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Synthesis of a Homologous Series of Side Chain Extended Orthogonally-Protected Aminooxy-Containing Amino Acids

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Abstract

Practical methodology is reported for the synthesis of a homologous series of side chain extended amino acids containing aminooxy functionality bearing orthogonal protection suitable for Fmoc peptide synthesis. These reagents may be useful for the preparation of libraries containing fragments joined by peptide linkers.

Keywords

oxime; peptidomimetic; aldehydes; combinatorial chemistry

Fragment-based approaches to drug discovery have gained increasing importance in the pharmaceutical industry.[1] Tethered libraries represent a subclass of fragment-based methodologies. However, to date, tethered libraries have typically employed structurally simple linker elements, consisting mainly of polymethylene segments bearing terminal aminoxy groups that serve as anchoring points for fragment attachment.[2] However, increased library complexity, leading potentially to enhanced bioactivites, may be possible by combining the structural diversity of peptide scaffolds together with linker-based functionalized oxime ethers. Amino acids **1a** - **1f** and **2a** were designed to serve as key components of linker-based peptide libraries by providing protected aminooxy groups appended onto the peptide backbone via methylene chains of increasing lengths (Figure 1). Final tethered products would be obtained by acid-catalyzed oxime ether deprotection and conjugation with fragment libraries. However, only the core structures of **1a**,[3] **1c**[4] and **1d**[5] are known in the literature and efficient syntheses of the series **1a** - **f** and **2a** have not yet been reported. Practical methodology for the preparation of **1a** - **f** and **2a** bearing orthogonal protection designed for Fmoc-based peptide synthesis was the focus of the current study.

Cross metathesis (CM) has found wide application for coupling through highly efficient carbon - carbon bond formation.[6] A CM approach for the synthesis of target compounds **1d** - **1f** required the *N*-Boc-protected aminooxy building blocks **4a** - **4c**,[7] which could be obtained by reaction of the corresponding bromides **3a** - **3c** with *N*-Boc hydroxylamine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2(Scheme 1).[8] The CM coupling of *N*-Fmoc-L-allylglycine *O*-benzyl ester (**5**)[6c] with five equivalents of the respective substrates **4a** - **4c** in refluxing CH_2Cl_2 using 5% Grubbs 2nd generation catalyst, [$((PCy_3)(Im(Mes)_2)Ru{=}CHPh)$][9] provided the reaction products **6a** - **6c** (Scheme 2), which were contaminated with small quantities of unwanted material resulting from homodimerization of reagent **4**. It was advantageous to subject the crude mixtures directly to hydrogenation to provide the easily purified final products **1d** - **1f**. Based on combined yields over two steps, it was found that CM

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efficiency improved with increasing substrate chain length (**1d**, 40% yield; **1e**, 53% yield and **1f**, 72% yield).

The shorter homologues **1a** - **1c** were prepared from L-serine (Ser), L-homoserine (HSer) and L-glutamic acid (Glu), respectively. Near quantitative conversion of Ser to *N*-Trt-L-serine *O*-methyl ester (*N*-Trt-Ser-OMe, **7**[10]) and reaction with *N*-hydroxyphthalimide under Mistunobu conditions, provided the globally-protected product **8** (79% yield, Scheme 3). Use of the *N*-Trt group was to minimize unwanted beta-elimination during the Mitsunobu reaction.[11] Having served its purpose, the *N*-Trt group was replaced by Cbz protection to provide intermediate **9** (84% yield).

Conversion of HSer to *N*-Cbz-L-homoserine *O*-methyl ester (*N*-Cbz-HSer-OMe, **10**) was by literature procedures,[12] while the side chain-elongated analogue **12** was obtained from commercially available *N*-Cbz-Glu-OBn (**11**)[13] by reduction of the free carboxylic acid group (Scheme 4).[14] When direct transformation of **10** to the phthalimidooxy product **15** under Mitsunobu conditions was accompanied by poor yields and the formation of cyclized by-products, the alcohols **10** and **12** were converted in quantitative yield to the corresponding mesyl esters (**13** and **14**[15]). Subsequent nucleophilic displacement using *N*-hydroxyphthalimide provided **15** and **16**, respectively in approximately 60 - 70% yields.[12]

To complete the synthesis of the target compounds the phthalimidooxy intermediates **9**, **15** and **16** were cleaved by treatment with methylhydrazine in CH_2Cl_2 at 0° C, then protected as their *N*-Boc derivatives (**17** - **19**) by reaction with Boc anydride (near quantitative yields for two steps, Scheme 5). Cleavage of the amino acid methyl esters of **17** and **18** (LiOH in aqueous THF) followed by Cbz hydrogenolysis and reprotection as the *N*-Fmoc derivatives provided final products **1a** and **1b** in 58% and 81% yields over three steps. Hydrogenation of **19** and *N*-Fmoc reprotection gave **1c** directly (47% yield). To determine whether the DBU/DMF conditions resulted in racemization at the α-amino center, **1c** was evaluated for enantiomeric purity and found to exhibit an ee value greater than 98%.

Use of the Boc-protected aminooxy-containing analogue **1a** in solid-phase peptide synthesis is potentially limited by β-elimination during piperidine-mediated Fmoc deprotection. Indeed, attempted incorporation on NovaSyn® TGR resin of **1a** in place of threonine in the Tsg101-binding peptide, FITC-Ava-PEPTAPPEE-amide[16] gave only product resulting from β-elimination.[17] Therefore, the aminooxy Boc protecting group in **1a** was replaced with the more electron donating 4-methyl-triphenylmethyl (Mtt) (final product **2a**). This was achieved by deprotection of phthalamide **9** using methyl hydrazine in CH_2Cl_2 followed by reaction with Mtt-Cl [$N(i\text{-}Pr)_2Et$, CH_2Cl_2] to give the corresponding Mtt-protected aminooxy analogue **20** in quantitative yield (Scheme 6). Conversion to the *N*-Fmoc final product **2a** was as described above for the preparation of target compounds **1a** - **1c**. Repeating the synthesis of the Tsg101-binding peptide described above using **2a** rather than **1a** gave the desired aminooxy-containing peptide as the major product with no β-elimination side product as detected by HPLC.[18] This homologous series of aminooxy-containing amino acids analogues are intended to serve as valuable building blocks for the post solid-phase construction of tethered oxime-based peptide libraries.

General Procedure for the Preparation of 4a - 4c

To a mixture of alkenyl bromide (**3a** - **3c**) (10 mmol) and *N*-Boc-hydroxylamine (25 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C, was added DBU (7.5 mL) carefully. The reaction mixture was warmed to room temperature and stirred (overnight). The mixture was diluted with EtOAc (150 mL) and washed with H_2O (50 mL) then brine (50 mL) and purified by silica gel column chromatography (hexanes:EtOAc) to yield **4a** - **4c** as colorless oils.

N-(2-Propen-1-yloxy)-carbamic Acid 1,1-Dimethylethyl Ester (4a)[7]

Prepared from **3a** (1.18 g, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (brs, 1 H), 5.88 (m, 1 H), 5.28 - 5.18 (m, 2 H), 4.27 (dd, *J* = 6.2, 1.0 Hz, 2 H), 1.41 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.74, 132.52, 119.54, 81.37, 77.24, 28.08. IR (KBr) ν_{max}: 3290.9, 2979.5, 2361.4, 1717.3, 1456.0, 1368.3, 1249.7, 1165.8, 1107.9, 928.6. ESI-MS (+VE) *m/z*: 196.1 $(M + Na)^+$.

N-(3-Buten-1-yloxy)-carbamic Acid 1,1-Dimethylethyl Ester (4b)[7]

Prepared from 3b (1.10 g, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1 H), 5.74 (m, 1 H), 5.03 (m, 1 H), 4.96 (m, 1 H), 3.81 (t, *J* = 6.8 Hz, 2 H), 2.30 (m, 2 H), 1.39 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.90, 134.31, 116.64, 81.36, 75.45, 32.40, 28.09. IR (KBr) ν_{max}: 3297.7, 2979.5, 1721.2, 1478.2, 1368.3, 1245.8, 1165.8, 1108.9, 773.3. ESI-MS (+VE) *m/z*: 210.1 $(M + Na)^+$.

N-(4-Penten-1-yloxy)-carbamic Acid 1,1-Dimethylethyl Ester (4c)[7]

Prepared from **3c** (1.20 g, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1 H), 5.71 (m, 1 H), 4.92 (m, 1 H), 4.86 (m, 1 H), 3.75 (t, *J* = 6.6 Hz, 2 H), 2.04 (m, 2 H), 1.62 (m, 2 H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.90, 137.80, 114.90, 81.41, 75.96, 29.92, 28.13, 27.12. IR (KBr) ν_{max}: 3300.6, 2978.5, 1721.2, 1368.3, 1246.8, 1166.7, 1108.9, 912.2, 775.2. ESI-MS (+VE) *m/z*: 224.1 $(M + Na)^+$.

General Procedure for the Preparation of Final Products 1d - 1f

A solution of alkene **4a** - **4c** (5.0 eq.) and protected allylglycine **5**[6c] (1.0 eq) in anhydrous CH_2Cl_2 (20 mL) was degassed under argon (5 min), then Grubbs 2nd generation catalyst $[((PCy_3)(Im(Mes)_2)Ru{=}CHPh)]$[9] (0.05 eq.) was added and the mixture was refluxed (8 h). The solvent was evaporated by rotary evaporatoration and the residue was purified by silica gel column chromatography (hexanes:EtOAc) to yield crude **6a** - **6c** as colorless oils. Without further purification, a solution of **6** in EtOH (10 mL) was hydrogenated at room temperature under 1 atm H_2 over 10% Pd·C (10% by weight) (1 h). Catalyst was removed by filtration and the filtrate was concentrated and purified by silica gel column chromatography (CH_2Cl_2:MeOH) to yield **1d** - **1f** as white waxes.

(3*S*)-3-Carboxy-12,12-dimethyl-10-oxo-8,11-dioxa-2,9-diazadecanoic Acid 1-(9*H*-Fluoren-9-ylmethyl) Ester (1d)

Prepared from **4a** in 40% yield over 2 steps. $[\alpha]^{20}_D$ + 9.60 (c 0.70, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.55 Hz, 2 H), 7.59 (dd, *J* = 7.07, 4.13 Hz, 2 H), 7.38 (t, *J* = 7.46 Hz, 2 H), 7.29 (t, *J* = 7.45 Hz, 2 H), 7.18 (brs, 1 H), 5.67 (d, *J* = 7.95 Hz, 1 H), 4.40 - 4.38 (m, 3 H), 4.20 (m, 1 H), 3.84 (t, *J* = 5.97 Hz, 2 H), 1.91 (m, 1 H), 1.76 (m, 1 H), 1.68 - 1.59 (m, 3 H), 1.54 - 1.46 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.16, 157.53, 156.35, 143.75, 143.61, 141.24, 127.67, 127.04, 125.05, 119.93, 82.30, 76.09, 67.17, 53.60, 47.05, 31.71, 28.16, 27.24, 21.58. IR (KBr) ν_{max}: 3296.7, 2923.6, 1701.9, 1522.5, 1449.2, 1160.9, 909.3, 733.8. ESI-MS (+VE) *m/z*: 507.2 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{26}H_{32}N_2NaO_7$ $(M + Na)^+$: 507.2107, Found: 507.2104.

(3*S*)-3-Carboxy-13,13-dimethyl-11-oxo-9,12-dioxa-2,10-diazadecanoic Acid 1-(9*H*-Fluoren-9-ylmethyl) Ester, (1e)

Prepared from **4b** in 53% yield over 2 steps. $[\alpha]^{20}_{D}$ + 7.04 (c 0.48, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.55 Hz, 2 H), 7.66 - 7.50 (m, 3 H), 7.37 (m, 2 H), 7.29 (t, *J* = 7.41 Hz, 2 H), 5.54 (d, *J* = 8.13 Hz, 1 H), 4.55 - 4.30 (m, 3 H), 4.19 (m, 1 H), 3.82 (m, 2 H), 1.88 (m, 1 H), 1.72 (m, 1 H), 1.64 - 1.55 (m, 2 H), 1.46 (s, 9 H), 1.44 - 1.35 (m, 3 H), 1.30 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.23, 157.51, 156.22, 143.76, 143.61, 141.24, 127.69, 127.04, 125.04, 119.93, 82.16, 76.44, 67.11, 53.66, 47.06, 31.96, 28.17, 27.50, 25.30, 24.77. IR (KBr) ν_{max}: 3285.1, 2933.2, 2364.3, 1700.9, 1521.6, 1449.2, 1162.8, 909.3, 732.8. ESI-MS (+VE) *m/z*: 521.2 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{27}H_{34}N_2NaO_7$ $(M + Na)^+$: 521.2264, Found: 521.2261.

(3*S*)-3-Carboxy-14,14-dimethyl-12-oxo-10,13-dioxa-2,11-diazadecanoic Acid 1-(9*H*-Fluoren-9-ylmethyl) Ester (1f)

Prepared from **4c** in 72% yield over 2 steps. $[\alpha]^{20}_{D}$ + 7.67 (c 1.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.47 Hz, 2 H), 7.64 - 7.51 (m, 3 H), 7.37 (t, *J* = 7.32 Hz, 2 H), 7.29 (d, *J* = 2.29 Hz, 2 H), 5.55 (brs, 1 H), 4.57 - 4.27 (m, 3 H), 4.19 (t, *J* = 6.84 Hz, 1 H), 3.80 (t, *J* = 6.44 Hz, 2 H), 1.86 (m, 1 H), 1.70 (m, 1 H), 1.64 - 1.52 (m, 2 H), 1.52 - 1.41 (m, 10 H), 1.41 - 1.19 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.31, 156.15, 143.70, 141.24, 127.67, 127.04, 125.10, 119.93, 113.43, 81.82, 76.60, 67.04, 47.10, 32.13, 28.76, 28.20, 27.74, 25.49, 24.94. IR (KBr) ν_{max}: 3292.9, 2928.4, 1702.8, 1450.2, 1248.7, 1162.9, 1107.9, 909.3, 731.9. ESI-MS (+VE) *m/z*: $(M + Na)^+$ 535.2. HR-ESI/APCI MS calcd for $C_{28}H_{36}N_2NaO_7$ $(M + Na)^+$: 535.2420, Found: 535.2414.

N-(Triphenylmethyl)-L-serine Methyl Ester (7)[10]

To cooled MeOH (100 mL) at 0 °C was added acetyl chloride (10.0 mL) dropwise. The resulting solution was stirred (15 min) then L-Serine (5.0 g, 47.6 mmol) was added and the solution was stirred at reflux (2 h) then cooled to room temperature. The solvent was evaporated to provide H-Ser-OMe·HCl as a white solid (7.40 g, quantitative). To a suspension of this material (1.50 g, 9.74 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added triethylamine (3.0 mL, 21.4 mmol) and trityl chloride (2.90 g, 10.22 mmol) and the mixture was stirred at room temperature (overnight). The reaction mixture was diluted with EtOAc (200 mL), washed with H_2O (50 mL) and brine (50 mL) and purified by silica gel column chromatography (hexanes:EtOAc) to yield **7** as a white solid (3.20 g, 91% yield). $[\alpha]^{20}_{D}$ + 3.02 (c 1.80, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 - 7.44 (m, 6 H), 7.27 - 7.17 (m, 9 H), 3.69 (m, 1 H), 3.55 (m, 2 H), 3.27 (s, 3 H), 2.96 (brs, 1 H), 2.29 (brs, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.90, 145.57, 128.72, 127.91, 126.59, 70.94, 64.92, 57.78, 51.96. ESI-MS (+VE) m/z: 384.1(M + Na)+.

O-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-*N*-(triphenylmethyl)-L-serine Methyl Ester (8)

To a solution of alcohol **7** (2.80 g, 7.76 mmol), *N*-hydroxyphthalimide (2.53 g, 15.51 mmol) and triphenyl-phosphine (4.50 g, 17.06 mmol) in THF (100 mL) at 0 °C under argon was slowly added diethyl azodicarboxylate (DEAD) (40% in toluene, 7.80 mL, 17.06 mmol). The mixture was warmed to room temperature and stirred (overnight). The reaction mixture was diluted with EtOAc (200 mL), washed with H_2O (2 × 50 mL) and brine (50 mL) and dried (Na_2SO_4). Purification by silica gel column chromatography (hexanes:EtOAc) provided **8** as a white wax (3.10 g, 79% yield). $[\alpha]^{20}_{D}$ + 39.6 (c 1.34, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 7.83 - 7.81 (m, 2 H), 7.74 - 7.72 (m, 2 H), 7.55 - 7.52 (m, 6 H), 7.27 - 7.23 (m, 6 H),

7.19 - 7.15 (m, 3 H), 4.48 (dd, J = 9.2, 4.0 Hz, 1 H), 4.13 (dd, J = 9.2, 6.0 Hz, 1 H), 3.70 (m, 1 H), 3.42 (s, 3 H), 3.10 (d, J = 10.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.51, 162.94, 145.58, 134.46, 128.82, 128.72, 127.90, 126.49, 123.48, 80.18, 71.20, 55.74, 52.15. IR (KBr) νmax: 2360.4, 1735.6, 1026.9, 701.0. ESI (+VE) m/z: 529.1 (M + Na)+. HR-ESI/APCI MS calcd for $C_{31}H_{26}N_2NaO_5$ (M +Na) +: 529.1739, Found: 529.1760.

O-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-*N*-[(phenylmethoxy)carbonyl]-L-serine Methyl Ester (9)

To a solution of **8** (2.00 g, 3.95 mmol) in CH_2Cl_2 (50 mL) at room temperature was added 37% HCl (0.50 mL) and the suspension was stirred (1 h) then quenched by addition of $NaHCO_3$. The reaction mixture was extracted with CH_2Cl_2 and the combined organic layer was washed with brine and dried (Na_2SO_4). The solvent was removed by rotary evaporation and the residue was dissolved in THF (30 mL) with H_2O (10 mL). To this was added $NaHCO_3$ (498 mg, 5.92 mmol) and benzyl chloroformate (0.59 mL, 4.15 mmol) and the mixture was stirred at room temperature (overnight). THF was removed by rotary evaporation and the resulting aqueous phase was extracted with EtOAc and the combined organic layer was washed with H_2O (2 × 50 mL) and brine (50 mL) and dried (Na_2SO_4), then purified by silica gel column chromatography (hexanes:EtOAc) to yield **9** as a viscous colorless oil (1.32 g, 84% yield). $[\alpha]^{20}_D$ + 31.8 (c 0.75, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 - 7.73 (m, 2 H), 7.67 - 7.65 (m, 2 H), 7.30 - 7.20 (m, 5 H), 6.26 (d, J = 8.8 Hz, 1 H), 5.07 (s, 2 H), 4.73 (dd, J = 10.6, 3.0 Hz, 1 H), 4.57 (m, 1 H), 4.33 (dd, J = 10.6, 3.4 Hz, 1 H), 3.63 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 163.19, 156.11, 136.21, 134.76, 128.56, 128.45, 128.06, 127.97, 123.71, 77.61, 67.11, 53.45, 52.80. IR (KBr) νmax: 3366.1, 2954.4, 2363.3, 1727.9, 1516.7, 1211.1, 1054.9, 876.5, 698.1. HR-ESI/APCI MS cacld for $C_{20}H_{18}N_2NaO_7$ (M +Na) +: 421.1011, Found: 421.1007.

General Procedure for the Preparation of 13 and 14

To a solution of *N*-Cbz-L-homoserine *O*-methyl ester (**10**)[12] or 5-hydroxy-*N*-[(phenylmethoxy)carbonyl]-L-norvaline (**12**)[14] (1.0 eq.) in CH_2Cl_2 (30 mL) at 0 °C, was added triethylamine (1.5 equiv.) and methylsulfonyl chloride (1.2 equiv.) and the mixture was stirred (1 h). The mixture was diluted with CH_2Cl_2 (100 mL) then washed with H_2O (50 mL) and brine (50 mL) and dried (Na_2SO_4). Purificaiton by silica gel column chromatography (hexanes: EtOAc) provided **13** (from **10**) and **14** (from **12**) as viscous colorless oils.

N-Benzyloxycarbonyl-*O*-mesyl-*L*-serine Methyl Ester (13)

Prepared from **10** (2.70 g, quantitative yield). $[\alpha]^{20}_D$ + 5.0 (c 0.30, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.30 (m, 5 H), 5.61 (d, *J* = 6.0 Hz, 1 H), 5.11 (s, 2 H), 4.50 (m, 1 H), 4.28 (m, 2 H), 3.76 (s, 3 H), 2.96 (s, 3 H), 2.33 (m, 1 H), 2.14 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.73, 171.86, 155.93, 135.97, 128.55, 128.29, 128.12, 67.21, 65.69, 52.76, 50.76, 37.14, 31.79. IR (KBr) $\nu_{max:}$ 3327.6, 2940.0, 1696.1, 1538.0, 1341.3, 1172.5, 1005.7, 740.5. ESI-MS (+VE) *m/z*: 368.0 $(M + Na)^+$.

5-[(Methylsulfonyl)oxy]-*N*-[(phenylmethoxy)carbonyl]-*L*-norvaline Phenylmethyl Ester (14)

Prepared from **12** (3.95 g, quantitative yield). [Note: This material was used in the next step without further purification.] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (10H, m), 5.36 (1H, d, *J* = 8.0 Hz) 5.18 (2H, m), 5.11 (2H, s), 4.45 (1H, q, *J* = 8.0 Hz), 4.20 (2H, t, *J* = 8.0 Hz), 2.95 (3H, s), 2.01 (1H, m), 1.84-1.70 (3H, m). ESI-MS *m/z* 525.1 $(M + Na^+)$.

General Procedure for the Preparation of 15 and 16

To a solution of *N*-hydroxyphthalimide (2.0 equiv.) in DMF (10 mL) at room temperature was added a solution of DBU (2.0 equiv.) in DMF (10 mL). The mixture was cooled to 0 °C and stirred (30 min), then a solution of either **13** (to prepare **15**) or **14** (to prepare **16**) (1.0 eq.) in DMF (10 mL) was added dropwise. The mixture was warmed to room temperature and stirred (2 days). The solution was diluted with EtOAc (200 mL) and washed with H_2O (50 mL) and brine (50 mL) and dried (Na_2SO_4). Purification by silica gel column chromatography (hexanes:EtOAc) provided products **15** and **16** as white solids.

O-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-*N*-[(phenylmethoxy)carbonyl]-L-homoserine Methyl Ester (15)[12]

Prepared from **13** (2.20 g, 71% yield). $[\alpha]^{20}_D$ + 8.6 (c 1.95, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 - 7.74 (m, 2 H), 7.71 - 7.67 (m, 2 H), 7.31 - 7.22 (m, 5 H), 6.16 (d, *J* = 8.4 Hz, 1 H), 5.07 (s, 2 H), 4.58 (q, *J* = 6.8 Hz, 1 H), 4.26 (m, 2 H), 3.70 (s, 3 H), 2.27 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.02, 163.47, 156.14, 136.39, 134.60, 128.74, 128.40, 127.97, 127.91, 123.60, 74.98, 66.85, 52.55, 51.44, 30.19. IR (KBr) $\nu_{max:}$ 3331.4, 2364.3, 2343.1, 1725.0, 1682.6, 1529.3, 1216.9, 698.1. ESI (+VE) *m/z*: 435.1 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{21}H_{20}N_2NaO_7$ $(M + Na)^+$: 435.1168, Found: 435.1176.

5-[(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)oxy]-*N*-[(phenylmethoxy) carbonyl]-L-norvaline Phenyl-methyl Ester (16)

Prepared from **14** (2.70 g, 61% yield). $[\alpha]^{20}_D$-16.34 (c 0.35, $CHCl_3$). mp: 101 - 105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.80 (2H, m), 7.76-7.73 (2H, m), 7.40-7.29 (10H, m), 5.47 (1H, d, *J* = 8.0 Hz), 5.20 (2H, s), 5.10 (2H, s), 4.49 (1H, m), 4.19 (2H, m), 2.16 (1H, m), 2.02 (1H, m), 1.81 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ; 171.95, 163.57, 155.94, 136.23, 135.23, 134.45, 128.86, 128.56, 128.44, 128.38, 128.23, 128.06, 127.98, 123.50, 67.20, 66.91, 53.63, 28.83, 24.28. IR (neat) ν_{max} = 3324, 3066, 3033, 2965, 2936, 2361, 2336, 1725, 1698, 1539, 1276, 1186, 1127, 1018, 876, 736, 696. HR-ESI/APCI MS calcd for $C_{28}H_{26}N_2NaO_7$ $(M + Na)^+$: 525.1638, Found: 525.1641.

General Procedure for the Preparation of 17 - 20

To a solution of intermediates **9**, **15** or **16** (1.0 eq.) in CH_2Cl_2 (20 mL) at 0 °C was added methylhydrazine (1.5 equiv.) and the reaction mixture was stirred at 0 °C (1 h). The mixture was passed through celite and the filtrate was concentrated and dried under high vacuum (30 min). For product **20** see below.* For products **17** - **19**, the residue was re-dissolved in THF (50 mL) and to this was added triethylamine (2.0 equiv.) and Boc_2O (2.0 equiv.) and the solution was stirred at room temperature (overnight). The reaction mixture was diluted with EtOAc (200 mL) and washed with H_2O (2 × 50 mL) and brine (50 mL) and dried (Na_2SO_4). Purification by silica gel column chromatography (hexanes:EtOAc) provided **17** - **19** as viscous colorless oils. For product **20**, the residue above* prepared by treatment of **9** with methylhydrazine was dissolved in CH_2Cl_2 (10 mL) and to this was added diisopropylethylamine (DIPEA) (2.0 equiv.) and 4-methyltrityl chloride (1.2 equiv.) and the mixture was stirred room temperature (1 h). The mixture was diluted with EtOAc (100 mL) and washed with H_2O (50 mL) and brine (50 mL) and dried (Na_2SO_4). Purification by column chromatography (hexanes:EtOAc) using triethylamine-pretreated silica gel provided **20** as a white wax.

3-[[(1,1-Dimethylethoxy)carbonyl]aminooxy]-*N*-[(phenylmethoxy)carbonyl]-L-serine Methyl Ester (17)

Prepared from **9** (1.05 g, 95% yield). $[\alpha]^{20}_{D}$ - 11.7 (c 1.33, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1 H), 7.31 - 7.23 (m, 5 H), 6.18 (d, *J* = 8.4 Hz, 1 H), 5.09 (s, 2 H), 4.54 (m, 1 H), 4.19 (dd, *J* = 7.2 Hz, 4.4 Hz, 1 H), 4.03 (dd, *J* = 11.0, 3.4 Hz, 1 H), 3.70 (s, 3 H), 1.42 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.54, 156.87, 156.32, 136.24, 128.44, 128.06, 127.97, 82.30, 75.89, 67.00, 53.18, 52.60, 28.09. IR (KBr) $\nu_{max:}$ 3297.7, 2978.5, 2361.4, 1716.3, 1519.6, 1210.1, 1055.8, 775.2. ESI-MS (+VE) *m/z*: 391.1 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{17}H_{24}N_2NaO_7$ $(M + Na)_+$: 391.1481, Found: 391.1482.

4-[[(1,1-Dimethylethoxy)carbonyl]aminooxy]-*N*-[(phenylmethoxy)carbonyl]-L-homeserine Methyl Ester (18)

Prepared from **15** (0.90 g, 97% yield). $[\alpha]^{20}_{D}$ + 5.8 (c 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1 H), 7.37 - 7.26 (m, 5 H), 6.10 (d, *J* = 7.6 Hz, 1 H), 5.12 (s, 2 H), 4.52 (dd, *J* = 13.6, 6.4 Hz, 1 H), 4.00 - 3.87 (m, 2 H), 3.73 (s, 3 H), 2.12 (m, 2 H), 1.47 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.56, 156.94, 156.13, 136.34, 128.41, 128.00, 127.91, 81.88, 72.87, 66.81, 52.44, 51.62, 30.28, 28.12. IR (KBr) $\nu_{max:}$ 3308.3, 2977.6, 2361.4, 1703.8, 1527.4, 1246.7, 1162.9, 739.6. ESI-MS (+VE) *m/z*: 405.1 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{18}H_{26}N_2NaO_7$ $(M + Na)_+$: 405.1638, Found: 405.1647.

5-[[(1,1-Dimethylethoxy)carbonyl]aminooxy]-*N*-[(phenylmethoxy)carbonyl]-L-norvaline Phenylmethyl Ester (19)

Prepared from **16** (1.85 g, 80% yield). $[\alpha]^{20}_{D}$ -5.92 (c 0.58, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.28 (10H, m), 7.15 (1H, s), 5.56 (1H, d, *J* = 8.0 Hz), 5.22-5.13 (2H, m), 5.10 (2H, s), 4.42 (1H, q, *J* = 8.0 Hz), 3.81 (2H, t, *J* = 6.0 Hz), 2.23-1.92 (1H, m), 1.90-1.80 (1H, m), 1.72-1.60 (2H, m), 1.45 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$) 172.15, 157.00, 156.05, 136.22, 135.27, 128.56, 128.44, 128.39, 128.23, 128.07, 81.70, 75.74, 67.09, 66.93, 53.77, 29.00, 28.14, 27.57, 23.93. IR (neat) ν_{max} = 3310, 3065, 3034, 2976, 2940, 2881, 1711, 1523, 1454, 1367, 1247, 1165, 1110, 1044, 911, 734, 697. ESI-MS *m/z* 495.1 $(M + Na^+)$. HR-ESI/APCI MS calcd for $C_{25}H_{32}N_2NaO_7$ $(M + Na)^+$: 495.2107, Found: 495.2110.

3-[(Diphenyl-p-tolyl-methyl)-aminooxy]-*N*-[(phenylmethoxy)carbonyl]-L-serine Methyl Ester (20)

Prepared from **9** (1.31 g, quantitative yield). $[\alpha]^{20}_{D}$ + 6.5 (c 1.08, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 - 7.16 (m, 15 H), 7.13 (AB, J_{AB} = 8.4 Hz, 2 H), 7.05 (AB, J_{AB} = 8.4 Hz, 2 H), 6.69 (s, 1 H), 5.40 (d, *J* = 8.8 Hz, 1 H), 5.06 (m, 2 H), 4.52 (m, 1 H), 3.91 (dd, *J* = 10.8, 4.8 Hz, 1 H), 3.84 (dd, *J* = 10.8, 3.6 Hz, 1 H), 3.59 (s, 3 H), 2.28 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 156.0, 144.2, 141.1, 136.6, 136.3, 129.1, 129.0, 128.6, 128.5, 128.2, 128.1, 127.8, 127.0, 73.9, 67.0, 53.8, 52.5, 21.0. IR (KBr) $\nu_{max:}$ 3402.8, 2949.6, 1717.3, 1509.0, 1208.2, 1058.7, 698.1. ESI-MS (+VE) *m/z*: 547.2 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{32}H_{32}N_2NaO_5$ $(M + Na)^+$: 547.2209, Found: 547.2213.

General Procedure for the Preparation of Final Products 1a - 1c and 2a

To a solution of intermediates **17**, **18** or **20** (1.0 equiv.) in THF (10 mL) with H_2O (10 mL) at 0 °C, was added $LiOH{\cdot}H_2O$ (1.2 equiv.) and the mixture was stirred at 0 °C (2 h). THF was removed by rotary evaporation and the residual aqueous phase was washed with Et_2O then acidified to pH 3 - 4 by addition of 1 N HCl. (Note: For 20 saturated NH_4Cl was used rather

than 1 N HCl.) The mixtures were extracted with EtOAc (3 × 50 mL) and the combined EtOAc extracts were washed with H_2O (50 mL) and brine (50 mL) and dried (Na_2SO_4). The organic phase was concentrated and the residue was dissolved in MeOH (20 mL) and stirred with 10% Pd·C under 1 atm H_2 (2 h). (Note: The preparation of **1c** began at this point by the direct hydrogenation of **19**) The Pd·C was removed by filtration and the filtrate was concentrated and the residue was dissolved in dioxane (10 mL) and H_2O (10 mL). To this was added 9-fluorenylmethyl succinimidyl carbonate (FmocOSu) (1.1 equiv.) and $NaHCO_3$ (2.0 equiv.) and the mixture was stirred at room temperature (overnight). The reaction solution was acidified to pH 3 - 4 by addition of 1 N HCl. (Note: For **2a** saturated NH_4Cl was used rather than 1 N HCl.) The mixture was extracted with EtOAc (3 × 50 mL) and the combined EtOAc extract was washed with H_2O (50 mL) and brine (50 mL) and dried (Na_2SO_4). Purificaiton by silica gel column chromatography (CH_2Cl_2:MeOH) provided final products **1a** - **1c** and **2a** as viscous colorless oils. Lyophilization from CH_3CN/H_2O provided white powders, which were suitable for solid-phase applications.

(3*S*)-3-Carboxy-9,9-dimethyl-7-oxo-5,8-dioxa-2,6-diazadecanoic Acid 1-(9*H*-Fluoren-9-ylmethyl) Ester (1a)

Prepared from **17** (0.62 g, 58% yield over 3 steps). $[\alpha]^{20}$ + 4.4 (c 1.30, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (brs, 1 H), 7.30 (d, *J* = 7.6 Hz, 2 H), 7.65 - 7.50 (m, 2 H), 7.37 (t, *J* = 7.6 Hz, 2 H), 7.30 - 7.17 (m, 2 H), 6.48 (brs, 1 H), 4.54 (m, 1 H), 4.37 (m, 2 H), 4.28 (dd, *J* = 11.4, 3.4 Hz, 1 H), 4.22 (d, *J* = 7.2 Hz, 1 H), 4.02 (dd, *J* = 11.4, 5.4 Hz, 1 H), 1.46 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.29, 156.56, 143.70, 143.56, 141.25, 127.72, 127.07, 125.14, 119.95, 83.81, 75.79, 67.45, 52.73, 46.98, 28.02, 27.55. IR (KBr) $\nu_{max.}$ 3288.0, 2977.6, 2364.3, 1700.9, 1521.6, 1449.2, 1160.0, 734.7. ESI-MS (+VE) *m/z*: 465.1 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{23}H_{26}N_2NaO_7$(M +Na) $^+$: 465.1638, Found: 465.1636.

(3*S*)-3-Carboxy-10,10-dimethyl-8-oxo-6,9-dioxa-2,7-diazadecanoic Acid, 1-(9*H*-fluoren-9-ylmethyl) Ester (1b)

Prepared from **18** (1.60 g, 81% yield over 3 steps). $[\alpha]^{20}$ + 9.0 (c 0.79, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.6 Hz, 2 H), 7.69 - 7.60 (m, 2 H), 7.52 (m, 1 H), 7.36 (t, *J* = 7.4 Hz, 2 H), 7.27 (t, *J* = 7.6 Hz, 2 H), 6.67 (brs, 1 H), 4.55 (m, 1 H), 4.43 - 4.28 (m, 2 H), 4.20 (t, *J* = 7.4 Hz, 1 H), 3.96 (m, 2 H), 2.15 (m, 2 H), 1.47 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.64, 157.92, 156.86, 143.81, 143.67, 141.23, 127.68, 127.07, 125.21, 119.91, 106.60, 82.81, 73.12, 67.37, 51.77, 47.00, 29.68, 28.12, 27.54. IR (KBr) $\nu_{max.}$ 3296.7, 2977.6, 2355.6, 1700.9, 1523.5, 1160.9, 1106.9, 909.3, 735.7. ESI-MS (+VE) *m/z*: 479.1 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{24}H_{28}N_2NaO_7$ (M +Na) $^+$: 479.1794, Found: 479.1808.

(3*S*)-3-Carboxy-11,11-dimethyl-9-oxo-7,10-dioxa-2,8-diazadecanoic Acid 1-(9*H*-Fluoren-9-ylmethyl) Ester (1c)

Prepared from **19** (0.82 g, 47% yield over 2 steps). $[\alpha]^{20}$ + 0.152 (c 0.77, $CHCl_3$). mp: 55 - 65 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.6 Hz), 7.62-7.56 (2H, m), 7.39 (2H, t, *J* = 6.8 Hz), 7.30 (2H, t, *J* = 7.2 Hz), 5.74 (1H, s), 4.56-4.32 (3H, m), 4.22 (1H, t, *J* = 6.8 Hz), 3.91 (2H, s), 2.16-2.04 (1H, m), 1.96-1.84 (1H, m), 1.78-1.68 (2H, m), 1.47 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.44, 156.49, 143.85, 141.20, 127.63, 127.04, 125.14, 119.88, 82.09, 75.80, 67.12, 53.72, 47.05, 38.33, 30.89, 28.76, 28.17, 23.90. IR (neat) ν_{max} = 3295, 2977, 2941, 2879, 1710, 1525, 1451, 1368, 1249, 1165, 1110, 1043, 758, 739, 699. ESI-MS *m/z* 493.1 $(M + Na^+)$. HR-ESI/APCI MS calcd for $C_{25}H_{30}N_2NaO_7$ (M + Na) $^+$: 493.1951, Found: 493.1957.

3-[(Diphenyl-p-tolyl-methyl)-aminooxy]-*N*-[[(9*H*-fluoren-9-ylmethyl)oxy] carbonyl]-L-serine (2a)

Prepared from **20** (0.40 g, 58% yield over 3 steps). $[\alpha]^{20}_{D}$ - 9.4 (c 0.75, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (dd, *J* = 7.4, 2.2 Hz, 1 H), 7.71 (t, *J* = 5.4 Hz, 1 H), 7.65 (m, 1 H), 7.45 - 7.16 (m, 16 H), 7.09 - 7.00 (m, 5 H), 5.02 (dd, *J* = 16.2, 12.6 Hz, 1 H), 4.32 - 4.18 (m, 3 H), 3.84 (m, 1 H), 3.60 (t, *J* = 9.6 Hz, 1 H), 2.25 (s, 1.6 H), 2.21 (s, 1.4 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 156.6, 145.1, 144.3, 142.0, 141.2, 137.4, 136.0, 129.2, 128.8, 128.5, 128.2, 128.1, 127.9, 127.5, 127.0, 125.7, 120.5, 74.1, 73.4, 66.3, 65.9, 54.3, 47.0, 21.0. IR (KBr) $\nu_{max:}$ 3404.7, 2365.3, 1706.7, 1508.1, 1229.4, 1056.8, 700.0. ESI-MS (+VE) *m/z*: 621.2 $(M + Na)^+$. HR-ESI/APCI MS calcd for $C_{38}H_{34}N_2NaO_5$ $(M + Na)^+$: 621.2365, Found: 621.2368.

Determination of Enatiomeric Purity of 1c

Phenylalanine dipeptides were prepared by solid-phase techniques (Figure 2), and the resulting diastereomers were separated by reverse-phase HPLC (Figure 3). Analysis of the racemic D, L-phenylalanine containing dipeptides (**20**) indicated good separation of diastereomers. The dipeptide **21** prepared using enantiomerically pure L-phenylalanine did not show detectable diastereomeric contamination, indicating that the ee value of **1c** is > 98%.

Acknowledgments

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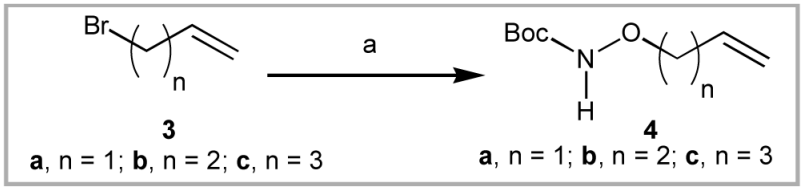
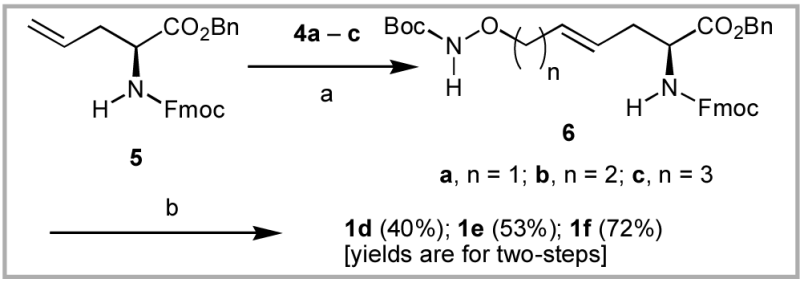
(17). The major fraction obtained by HPLC purification of the peptide resulting using **1a** to replace the Thr residue, provided a mass spectral molecular ion (1422.4) that was 31 amu lower than expected for the correct product (1453.5). This is consistent with beta elimination of HO-NH_2 followed by

reduction of the resulting double bond during under the reducing conditions of resin cleavage using triethylsilane

(18). The major fraction obtained by HPLC purification of the peptide resulting from the use of **2a** to replace the Thr residue, provided mass spectral molecular ions consistent with the desired peptide product [m/z 1454.5, (M+H) and 1476.5 (M+Na)]

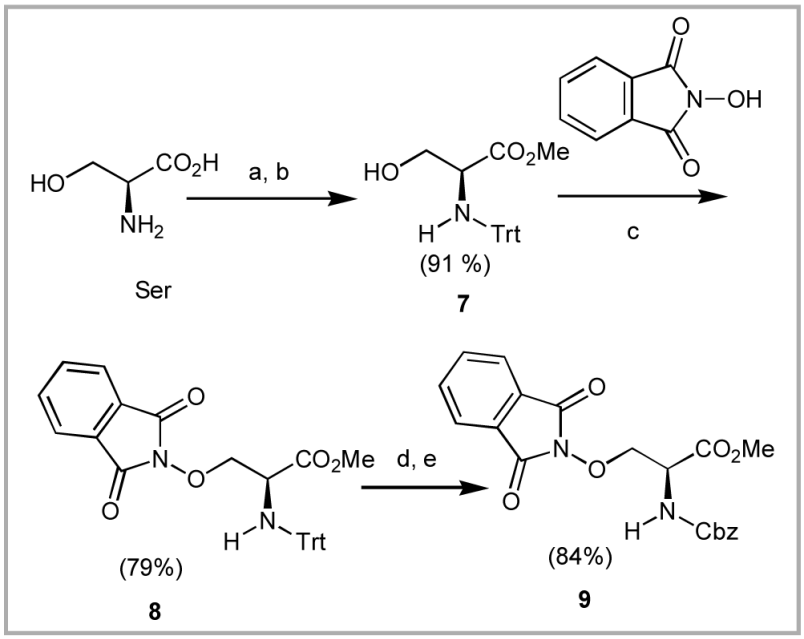


Scheme 1. Reagents and conditions
(a) Boc-NH-OH, DBU, CH_2Cl_2.



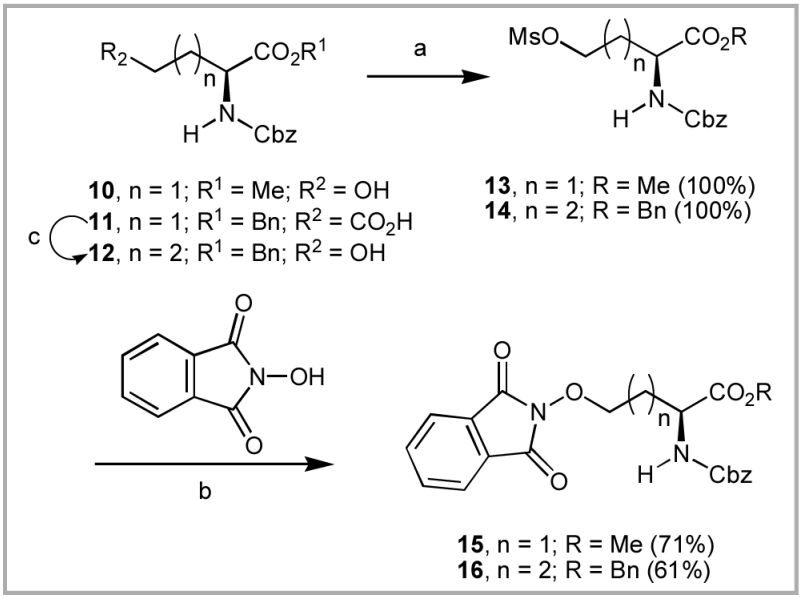
Scheme 2. Reagents and conditions

(a) Grubbs 2nd generation catalyst, CH_2Cl_2, Δ; (b) H_2, Pd·C, EtOH.



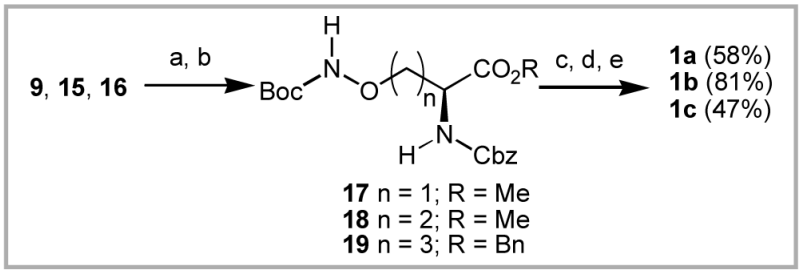
Scheme 3. Reagents and conditions

(a) HCl, MeOH; (b) TrtCl, NEt_3; (c) Ph_3P, DEAD; (d) HCl (37%), CH_2Cl_2; CbzCl, THF, H_2O.



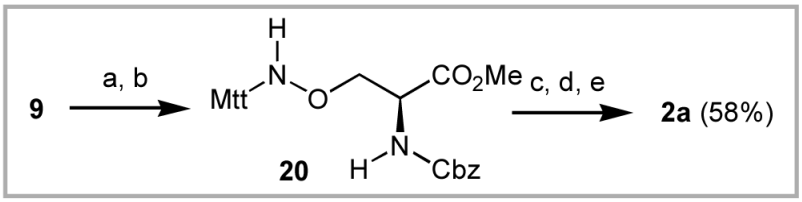
Scheme 4. Reagents and conditions

(a) MsCl, NEt_3, CH_2Cl_2; (b) DBU, DMF; (c) $ClCO_2Et$, 4-Methyl morpholine, THF followed by $NaBH_4$, MeOH.



Scheme 5. Reagents and conditions

(a) $MeNHNH_2$, CH_2Cl_2; (b) Boc_2O, NEt_3, THF; (c) LiOH, THF, H_2O; (d) H_2, Pd·C, MeOH; (e) FmocOSu, dioxane.



Scheme 6. Reagents and conditions

a) $MeNHNH_2$, CH_2Cl_2; (b) MttCl, N(*i*-Pr)$_2$Et, CH_2Cl_2; (c) LiOH, THF, H_2O; (d) H_2, Pd·C, MeOH; (e) FmocOSu, dioxane.

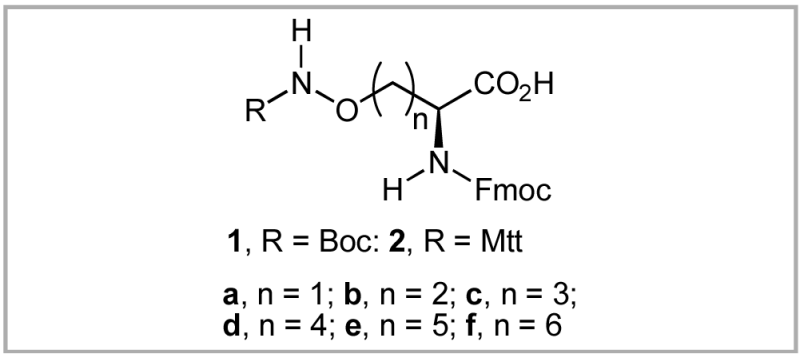


Figure 1.
Structures of protected aminooxy amino acid analogues.

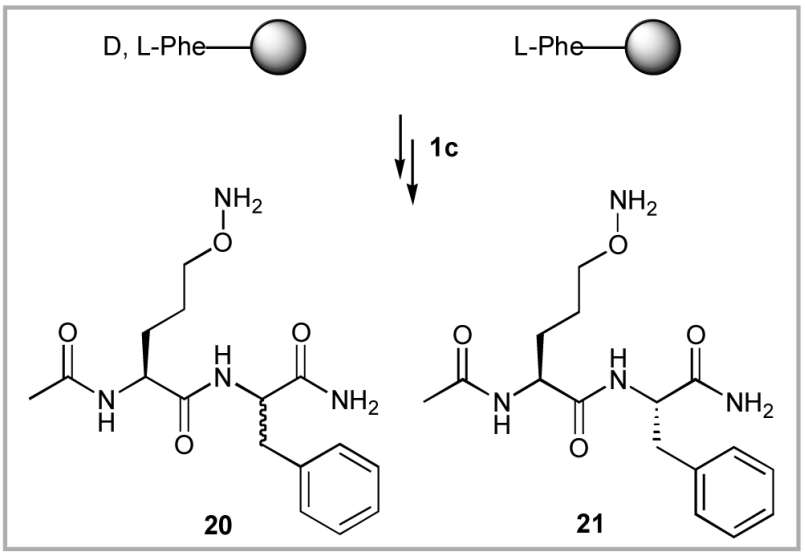


Figure 2.
Determination of enantiomeric puriy of **1c**.

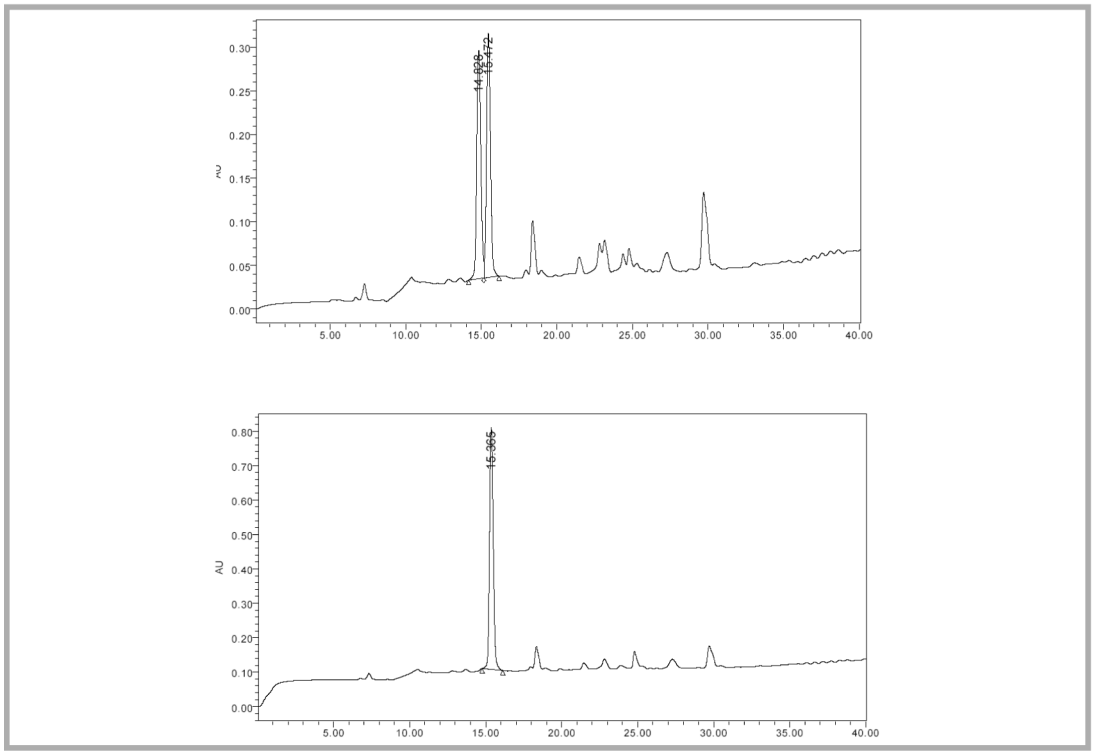


Figure 3.
HPLC chromatograms of **20** (top) and **21** (bottom). Identity of the 3 major peaks were confirmed by ESI mass spectra [337.1$(M+H)^+$ and 359.1 $(M+Na)^+$].