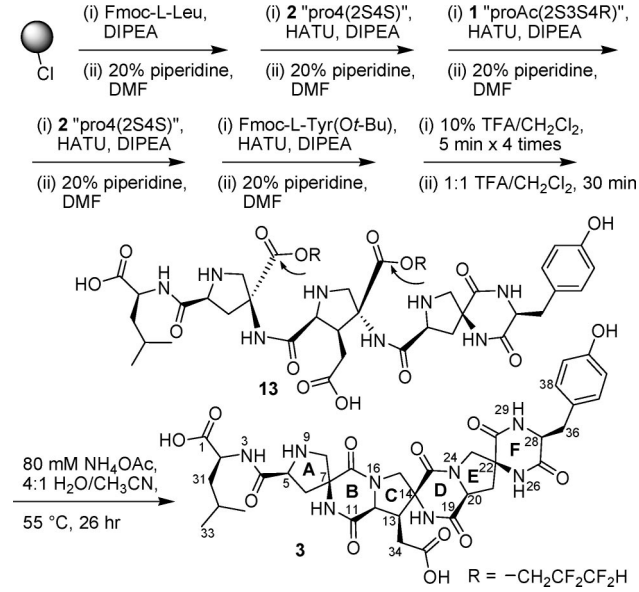
SCHEME 2. Synthesis of ProAc(2S3S4R) Monomer



We achieved this by reducing the ketone **8b** to the trichloromethylcarbinol[30] **9**. The reaction of **8b** with trichlormethyl anion yielded only the single diasteremer **9** as evidenced by RP-HPLC-MS and 1D proton NMR. Intermediate **9** was then treated with a basic solution of azide to affect a Jocic rearrangement[31] with reaction conditions similar to those developed by Corey−Link,[32] which have been previously used by us[17] to synthesize quarternary amino acid derivatives. The Jocic rearrangement produced the azido acid **10** (Scheme 2).

The azide **10** was then reduced to the amine by using Zn/AcOH followed by protection with 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-Su) to form **11**. The Fmoc protected amino acid **11** was purified by silica gel chromatography and a portion was crystallized from hexanes/ethyl acetate. The X-ray determined structure revealed that the stereochemistry was as shown for compound **11** and consistent with our tentative assignment using NMR. Compound **11** was then treated with 1,3-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (DMAP) and 2,2,3,3-tetrafluoropropanol to yield ester **12**. Finally the Pf protecting group was exchanged for a Boc protecting group while simultaneously removing the benzyl protecting group to unmask the carboxylic acid for coupling. Use of *N*,*N*-diisopropylethylamine (DIPEA) as an additive in this reaction reduced the reaction time from a week to less than a day. After final purification we obtained the desired monomer **1** as a white foam in an overall 38% yield from the ketone **8b** (Scheme 2). Only three purification columns are required through the synthesis from **6**. The synthesis we describe is well optimized and highly reproducible for the scale of 850 mg of compound **1**, which was enough for initial investigations of compatibility of **1** in solid phase synthesis (for one solid phase synthesis we need 30 mg of **1**, and thus we have enough for 28 couplings). The synthesis of **1** identified that the enolate alkylation to form **8a** and **8b** and the trichloromethyl anion reduction to form **9** are potential bottlenecks that we are working to scale up.

SCHEME 3. Assembly of a Short Oligomer with ProAc(2S3S4R)[a]



[a] The hydrogens on compound **3** are labeled with the number of the heavy atom to which they are attached. The hydrogens of methylenes are labeled "α" if they go into the page and "*β*" if they come out.

We incorporated **1** into a heterosequence of three bis-amino acid monomers using solid phase synthesis protocols that we have previously developed for synthesizing bis-peptides.[16,18] The oligomeric sequence (L)-Leu-pro4(2S4S)-proAc(2S3S4R)-pro4(2S4S)-(L)-Tyr was assembled on 2-chlorotritylchloride resin[33] (50 mg scale). The leucine residue was used to enhance the lipophilicity of the oligomer and the tyrosine residue provides a UV-chromophore to facilitate purification and characterization. After the attachment of leucine to the resin,[34] each subsequent residue was activated as the 1-hydroxy-7-azabenzotriazole (-OAt) ester[35] for coupling to the growing oligomer on solid support. Quantitative coupling of the first pro4(2S4S) monomer was achieved through double coupling of 2 equiv with respect to the resin loading. The next coupling of proAc(2S3S4R) to the pro4(2S4S) monomer proved to be difficult. After screening a range of coupling methods and reagents we concluded that our general coupling method that involves activating monomer as the -OAt ester gives the best results (up to 45% after double coupling with 3 equiv for 4 h). This may be due to the additional hindrance of the *t*-Bu protected acetyl side chain. The subsequent coupling of pro4(2S4S) monomer to proAc(2S3S4R) monomer was quanititaive. After coupling tyrosine and removal of the terminal Fmoc group, the oligomer was cleaved from the resin with trifluoroacetic acid (TFA)/CH_2Cl_2. After removal of TFA, the oligomer **13** was lypholized to remove any traces of TFA (Scheme 3).

To form the remaining two diketopiperazine (DKP) rings, **13** was dissolved in an 80 mM solution of ammonium acetate in 20% acetonitrile/water and heated in an oven at 55 °C. The reaction progress was monitored by RP-HPLC-MS. After 26 h, analysis of the reaction mixture revealed a major peak for the desired product **1** and no trace of **13** or any intermediates. The crude oligomer was purified by preparative C_{18} reverse-phase

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HPLC and after lypholization, **3** was obtained as a white powder that we used for NMR studies and high-resolution mass spectrometry (Scheme 3).

For NMR experiments, the oligomer **3** was dissolved in 350 μL of dimethyl sulfoxide-d_6 and 5 μL of trifluoroacteic acid to protonate all basic groups. We carried out a series of two-dimensional NMR experiments (COSY, TOCSY, ROSEY, HMQC, and HMBC) and assigned the hydrogen and carbon chemical shifts using SPARKY.[36] The NMR data confirmed the expected connectivity and stereochemistry.

We carried out a stochastic conformational[37] search in vacuo using the AMBER94[38] force field within the molecular mechanics package MOE.[39] The lowest energy conformation is shown in Figure 2; ROESY correlations between non-*J*-coupled protons are superimposed on the structure sorted by intensity, classified as strong, medium, and weak. The conformational search revealed two closely spaced lowest energy conformers (separated by 0.22 kcal/mol) differing in conformation of the pyrolidine ring A. The ROESY correlations for pyrolidine ring A were consistent with this in that they did not support a single conformation.

In the case of pyrolidine ring C, amide proton H18 shows a strong correlation with H13 but only a medium correlation with H15α. There is a medium intensity correlation between H15α and H12. We also observe a medium strength correlation between H20 and H15β. These correlations are in agreement with the structure shown.

The ROESY correlations of pyrrolidine ring E are consistent with the envelop conformation shown—it is identical with the envelop conformation that we have previously seen for a pro4(2S4S) monomer followed by an (*S*)-tyrosine.[18,19] The amide proton H26 shows a strong correlation with H21β and a medium correlation with H23β. A weak correlation of H20 with H23α further supports this conformation. We did not observe ROESY correlations between the tyrosine aromatic hydrogens and H23α and H23β as seen in previous structures;[18,19] this may be because this structure was determined in DMSO rather than water, which would promote a hydrophobic interaction between the aromatic tyrosine and the methylene 23.

The acetyl side chain appears to prefer a single rotamer because we observe a 3 Hz (gauche) and 11.5 Hz (anti) $J_{1,3}$ coupling constant between H13 to H34α and H13 to H34β. We cannot absolutely assign the chemical shift of H34α and H34β and so there are two possible conformations consistent with these chemical shifts. The conformation shown in Figure 2 is the lower energy rotamer by 3.2 kcal/mol.

In conclusion, we have developed a synthesis for a carboxylate functionalized bis-amino acid, proAc(2S3S4R). We demonstrated that it can be incorporated into bis-peptides, determined the structure of a short bis-peptide oligomer that contains it, verified its stereochemical configuration, and determined its conformational preference. The synthesis of this monomer is general and can be used to incorporate other functional groups.

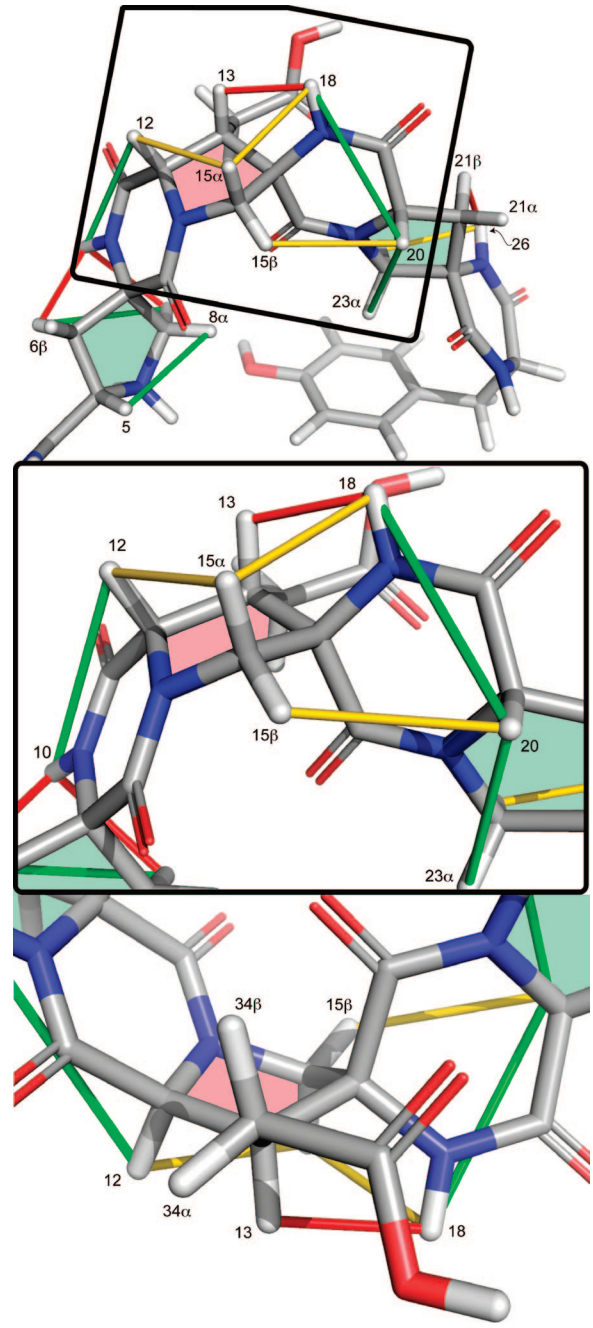

FIGURE 2. The AMBER94 energy minimized structure of bis-peptide **3** that is most consistent with the superimposed ROESY correlations. The sequence is (L)-Leu-pro4(2S4S)-proAc(2S3S4R)-pro4(2S4S)-(L)-Tyr and the pyrrolidine rings are shaded green, red, and green, respectively. The inset figures are a close-up of the second monomer (proAc(2S3S4R), red). The colors of the ROESY correlation lines are color-coded based on their intensity (red = strong, yellow = medium, green = weak).

Experimental Section

General HPLC-MS Procedures. HPLC-MS analysis was performed on a C_{18} reverse-phase column (3.5 μm packing, 4.6 mm × 150 mm) coupled to a mass spectrometer (ESI source). Purification by preparative HPLC was carried out on a semiprep C_{18} column (5 μm packing, 19 mm × 100 mm). HPLC mobile phase: Solvent A "water (0.1% formic acid)", Solvent B "acetonitrile (0.05% formic acid)". Method 05_95 indicates that a gradient was run with initial composition of solvent A 95% and B 5% to solvent A 5% and B 95% over 30 min. Method 05_100xx indicates

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that a gradient was run with initial composition of solvent A 95% and B 5% to solvent B 100% over 32 min and solvent B 100% was continued for an additional 8 min. The analytical HPLC-MS was run at a flow rate of 0.8 mL/min while the preparative HPLC was run at 18 mL/min of mobile phase.

(2*S*,3*S*,4*S*)-Benzyl 3-((*tert*-Butoxycarbonyl)methyl)-4-(trichloromethyl)-4-hydroxy-1-(9-phenyl-9*H*-fluoren-9-yl)pyrrolidine-2-carboxylate (9). The pure alkylated ketone **8b** (1.06 g, 1.85 mmol) was placed in an oven-dried 50-mL round-bottomed flask and dissolved in dry THF (18.5 mL) under argon. Dry $CHCl_3$ (9.2 mL) was added and the solution was cooled to −78 °C in an acetone/dry ice bath. After equilibrating for 30 min, a 1 M solution of LiHMDS in THF (3.7 mL, 2 equiv) was slowly added to the reaction mixture via syringe pump (0.5 mL/min). The stirring was continued for an additional 2 h after which the reaction was quenched by a quick addition of saturated NH_4Cl solution (5 mL, at room temperature) to the reaction mixture at −78 °C. The quenched reaction mixture was allowed to warm to room temperature. The resulting suspension was further diluted with water (20 mL) and extracted with EtOAc (250 mL). The organic layer was washed with 1:1 brine/water (25 mL) followed by brine (25 mL). After drying over anhydrous Na_2SO_4 for 30 min, the organic layer was decanted and concentrated to dryness by rotary evaporation under reduced pressure. The crude product was purified by automated Flash Chromatography (3 × 50 g columns connected in series packed with oven-dried silica; Solvent A: hexanes; Solvent B: EtOAc; gradient elution: 0−5 CVs "solvent A 100%" followed by 5−75 CVs "solvent B 0% to 40%"). The fractions were analyzed by HPLC. On the basis of the analysis, the relevant fractions were divided into three parts (pure **9**, pure **8b**, and mixture of **9**, **8b**, and **8a**) and pooled together separately. The three pools were concentrated by rotary evaporation and the resulting oils were further dried under reduced pressure overnight to yield pure product **9** (0.81 g, 1.17 mmol, 63%), recovered ketone **8b** (0.15 g, 0.26 mmol, 14%), and the mixture of **9**, **8b**, and isomerized ketone **8a** (0.17 g, ∼15%), all as white foams (overall recovery ∼92%). The stereochemistry for **9** was back-assigned after the determination of the stereochemistry at the quaternary center of intermediate **11** by X-ray crystallography.

HPLC-MS. Method: 05_100xx; UV detection at 301 nm; **9** t_R = 32.8 min, *m*/*z* (ion) 692.2 (100%), 694.2 (95.9%) (M + H^+ expected 692.2 and 694.2, characteristic for the $-CCl_3$ group). **^{1}H NMR** (500.1 MHz, $CDCl_3$): δ 7.62 (dd, J = 7.0, 7.0 Hz, 2H), 7.52−7.18 (m, 14H), 7.09 (m, 2H), 5.31 (s, 1H), 4.78 (d, J = 12.4 Hz, 1H), 4.51 (d, J = 12.4 Hz, 1H), 3.83 (d, J = 9.4 Hz, 1H), 3.81 (d, J = 10.5 Hz, 1H), 3.62 (d, J = 10.5 Hz, 1H), 3.34 (ddd, J = 9.4, 8.2, 5.0, Hz, 1H), 2.89 (dd, J = 18.0, 5.0 Hz, 1H), 1.91 (dd, J = 18.0, 9.1 Hz, 1H), 1.16 (s, 9H). **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 176.1, 170.1, 146.3, 146.1, 141.5, 140.6, 140.3, 134.5, 128.9, 128.9, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 127.8, 127.6, 127.2, 127.0, 125.9, 120.4, 120.2, 103.1, 88.2, 80.9, 75.5, 67.3 (CH_2), 65.2 (CH), 59.5 (CH_2), 42.8 (CH), 31.8 (CH_2), 27.8 (CH_3, 3C). **IR** (thin film): 3391, 3062, 2976, 2936, 2877, 1727, 1451, 1368, 1257, 1156, 1065, 909, 794, 735. $[\alpha]^{27}_D$ −97.3 (*c* 0.42, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{38}H_{36}Cl_3NO_5Na^+$ (M + Na^+) 714.1557, found 714.1564.

(2*S*,3*S*,4*R*)-2-((Benzyloxy)carbonyl)-3-((*tert*-butoxycarbonyl)methyl)-4-azido-1-(9-phenyl-9*H*-fluoren-9-yl)pyrrolidine-4-carboxylic Acid (10). The pure intermediate **9** (0.83 g, 1.2 mmol) was placed in a 50-mL round-bottomed flask and dissolved in dioxane (12 mL). The reaction flask was cooled in an ice bath for 2 min and a solution (precooled to 0 °C) of NaN_3 (390 mg, 6 mmol, 5 equiv) and NaOH (240 mg, 6 mmol, 5 equiv) in water was added drop by drop. Additional dioxane (3−4 mL) was added to dissolve any starting material that precipitates out upon addition of aqueous solution. The stirring was continued and the reaction progress was monitored by HPLC: a small volume (5 μL) was withdrawn from the reaction flask, diluted with MeOH (45 μL), and filtered through Spin-X centrifuge filter. We injected a portion of this solution (5 μL) into HPLC-MS for analysis (method: 05_100xx, UV detection at 301 nm; **10** t_R = 30.4 min, *m*/*z* (ion) 645.2 and 667.2 (M + H^+ expected 645.3 and M + Na^+ expected 667.3), **9** t_R = 32.7 min). After completion (ca. 9 h, as indicated by the absence of the peak corresponding to **9**), the reaction was quenched by the addition of saturated NH_4Cl solution (10 mL). The resulting mixture was further diluted with water (50 mL) and extracted with EtOAc (500 mL). The organic layer was washed with 1:1 brine/water (50 mL) followed by brine (25 mL). After drying over anhydrous Na_2SO_4 for 30 min, the organic layer was decanted and concentrated to dryness by rotary evaporation under reduced pressure. Any residual solvent was removed by drying under reduced pressure to yield crude product **10** as a yellow foam (0.81 g). This crude product was taken to the next step without any further purification. For characterization, the above reaction was repeated at 0.30 mmol scale and the resulting crude product was purified by automated flash chromatography (50 g silica columns; solvent A: hexanes with 0.1% AcOH, solvent B: EtOAc with 0.1% AcOH; gradient elution: 0−5 CVs "solvent B 10%" followed by 5−45 CVs "solvent B 10% to 60%"). The desired fractions were pooled together and concentrated by rotary evaporation. To remove residual acetic acid, the purified product was redissolved in EtOAc (200 mL) and washed with water (2 × 20 mL). The organic layer was further washed with brine (20 mL) and after drying over anhydrous sodium sulfate for 20 min, solvent was removed by rotary evaporation under reduced pressure. The resulting oil was further dried under reduced pressure overnight to yield the desired product **10** (172 mg, 0.26 mmol, 87%) as a white foam.

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.75 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 7.4 Hz, 1H), 7.50−7.06 (m, 16H), 4.69 (d, J = 12.4 Hz, 1H), 4.49 (d, J = 12.4 Hz, 1H), 3.86 (d, J = 9.6 Hz, 1H), 3.55 (d, J = 10.0 Hz, 1H), 3.22 (d, J = 10.0 Hz, 1H), 2.78 (ddd, J = 10.2, 9.6, 4.0 Hz, 1H), 2.44 (dd, J = 17.5, 4.0 Hz, 1H), 2.16 (dd, J = 17.5, 10.2 Hz, 1H), 1.17 (s, 9H). **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 173.8, 170.1, 169.7, 145.4, 143.5, 141.6, 140.6, 140.0, 134.5, 129.5, 129.2, 128.9, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 127.3, 127.1, 126.9, 120.7, 120.1, 81.5, 76.2, 70.9, 67.5 (CH_2), 61.6 (CH), 55.9 (CH_2), 46.3 (CH), 32.9 (CH_2), 27.8 (CH_3, 3C). **IR** (thin film): 3061, 3034, 2978, 2936, 2107, 1730, 1451, 1368, 1255, 1157, 909, 737. $[\alpha]^{27}_D$ +124 (*c* 0.47, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{38}H_{36}N_4O_6Na^+$ (M + Na^+) 667.2533, found 667.2458.

(2*S*,3*S*,4*R*)-2-((Benzyloxy)carbonyl)-3-((*tert*-butoxycarbonyl)methyl)-4-amino-1-(9-phenyl-9*H*-fluoren-9-yl)pyrrolidine-4-carboxylic Acid (s1). The crude product **10** obtained in the preceding step (0.81 g, all of it) was transferred to a 50-mL round-bottomed flask and dissolved in dry THF (12 mL). AcOH (12 mL) was added to this solution followed by 0.16 g of Zn dust (20 wt % of **10**). The reaction mixture was stirred at rt and the reaction progress was monitored by HPLC: a small volume (10 μL) was withdrawn from the reaction flask, diluted with MeOH (250 μL), and filtered through Spin-X centrifuge filter. We injected a portion of this solution (5 μL) into HPLC for analysis (method: 05_100xx, UV detection at 301 nm; **s1** t_R = 20.2 min, *m*/*z* (ion) 619.2 and 641.2 (M + H^+ expected 619.3 and M + Na^+ expected 641.3), **10** t_R = 30.4 min). After completion (ca. 3 h, as indicated by the absence of a peak corresponding to **10**) the reaction mixture was filtered through a filter paper, using a Buchner funnel under reduced pressure. The residual zinc was washed 2−3 times with a THF/AcOH (1:1) mixture to extract any trapped product. All the filtrates were combined and concentrated by rotary evaporation under reduced pressure. To remove residual acetic acid, the residual solid was dissolved in CH_2Cl_2 (5 mL) and diluted with hexanes (20 mL), then the solvent was removed by rotary evaporation under reduced pressure. This process was repeated one more time to ensure complete removal of acetic acid. The resulting glue-like solid was further dried under reduced pressure overnight to yield crude product **s1** as a white foam (0.75 g) that was taken to the next step as such. For characterization, a small part of this crude product **s1** (35 mg) was dissolved in 1:1 acetonitrile/water (500 μL) and

purified by preparative HPLC (method: 05_95, UV detection at 254 nm, t_R = 18.1 min). The products containing fractions from all three injections were pooled and the solvent was removed by lyophilization to yield pure product **s1** as fluffy white powder (ca. 30 mg), which was used for the characterization tests.

^{1}H NMR (500.1 MHz, $(CD_3)_2CO$): δ 7.80−7.15 (m, 18H), 4.61 (d, J = 12.6 Hz, 1H), 4.57 (d, J = 12.6 Hz, 1H), 3.93 (d, J = 10.6 Hz, 1H), 3.74 (d, J = 8.2 Hz, 1H), 3.49 (d, J = 10.6 Hz, 1H), 2.79 (ddd, J = 8.8, 8.2, 6.6 Hz, 1H), 2.59 (dd, J = 18.0, 8.8 Hz, 1H), 2.34 (dd, J = 18.0, 6.6 Hz, 1H), 1.302 (s, 9H). **^{13}C NMR** (125.8 MHz, $(CD_3)_2CO$): δ 206.5, 206.3, 206.2, 206.0, 176.1, 172.4, 171.7, 148.5, 147.9, 144.4, 141.4, 141.1, 137.1, 129.6, 129.3, 129.1, 129.1, 129.1, 128.7, 128.5, 128.2, 128.2, 127.9, 126.8, 121.1, 120.7, 81.0, 76.3, 70.3, 66.4 (CH_2), 64.1 (CH), 60.3 (CH_2), 48.5 (CH), 32.7 (CH_2), 28.2 (CH_3, 3C). **IR** (thin film): 3414, 2978, 1720, 1501, 1256, 1155, 909, 735. $[\alpha]^{27}_D$ +19.6 (*c* 0.28, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{38}H_{39}N_2O_6^+$ ($M + H^+$) 619.2808, found 619.2825.

(2*S*,3*S*,4*R*)-2-((Benzyloxy)carbonyl)-3-((*tert*-butoxycarbonyl)-methyl)-4-((9*H*-fluoren-9-yl)methoxycarbonylamino)-1-(9-phenyl-9*H*-fluoren-9-yl)pyrrolidine-4-carboxylic Acid (11). The crude amino acid **s1** obtained in the preceding step (0.71 g) was transferred to a 50-mL round-bottomed flask and dissolved in dioxane (about 12 mL). This was followed by the addition of water (6 mL) and sodium carbonate (0.25 g, 2.4 mmol, 2 equiv assuming 100% conversion in the previous 2 steps). More dioxane (5−6 mL) was added to ensure that any precipitated starting material goes back into the solution. To this reaction mixture was added Fmoc-Su in one portion. At this stage the reaction mixture was inhomogeneous. The reaction progress was monitored by HPLC: a small volume (10 μL) was withdrawn from the reaction flask, diluted with MeOH (200 μL), and filtered through a Spin-X centrifuge filter. We injected a portion of this solution (5 μL) into HPLC for analysis (method: 05_100xx, UV detection at 301 nm; **11** t_R = 32.4 min, *m*/*z* (ion) 842.3 ($M + H^+$ expected 842.3), **s1** t_R = 22.4 min). After completion (ca. 17 h, as indicated by the absence of the peak corresponding to **s1**), the reaction mixture was quenched by the dropwise addition of 2 N HCl solution until the solution was at pH ∼4 as monitored by pH paper. The resulting clear solution was extracted with EtOAc (200 mL + 50 mL). The combined organic layer was washed with 1:1 brine/water (50 mL) followed by brine (25 mL). After being dried over anhydrous Na_2SO_4 for 30 min, the organic layer was decanted and concentrated by rotary evaporation under reduced pressure. The crude product was purified by automated flash chromatography (50 g silica column; solvent A: hexanes with 0.1% AcOH, solvent B: EtOAc with 0.1% AcOH; gradient elution: 0−5 CVs "solvent B 10%" followed by 5−55 CVs "solvent B 10% to 60%"). The desired fractions were pooled together and concentrated by rotary evaporation. To remove the traces of acetic acid, the residual solid was dissolved in CH_2Cl_2 (5 mL) and diluted with hexanes (20 mL), then the solvent was removed by rotary evaporation under reduced pressure. This process was repeated one more time to ensure the complete removal of acetic acid. The resulting oil was further dried under reduced pressure overnight to yield pure product **11** (0.78 g, 0.93 mmol, 81% over three steps, based on **9**) as a white foam. A portion of this product was then recrystallized from ethyl acetates/hexanes to yield small, colorless granular crystals of **11** which were used for NMR and other analyses. X-ray structure determination of these crystals revealed that the stereochemistry of the intermediate **11** is *S* at position 3 and *R* at position 4 (Figure 26 in the Supporting Information as well as crystallographic data in CIF format).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 12.470 (br s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 7.3 Hz, 2H), 7.61−7.17 (m, 22H), 7.128 (t, J = 7.4 Hz, 1H), 6.938 (s, 1H), 4.78 (d, J = 12.0 Hz, 1H), 4.42 (m, 1H), 4.34 (d, J = 12.0 Hz, 1H), 4.22 (t, J = 7.1 Hz, 1H), 4.16 (d, J = 8.8 Hz, 1H), 4.10 (m, 1H), 3.57 (d, J = 10.5 Hz, 1H), 3.35 (d, J = 10.5 Hz, 1H), 3.09 (m, 1H), 2.11 (dd, J = 18.0, 10.5 Hz, 1H), 1.91 (br d, J = 17.6 Hz, 1H), 1.331 (s, 9H). **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 172.0, 171.2, 170.8, 155.8, 145.2, 144.1, 143.9, 142.4, 141.9, 141.3, 139.9, 139.7, 134.6, 129.8, 129.3, 129.1, 129.0, 128.8, 128.7, 128.6, 128.3, 128.0, 127.9, 127.7, 127.7, 127.3, 127.2, 127.1, 126.9, 125.5, 125.3, 120.6, 120.0, 82.2, 76.7, 67.2 (CH_2), 67.1 (CH_2), 65.0, 60.6 (CH), 55.8 (CH_2), 47.1 (CH), 42.2 (CH), 33.3 (CH_2), 27.9 (CH_3, 3C). **IR** (thin film): 3339, 3063, 3034, 2978, 1728, 1503, 1450, 1256, 1156, 1056, 909, 737. $[\alpha]^{27}_D$ +143 (*c* 0.54, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{53}H_{49}N_2O_8^+$ ($M + H^+$) 841.3489, found 841.3497.

(2*S*,3*S*,4*R*)-2-Benzyl 4-(2,2,3,3-Tetrafluoropropyl) 3-((*tert*-Butoxycarbonyl)methyl)-4-(9*H*-fluoren-9-yl)methoxycarbonylamino)-1-(9-phenyl-9*H*-fluoren-9-yl)pyrrolidine-2,4-dicarboxylate (12). To a solution of **11** (0.88 g, 1.05 mmol) in dry CH_2Cl_2 (21 mL, 20 mL per mmol) in a 50-mL round-bottomed flask was added DMAP (13 mg, 0.1 mmol, 0.1 equiv) and 2,2,3,3-tetraflouropropanol (283 μL, 3.15 mmol, 3 equiv). The resulting solution was cooled in an ice bath under argon. To this cooled solution was added DCC (433 mg, 2.1 mmol, 2 equiv) in one portion. The reaction mixture was allowed to warm to room temperature and the stirring was continued overnight. The reaction progress was monitored by HPLC: a small volume (10 μL) was withdrawn from the reaction flask, diluted with MeOH (200 μL), and filtered through a Spin-X centrifuge filter. We injected a portion of this solution (10 μL) into HPLC for analysis (method: 05_100xx, UV detection at 301 nm; **12** t_R = 34.4 min, **11** t_R = 32.4 min). After completion (ca. 18 h, as indicated by the absence of the peak corresponding to **11**), the reaction was quenched by the addition of acetic acid (200 μL) and concentrated to dryness by rotary evaporation. The residual solid was dissolved in 30% EtOAc/hexanes (100 mL) and filtered through a sintered funnel to remove the dicyclohexylurea byproduct. The filtrate was concentrated by rotary evaporation to yield the crude product **12** as a yellow foam. The crude product was purified by automated flash chromatography (2 × 40 g silica columns connected in series; solvent A: hexanes, solvent B: EtOAc; gradient elution: 0−5 CVs "solvent A 100%" followed by 5−45 CVs "solvent B 0% to 30%"). The desired fractions were pooled and concentrated by rotary evaporation. The resulting oil was further dried under reduced pressure overnight to yield **12** as a white foam (0.89 g, 0.93 mmol, 89%).

^{1}H NMR (500.1 MHz, $CDCl_3$): δ 7.79 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 7.6 Hz, 2H), 7.64−7.17 (m, 23H), 7.06 (t, J = 7.4 Hz, 1H), 6.16 (br t, J = 52.6 Hz, $-CF_2CF_2H$, 1H), 4.81 (d, J = 12.0 Hz, 1H), 4.72−4.64 (m, 1H), 4.59 (d, J = 9.1 Hz, 1H), 4.43 (d, J = 12.0 Hz, 1H), 4.35−4.28 (m, 2H), 4.17−4.12 (m, 2H), 3.12 (d, J = 10.0 Hz, 1H), 3.06 (d, J = 10.0 Hz, 1H), 2.66−2.61 (m, 1H), 2.34 (dd, J = 18.3, 11.4 Hz, 1H), 2.05 (br d, J = 17.5 Hz, 1H), 1.384 (s, 9H). **^{13}C NMR** (125.8 MHz, $CDCl_3$): δ 172.9, 169.6, 156.0, 148.0, 144.1, 143.9, 142.3, 141.4, 141.2, 139.3, 135.2, 129.1, 128.9, 128.8, 128.6, 128.6, 127.9, 127.8, 127.5, 127.3, 127.3, 127.1, 127.1, 125.3, 125.2, 120.4, 120.1, 120.0, 109.0 (tt, ^1J = 249 Hz, ^2J = 33 Hz, CH), 82.4, 76.1, 67.2 (CH_2), 66.7 (CH_2), 64.2, 61.5 (CH), 60.6 (t, ^2J = 30 Hz, CH_2), 56.7 (CH_2), 47.1 (CH), 44.6 (CH), 32.4 (CH_2), 28.0 (CH3, 3C). **IR** (thin film): 3325, 3061, 3034, 2978, 1741, 1509, 1260, 1164, 909, 737. $[\alpha]^{27}_D$ +123 (*c* 0.37, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{56}H_{50}F_4N_2O_8Na^+$ ($M + Na^+$) 977.3401, found 977.3438.

(2*S*,3*S*,4*R*)-4-((2,2,3,3-Tetrafluoropropoxy)carbonyl)-1-(*tert*-butoxycarbonyl)-3-((*tert*-butoxycarbonyl)methyl)-4-(9*H*-fluoren-9-yl)methoxycarbonylamino)pyrrolidine-2-carboxylic Acid (1). Ester **12** (1.36 g, 1.42 mmol) was transferred to a 100-mL round-bottomed flask and dissolved in dry THF (28 mL). Solid Boc_2O (1.0 g, 4.3 mmol) was added to this solution followed by careful addition of 10 wt % Pd/C (136 mg, 10 wt % of **12**) in one portion. The reaction flask was degassed under reduced pressure and backfilled with H_2 gas several times. After 1 h of stirring, a catalytic amount of DIPEA (25 μL, 10 mol% of **12**) was added to speed up the reaction. The process of degassing and backfilling with H_2 gas was repeated and the stirring was continued overnight. The reaction progress was monitored by HPLC: a small volume (10 μL) was withdrawn from the reaction flask, diluted with MeOH (200 μL)

and filtered through a Spin-X centrifuge filter. We injected a portion of this solution (10 μL) into HPLC for analysis (method: 05_100xx, UV detection at 301 nm; **1** t_R = 27.7 min, **12** t_R = 34.4 min). After completion (ca. 23 h, as indicated by the absence of the peaks corresponding to **12** or other intermediates), the reaction mixture was mixed with Celite and the solvent was removed by rotary evaporation under reduced pressure. The resulting solid mixture was transferred to a 40 g empty loading column and further dried overnight under reduced pressure to remove any residual solvent. This was followed by purification by automated flash chromatography (2 × 40 g silica columns connected in series; solvent A: CH_2Cl_2 with 0.1% AcOH, solvent B: 5% MeOH in CH_2Cl_2 with 0.1% AcOH; gradient elution: 0−10 CVs "solvent A 100%" followed by 10−50 CVs "solvent B 0% to 100%"). The desired fractions were pooled and concentrated by rotary evaporation. To remove residual acetic acid, the purified product was dissolved in EtOAc (200 mL) and washed with water (2 × 20 mL). The combined organic layer was further washed with brine (20 mL) and after drying over anhydrous sodium sulfate for 20 min, the solvent was removed by rotary evaporation under reduced pressure. The resulting oil was further dried under reduced pressure overnight to yield **1** as a white foam (0.85 g, 1.17 mmol, 83%).

^{1}H NMR (500.1 MHz, 77 °C $(CD_3)_2SO$): δ 12.32 (br s, 1H), 8.141 (s, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.67 (d, J = 7.6 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.33 (m t, J = 7.6, Hz, 2H), 6.39 (tt, J = 52.2, 5.1 Hz, $-CF_2CF_2H$, 1H), 4.58−4.40 (m, 3H), 4.36−4.30 (m, 2H), 4.21 (t, J = 6.8 Hz, 1H), 4.10 (d, J = 11.5 Hz, 1H), 3.68 (d, J = 11.5 Hz, 1H), 3.24 (m, 1H), 2.52 (dd, J = 17.5, 4.7 Hz, 1H), 2.31 (dd, J = 17.5, 9.6 Hz, 1H), 1.42 (s, 9H), 1.38 (s, 9H). **^{13}C NMR** (125.8 MHz, 20 °C $(CD_3)_2SO$): mixture of rotamers δ 171.1 and 170.6, 169.6 and 169.5, 168.7 and 168.6, 155.8, 153.5 and 152.9, 143.7 and 143.5, 140.7, 127.7, 127.1, 125.1, 120.2, 109.0 (t, J = 249 Hz, CH), 80.4 and 80.3, 79.6, 79.5, 65.9 (CH_2), 60.3 and 60.0 (CH), 60.3 (CH_2), 53.6 and 53.0 (CH_2), 46.5 (CH), 43.5 and 42.8 (CH), 32.2 and 32.1 (CH_2), 28.0 and 27.8 (CH_3, 3C), 27.7 (CH_3, 3C). **IR** (thin film): 3318, 3061, 3034, 2978, 1742, 1519, 1260, 1156, 909, 737. $[\alpha]^{27}_D$ +75.9 (*c* 0.34, $CHCl_3$). **HRMS-ES** (*m*/*z*): calcd for $C_{35}H_{40}F_4N_2O_{10}Na^+$ (M + Na^+) 747.2517, found 747.2444.

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Supporting Information Available: Other experimental procedures for the synthesis, solid phase assembly of oligomer **3**, relevant NMR spectra, and crystallographic data for intermediate **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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