Nitrone proteting groups for enantiopure *N*-hydroxy amino acids and synthesis of N-terminal peptide hydroxylamines for chemoselective ligations

S. Irene Medina, Jian Wu, and Jeffrey W. Bode*

Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104–6323

Supporting Information

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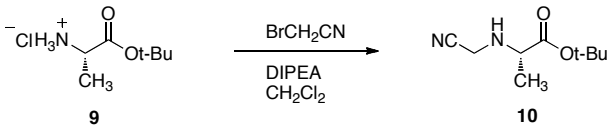
1. General Methods

All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of nitrogen. CH_2Cl_2 was distilled over CaH_2. THF was distilled from Na/benzophenone. CH_3OH and DMF were dried by passage over molecular sieves under Ar atmosphere. *N*,*N*-Diisopropylethylamine (DIPEA) was distilled from CaH_2. Other reagents were used without further purification. Oxone® was purchased from Alfa Aesar. Thin layer chromatography (TLC) was performed on Merck precoated plates (silica gel 60 F_{254}, Art 5715, 0.25 mm) and was visualized by fluorescence quenching under UV light or by staining with potassium permanganate or ninhydrin. Preparative thin-layer chromatography (PTLC) was performed using plates prepared from silica gel EMD 60 PF254 (Art 7749). Column chromatography was performed on E. Merck Silica Gel 60 (230–400 Mesh) using a forced flow of 0.5–1.0 bar. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on a Bruker Avance II 500 spectrometer. Chemical shifts are expressed in parts per million (PPM) downfield from residual solvent peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a JASCO FT/IR-430 spectrophotometer and are reported as wavenumber (cm^{-1}). Optical rotations $[\alpha]_D^T$ were measured at temperature T on a Jasco P–2000 polarimeter operating at the sodium D line with a 100 mm path length cell, and are reported as follows: (concentration (g/ml x 100), solvent). Reverse phase HPLC was performed using the following columns: YMC R-ODS-10A C-18 (250 x 4.6 mm for analytical and 250 x 20 mm for preparative), or Zorbax Eclipse XDB-C8 4.6 × 150 mm. All separations utilized a gradient of iPrOH or CH_3CN and millipore H_2O, each containing 0.1 % TFA. Peptides were prepared by standard Fmoc manual solid-phase synthesis protocols using Rink amide MBHA resin with a loading of 0.70 mmol/g. All amino acids were purchased from Nova Biochem, and peptide couplings were monitored by Kaiser ninhydrin test.[1]

(1) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. "Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides." *Anal. Biochem*. **1970**, *34*, 595.

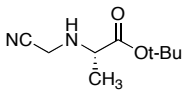
2. Experimental Procedure and Characterization of Data

2.1 General procedure for cyanomethylation of amino acid esters



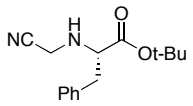
9 **10**

The following procedure is representative. *N*-cyanomethyl amino acid esters were prepared according to modified literature procedures.[2] A solution (0.1 M) of (*S*)-1-(*tert*-butoxy)-1-oxopropan-2-aminium chloride (0.516 g, 2.85 mmol, 1.00 equiv) in CH_3CN (27.0 mL) was treated with $BrCH_2CN$ (0.330 mL, 3.13 mmol, 1.10 equiv) and DIPEA (1.00 mL 5.74 mmol, 2.00 equiv), and the reaction was stirred at rt overnight. The mixture was transferred to a separatory funnel and saturated aq $NaHCO_3$ (100 mL) was added. The solution was extracted with CH_2Cl_2 (2 x 100 mL), the organic phases were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by flash chromatography on silica (20% EtOAc in hexanes) to provide compound **10** as a colorless oil (0.51 g, 97% yield)

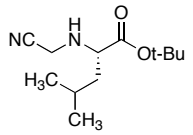


(*S*)-*tert*-butyl 2-(cyanomethylamino)propanoate (10). $[\alpha]_D^{20}$ –75.0 (*c* 0.04, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.60 (d, 2H, *J* = 1.5 Hz, $NCCH_2$), 3.38 (q, 1H, *J* = 7.0 Hz, NCH), 1.48 (s, 9H, $OCMe_3$), 1.30 (d, 3H, *J* = 7.0 Hz, Me); ^{13}C NMR ($CDCl_3$) δ 173.4, 117.7, 82.1, 56.0, 35.6, 28.2, 18.7; IR (thin film) ν 3341.5, 2980.4, 2936.5, 1726.9, 1453.5, 1370.6, 1253.5, 1156.1 cm^{-1}; HRMS (ESI) calcd for $C_9H_{16}N_2O_2$ Na $[M+Na]^+$ *m/z*: 207.1109, found 207.1152.

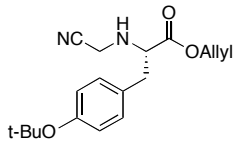
(2) Tokuyama, H.; Kuboyama, T.; Amano, A.; Yamashita, T.; Fukuyama, T. "A Novel Transformation of Primary Amines to *N*-Monoalkylhydroxylamines." *Synthesis*, **2000**, 1299–1304. (b) Tokuyama, H.; Kuboyama, T.; Fukuyama, T. "Transformation of Primary Amines to *N*-Monoalkylhydroxylamines: *N*- Hydroxy-(S)-1-Phenylethylamine Oxalate." *Org. Synth.*, **2003**, *80*, 207–212.



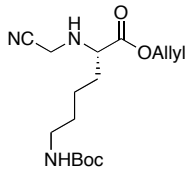
(*S*)-*tert*-butyl 2-(cyanomethylamino)-3-phenylpropanoate. Cyanomethylation of (*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-aminium chloride (1.00 g, 3.89 mmol, 1.00 equiv) was executed according to the general procedure to afford the cyanomethylated product as a colorless oil (0.98 g, 97% yield). $[\alpha]_D^{20}$ +2.5 (*c* 0.20, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.31–7.21 (m, 5H, Ph), 3.58–3.55 (m, 1H, NCH), 3.51 (d, 2H, *J* = 7.5 Hz, $NCCH_2$), 3.01 (dd, 1H, *J* =14.0, 6.0 Hz, $PhCH_2$), 2.90 (dd, 1H, *J* = 13.5, 7.5 Hz, $PhCH_2$), 1.89 (br, 1H), 1.42 (s, 9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 172.2, 136.7, 129.4, 128.6, 127.0, 117.5, 82.3, 61.6, 39.4, 36.0, 28.0; IR (thin film) ν 3337.2, 2979.0, 2933.2, 1725.5, 1455.5, 1368.7, 1153.7 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{25}N_2O_3$ $[M+H]^+$ *m/z*: 317.1874, found 317.1865.



(*S*)-*tert*-butyl 2-(cyanomethylamino)-4-methylpentanoate. Cyanomethylation of (*S*)-1-(*tert*-butoxy)-4-methyl-1-oxopentan-2-aminium (2.50 g, 10.7 mmol, 1.00 equiv) was executed according to the general procedure, and the crude oil was purified by flash chromatography on silica (15% EtOAc in hexanes) to afford the cyanomethylated product as a colorless oil (2.40 g, 99% yield). $[\alpha]_D^{20}$ –35.4 (*c* 1.70, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.61–3.50 (m, 2H, $NCCH_2$), 3.25 (br s, 1H, NCH), 1.79–1.75 (br m, 2H, CH_2), 1.50–1.39 (m, 10H, CH and $OCMe_3$), 0.93 (t, 6H, *J* = 6.0 Hz, 2 x Me); ^{13}C NMR ($CDCl_3$) δ 173.9, 117.8, 82.1, 59.6, 42.5, 36.2, 28.2, 25.0, 22.9, 22.2; IR (thin film) ν 3336.7, 2958.7, 2935.6, 1725.9, 1473.3, 1368.7, 1150.8 cm^{-1}; HRMS (ESI) calcd for $C_{12}H_{23}N_2O_2$ $[M+H]^+$ *m/z*: 227.1760, found 227.1756.

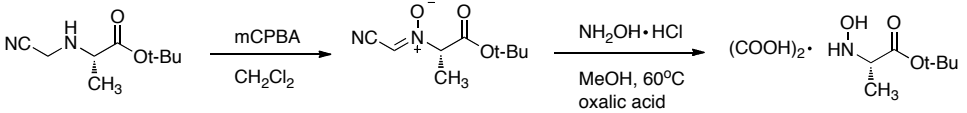


(*S*)-allyl 3-(4-*tert*-butoxyphenyl)-2-(cyanomethylamino)propanoate. Cyanomethylation of (*S*)-allyl 2-amino-3-(4-(*tert*-butoxy)phenyl)propanoate (10.5 g, 38.1 mmol, 1.00 equiv) was executed according to the general procedure, and the crude oil was purified by flash chromatography on silica (15% EtOAc in hexanes) to afford a colorless oil (10.4 g, 86% yield). $[\alpha]_D^{20}$ –4.0 (*c* 0.15, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.07 (d, 2H, *J* = 8.0 Hz, Ar), 6.91 (d, 2H, *J* = 7.5 Hz, Ar), 5.89–5.81 (m, 1H, OCH_2CHCH_2), 5.31–5.23 (m, 2H, $CHCH_2$), 4.60 (d, 2H, *J* = 5.5 Hz, OCH_2), 3.66 (br, 1H, NHCH), 3.54 (s, 2H, $NCCH_2$), 3.04 (dd, 1H, *J* = 14.0, 6.0 Hz, $PhCH_2$), 2.90 (dd, 1H, *J* = 14.0, 6.0 Hz, $PhCH_2$), 1.87 (br s, 1H), 1.32 (s, 9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 172.7, 154.6, 131.6, 131.0, 129.8, 124.4, 119.2, 117.3, 78.6, 66.0, 61.3, 38.7, 36.1, 28.9; IR (thin film) ν 3341.5, 2977.5, 2936.5, 1734.7, 1507.1, 1366.3, 1236.6, 1162.9; HRMS (ESI) calcd for $C_{18}H_{25}N_2O_3$ $[M+H]^+$ *m/z*: 317.1874, found 317.1865.



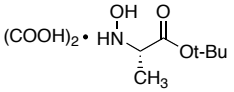
(*S*)-allyl 6-(*tert*-butoxycarbonylamino)-2-(cyanomethylamino)hexanoate. Cyanomethylation of (*S*)-allyl 2-amino-6-((*tert*-butoxycarbonyl)amino)hexanoate (7.24 g, 25.3 mmol, 1.00 equiv) was executed according to the general procedure, and the crude oil was purified by flash chromatography on silica (15% EtOAc in hexanes) to afford the product as a colorless oil (7.13 g, 86% yield). $[\alpha]_D^{20}$ –17.5 (*c* 0.24, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.97–5.89 (m, 1H, OCH_2CHCH_2), 5.36–5.27 (m, 2H, $CHCH_2$), 4.65 (d, 2H, *J* = 5.5 Hz, OCH_2), 4.55 (br, s, 1H, NHCO), 3.64–3.55 (m, 2H, $NCCH_2$), 3.38 (br s, 1H, NHCH), 3.10 (d, 2H, *J* = 6.5 Hz, CH_2), 1.91 (br s, 1H), 1.76–1.71 (m, 1H, CH_2), 1.67–1.60 (m, 1H, CH_2), 1.51–1.38 (m, 13H, 2 x CH_2 and $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 173.5, 156.1, 131.7, 119.3, 117.5, 79.3, 66.0, 60.2, 40.3, 36.1, 32.6, 29.8, 28.6, 22.8; IR (thin film) ν 3345.4, 2937.0, 1731.7, 1702.3, 1520.1, 1175.8 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{27}N_3O_4Na$ $[M+Na]^+$ *m/z*: 348.1899, found 348.1893.

2.2 General procedure for the preparation of *N*-hydroxyamino acid ester oxalates[2]



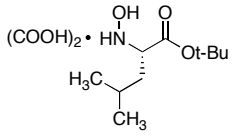
10 **11** **12**

(*S*)-*tert*-butyl 2-(cyanomethylamino)propanoate **(10)** (1.09 g, 5.92 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (20 mL), stirred, and cooled to 0 °C. To the cooled reaction mixture, *m*CPBA (2.55 g, 14.8 mmol, 2.00 equiv) was added in several in portions over 30 min. The solution was allowed to warm to rt and stirred for 45 min. The reaction was quenched by addition of saturated aq $Na_2S_2O_3$ (15 mL) and saturated aq $NaHCO_3$ (25 mL) and the resulting biphasic mixture stirred for an additional 30 min. The mixture was then transferred to a separatory funnel and the organic phase removed. The aqueous phase was extracted with CH_2Cl_2 (2 x 30 mL), the organic phases were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to provide the cyano-nitrone as a yellow oil. The yellow oil was immediately dissolved in MeOH (57.7 mL), hydroxylamine hydrochloride (2.05 g, 29.6 mmol, 5.00 equiv) was added to the solution, and stirred overnight at 60 °C. The reaction was allowed to cool to rt and CH_2Cl_2 (20 mL) was added to provide a white precipitate which was removed by vacuum filtration. Concentration of the filtrate in vacuo afforded a yellow oil. The oil was then dissolved CH_2Cl_2 (20 mL), transferred to a separatory funnel and extracted with saturated aq $NaHCO_3$ (30 mL). The aqueous phase was washed with CH_2Cl_2 (2 x 20 mL), the organic phases were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to a reduced volume. Oxalic acid (1.45 g, 1.18 mmol, 2.00 equiv) in MeOH (2 mL) was added to the solution, followed by cold Et_2O (50 mL), producing a white precipitate which was collected by vacuum filtration and rinsed several times with cold Et_2O to afford compound **12** as a white solid (0.768 g, 53% yield).

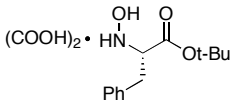


(*S*)-*tert*-butyl 2-(hydroxyamino)propanoate oxalate (12). mp 138–140 °C; $[\alpha]_D^{20}$ –19.3 (*c* 0.15, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.70 (br s, 3H, OH), 3.71–3.68 (m, 1H, NHCH), 1.42 (s, 9H, $OCMe_3$),

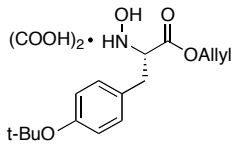
1.18 (d, 3H, J = 6.5 Hz, Me); ^{13}C NMR (DMSO-d_6) δ 170.8, 163.2, 81.4, 59.8, 27.7, 13.5; IR (KBr) ν 3424.4, 2296.3, 2937.0, 1747.1, 1736.1, 1631.9, 1217,8, 1157.5; ESI calcd for $C_7H_{16}NO_3$ $[M+H]^+$ *m/z*: 162.1, found 162.5.



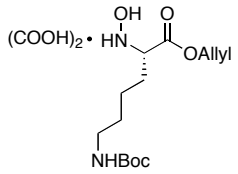
(*S*)-*tert*-butyl 2-(hydroxyamino)-4-methylpentanoate oxalate (14). (*S*)-*tert*-butyl 2-(cyanomethylamino)-4-methylpentanoate (0.550 g, 2.28 mmol, 1.00 equiv) was converted to the corresponding *N*-hydroxylamine oxalate according to the general procedure, and isolated as a white solid (0.650 g, 97% yield). mp 97–100 °C; $[\alpha]_D^{20}$ +1.7 (*c* 0.34, MeOH); ^{1}H NMR (D_3COD) δ 3.94 (t, 1H, J = 7.0 Hz, NHCH), 1.80–1.77 (br m, 1H, *CH*Me$_2$), 1.70–1.68 (br m, 2H, iPr*CH$_2$*), 1.53 (s, 9H, OCMe$_3$), 0.99 (t, 6H, J = 5.5 Hz, 2 x Me); ^{13}C NMR (D_3COD) δ 169.2, 165.0, 85.0, 64.3, 37.5, 28.2, 26.1, 23.2, 22.0; IR (KBr) ν 3426.4, 2963.5, 1743.8, 1596.2, 1253.9, 1158.0 cm^{-1}; ESI cald for $C_{10}H_{22}NO_3$ $[M+H]^+$ *m/z*: 204.2, found 204.5.



(*S*)-*tert*-butyl 2-(hydroxyamino)-3-phenylpropanoate oxalate (15). (*S*)-*tert*-butyl 2-(cyanomethylamino)-3-phenylpropanoate (2.72 g, 10.5 mmol, 1.0 equiv) was converted to the corresponding *N*-hydroxylamine oxalate according to the general procedure and isolated as a white solid (3.21 g, 93% yield). mp 89–92 °C; $[\alpha]_D^{20}$ +16.7 (*c* 0.12, MeOH); ^{1}H NMR (D_3COD) δ 7.34–7.26 (m, 5H, Ph), 4.20–4.17 (br m, 1H, NHCH), 3.31-3.26 (m, 1H, Ph*CH$_2$*), 3.03 (dd, 1H, J = 13.5, 9.5 Hz, Ph*CH$_2$*), 1.33 (s, 9H, OCMe$_3$); ^{13}C NMR (D_3COD) δ 168.6, 164.4, 135.8, 130.6, 129.7, 128.5, 84.8, 66.7, 34.7, 28.0; IR (thin film) ν 2980.4, 2561.0, 2492.5, 1740.9, 1642.5, 1253.9, 1155.1, 1253.9, 1155.1 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{19}NO_3Na$ $[M + Na]^+$ *m/z* 260.1263 found 260.1241.

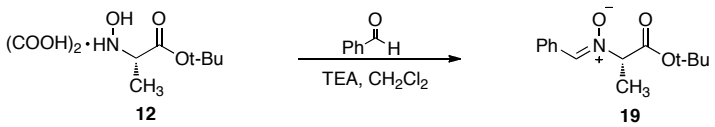


(*S*)-allyl 3-(4-*tert*-butoxyphenyl)-2-(hydroxyamino)propanoate oxalate (17). (*S*)-allyl 3-(4-*tert*-butoxyphenyl)-2-(cyanomethylamino)propanoate (1.02 g, 3.10 mmol, 1.00 equiv) was converted to the corresponding *N*-hydroxylamine oxalate according to the general procedure and isolated as a white solid (1.17 g, 98% yield). mp 105.0–106.5 °C; $[\alpha]_D^{20}$ –3.2 (*c* 0.19, MeOH); ^{1}H NMR (CD_3OD) δ 7.13 (d, 2H, *J* = 8.0 Hz, Ar), 6.93 (d, 2H *J* = 8.5 Hz, Ar), 5.86–5.78 (m, 1H, OCH_2CHCH_2), 5.27–5.17 (m, 2H, $CHCH_2$), 4.59–4.58 (m, 2H, OCH_2), 4.04 (t, 1H, *J* = 7.0 Hz, NHCH), 3.05 (dd, 1H, *J* = 14.0, 6.5 Hz, $ArCH_2$), 3.00–2.94 (m, 1H, $ArCH_2$), 1.32 (s, 9H, $OCMe_3$); ^{13}C NMR (CD_3OD) δ 169.1, 163.1, 154.7, 131.4, 129.8, 124.2, 118.1, 115.3, 78.4, 66.1, 65.6, 33.0, 27.9; IR (thin film) ν 3424.9, 3258.6, 2977.0, 1739.9, 1507.1, 1365.8, 1236.6, 1162.8 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{23}NO_4$ $[M+H]^+$ *m/z*: 294.1705, found 294.1707.

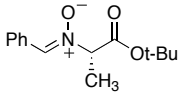


(*S*)-allyl 6-(*tert*-butoxycarbonylamino)-2-(hydroxyamino)hexanoate oxalate (18). (*S*)-allyl 6-(*tert*-butoxycarbonylamino)-2-(cyanomethylamino)hexanoate (3.22 g, 9.90 mmol, 1.00 equiv) was converted to the corresponding *N*-hydroxylamine oxalate according to the general procedure, and isolated as a white solid (3.49 g, in 90% yield). mp 105–106.5 °C; $[\alpha]_D^{20}$ –1.1 (*c* 0.54, MeOH); ^{1}H NMR (CD_3OD) δ 6.01–5.93 (m, 1H, OCH_2CHCH_2), 5.37 (dd, 1H, *J* = 15.5, 1.5 Hz, $CHCH_2$), 5.26 (d, 1H, *J* = 10.5 Hz, $CHCH_2$), 4.70 (d, 2H, *J* = 5.5 Hz, OCH_2), 3.84 (br s, 1H, NHCH), 3.03 (t, 2H, *J* = 6.7 Hz, $NHCH_2$), 1.76 (br d, 2H, CH_2), 1.43–1.36 (m, 13H, 2 x CH_2 and $OCMe_3$); ^{13}C NMR (CD_3OD) δ 171.0, 164.4, 157.3, 131.8, 118.0, 78.6, 65.8, 64.5, 39.6, 29.4, 27.8, 27.5, 22.6; IR (thin film) ν 3390.2, 2936.5, 2975.6, 1746.2, 1692.7, 1522.0, 1263.2, 1175.4 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{26}N_2O_5Na$ $[M+Na]^+$ *m/z*: 325.1739, found 325.1734.

2.3 General procedure for the preparation of *N*-benzylidene nitrones

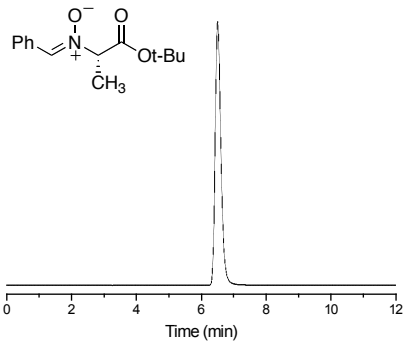


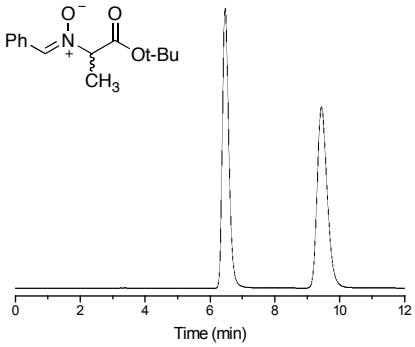
The following procedure is representative. (*S*)-*tert*-butyl 2-(hydroxyamino)propanoate oxalate **12** (0.25 g, 0.99 mmol, 1.0 equiv) was dissolved in CH_2Cl_2, (9 mL). To this solution, benzaldehyde (0.15 mL, 1.5 mmol, 1.5 equiv) and triethylamine (0.41 mL, 3.0 mmol, 3.0 equiv) were added and the mixture stirred overnight. The reaction was transferred to a separatory funnel, and H_2O (40 mL) was added. The organic phase was removed; the aqueous solution was extracted with CH_2Cl_2 (2 x 40 mL), and the organic phases were combined and washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica (30% EtOAc in hexanes), to afford compound **19** as white foam (0.20 g, 81% yield).

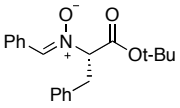


(*S*)-*N*-benzylidene-1-*tert*-butoxy-1-oxopropan-2-amine oxide (19). $[\alpha]_D^{20}$ –23.7 (*c* 0.16, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.25–8.23 (m, 2H, Ph), 7.43 (s, 1H, Ph*CH*NO), 7.40–7.39 (m, 3H, Ph), 4.65 (q, 1H, *J* = 7.0 Hz, NCH), 1.72 (d, 3H, *J* = 7.0 Hz, Me), 1.46 (s, 9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 167.2, 134.7, 130.6, 130.4, 128.8, 128.5, 82.9, 74.0, 27.9, 15.6; IR (thin film) ν 2980.4, 29.36.5, 1736.5, 1585.2, 1453.5, 1156.1 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{19}NO_3Na$ $[M+Na]^+$ *m/z*: 272.1263, found 272.1271.

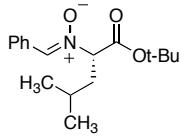
HPLC: ChiralPak AS-H chiral column, flow rate = 1 mL/min, 10% iPrOH in hexanes



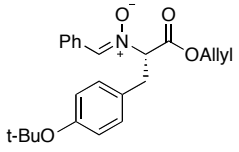




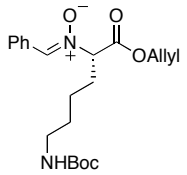
(*S*)-*N*-benzylidene-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-amine oxide. (*S*)-*tert*-butyl 2-(hydroxyamino)-3-phenylpropanoate oxalate **(15)** (0.16 g, 0.48 mmol, 1.0 equiv) was converted to the corresponding *N*-benzylidene nitrone according to the general procedure, and was purified by flash column chromatography on silica (15% EtOAc in hexanes) to provide the title compound as a white solid (0.150 g, 96% yield). mp 150–153 °C; $[\alpha]_D^{20}$ –238.0 (*c* 1.01, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 8.17–8.16 (m, 2H, Ph), 7.41–7.39 (m, 3H, Ph), 7.25–7.24 (m, 5H, Ph), 7.13 (s, 1H, Ph*CH*NO), 4.62 (dd, 1H, *J* = 9.5, 4.5 Hz, NCH), 3.66 (dd, 1H, *J* = 14.5, 9.5 Hz, $PhCH_2$), 3.34 (dd, 1H, *J* = 14.5, 9.5 Hz, $PhCH_2$), 1.48 (s, 9H, $OCMe_3$); ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.1, 136.7, 135.8, 130.6, 130.3, 129.2, 128.8, 128.7, 128.5, 127.1, 83.3, 80.3, 35.4, 27.9; IR (thin film) ν 2983.8, 2933.2, 1727.9, 1584.7, 1295.4, 1151.2; HRMS (ESI) calcd for $C_{17}H_{25}NO_3Na$ $[M+H]^+$ *m/z*: 314.1732, found 314.1718.



(*S*)-*N*-benzylidene-1-*tert*-butoxy-4-methyl-1-oxopentan-2-amine oxide. (*S*)-*tert*-butyl 2-(hydroxyamino)-4-methylpentanoate oxalate **(14)** (0.96 g, 3.30 mmol, 1.00 equiv) was converted to the corresponding *N*-benzylidene nitrone according to the general procedure, and purified by flash column chromatography on silica (15 % EtOAc in hexanes) to provide the title compound as a white solid (0.83 g, 86 % yield). mp 136–140 °C; $[\alpha]_D^{20}$ –79.6 (*c* 0.24, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.25 (t, 2H, *J* = 4.0 Hz, Ph), 7.42–7.40 (m, 4H, Ph and Ph*CH*NO), 4.57–4.54 (m, 1H, CH), 2.28–2.22 (m, 1H, iPrCH_2), 1.86–1.80 (m, 1H, iPrCH_2), 1.69–1.63 (m, 1H, *CH*Me_2), 1.47 (s, 9H, $OCMe_3$), 0.98 (d, 6H, *J* = 6.5 Hz, CH*Me*$_2$); ^{13}C NMR ($CDCl_3$) δ 167.2, 135.2, 130.6, 130.5, 128.9, 128.6, 83.0, 38.3, 28.0, 24.9, 23.0, 21.9; IR (thin film) ν 2982.3, 2956.8, 2933.6, 1734.6, 1577.9, 1369.2, 1154.1; HRMS (ESI) calcd for $C_{17}H_{25}NO_3Na$ $[M+Na]^+$ *m/z*: 314.1732, found 314.1718.

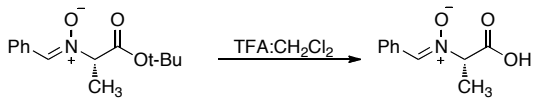


(*S*)-1-(allyloxy)-*N*-benzylidene-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-amine oxide. (*S*)-allyl 3-(4-*tert*-butoxyphenyl)-2-(hydroxyamino)propanoate oxalate **(17)** (2.0 g, 5.2 mmol, 1.0 equiv) was dissolved in DMF (13 mL) and converted to the corresponding nitrone according to the general procedure. The product was purified by flash column chromatography on silica (15% EtOAc in hexanes) to afford the title compound as a colorless oil (1.51 g, 75% yield). $[\alpha]_D^{20}$ –198.1 (*c* 0.27, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, 2H, *J* = 7.5, 2.0 Hz, Ph), 7.39–7.37 (m, 3H, Ph), 7.11 (d, 2H, *J* = 8.5 Hz, Ar), 7.05 (s, 1H, Ph*CH*NO), 6.84 (d, 2H, *J* = 8.5 Hz, Ar), 5.93–5.85 (m, 1H, OCH_2CHCH_2), 5.35–5.22 (m, 2H, $CHCH_2$), 4.77–4.64 (m, 3H, OCH_2 and NCH), 3.65 (dd, 1H, *J* = 14.0, 10.0 Hz, $PhCH_2$), 3.34 (dd, 1H, *J* = 14.5, 4.5 Hz, $PhCH_2$), 1.25 (s, 9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 166.6, 154.5, 136.5, 131.3, 131.2, 130.8, 130.0, 129.6,128.9, 128.6, 124.6, 119.2, 79.8, 78.6, 66.8, 34.7, 28.9; IR (thin film) ν 2977.1, 1747.7, 1506.6, 1365.3, 1236.6, 1161.42; HRMS (ESI) calcd for $C_{23}H_{28}NO_4$ $[M+H]^+$ *m/z*: 382.2018, found 382.2034.



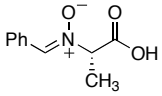
(*S*)-1-(allyloxy)-*N*-benzylidene-6-(*tert*-butoxycarbonylamino)-1-oxohexan-2-amine oxide. (*S*)-allyl 6-(*tert*-butoxycarbonylamino)-2-(hydroxyamino)hexanoate oxalate **(18)** (2.06 g, 5.25 mmol, 1.00 equiv) was dissolved in DMF (26 mL) and converted to the corresponding *N*-benzylidene nitrone according to the general procedure, and purified by flash chromatography on silica (45% EtoAc in hexanes) to afford compound the title compound as white foam (1.64 g, 80 % yield). $[\alpha]_D^{20}$ –52.1 (*c* 0.52, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.26–8.25 (m, 2H, Ph), 7.46–7.43 (m, 4H, Ph and Ph*CH*NO), 5.93–5.85 (m, 1H, OCH_2CHCH_2), 5.32 (d, 1H, *J* = 17 Hz, $CHCH_2$), 5.23 (d, 1H, *J* = 10.5, $CHCH_2$), 4.73–4.64 (m, 2H, OCH_2), 4.58–4.55 (m, 2H, NHCO and NO*CH*), 3.13–3.12 (m, 2H, $NHCH_2$), 2.44–2.36 (m, 1H, $CHCH_2$), 2.14–2.07 (m, 1H, $CHCH_2$), 1.57–1.52 (m, 3H, CH_2 x 2), 1.39 (br s, 10H, CH_2 and $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 167.2, 156.1, 136.2, 131.3, 131.0, 130.2, 129.0, 128.7, 119.3, 78.1, 66.7, 40.2, 29.7, 28.7, 28.6, 28.5, 23.1; IR (thin film) ν 2975.6, 2931.7, 1746.2, 1697.5, 1522.0, 1170.5 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{30}N_2O_5Na$ $[M+Na]^+$ *m/z*: 413.2052, found 413.2040.

2.4 General procedure for deprotection of *N*-benzylidene amino acid ester nitrones

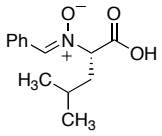


19 **20**

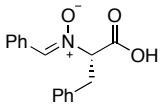
The following procedure is representative. (*S*)-*N*-benzylidene-1-*tert*-butoxy-1-oxopropan-2-amine oxide (**19)** (0.491 g, 1.98 mmol, 1.00 equiv) was dissolved in a 1:1 solution of CH_2Cl_2/TFA (20 mL) and stirred for 2 h at rt. The solvent was reduced to minimum volume, and cold Et_2O (15 mL) was added to the solution to provide a white precipitate. The precipitate was collected by vacuum filtration, and washed several times with Et_2O to afford compound **20** (0.30 g, 78% yield).



(*S*)-*N*-benzylidene-1-carboxyethanamine oxide (20). mp 141–142 °C; $[\alpha]_D^{20}$ –23.39 (*c* 1.03, MeOH); ^{1}H NMR (CD_3OD) δ 8.28 (dd, 2H, *J* = 7.5, 1.5 Hz, Ph), 7.96 (s, 1H, Ph*CH*NO), 7.50–7.46 (m, 3H, Ph), 5.03 (q, 1H, *J* = 7.0 Hz, CH), 1.71 (d, 3H, *J* = 7.0 Hz, Me); ^{13}C NMR (CD_3OD) δ 171.0, 140.0, 132.6, 131.4, 130.7, 129.6, 73.5, 15.6; IR (KBr) ν 3436.0, 29.0, 1752.0, 1633.4, 1148.4, 1082.8; HRMS (ESI) cald for $C_{10}H_{12}NO_3$ $[M+H]^+$ *m/z*: 194.0817, found 194.0819.



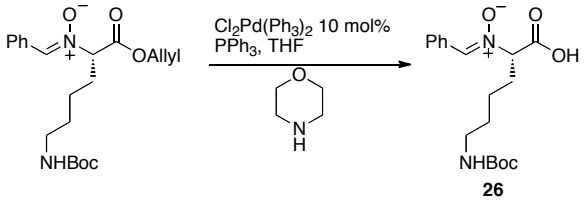
(*S*)-*N*-benzylidene-1-carboxy-3-methylbutan-1-amine oxide (23). (*S*)-*N*-benzylidene-1-*tert*-butoxy-4-methyl-1-oxopentan-2-amine oxide (0.340 g, 1.17 mmol, 1.00 equiv) was dissolved in 1:1 CH_2Cl_2/TFA (12 mL) and stirred for 2h at rt. The solution was concentrated in vacuo and compound **23** was isolated without further purification as a colorless oil (0.230 g, 84% yield). $[\alpha]_D^{20}$ –86.9 (*c* 0.16, MeOH); ^{1}H NMR (CD_3OD) δ 8.29 (d, 2H, *J* = 6.5 Hz, Ph), 8.00 (s, 1H, Ph*CH*NO), 7.51–7.47 (m, 3H, Ph), 4.96 (dd, 1H, *J* = 10.5, 4.5 Hz, CH), 2.32–2.26 (m, 1H, iPrCH_2), 1.86–1.80 (m, 1H, iPrCH_2), 1.62–1.57 (m, 1H, *CH*Me_2), 1.02–1.00 (m, 6H, Me x 2); ^{13}C NMR (CD_3OD) δ 169.8, 139.5, 131.3, 130.1, 129.4, 128.4, 75.4, 37.4, 24.7, 22.2, 20.5; IR (thin film) ν 2959.2, 1672.4, 1458.4, 1199.9; HRMS (ESI) calcd for $C_{13}H_{16}NO_3$ $[M-H]^-$ *m/z*: 234.1, found 234.6.



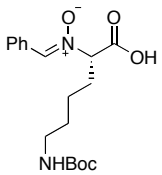
(*S*)-*N*-benzylidene-1-carboxy-2-phenylethanamine oxide (24). (*S*)-*N*-benzylidene-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-amine oxide (0.15 g, 0.42 mmol, 1.0 equiv) was dissolved in 1:1 CH_2Cl_2/TFA (4 mL) and stirred for 2 h at rt. The product was isolated according to the general procedure to provide compound **24** as a white solid (0.06 g, 53% yield). mp 155–157 °C; $[\alpha]_D^{20}$ –84.4 (*c* 0.32, MeOH); ^{1}H NMR (CD_3OD) δ 8.10 (d, 2H, *J* = 7.5 Hz, Ph), 7.45–7.37 (m, 4H, Ph and Ph*CH*NO), 7.26–7.16 (m, 5H, Ph), 4.88 (dd, 1H, *J* = 10.5, 4.0 Hz, CH), 3.54 (m, 1H, PhCH_2), 3.37 (dd, 1H, *J* = 14.3, 4.7 Hz, PhCH_2); ^{13}C NMR (CD_3OD) δ 169.5, 140.2, 137.4, 132.1, 130.4, 129.7, 129.3, 129.2, 127.8, 79.3, 35.9; IR (KBr)

ν 3438.9, 3029.1, 2863.2, 1725.9, 1456.4, 1229.8, 1130.5; HRMS (ESI) calcd for $C_{16}H_{16}NO_3$ $[M+H]^+$ *m/z*: 270.1130, found 270.1117.

General procedure for removal of allyl group



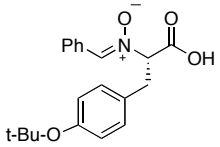
Removal of the allyl group was performed according to the published procedure.[3] To a round bottom flask charged with a magnetic stir bar, $Cl_2Pd(PPh_3)_2$ (0.53 g, 0.13 mmol, 10 mol%) PPh_3 (0.11 g, 0.41 mmol, 30 mol%), and THF (7 mL) were stirred under nitrogen for 40 min. (*S*)-1-(allyloxy)-*N*-benzylidene-6-(*tert*-butoxycarbonylamino)-1-oxohexan-2-amine oxide was placed under an inert atmosphere of nitrogen and dissolved in THF (6 mL). The solution was add to the palladium slurry with a syringe and morpholine (1.2 mL) was added dropwise to the reaction mixture. After stirring 2 h at rt, the solution was concentrated in vacuo and the resultant yellow solid dissolved in H_2O (50 mL) and washed with CH_2Cl_2 (3 x 50mL). The aqueous solution was then acidified with 1N HCl to a pH ~ 3 and extracted with CH_2Cl_2 (3 x 70mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was recrystallized from Et_2O to provide **26** as a white solid in (0.23 g, 50% yield).



(*S*)-*N*-benzylidene-5-(*tert*-butoxycarbonylamino)-1-carboxypentan-1-amine oxide (26). mp 138–140 °C; $[\alpha]_D^{20}$ –22.8 (*c* 0.14, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 2H, *J* = 7.5 Hz, Ph), 7.58–7.48 (m, 4H, Ph and Ph*CH*NO), 4.52–4.49 (m, 1H, CH), 3.10 (br s, 2H, NH*CH*$_2$), 2.35–2.31 (m, 1H, CH*CH*$_2$), 2.13

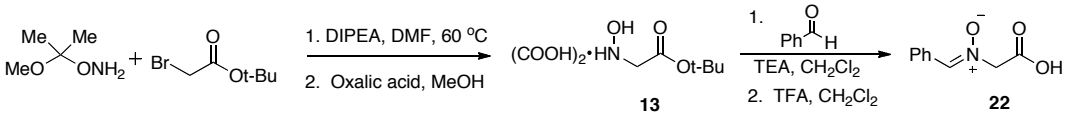
(3) Kunz, H.; Waldmann, H. "The Allyl Group as a Mildly and Selectively Removable Carboxy-Protecting Group for the Synthesis of Labile *O*-Glycopeptides." *Angew. Chem. Int. Ed.*, **1984**, *23*, 71–72.

(br d, 1H, CH*CH*$_2$), 1.56–1.53 (m, 2H, CH_2), 1.48–1.40 (m, 11H, CH_2 and OC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 169.4, 156.1, 139.7, 132.5, 130.4, 128.9, 128.7, 79.2, 75.2, 40.1, 31.3, 29.4, 28.4, 23.1; IR (thin film) ν 3340.5, 2932.2, 1687.4, 1520.1, 1165.7 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{26}N_2O_5Na$ $[M+Na]^+$ *m/z*: 373.1739, found 373.1731.



(*S*)-*N*-benzylidene-2-(4-*tert*-butoxyphenyl)-1-carboxyethanamine oxide (25). (*S*)-1-(allyloxy)-*N*-benzylidene-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-amine oxide (1.34 g, 3.51 mmol, 1.00 equiv) was converted to the carboxylic acid according to the general procedure to afford compound **25** as a white solid (0.710 g, 59% yield). mp 130–132 °C; $[\alpha]_D^{20}$ –211.2 (*c* 0.16, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 2H, *J* = 7.5 Hz, Ph), 7.50–7.38 (m, 3H, Ph), 7.08 (d, 2H, *J* = 8.0 Hz, Ar), 6.83 (d, 2H, *J* = 8.5 Hz, Ar), 6.77 (s, 1H, Ph*CH*NO), 4.46 (dd, 1H, *J* = 11.5, 3.5 Hz, CH), 3.49–3.44 (m, 1H, Ar*CH*$_2$), 3.36 (dd, 1H, *J* = 10.5, 3.5 Hz, Ar*CH*$_2$), 1.22 (s, 9H, OC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 168.9, 155.1, 140.5, 132.8, 130.5, 130.2, 129.6, 128.9, 128.0, 124.8, 78.8, 76.3, 37.8, 28.8; IR (thin film) ν 2976.1, 1734.6, 1506.6, 1450.6, 1237.1, 1160.4; HRMS (ESI) calcd for $C_{20}H_{24}NO_4$ $[M+H]^+$ *m/z*: 342.1705, found 342.1715.

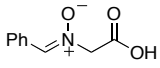
2.5 Preparation of *N*-benzylidene-1-carboxymethanamine oxide



***N*-benzylidene-1-carboxymethanamine oxide (22).** Prepared using the following 4 step procedure starting with *O*-(2-methoxyisopropyl)hydroxylamine[4] (4.5 g, 43.0 mmol, 1.0 equiv) and tert-butyl bromoacetate (7.6 mL, 52.0 mmol, 1.2 equiv) were dissolved in 8 mL of DMF, DIPEA (11.0 mL, 86.0 mmol, 2.0 equiv) was added and the solution stirred at 60 °C overnight. The solution was partitioned between saturated aq $NaHCO_3$ (100 mL) and CH_2Cl_2. The organic layer was removed and the aqueous phase extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were washed with brine,

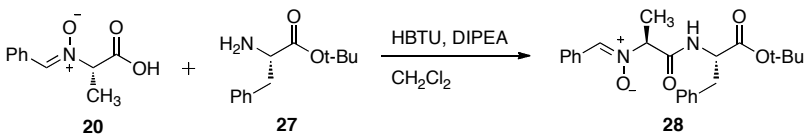
(4) Mori, K.; Koseki, K. "Synthesis of Trichostatin A, a Potent Differentiation Inducer of Friend Leukemic Cells, and its Antipode." *Tetrahedron*, **1988**, *44*, 6013–6020.

dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by flash chromatography on silica (30% EtOAc in hexanes) to afford *tert*-butyl 2-(((2-methoxypropan-2-yl)oxy)amino)acetate as a yellow oil (5.2 g, 55% yield). Deprotection of the hydroxylamine was achieved by stirring *tert*-butyl 2-(((2-methoxypropan-2-yl)oxy)amino)acetate for 2 h at rt with oxalic acid (8.6 g, 68 mmol, 3.0 equiv) in MeOH (100 mL, 0.2 M). Removal of the solvent, followed by recrystallization from Et_2O provided *tert*-butyl 2-(hydroxyamino)acetate oxalate as a white solid (3.0 g, 55% yield). Transformation to the corresponding *N*-benzylidine nitrone was achieved by stirring *tert*-butyl 2-(hydroxyamino)acetate oxalate (0.23 g, 0.96 mmol, 1.00 equiv), benzylaldehyde (0.12 mL, 1.2 mmol, 1.2 equiv), NEt_3 (0.15 mL, 1.06 mmol, 1.1 equiv), and CH_2Cl_2 (5.0 mL, 0.2 M) overnight. The reaction was transferred to a separatory funnel containing 30 mL of H_2O. The organic phase was removed and washed twice with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The yellow solid was recrystallized from Et_2O to provide *N*-benzylidene-2-*tert*-butoxy-2-oxoethanamine oxide (0.22 g, 99% yield). *N*-benzylidene-2-*tert*-butoxy-2-oxoethanamine oxide (0.22 g, 0.95 mmol, 1.00 equiv) was dissolved in a solution of 1:1 TFA/CH_2Cl_2 (10 mL, 0.1 M) and stirred at rt for 2 h. The solvent was reduced to 2.0 mL and cold Et_2O was added to precipitate **22** as white solid (0.16 g, 93% yield).



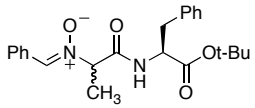
***N*-benzylidene-1-carboxymethanamine oxide (22).** mp 180–181 °C; ^{1}H NMR (CD_3OD) δ 8.28 (dd, 2H, J = 7.0, 4.0 Hz, Ph), 7.88 (s, 1H, Ph*CH*NO), 7.51–7.48 (m, 3H, Ph), 4.82 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 167.7 135.7, 130.7, 130.2, 128.4, 128.0, 67.7; IR (thin film) ν 3429.3, 1723.5, 1232.2, 1163.3, 1159.0 cm^{-1}; HRMS (ESI) calcd for $C_9H_{11}NO_3$ $[M+H]^+$ *m/z*: 180.0661, found 180.0694.

2.6 Preparation of nitrone dipeptide for epimerization studies

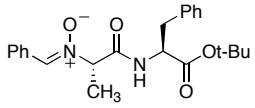


The following procedure is representative. To a solution of (*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-aminium chloride (0.036 g, 0.180 mmol, 1.00 equiv) in CH_2Cl_2 (2.0 mL), (*S*)-*N*-benzylidene-1-

carboxyethanamine oxide (0.045 g, 0.23 mmol, 1.30 equiv), HBTU (0.081 g, 0.22 mmol, 1.2 equiv), and DIPEA (0.10 mL, 0.54 mmol, 3.0 equiv) were added and stirred at rt overnight. The solution was transferred to a separatory funnel containing saturated aq $NaHCO_3$ (30 mL) and CH_2Cl_2 (30 mL). The organic phase was removed and the aqueous solution was extracted with CH_2Cl_2 (2 x 30 mL). The organic phases were combined and washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by PTLC (1:1 EtOAc/hexanes) to provide **29** as white foam (0.046 g, 50% yield).



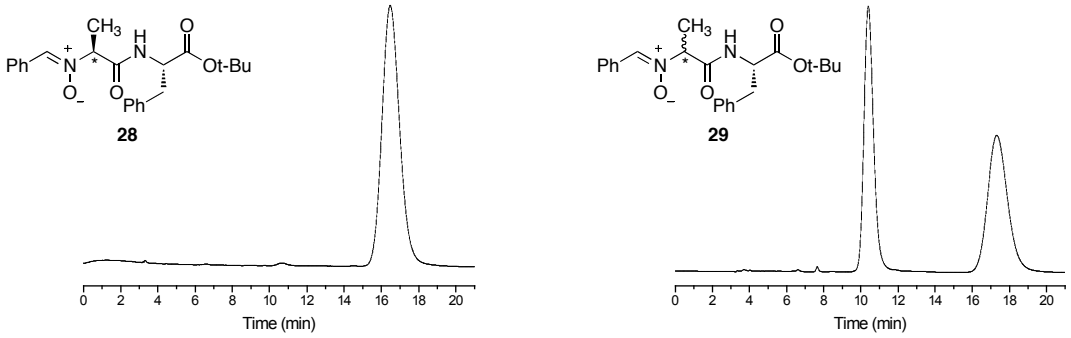
(*S*)-*N*-benzylidene-1-(1-*tert*-butoxy-1-oxo-3-phenylpropan-2-ylamino)-1-oxopropan-2-amine oxide (29). ^{1}H NMR ($CDCl_3$) δ 8.46 (t, 2H, *J* = 8.0 Hz, Ph), 8.27–8.23 (m, 4H, Ph), 7.49–7.43 (m, 8H,Ph and Ph*CH*NO), 7.22–7.18 (m, 3H, Ph), 7.04–6.93 (m, 5H,Ph), 4.68–4.49 (m, 2H, CH), 3.19–3.15 (dd, 1H, *J* = 14.0, 5.5 Hz, CH), 3.11–3.08 (dd, 1H, *J* = 13.5, 5.0 Hz, CH_2), 3.05–3.01 (dd, 1H, *J* = 14.0, 7.5 Hz, CH_2), 2.96–2.92 (dd, 1H, *J* = 13.5, 7.5 Hz, CH_2), 1.78 (d, 3H, *J* = 6.5 Hz, Me), 1.67 (d, 3H, *J* = 7.0 Hz, Me), 1.43 (s, 9H, $OCMe_3$), 1.34 (s, 9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.8, 168.4, 168.1, 136.4, 136.3, 136.2, 136.1, 131.4, 131.3, 130.0, 129.9, 129.6, 129.5, 129.4, 128.8, 128.7, 128.5, 128.4, 127.0, 126.8, 82.3, 82.2, 74.3, 74.2, 53.9, 53.8, 38.1, 38.0, 28.1, 28.0, 17.5, 17.3; IR (thin film) ν 2975.6, 2931.7, 1734.1, 1676.8, 1528.7, 1150.8.



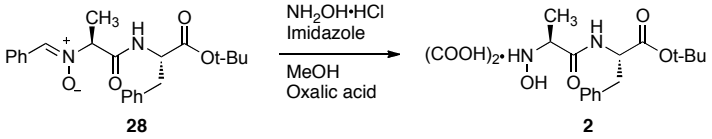
(*S*)-*N*-benzylidene-1-((*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-ylamino)-1-oxopropan-2-amine oxide (28). (*S*)-*N*-benzylidene-1-carboxyethanamine oxide (**20)** (0.300 g, 1.55 mmol, 1.20 equiv) was coupled to (*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-aminium chloride according to the general procedure. The product was purified by flash column chromatography on silica (60% EtOAc in hexanes) to provide **28** as white foam (0.300 g, 59% yield). $[\alpha]_D^{20}$ +46.4 (*c* 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.45 (d, 1H, *J* = 7.5 Hz, Ph), 8.24 (d, 2H, *J* = 7.5 Hz, Ph), 7.48–7.43 (m, 4H, Ph and Ph*CH*NO), 7.24–7.18 (m, 4H, Ph), 4.68 (q, 1H, *J* = 7.0 Hz, CH), 4.55 (q, 1H, *J* = 7.0 Hz, CH), 3.17 (dd, 1H, *J* = 14.0, 5.5 Hz, CH_2), 3.03 (dd, 1H, *J* = 14.0, 7.0 Hz, CH_2), 1.67 (d, 3H, *J* = 7.0 Hz, Me), 1.34 (s,

9H, $OCMe_3$); ^{13}C NMR ($CDCl_3$) δ 169.8, 168.1, 136.3, 136.1, 131.3, 129.9, 129.6, 129.4, 128.7, 128.5, 127.0, 82.3, 74.2, 53.9, 38.2, 28.0, 17.5; IR (thin film) ν 3271.6, 2978.5, 2937.0, 1734.1, 1676.3, 1528.7, 1151.7 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{27}N_2O_4$ $[M-H]^-$ *m/z*: 395.1971, found 395.1968.

HPLC: ChiralPak AS-H chiral column, flow rate = 1 mL/min, 10% iPrOH in Hexanes

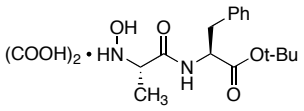


2.7 Hydrolysis of N-benzylidene nitrone dipeptides



(*S*)-*N*-benzylidene-1-((*S*)-1-*tert*-butoxy-1-oxo-3-phenylpropan-2-ylamino)-1 oxopropan-2-amine oxide **(28)** (50.0 mg, 0.130 mmol, 1.00 equiv) was dissolved in MeOH (1.3 mL), to this solution hydroxylamine hydrochloride (41.0 mg, 0.630 mmol, 5.00 equiv) was added and the reaction stirred overnight at 40 °C. The reaction was allowed to cool to rt and CH_2Cl_2 (3.0 mL) was added to induce a white precipitate. The solution was filtered, the precipitate was washed with CH_2Cl_2 and the filtrate was concentrated in vacuo to a reduced volume (ca. 1.0 mL). The solution was transferred to a separatory funnel containing saturated aq $NaHCO_3$ (30 mL) and CH_2Cl_2 (30 mL). The organic phase was removed and the aqueous phase extracted with CH_2Cl_2 (2 x 30mL). The organic phases were combined, washed with brine, dried over Na_2SO_4, and filtered. To the filtrate a solution of oxalic acid (0.032 g, 0.250 mmol, 2.00 equiv) in MeOH (1.0 mL) was added and the solution concentrated in vacuo to a reduced

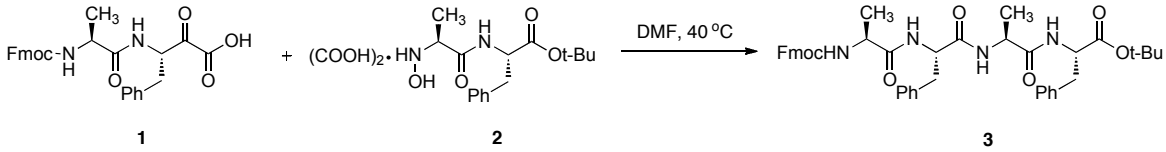
volume, followed by additon of cold Et_2O to produce a white precipitate. The precipitate was isolated by vacuum filtration, and washed with Et_2O to afford **2** as a white solid in (0.039 g, 77% yield).



(*S*)-*tert*-butyl 2-((*S*)-2-(hydroxyamino)propanamido)-3-phenylpropanoate oxalate (2). $[\alpha]_D^{20}$ +1.5 (*c* 0.20, MeOH); mp = 138–140 °C; ^{1}H NMR (CD_3OD) δ 7.30–7.22 (m, 5H, Ph), 4.48 (t, 1H, *J* = 7.0 Hz, CH), 3.83 (d, 1H, *J* = 6.5 Hz, CH), 3.12 (dd, 1H, *J* = 13.5, 6.2 Hz, Ph*CH*$_2$), 3.02 (dd, 1H, *J* = 14.0, 8.2 Hz, Ph*CH*$_2$), 1.30 (s, 9H, $OCMe_3$); ^{13}C NMR (CD_3OD) δ 171.7, 170.9, 165.6, 138.0, 130.4, 129.5, 127.9, 83.2, 61.1, 55.8, 38.3, 28.2, 14.3; IR (KBr) ν 3424.4, 3360.3, 2976.5, 2932.2, 1729.8, 1681.1, 1158.5 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{24}N_2O_4Na$ $[M+Na]^+$ *m/z*: 331.1634, found 331.1628.

2.8 Epimerization studies of chemoselective ligation

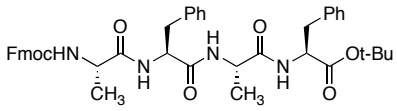
The preparation and characterization of compound **1** was published previously.[5]



1 **2** **3**

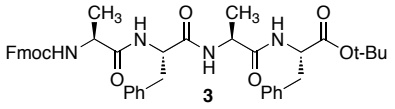
(*S*)-*tert*-butyl 2-((*S*)-2-(hydroxyamino)propanamido)-3-phenylpropanoate oxalate **(2)** (0.050 g, 0.130 mmol, 1.00 equiv) was added to the freshly prepared solution of (*S*)-3-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-2-oxo-4-phenylbutanoic acid **(1)** in DMF (1.0 mL) and stirred at 40 °C overnight. The reaction was transferred to a separatory funnel containing saturated aq $NaHCO_3$ (10 mL) and CH_2Cl_2 (10 mL) and the organic layer was removed. The aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL), the organic phases combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by flash column chromatography on silica (60% EtOAc in hexanes) to afford compound **3** as white foam (0.047 g, 50% yield).

(5) Ju, L.; Lippert, A. R.; Bode, J. W. "Stereoretentive Synthesis and Chemoselective Amide-Forming Ligations of C-Terminal Peptide α-Ketoacids." *J. Am. Chem. Soc.*, **2008**, *130*, 4253–4255.



(5*S*,8*S*,11*S*,14*S*)-*tert*-butyl 8,14-dibenzyl-1-(9*H*-fluoren-9-yl)-5,11-dimethyl-3,6,9,12-tetraoxo-2-oxa-4,7,10,13-tetraazapentadecan-15-oate (3). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, 2H, *J* = 7.5 Hz, Ar), 7.57 (t, 2H, *J* = 6.5 Hz, Ar), 7.4 (t, 2H, *J* = 7.5 Hz, Ar), 7.33–7.30 (m, 2H, Ar), 7.23–7.13 (m, 10H, Ph), 6.96 (br s, 1H, NH), 6.86 (br d, 1H, *J* = 6.5, NH), 6.74 (d, 1H, *J* = 6.0 Hz, NH), 5.38 (br d, 1H, *J* = 5.5 Hz, NH), 4.71–4.65 (m, 2H, CH x 2), 4.55–4.52 (br m, 1H, CH), 4.42–4.39 (m, 1H, CH), 4.24–4.15 (m, 3H, CH_2 and CH), 3.06–3.03 (m, 4H, CH_2 x 2), 1.36 (s, 9H, $OCMe_3$), 1.32–1.25 (m, 6H, Me x 2); ^{13}C NMR ($CDCl_3$) δ 172.4, 171.4, 170.4, 170.3, 156.3, 143.7, 141.5, 136.5, 136.3, 129.6, 129.3, 128.8, 128.4, 128.0, 127.2, 127.0, 125.2, 125.1, 120.2, 82.3, 67.3, 54.4, 53.9, 51.1, 49.0, 47.2, 38.2, 28.0, 18.8, 18.5; IR (thin film) ν 3281.2, 3063.3, 2975.6, 2931.7, 1634.3, 1527.8; HRMS (ESI) calcd for $C_{43}H_{48}N_4O_7Na$ $[M+Na]^+$ *m/z*: 755.3421, found 755.3422.

HPLC: ChiralPak AS-H chiral column, flow rate = 1 mL/min, 15% MeOH in CO_2.



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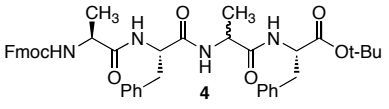

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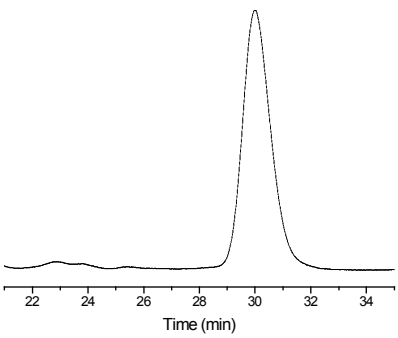
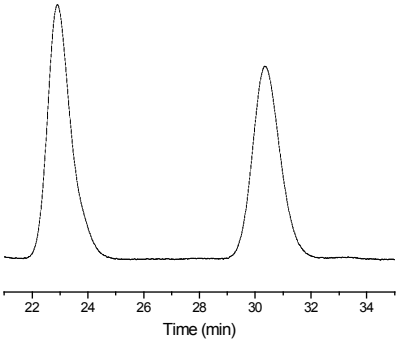
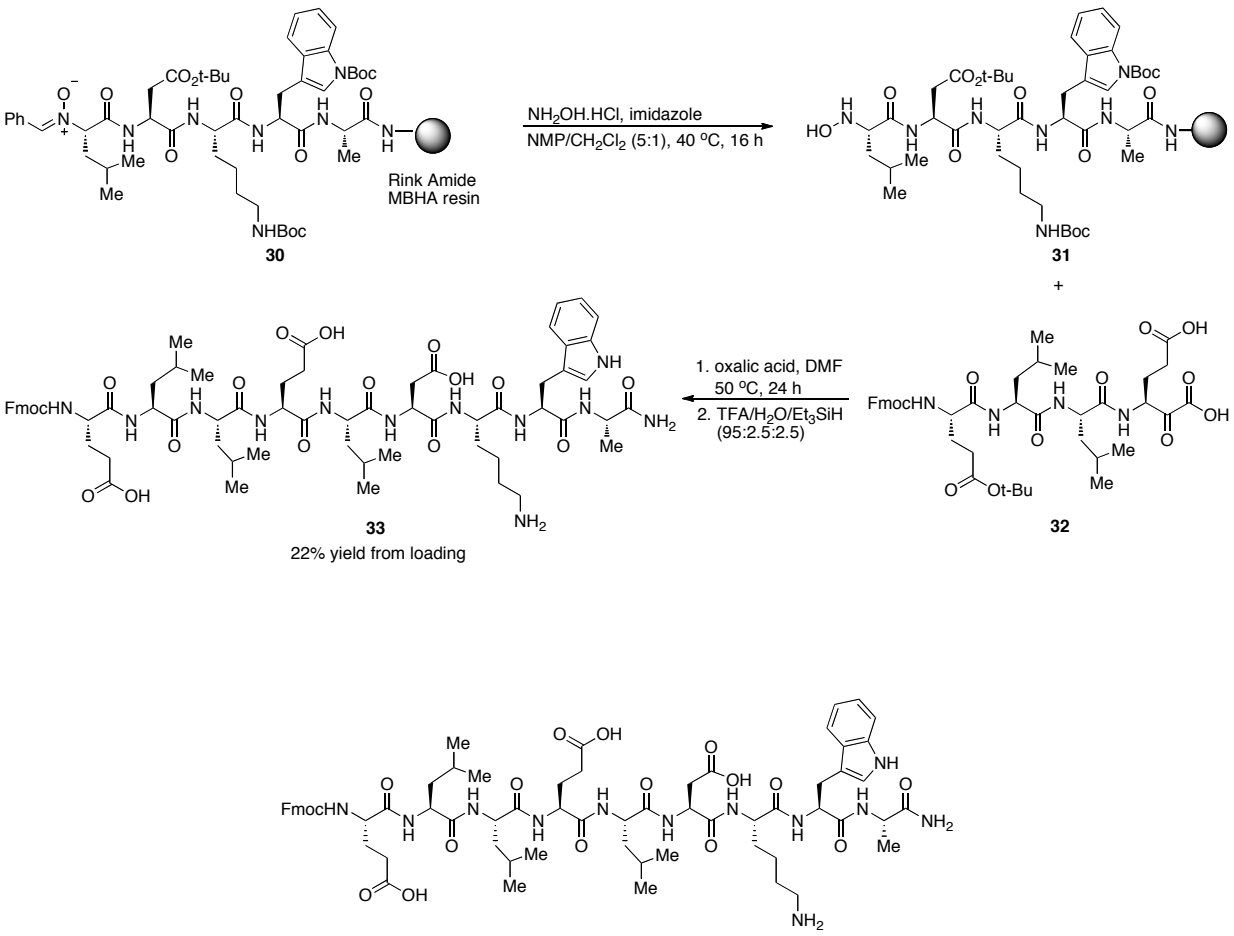




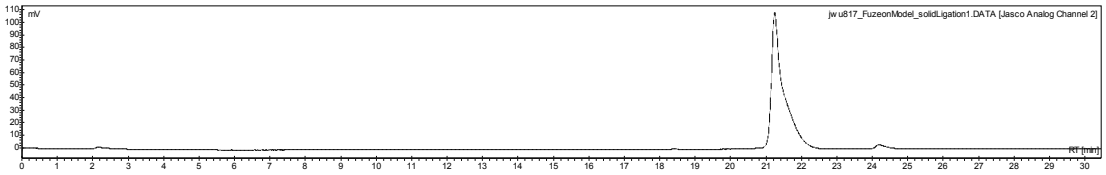
2.9 Synthesis of resin-bound N-terminal hydroxylamines



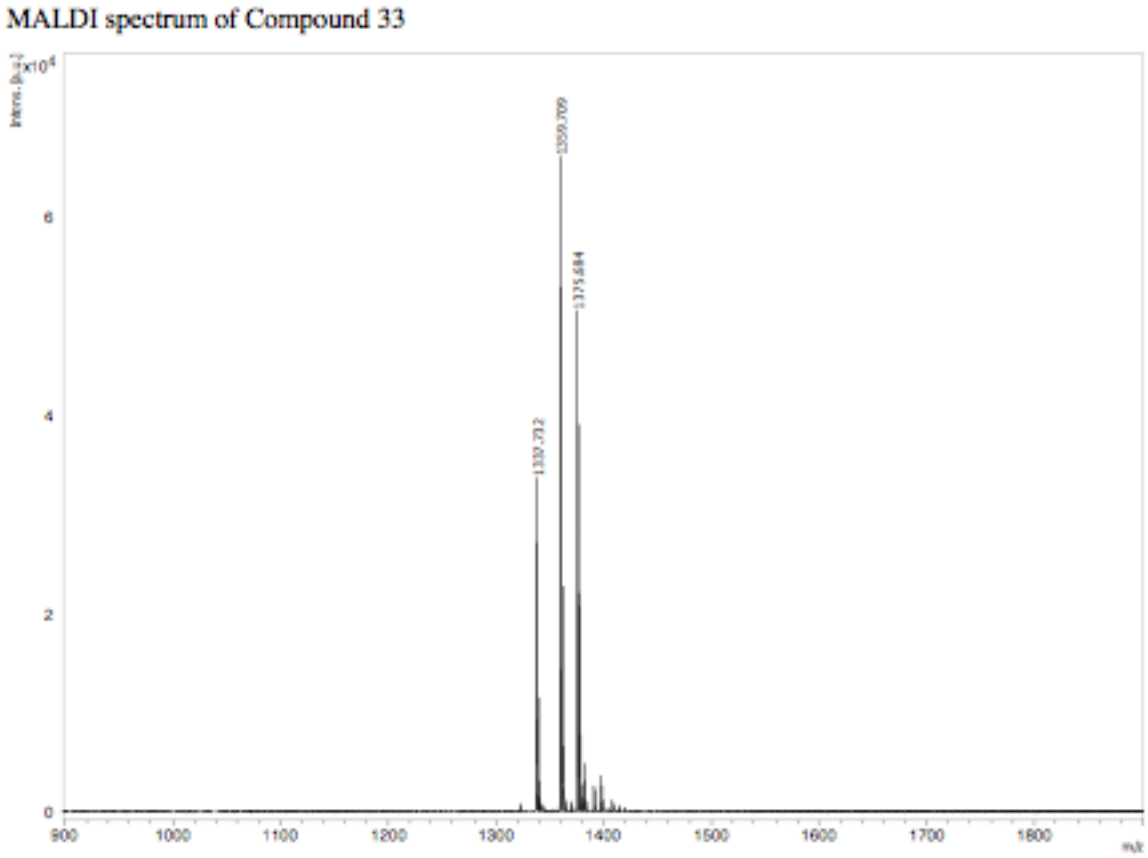
Fmoc-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Tyr-Ala-NH_2 (33). Resin-bound peptide **30** (20 mg, 0.010 mmol, 1 equiv) was added to a mixture (0.3 mL) of hydroxylamine hydrochloride (1.25 g) and imidazole (0.92 g) in NMP/CH_2Cl_2 (5 mL / 1mL), the resulting suspension was shaken at 40 °C for 16 h. The resin was filtered and rinsed with NMP and CH_2Cl_2 and dried in vacuo. The dry resin was mixed with crude (*S*)-3-((5*S*,8*S*,11*S*)-5-(3-(*tert*-butoxy)-3-oxopropyl)-1-(9*H*-fluoren-9-yl)-8,11-diisobutyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecanamido)-2-oxohexanedioic acid (**32)** (0.02 mmol, 2 equiv) and oxalic acid (3 mg, 0.02 mmol, 2 equiv) in DMF (0.2 mL). The resulting suspension was shaken at 50 °C for 24 h. The resin was filtered and washed with DMF and CH_2Cl_2. The crude peptide was cleaved from the resin by a solution of TFA/Et_3SiH/H_2O (95/2.5/2.5, 0.5 mL) for 1.5 h. The resin was filtered and

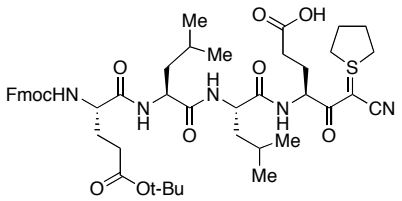
rinsed with CH_2Cl_2 and TFA. The combined filtrate was concentrated in vacuo, crude ligation product was precipitated and triturated with chilled Et_2O. A portion (7 mg) of crude ligation product was purified by preparative reverse phase HPLC using a linear gradient of 25–55% CH_3CN in H_2O with 0.1% TFA over 30 min with a flow rate of 20 mL/min, subsequent lyophilization afforded the ligated product **33** (3.0 mg, 22% from resin loading) as a white solid.

a) HPLC trace of purified ligation product: gradient 5–85% CH_3CN in H_2O with 0.1% TFA over 25 min with a flow rate of 1.0 ml/min, 280 nm, Zorbax Eclipse XDB-C8 4.6 × 150 mm column.

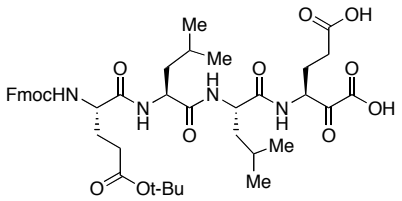


b) MALDI MS (*m/z*) [MH^+] calcd for $C_{67}H_{93}N_{12}O_{17}$, 1337.68, found 1337.73; [MNa^+] calcd for $C_{67}H_{92}N_{12}NaO_{17}$, 1359.66, found 1359.71; [MK^+] calcd for $C_{67}H_{92}KN_{12}O_{17}$, 1375.63, found 1375.68.





FmocGlu(O^{t}Bu)-Leu-Leu-Glu-α-cyanosulfur-ylide. The peptide was prepared on 2-chlorotrityl resin (NovaBiochem, initial loading 1.3 mmol/g) using standard Fmoc SPPS protocols. To 2-chlorotrityl resin (200 mg, 0.260 mmol, 1.0 equiv) in a centrifuge tube was added 2 mL of CH_2Cl_2 and allowed to pre-swell for 10 min. Then Fmoc-Leu-Glu(OH)-α-cyanosulfur-ylide (230 mg, 0.390 mmol, 1.5 equiv) and DIPEA (0.170 mL, 1.04 mmol, 4.0 equiv) were added to the mixture and the tube was shaken at room temperature for 1.5 h. The resin was filtered and washed with CH_2Cl_2 and DMF. The resin was dried under vacuum to give dipeptide loaded 2-chlorotrityl resin (340 mg), followed by Fmoc deprotection with 10% piperidine in DMF (3 mL) solution for 10 min. The resin was filtered off and washed thoroughly with DMF, iPrOH and CH_2Cl_2. The following coupling reaction was carried out using 4 equiv of the Fmoc protected amino acid, 4 equiv of HBTU, 4 equiv of HOBt·H_2O, and 6 equiv of DIPEA in CH_2Cl_2 (active esters were formed in solution phase at 0 °C for 20 min. After each coupling reaction (2 h at rt), the solution was removed by filtration and the resin washed with DMF and CH_2Cl_2. After the final coupling, the resin was cleaved with a solution of CH_2Cl_2/CF_3CH_2OH/HOAc (7/2/1) and purified by PTLC (66% yield from loading). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.5 Hz, 2H, Ar), 7.66 (d, *J* = 7.0 Hz, 2H, Ar), 7.38 (t, *J* = 7.5 Hz, 2H, Ar), 7.31 (t, *J* = 7.5 Hz, 2H, Ar), 4.64 (s, 1H, CH), 4.45-4.32 (m, 4H, 2 x CH and CH_2), 4.22 (t, *J* = 6.5 Hz, 1H, CH), 4.16-4.12 (m, 1H, CH), 3.57-3.48 (m, 2H, CH_2), 3.32-3.22 (m, 2H, CH_2), 2.50-2.40 (m, 2H, CH_2), 2.32 (t, *J* = 7.5 Hz, 2H, CH_2), 2.27-2.20 (m, 2H, CH_2), 2.15-1.82 (m, 7H, CH_2), 1.70-1.55 (m, 6H, CH_2 x 3), 1.44 (s, 9H, OC*Me*$_3$), 0.95-0.84 (m, 12H, Me x 6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 155.6, 142.4, 142.3, 139.7, 125.9, 125.3, 123.4, 118.0, 78.9, 65.2, 55.0, 53.4, 52.9, 50.3, 50.2, 45.5, 44.0, 43.7, 38.9, 38.6, 31.8, 29.8, 27.3, 26.6, 25.5, 23.0, 22.9, 20.7, 20.6, 19.1, 19.0; HRMS (ESI) (*m/z*) $[M+Na]^+$ calcd for $C_{47}H_{63}N_5NaO_{10}S$, 912.4193, found 912.4180.

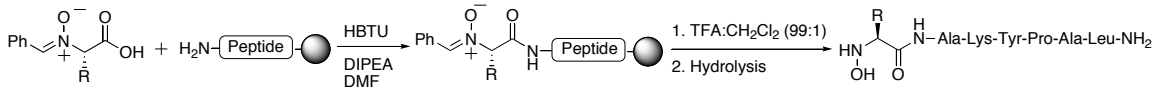


(*S*)-3-((5*S*,8*S*,11*S*)-5-(3-(*tert*-butoxy)-3-oxopropyl)-1-(9*H*-fluoren-9-yl)-8,11-diisobutyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecanamido)-2-oxohexanedioic acid (32) The peptide sulfur ylide FmocGlu(O^{t}Bu)-Leu-Leu-Glu-α-cyanosulfur-ylide (18 mg, 0.020 mmol, 1 equiv) was dissolved in 1:1 THF/H_2O (0.4 mL). Oxone (25 mg, 0.040 mmol, 2 equiv) was added and the slurry was stirred at rt for 40 min. Upon observing of the disappearance of the sulfur ylide, $(CH_3)_2S$ (0.03mL, 0.4 mmol, 20 equiv) was added to quench excess Oxone and stirred at rt for 5 min. Following the addition of DMF (0.2 mL) to the solution, THF, water and $(CH_3)_2S$ were removed in vacuo. The crude α-ketoacid was obtained as an approximately 0.1 M solution in DMF, which was directly used for the ligation without further purification.

2.10 Preparation of unprotected N-terminal hydroxylamine peptides

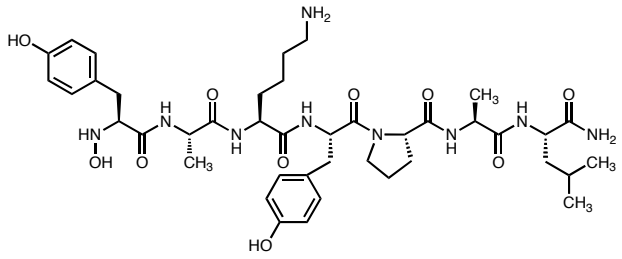
Standard protocol for the synthesis of peptides on solid support: All peptides were synthesized on rink amide MBHA resin with a loading of 0.70 mmol/g using standard Fmoc synthesis protocol. All couplings were carried out with Fmoc amino acids (4.0 equiv), HBTU (3.9 equiv), and DIPEA (8.0 equiv) in DMF. Prior to all coupling reactions, the resin was swelled in CH_2Cl_2 for 5 min. All Fmoc deprotections were carried out with a solution of 20% piperdine in DMF. All couplings were monitored by the Kaiser ninhydrin test.

General procedure for the synthesis of N-terminal peptide hydroxylamines

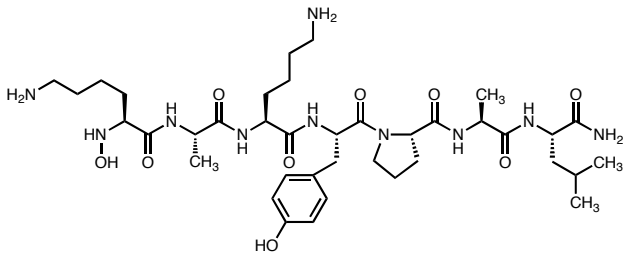


The following procedure is representative. Peptide (0.110 g) attached to rink amide resin was swelled in CH_2Cl_2 (2.0 mL) for 5 min. (*S*)-*N*-benzylidene-2-(4-*tert*-butoxyphenyl)-1-carboxyethanamine oxide (**25)** (0.110 g, 0.310 mmol, 4.00 equiv) and HBTU (0.120 g, 0.310 mmol, 3.90 equiv) were dissolved in DMF (1.00 mL) and DIPEA (0.110 mL, 0.630 mmol, 8.00 equiv) was added to the solution. After 5

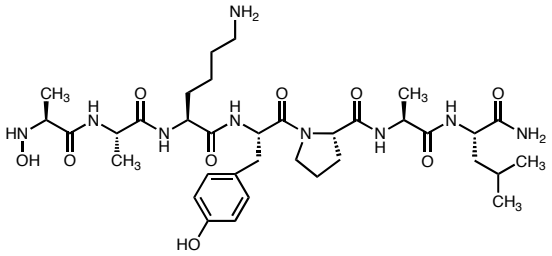
min, the resin was drained and the solution containing compound **25** was added to the resin; the solution was agitated until reaction completion was confirmed by Kaiser test. The resin was consecutively rinsed several times with DMF, CH_2Cl_2, DMF, and MeOH. The resin was dried under vacuum, placed in a glass vial and treated with a solution of 99% TFA in CH_2Cl_2. After agitation for 30 min, the resin was filtered and washed with TFA (1.0 mL). The filtrate was placed under a stream of N_2 and reduced to a volume of 1.0 mL. Cold Et_2O was added to the solution, inducing a white precipitate that was collected by vacuum filtration and rinsed several times with cold Et_2O to provide the *N*-benzylidne nitrone peptide as a white solid (0.050 g). The peptide (0.025 g) was dissolved in a solution of 5% TFA in H_2O. The solution was passed through a column of C-18 silica layered with hydroxylamine Wang resin (0.134 g, 2.00 mmol/g, 10.0 equiv) several times. The resulting solution was then lyophilized and the crude hydroxylamine was purified by preparative HPLC using a gradient of 5–50% iPrOH in H_2O over 30 min with a flow rate of 10.0 mL/min and monitored at 220 nm. Compound **34** was isolated as a white solid (0.015 g, 66% yield).



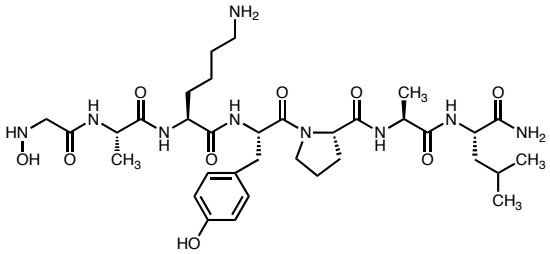
α-*N*-hydroxy-Tyr-Ala-Lys-Pro-Ala-Leu-NH_2 (34). ^{1}H NMR (D_3COD) δ 7.10–7.02 (m, 4H), 6.73–6.69 (m, 4H), 4.59 (br m, 1H), 4.47 (br m, 1H), 4.38–4.21 (m, 4H), 4.13 (q, 1H, J = 7.0 Hz), 3.88–3.74 (m, 2H), 3.57–3.51 (m, 2H), 3.17–3.00 (m, 2H), 2.94–2.80 (m, 6H), 2.15 (br m, 1H), 2.00–1.96 (m, 3H), 1.71–1.56 (m, 8H), 1.40–1.29 (m, 7H), 1.18–1.16 (m, 2H), 0.97–0.91 (m, 6H); HPLC retention time: 13.3 min. at 220 nm, column: YMC R-ODS-10A 250 x 20 mm, flow rate: 1 mL/min, gradient: 5–50% iPrOH in H_2O over 30 minutes. HRMS (ESI) calcd for $C_{41}H_{62}N_9O_{10}$ $[M+H]^+$ 840.4620, found 840.4611.



α-*N*-hydroxy-Lys-Ala-Lys-Tyr-Pro-Ala-Leu-NH$_2$ (35). (*S*)-*N*-benzylidene-5-(*tert*-butoxycarbonylamino)-1-carboxypentan-1-amine oxide (**26**) (0.049 g, 0.140 mmol, 4.00 equiv) was coupled to 0.05 g of peptide bound resin to provide the corresponding *N*-benzylidne nitrone peptide (0.012 g), of which 0.011 g was hydrolyzed according to the general procedure to afford **35** (3.0 mg, 32% yield). ^{1}HNMR (D_3COD) δ 7.10–7.05 (m, 2H), 6.74–6.69 (m, 2H), 4.58 (br m, 1H), 4.51 (br m, 1H), 4.38–4.17 (m, 4H), 3.94–3.87 (m, 1H), 3.73–3.45 (m, 3H), 3.06–2.86 (m, 6H), 2.19 (br m, 1H), 2.04–1.99 (m, 3H), 1.87–1.36 (m, 22H), 1.16 (d, 2H, J = 6.5 Hz), 0.97–0.90 (m, 6H). HPLC retention time: 7.5 min. at 220 nm, column: YMC R-ODS-10A 250 x 20 mm, flow rate: 1 mL/min, gradient: 5-50% iPrOH in H_2O over 30 min; ESI calcd for $C_{38}H_{65}N_{10}O_9$ $[M+ H]^+$ *m/z*: 805.48, found 805.90.

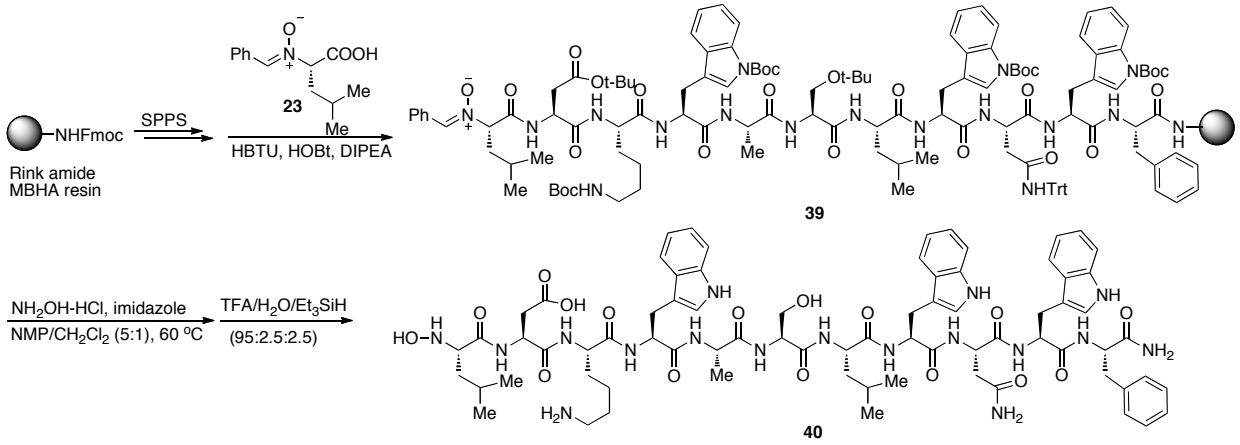


α-*N*-hydroxy-Ala-Ala-Lys-Tyr-Pro-Ala-Leu-NH$_2$ (36). (*S*)-*N*-benzylidene-1-carboxyethanamine oxide (**20**) (0.10 g, 0.52 mmol, 3.0 equiv) was coupled to 0.25 g of resin bound peptide to provide the corresponding *N*-benzylidene nitrone peptide (0.043 g). The *N*-benzylidne nitrone peptide (4.0 mg) was hydrolyzed according to the general procedure to afford compound **36** as a white solid (1.0 mg, 36% yield). ^{1}H NMR (D_3COD) δ 7.11–7.05 (m, 2H), 6.74–6.69 (m, 2H), 4.79–4.76 (m, 2H) 4.60 (br m, 1H), 4.404.23 (m, 5H), 3.85–3.75 (m, 2H), 3.57–3.44 (m, 2H), 3.17–3.16 (m, 1H), 3.07–3.06 (m, 1H), 3.00–2.79 (m, 4H), 2.18–2,16 (m, 1H), 2.01–1.94 (m, 3H), 1.70–1.63 (m, 7H), 1.45–1.29 (m, 11H), 1.12 (br s, 1H), 0.97–0.91 (m, 6H); HPLC retention time: 16.0 min, at 220 nm, column: YMC R-ODS-10A 250 x 20 mm, flow rate: 1 mL/min, gradient: 5-50% iPrOH in H_2O over 30 min.; HRMS (ESI) calcd for $C_{35}H_{57}N_9O_9Na$ $[M+Na]^+$ *m/z*: 770.4176, found 770.4183.



α-*N*-hydroxy-Gly-Ala-Lys-Tyr-Pro-Ala-Leu-NH_2 (37). *N*-benzylidene-1-carboxymethanamine oxide (**22**) (0.150 g, 0.840 mmol) was coupled to 0.040 g of resin bound peptide of which 0.010 g of the *N*-benzylidene nitrone was hydrolyzed according to the general procedure to afford compound **34** as a white solid (1.0 mg, 11% yield). ^{1}H NMR (D_3COD) δ 7.12–7.04 (m, 2H), 6.73–6.69 (m, 2H), 4.59 (br m, 1H), 4.38–4.23 (m, 4H), 3.91–3.85 (m, 1H), 3.74 (br m, 1H), 3.51–3.39 (m, 2H), 3.39 (s, 1H), 3.17–3.16 (m, 1H), 3.08 (br m, 1H), 2.94–2.80 (m, 3H), 2.17 (br s, 1H), 2.00 (br m, 2H), 1.67–1.57 (m, 7H), 1.50–1.33 (m, 8H), 1.19–1.12 (m, 1H), 0.970.92 (m, 6H); HPLC retention time: 14.2 min. at 220 nm, column: YMC R-ODS-10A 250 x 20 mm, flow rate: 1 mL/min, gradient: 5-50% iPrOH in H_2O over 30 min.; HRMS (ESI) calcd for $C_{34}H_{55}N_9O_9Na$ $[M+ Na]^+$ *m/z*: 756.4020, found 756.4022.

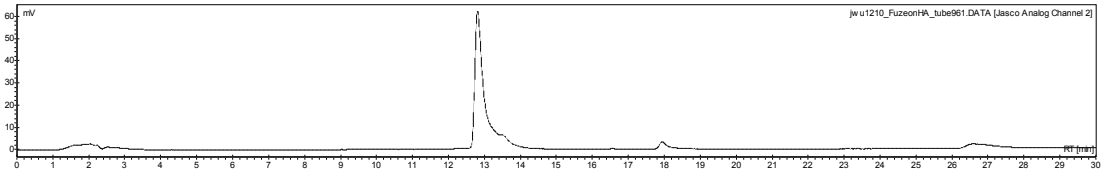
Preparation of 11 mer Hydroxylamine



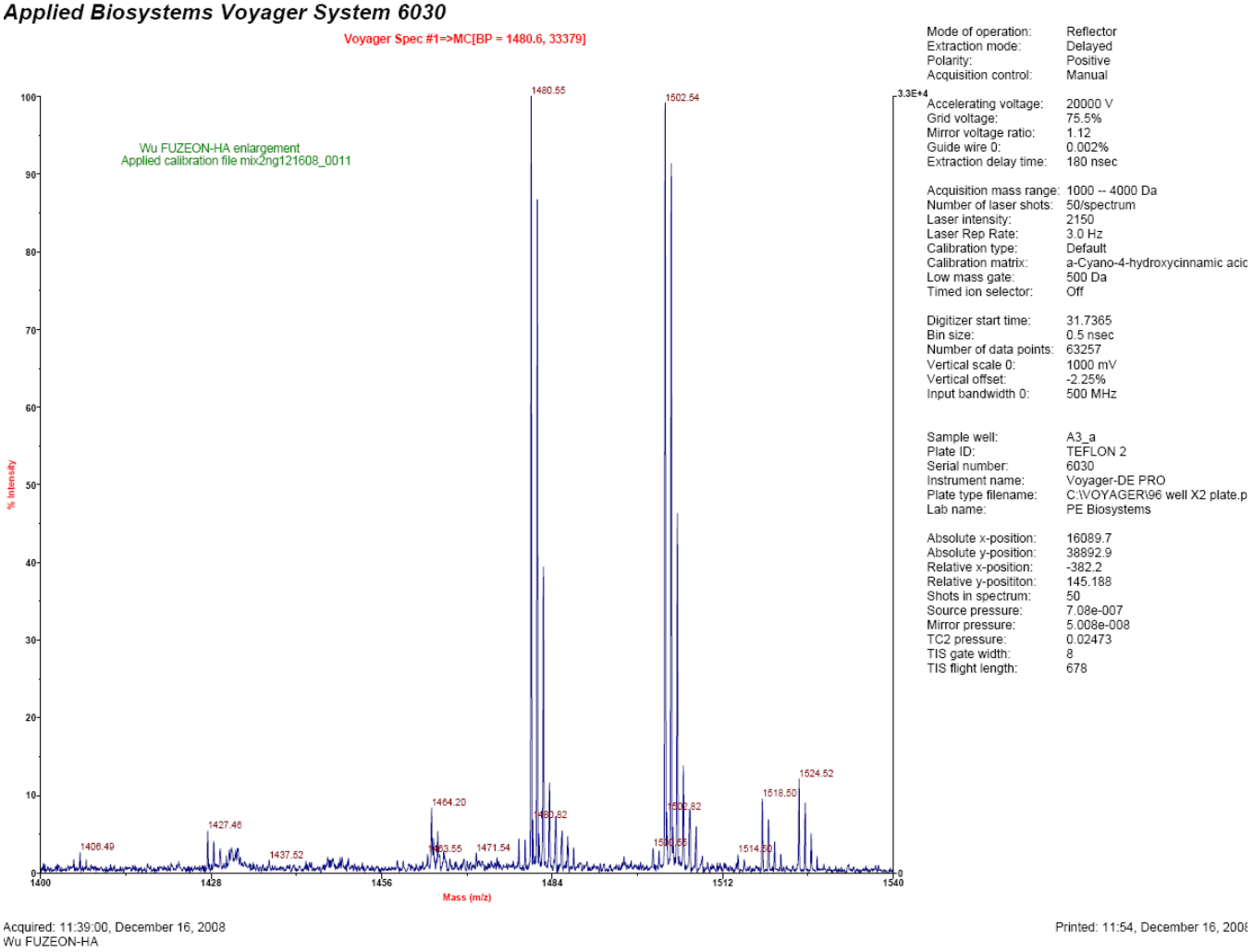
The mixture of resin-bound peptide **39** (40 mg, 0.016mmol) was mixed with a solution (0.6 mL, NH_2OH/imidazole/NMP/CH_2Cl_2 = 1.25 g : 0.918 g : 5 mL : 1 mL) and the resulting suspension was shaken at 60 ^oC for 16 h. The resin was filtered, rinsed with NMP and CH_2Cl_2, and dried in vacuo. The dry resin (38 mg) was mixed with a solution of TFA/TIPS/H_2O (95/2.5/2.5, 0.6 mL), and the resulting suspension was shaken under argon at rt for 1.5 h. The resin was filtered, washed with CH_2Cl_2 and TFA, followed by concentration of the filtrate to provide crude hydroxylamine **40** as an oil. Cold Et_2O was

added to the crude oil to provide a white precipitate, which was isolated by vacuum filtration to afford 17 mg of crude hydroxylamine **40.** Hydroxylamine **40** was purified by preparative reverse phase HPLC using a linear gradient of 25-55% CH_3CN in H_2O with 0.1% TFA over 30 min with a flow rate of 20 mL/min, subsequent lyophilization afforded hydroxylamine **40** (3.5 mg, 21% from crude) as a white solid.

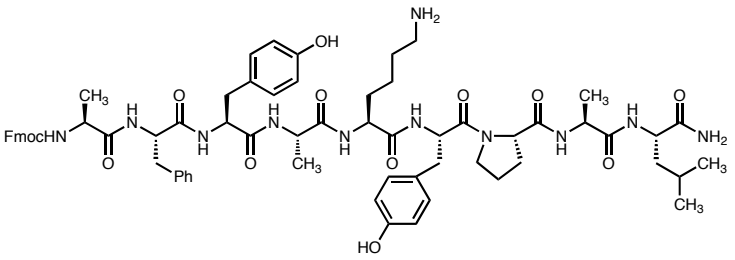
a) HPLC trace of purified hydroxylamine peptide: gradient 30–60% CH_3CN over 30 min at a flow rate of 1.0 ml/min, 280 nm, Zorbax Eclipse XDB-C8 4.6 × 150 mm column.



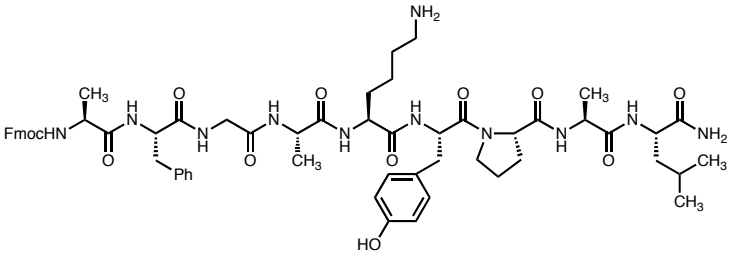
b) MALDI (*m/z*) $[M+H]^+$ calcd for $C_{74}H_{98}N_{17}O_{16}$, 1480.7, found 1480.6; $[M+Na]^+$ calcd for $C_{74}H_{97}N_{17}NaO_{16}$, 1502.7, found 1502.5.



2.11 Chemoselective ligation of peptide fragments

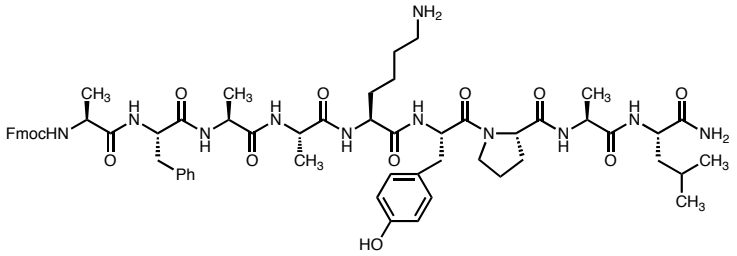


Fmoc-Ala-Phe-Tyr-Ala-Lys-Tyr-Pro-Ala-Leu-NH_2 (45). Hydroxylamine **34** (11.5 mg, 0.013 mmol, 1.0 equiv), and α-ketoacid **5** (0.010 g, 0.020 mmol, 1.5 equiv) were dissolved in 0.40 mL of 90% DMF in H_2O, and stirred overnight at 40 °C. The reaction was allowed to cool to room temperature and concentrated in vacuo. The ligated product was purified by preparative HPLC with a gradient of 40–50% iPrOH in H_2O over 30 min with a flow of 10 mL/min, and monitoring at 254 nm. Compound **45** was isolated as a white solid (0.007 g, 42% yield). ^{1}H NMR (D_3COD) δ 8.18–8.04 (m, 2H), 7.81–7.77 (m, 2H), 7.68–7.64 (m, 2H), 7.40–7.00 (m, 12H), 6.71–6.68 (m, 4H), 4.50–4.18 (m, 9H), 3.99–3.98 (m, 1H), 3.61 (br m, 1H), 3.49–3.45 (m, 1H), 3.05–2.85 (m, 7H), 19.4 (br m, 2H), 1.84 (br m, 1H), 1.68–1.57 (m, 7H), 1.40–1.15 (m, 12H), 0.95–0.91 (m, 6H); HPLC retention time: 12.4 min at 254 nm, column: YMC R-ODS-10A 250 x 20 mm, flow rate: 1 mL/min, gradient: 40–50% iPrOH in H_2O over 32 min; HRMS (ESI) calcd for $C_{68}H_{86}N_{11}O_{13}$ $[M+H]^+$ *m/z*: 1264.6407, found 1264.6427.

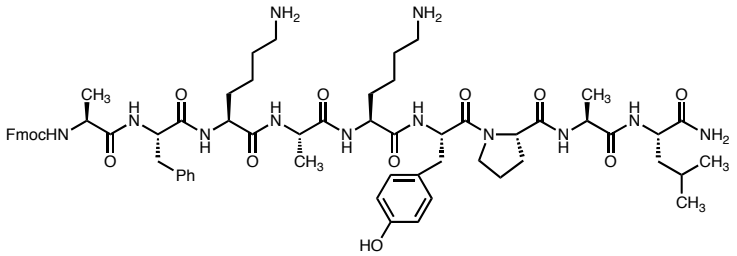


Fmoc-Ala-Phe-Gly-Ala-Lys-Tyr-Pro-Ala-Leu-NH_2 (47). Hydroxylamine **37** (4.3 mg, 5.8 μmol, 1.0 equiv) and α-ketoacid **5** (4.0 mg, 8.8 μmol, 1.5 equiv) were dissolved in 0.5 mL of 90% DMF in H_2O and oxalic acid (1.8 mg, 20.0 μmol, 3.4 equiv) was added. The solution was stirred overnight at 40 °C. The reaction was allowed to cool to rt and concentrated in vacuo. The crude product was purified by preparative HPLC using a gradient of 40–50% iPrOH in H_2O over 30 min with a flow of 10 mL/min, monitoring at 254 nm, to provide the product as a white solid (0.002 g, 36% yield). ^{1}H NMR (D_3COD)

δ 7.82–7.81 (m, 2H), 7.68–7.66 (m, 2H), 7.42–7.03 (m, 9H), 6.73–6.68 (m, 2H), 4.60 (br s, 1H), 4.48–47 (m, 1H), 4.39–4.18 (m, 7H), 4.03–3.90 (m, 3H), 3.83–3.78 (m, 3H), 3.72–3.58 (m, 3H), 3.48–3.45 (m, 2H), 3.17–3.16 (m, 1H), 3.05–2.97 (m, 2H), 2.90–2.87 (m, 3H), 2.21–2.16 (m, 1H), 1.97–1.90 (m, 3H), 1.67–1.58 (m, 7H), 1.40–1.29 (m, 10H), 1.21–1.13 (m, 3H), 0.96–0.89 (m, 6H); HPLC retention time: 15.7 min at 254 nm, column: YMC R-ODS-10A 250 x 20 mm flow rate: 1 mL/min, gradient: 40–50% IPROH in H_2O over 32 min; HRMS (ESI) calcd for $C_{61}H_{80}N_{11}O_{12}$ $[M+H]^+$ *m/z*: 1158.5988, found 1158.6036.



Fmoc-Ala-Phe-Ala-Ala-Lys-Tyr-Pro-Ala-Leu-NH_2 (46). Hydroxylamine **36** (1.6 mg, 2.1 μmol, 1.0 equiv) and α-ketoacid **5** (2.0 mg, 4.2 μmol, 2.0 equiv) were dissolved in 1.0 mL of DMF and oxalic acid (1.0 mg, 11.0 μmol, 5.3 equiv) was added to the solution and stirred overnight at 40 °C. The reaction was allowed to cool to rt and concentrated in vacuo. The crude product was purified by preparative HPLC using a gradient of 40–50% iPrOH in H_2O over 30 min with a flow of 10 mL/min, monitoring at 254 nm to provide **36** as a white solid (0.001 g, 48% yield). ^1H NMR (CD_3OD) δ 7.81–7.66 (m, 4H), 7.41–7.06 (m, 10H), 6.69–6.68 (m, 3H), 4.36–4.22 (m, 7H), 3.64 (br m, 1H), 3.54 (s, 3H), 3.02 br m, 2H), 2.87 (m, 4H), 1.95–1.62 (m, 9H), 1.38–1.21 (m, 10H), 1.12 (br m, 2H), 0.94–0.91 (m, 6H); HPLC retention time: 12.4 min, at 254 nm, column: YMC R-ODS-10A 250 x 20 mm, gradient: 40–50% IPROH in H_2O, flow rate: 1 mL/min; ESI calcd for $C_{62}H_{82}N_{11}O_{12}$ $[M+H]^+$ *m/z*: 1173.8 found 1173.4.



Fmoc-Ala-Phe-Lys-Ala-Lys-Tyr-Pro-Ala-Leu-NH_2 (48). Hydroxylamine **35** (2.6 mg, 2.9 μmol, 1.0 equiv) and α-ketoacid **5** (4.1 mg, 8.4 μmol, 2.9 equiv) were dissolved in 0.90 mL of DMF and oxalic acid (8.00 mg, 88.9 μmol, 30.6 equiv) was added to the solution and stirred overnight at 40 °C. The reaction was allowed to cool to rt and concentrated in vacuo. The crude product was purified by preparative HPLC using a gradient of 40–50% iPrOH in H_2O over 30 min with a flow rate of 10mL/min, monitoring at 254 nm to provide the title compound **48** (0.60 mg, 17% yield). ^{1}H NMR (CD_3OD) δ 7.80 (m, 2H), 7.65 (m, 2H), 7.40–7.06 (m, 10H), 6.68 (m, 2H), 4.31 (m, 7H), 3.57 (br s, 2H), 3.00 (br s, 2H), 2.85 (br m, 4H), 1.96 (br s, 3H), 1.58 (br m, 7H), 1.37–1.12 (m, 14H), 0.90 (m, 7H); HPLC retention time: 11.2 min, column: YMC R-ODS-10A 250 x 20 mm at 254 nm, flow rate: 1 mL/min, gradient: 40–50% iPrOH in H_2O over 32 min; ESI calcd for $C_{65}H_{89}N_{12}O_{12}$ $[M+H]^+$ *m/z:* 1230.5 found 1230.4.