Synthesis of N-Hydroxy Peptides: Chemical Ligation of O-Acyl Hydroxamic Acids

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SUPPORTING INFORMATION AVAILABLE

N-tert-Butyl-O-benzoylhydroxylamine. (precursor to 1a)

Following the procedure of Alewood,[1] tert-butylamine (5.21 mL, 49.54 mmol) was added slowly over 3 min to a solution of freshly-recrystallized dibenzoylperoxide (5.33 g, 22.02 mmol) in benzene (30 mL). After addition, the solution was heated to 45 °C. A further portion of tert-butylamine (4.05 mL, 38.53 mmol) was added after 1 h. The viscous mixture was left at 45 °C overnight, then cooled to room temperature. The suspension was taken up with diethyl ether and filtered. The pale yellow filtrate was stirred at room temperature whilst a solution of $FeCl_2$ [5 g in H_2O (4.5 mL) : HCl (conc.) 2.1 mL] was added slowly, until precipitation of the dark brown insoluble Fe(III) salts ceased. The mixture was washed with $NaHCO_3$ (sat. aq.), water, brine and dried over $MgSO_4$. Evaporation gave a yellow oil, and chromatography [7:1 Hexane : EtOAc] provided the product as a pale yellow oil (3.63 g, 85 % yield).

^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 8.05 (d, 2H, J = 7.1, phenyl CH), 7.59 (m, 1H, phenyl CH), 7.47 (m, 2H, phenyl CH), 1.24 (s, 9H, $C(CH_3)_3$).

N-tert-Butyl-N-(N'-[[(9-fluorenylmethyl)oxy]carbonyl]-glycinoyl)-O-benzoyl hydroxylamine. (1a)

To a room temperature solution of Fmoc-L-glycine (979 mg, 3.29 mmol) in dichloromethane (4 mL) and THF (1 mL) was added DMF (26 μL) followed by oxalyl chloride (575 μL, 6.59 mmol). Once the effervescence had subsided, the solution was heated to reflux for 1.5 h. The yellow solution was evaporated in vacuo and the yellow residue azeotroped with benzene (2x), before being dissolved in benzene (6 mL). To this solution was added a solution of N-tert-butyl-O-benzoylhydroxylamine (0.70 g, 3.62 mmol) in benzene (4 mL), followed by pyridine (522 μL, 6.59 mmol). The mixture was heated to reflux overnight, and then cooled. The orange suspension was taken up in ethyl acetate and 10% HCl (aq.), separated, and the organic layer washed with brine and then dried over $MgSO_4$. Evaporation gave an orange oil that was purified by chromatography [3:1 Hexane : EtOAc] to give the product **1a** as a pale yellow oil that solidified on storage (1.27 g, 82 %).

IR (υ max, cm^{-1}) : 3422, 3019, 1767, 1721, 1677.

(1) Alewood, P. F.; Calder, I. C.; Richardson, R. L. Synthesis **1981**, 121-122.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 8.14 (d, 2H, J = 8.0, phenyl CH), 7.78 (d, 2H, J = 7.5, phenyl CH), 7.71 (t, 1H, J = 7.8, phenyl CH), 7.64 (d, 2H, J = 7.0, phenyl CH), 7.55 (t, 2H, J = 7.5, phenyl CH), 7.42 (t, 2H, J = 7.3, phenyl CH), 7.34 (t, 2H, J = 7.3, phenyl CH), 5.69 (overlapping t, 1H, NH), 4.38 (d, 2H, J = 7.5, CH_2NH), 4.26 (m, 2H, $CHCH_2O$), 3.78 (dd, 1H, J = 17.8, 2.8, $CHCH_2O$), 1.57 (s, 9H, $C(CH_3)_3$).

^{13}C-NMR (125 MHz, APT, ppm) : δ 169.7 (C=O), 165.4 (C=O), 156.4 (C=O), 144.1 (2 x ipso C), 141.4 (2 x ipso C), 134.9 (ipso C), 130.3+129.2+127.8+127.2+125.3+120.0 (13 x phenyl CH), 67.3 ($CHCH_2O$), 63.7 ($C(CH_3)_3$), 47.3 ($CHCH_2O$), 43.4 (CH_2N), 27.4 ($C(CH_3)_3$).

N-tert-Butyl-N-(N'-[[(9-fluorenylmethyl)oxy]carbonyl]-L-alanoyl)-O-benzoyl hydroxylamine. (1b)

To a room temperature solution of Fmoc-L-alanine (1.04 g, 3.35 mmol) in dichloromethane (5 mL) was added DMF (26 μL) followed by oxalyl chloride (584 μL, 6.69 mmol). Once the effervescence had subsided, the yellow solution was heated to reflux for 1.5 h. After cooling, the solution was evaporated in vacuo and the solid residue azeotroped with benzene, before being dissolved in benzene (6 mL). To this yellow solution was added a solution of N-tert-butyl-O-benzoylhydroxylamine (0.71 g, 3.68 mmol) in benzene (5 mL), followed by pyridine (541 μL, 6.69 mmol). The mixture was heated to reflux overnight and then cooled. The colorless suspension was taken up in ethyl acetate and 10% HCl (aq.), separated, and the organic layer washed with brine and then dried over $MgSO_4$. Evaporation gave a viscous, colorless oil that was purified by flash chromatography [4:1 Hexane : EtOAc] to give **1b** as a colorless oil (1.56 g, 96 %).

IR (υ max, cm^{-1}) : 3424, 3019, 1768, 1720, 1666.

^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 8.2-7.3 (m, 13H, phenyl CH), 5.9 (d, 1H, J = 7.5 NH), 4.3 (d, 2H, J = 8.3, $CHCH_2O$), 4.2 (m, 2H, $CHCH_2O$+$CHCH_3$), 1.55 (s, 9H, $C(CH_3)_3$), 1.4 (d, 3H, J = 6.8, $CHCH_3$).

^{13}C-NMR (62.5 MHz, APT, ppm) : δ 172.8 (C=O), 165.1 (C=O), 155.1 (C=O), 144.0+143.8+141.2 (5 x ipso C), 134.7+130.1+129.0+127.6+127.5+127.1+125.2+119.9 (13 x phenyl CH), 66.8 ($CHCH_2O$), 63.6 ($C(CH_3)_3$), 48.9 ($CHCH_3$), 47.1 ($CHCH_2O$), 27.3 ($C(CH_3)_3$), 18.7 ($CHCH_3$).

N-tert-Butyl-N-[N'-benzoylglycinoyl]-hydroxylamine. (2a)

FMOC-Gly-N(^{t}Bu)OBz **1a** (1.07 g, 2.26 mmol) was dissolved in a commercially available solution of dimethylamine in THF (2M, 6.0 mL, 12 mmol) at room temperature and left overnight. Evaporation left a light orange solid that was purified by chromatography [3:1 to 0:1 Hexane : EtOAc] to give the product **2a** as a white solid (501 mg, 88 %).

IR (υ max, cm^{-1}) : 3200 (br), 3019, 1634, 1578.

^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 7.83-7.41 (m, 6H, phenyl CH + NH), 4.41 (d, 2H, J = 4.4, CH_2NH), 1.48 (s, 9H, $C(CH_3)_3$).
^{13}C-NMR (62.5 MHz, APT, ppm) : δ 169.2 (C=O), 168.2 (C=O), 133.2 (ipso C), 131.9+127.9+127.1 (5 x phenyl CH), 61.6 ($C(CH_3)_3$), 43.2 (CH_2N), 27.3 ($C(CH_3)_3$).

N-tert-Butyl-N-[N'-benzoyl-L-alaninoyl]-hydroxylamine. (2b)
FMOC-L-Ala-N(^{t}Bu)OBz **1b** (1.32 g, 2.72 mmol) was dissolved in a commercially available solution of dimethylamine in THF (2M, 13.6 mL, 27.15 mmol) at room temperature and left stirring overnight. Evaporation left a yellow/orange solid that was purified by chromatography [3:1 to 0:1 Hexane : EtOAc] to give the product **2b** as a white solid (598 mg, 83 %).
IR (υ max, cm^{-1}) : 3050 (br), 3019, 1631.
^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 9.8 (br s, 1H), 7.80 (m, 2H, phenyl CH), 7.62 (d, 1H, J = 7.2), 7.4 (m, 3H, phenyl CH), 5.2 (p, 1H, J = 6.9, $CHCH_3$), 1.50 (d, 3H, J = 6.9, $CHCH_3$), 1.48 (s, 9H, $C(CH_3)_3$).
^{13}C-NMR (62.5 MHz, APT, ppm) : δ 172.4 (C=O), 167.2 (C=O), 133.4 (ipso C), 131.8+128.5+127.1 (4 x phenyl CH), 61.6 ($C(CH_3)_3$), 48.0 ($CHCH_3$), 27.3 ($C(CH_3)_3$), 17.7 ($CHCH_3$).

N-tert-Butyl-N-[N'-benzoyl-L-alaninoyl]-nitroxide. [Bz-L-Ala-N(^{t}Bu)O•]. (3)
A solution of Bz-L-Ala-N(^{t}Bu)OH **2b** (100 mg, 0.38 mmol) in dichloromethane (5 mL) was shaken with a saturated solution of $K_3Fe(CN)_6$ in 2M NaOH (aq.) (10 mL, excess) for 1 min. The deep blue organic layer was isolated and washed with 10% NaOH (aq.), brine and dried over $MgSO_4$. Evaporation gave a blue solid that was purified by chromatography [6:1 to 2:1 Hexane : EtOAc] to give the acyl nitroxide **3** as a blue solid (73 mg, 73 %). This radical could be stored in the freezer, but was often utilized immediately following chromatographic purification.
TLC : 2:1 hexane:ethyl acetate, UV, PMA vanillin, R_f=0.47.
IR (υ max, cm^{-1}) : 3433, 3019, 1702, 1662.
^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ v. broad, indistinct peaks at approx. 7.93, 7.54, 1.53, 1.33.

N-tert-Butyl-N-[N'-benzoyl-L-alaninoyl]-O-benzylhydroxylamine. (4)
A solution of Bz-L-Ala-N(^{t}Bu)O• **3** (72 mg, 0.27 mmol) in toluene (4 mL) was degassed and stirred at room temperature overnight. A very faint blue color of the nitroxide remained, which dissipated upon heating to reflux. The solution was evaporated to give a very pale yellow oil. ^{1}H NMR indicated a 1.5:1 ratio of hydroxylamine **2b** to O-benzylated adduct **4**. Chromatography [6:1 to 2:1 Hexane : EtOAc] yielded the O-benzylated product **4** as a white solid (32 mg, 33 %).

IR (υ max, cm^{-1}) : 3018, 1648, 1603.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.86 (d, 2H, J = 7.5 phenyl CH), 7.54-7.40 (m, 8H, phenyl CH), 7.20 (br d, J = 6.5, NH), 5.28 (p, 1H, J = 7.1, $CHCH_3$), 5.13-5.00 (dd, 2H, J = 51.2, 9.8, $PhCH_2O$), 1.56 (s, 9H, $C(CH_3)_3$), 1.41 (d, 3H, J = 6.5, $CHCH_3$).

^{13}C-NMR (125 MHz, APT, ppm) : δ 176.0 (C=O), 166.7 (C=O), 134.3+134.1 (2 x ipso C), 131.6+129.3+129.2+129.1+129.0+128.9+128.6+127.2 (10 x phenyl CH), 80.7 ($PhCH_2O$), 63.0 ($C(CH_3)_3$), 47.9 ($CHCH_3$), 28.0 ($C(CH_3)_3$), 18.3 ($CHCH_3$).

N-tert-Butyl-N-[N'-acetyl-L-alaninoyl]-O-[α-phenethyl] ether. (5 as a 1:1 mixture of diastereomers)

A solution of Bz-L-Ala-N(^{t}Bu)O• **3** (29 mg, 0.11 mmol) was dissolved in pre-distilled ethylbenzene (2 mL) and stirred at room temperature overnight. The blue color of the acyl nitroxide dissipated. The colorless solution was evaporated under high vacuum and the crude product analyzed by ^{1}H NMR. Integration of the signals corresponding to both the benzylic methine protons, and the benzylic methyl groups indicated a 1:1 mixture of diastereomeric phenethyl adducts of **5**, in addition to the reduced hydroxylamine **2b**. Chromatography (6:1 to 3:1 Hexane : EtOAc) gave the diastereomeric mixture of **5** (12 mg, 30% [60% based on NMR ratio]) as a colorless oil.

TLC : 3:1 Hexane : EtOAc, R_f = 0.4. UV, vanillin or PMA stain.

IR (υ max, cm^{-1}) : 3423, 3021, 1646, 1580.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.75-7.20 (m, 11H), 6.71 (d, 1H, J = 5.5, CHNH), 5.49 (apparent p, 1H, J = 7.0, $NHCHCH_3$ one diastereomer), 5.06 (q, 1H, J = 6.7, $PhCHCH_3$ one diastereomer), 4.99 (q, 1H, J = 6.7, $PhCHCH_3$ other diastereomer), 4.49 (apparent p, 1H, J = 7.0, $NHCHCH_3$ other diastereomer), 1.71 (d, 3H, J = 7.0, $PhCHCH_3$ one diastereomer), 1.70 (d, 3H, J = 7.0, $PhCHCH_3$ other diastereomer), 1.57 (s, 9H, $C(CH_3)_3$ one diastereomer), 1.42 (d, 3H, J = 6.0 $NHCHCH_3$ other diastereomer), 1.39 (s, 9H, $C(CH_3)_3$ other diastereomer), 1.20 (d, 3H, J = 6.5, $NHCHCH_3$ other diastereomer).

^{13}C-NMR (125 MHz, APT, ppm) : δ 177.7 & 177.2 (both diastereomers C=O), 166.4 & 165.8 (both diastereomers C=O), 134.8 & 134.4 (both diastereomers ipso-C), 131.6 & 131.2 (both diastereomers ipso-C), 129.2 - 126.9 (both diastereomers 10 x Phenyl-CH), 85.6 & 85.2 (both diastereomers PhCHCH3), 63.3 & 63.2 (both diastereomers $C(CH_3)_3$), 48.5 & 48.2 (both diastereomers $NHCHCH_3$), 28.3 & 28.1 (both diastereomers $C(CH_3)_3$), 19.1 & 18.2 & 18.1 & 17.8 (both diastereomers $NHCHCH_3$ & $PhCHCH_3$).

N-tert-Butyl-O-[N'-acetyl-glycinoyl]-hydroxylamine. (7a)

To a 0 °C solution of N-acetyl-glycine (500 mg, 4.27 mmol) in dry THF (10 mL) was added 1,1'-carbonyldiimidazole (762 mg, 4.70 mmol) in one portion. The suspension was stirred for

1h at 0 °C before a solution of tert-butyl hydroxylamine[2] (381 mg, 4.27 mmol) in dry THF (10 mL) was added slowly. The mixture was stirred at 0 °C for 1h then at room temperature overnight. Ethyl acetate (20 mL) and 10% aq. HCl (20 mL) were added. The aqueous layer was extracted with further ethyl acetate and the combined organic layers washed with saturated brine and dried ($MgSO_4$). Evaporation gave a yellow oil that was purified by chromatography [3:1 to 1:1 Hexane : EtOAc] to give the ester **7a** as a colorless oil (335 mg, 42%).

IR (υ max, cm^{-1}) : 3019, 1751, 1676.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.17 (br. s, 1H, NH), 7.01 (br. s, 1H, NH), 3.89 (d, 2H, J = 5.0, CH_2NH), 1.88 (s, 3H, $CH_3C=O$), 0.99 (s, 9H, $C(CH_3)_3$).

^{13}C-NMR (125 MHz, APT, ppm) : δ 171.0 (C=O), 170.2 (C=O), 56.0 ($C(CH_3)_3$), 40.2 (CH_2NH), 26.4 ($C(CH_3)_3$), 22.6 ($CH_3C=O$).

N-tert-Butyl-O-[N'-benzoylglycinyl-glycinoyl]-hydroxylamine. (7b)

To a suspension of benzoylglycinyl-glycine (300 mg, 1.27 mmol) and tert-butyl hydroxylamine[2] (113 mg, 1.27 mmol) in dichloromethane (15 mL) was added DMF (2 mL) to aid dissolution followed by EDCI (243 mg, 1.27 mmol). The mixture was stirred overnight at room temperature. A white suspension resulted that was taken up in EtOAc and washed with saturated aq. $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated to give **7b** as a light brown oil (279 mg, 71% crude yield). TLC (1:3 Hexane : EtOAc) showed only the desired product.

^{1}H-NMR (500 MHz, CD_3OD, ppm) : δ 7.85 (m, 1H, NH), 7.79 (m, 2H, phenyl CH), 7.40 (m, 1H, phenyl CH), 7.31 (m, 2H, phenyl CH), 7.07 (br s, 1H, NH), 4.11 (d, 2H, J = 5.5, gly-CH_2), 4.01 (d, 2H, J = 6.0, gly-CH_2), 1.05 (s, 9H, ^{t}Bu).

^{13}C-NMR (125 MHz, APT, ppm) : δ 170.2 & 170.0 & 168.1 (3 x C=O), 133.5 (ipso-C), 131.8 (phenyl CH), 128.5 (2 x phenyl CH), 127.3 (2 x phenyl CH), 56.1 (^{t}Bu C), 43.5 (gly-CH_2), 40.3 (gly-CH_2), 26.4 (^{t}Bu-CH_3).

N-tert-Butyl-O-[N'-tertbutyloxycarbonylglycinyl-glycinoyl]-hydroxylamine. (7c)

To a stirred room temperature suspension of tert-butyloxycarbonylglycinyl-glycine (300 mg, 1.29 mmol) in dichloromethane (15 mL) was added DMF (1 mL) to aid dissolution, followed by tert-butyl hydroxylamine[2] (127 mg, 1.42 mmol) and EDCI (247 mg, 1.29 mmol). As EDCI was slowly added, a solid precipitated out of the clear yellow reaction mixture. After stirring overnight at room temperature, the residue was dissolved in EtOAc, washed with saturated aq. $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography [1:2 Hexane : EtOAc] to give **7c** (293 mg, 75% yield).

TLC : 1:2 hexane: ethyl acetate, not UV active, I_2, Vanillin, R_f=0.40.

IR (υ max, cm^{-1}) : 3436, 1686, 1508.

([2]) Kofron, W. G.; Baclawski, L. Org. Synth. **1972,** 52, 78.

^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 7.11 (s, 1H, NH), 5.55 (s, 1H, NH), 4.08 (d, 2H, J = 5.6, gly-CH_2), 3.83 (d, 2H, J = 5.3, gly-CH_2), 1.41 (s, 9H, BOC-tBu-CH_3), 1.11 (s, 9H, NtBu-CH_3).

^{13}C-NMR (62.5 MHz, APT, ppm) : δ 170.2 (C=O), 169.9 (C=O), 156.2 (C=O), 80.3 (BOC-tBu-C), 56.1 (NtBu-C), 44.1 (gly-CH_2), 40.1 (gly-CH_2), 28.3 (BOC-tBu-CH_3), 26.4 (NtBu-CH_3).

N-tert-Butyl-O-[N'-benzyloxycarbonyl-L-leucinylglycinoyl]-hydroxylamine. (7d)

To a solution of CBz-L-leucinylglycine (300 mg, 0.93 mmol), in dichloromethane (15 mL) and DMF (1 mL) (to aid dissolution) was added tert-butyl hydroxylamine[2] (83 mg, 0.93 mmol) followed by EDCI (178 mg, 0.93 mmol). The clear colorless mixture was stirred overnight at room temperature. Evaporation of the solvent left a yellow, viscous residue, which was taken up in ethyl acetate and washed with saturated aq. $NaHCO_3$, brine, and dried over $MgSO_4$. The organic layer was evaporated to give a yellow oil (359 mg). The product was purified by flash column chromatography [3:2 Hexane : EtOAc] to give a light yellow oil (228 mg, 62% yield).

TLC : 3:2 hexane: ethyl acetate, not UV active, I_2, PMA, R_f=0.30.

IR (υ max, cm^{-1}) : 3155, 3020, 1819, 1794, 1736.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 8.28 (s, 5H, phenyl CH), 6.80 (d, 1H, J = 8.4, NH), 6.06 (m, 2H, gly-CH_2), 5.31 (m, 1H, NH), 5.01 (t, 2H, J = 11.4, CBz-CH_2), 2.58 (m, 3H, leu-CH_2 & CH), 2.10 (s, 9H, tBu), 1.89 (m, 6H, leu-CH_3).

^{13}C-NMR (125 MHz, APT, ppm) : δ 173.5 (C=O), 170.0 (C=O), 156.4 (C=O), 136 (ipso-C-O of CBz), 128.4 & 128.1 & 127.9 (CBz-phenyl-CH), 66.9 (CBz-CH_2), 55.9 (tBu-C), 53.4 (tBu-CH_3), 41.3 (gly-CH_2), 40.1 (leu-$CH_2CH(CH_3)_2$), 26.3 (tBu-CH_3), 24.5 & 22.9 (leu-$CH_2CH(CH_3)_2$), 21.8 ($CH_2CH(CH_3)_2$).

N-tert-Butyl-O-[N'-benzoyl-L-alaninoyl]-hydroxylamine. (7f)

To a stirred, room temperature solution of benzoyl-L-alanine (300 mg, 1.55 mmol) and tert-butyl hydroxylamine[2] (138 mg, 1.55 mmol) in dichloromethane (15 mL) was added EDCI (297 mg, 1.55 mmol) giving a clear colorless solution. After stirring overnight, the mixture was evaporated to give a pale yellow residue. The mixture was taken up in ethyl acetate and washed with saturated aq. $NaHCO_3$, brine, and dried over $MgSO_4$. A portion of the crude material was used in another reaction. The rest of the crude product was purified by flash chromatography to give the purified product as a white solid (258 mg, 63%) (note: yield reported taking into account the amount of product placed on the column).

TLC : 3:1 hexane: ethyl acetate, UV, I_2, PMA, vanillin, R_f=0.20.

IR (υ max, cm^{-1}) : 3155, 3020, 1819, 1794, 1736.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.74 (m, 2H, phenyl CH), 7.42 (m, 1H, phenyl CH), 7.34 (s, 2H, phenyl CH), 7.06 (s, 1H, Ala-NH), 4.82 (m, 1H, Ala-CH), 1.49 (m, 3H, Ala-CH_3), 1.10 (s, 9H, ^{t}Bu).

^{13}C-NMR (125 MHz, APT, ppm) : δ 173.2 (C=O), 167.2 (C=O), 133.8 & 131.8 & 128.6 & 127.2 (5x phenyl CH), 56.2 (tBu C), 47.8 (NHCH(CH_3), 26.5 (3xtBu CH_3), 18.4 (NHCH(CH_3).

N-tert-Butyl-O-[N'-benzyloxycarbonylglycinyl-L-alaninoyl]-hydroxylamine. (7g)

To a stirred room temperature solution of benzyloxycarbonylglycinyl-L-alanine (300 mg, 1.07 mmol) in dichloromethane (15 mL) and DMF (2 drops) was added tert-butyl hydroxylamine[2] (105 mg, 1.18 mmol) followed by EDCI (205 mg, 1.07 mmol), giving a white cloudy solution. After stirring overnight at room temperature, the solution was clear and colorless. The mixture was evaporated, and the residue was dissolved in EtOAc, washed with saturated aq. $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated to give **7g** (358 mg, 95 %).

TLC : 1:3 hexane: ethyl acetate, not UV active, PMA, Vanillin, R_f=0.45.

IR (υ max, cm^{-1}) : 2975, 1735, 1686.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.26 (m, 5H, CBz-phenyl CH), 7.12 (s, 1H, NH), 6.10 (s, 1H, NH), 5.03 (s, 2H, CBz-CH_2), 4.58 (app. pentet, 1H, J=14.6, Ala-CH), 3.83 (d, 2H, J = 5.3, gly-CH_2), 1.33 (d, 3H, J=7.2, Ala-CH_3), 1.0 (s, 9H, tBu).

^{13}C-NMR (125 MHz, APT, ppm) : δ 172.8 (C=O), 169.3 (C=O), 156.8 (C=O), 136.3 (CBz-ipso-C), 128.5 & 128.1 & 128.0 (CBz-phenyl-CH), 67.0 (CBz-CH_2), 5.1 (tBu-C), 47.2 (Ala-CH), 44.2 (gly-CH_2), 26.4 (tBu-CH_3), 17.9 (Ala-CH_3).

N-tert-Butyl-N-{N'-[(9-fluorenylmethyl)oxy]carbonyl]-L-alaninoyl}-O-[N''-acetyl-glycinoyl]-hydroxylamine. (9a)

To a stirred room temperature solution of FMOC-L-alanine (100 mg, 0.32 mmol) in dichloromethane (1 mL) with DMF (2 drops) was added a commercially available solution of thionyl chloride in dichloromethane (2M, 1.61 mL, 3.21 mmol). After 1.5 h, the mixture was evaporated and the yellow residue azeotroped with dichloromethane. The residue was dissolved in dry THF (2 mL) and to it was added pyridine (52 μL, 0.64 mmol) followed by a solution of Ac-Gly-O-NH(^{t}Bu) (**7a**) (60.5 mg, 0.32 mmol) in dry THF (2 mL). The mixture was heated to reflux for 4 h. The dark orange mixture was decanted from the black solid, and evaporated. Chromatography [1:1 to 1:3 Hexane : EtOAc] gave the product **9a** as a colorless oil (76 mg, 49%).

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) : δ 7.78 (d, 2H, J = 7.0), 7.60 (d, 2H, J = 6.5), 7.42 (t, 2H, J = 7.3), 7.33 (t, 2H, J = 7.5), 6.49 & 6.21 (br. s, 1H, CH_2NH rotamers), 5.72 & 5.45 (d, 1H, CHNH rotamers), 4.42 - 4.01 (m, 6H), 2.02 (s, 3H, CH_3C=O), 1.46 & 1.44 (s, 9H, C(CH_3)$_3$ rotamers), 1.32 & 1.28 (d, 3H, CH_3CH rotamers).

^{13}C-NMR (125 MHz, APT, ppm) : δ 172.9 (C=O), 170.8 (C=O), 169.6 (C=O), 155.7 (C=O), 143.9 & 143.8 (2 x ipso-C), 141.4 (2 x ipso-C), 127.9 & 127.2 & 125.2 & 120.1 (8 x

phenyl-CH), 67.2 ($CHCH_2O$), 63.8 ($C(CH_3)_3$), 48.5 & 48.1 ($CHCH_3$ rotamers), 47.2 ($CHCH_2O$), 39.9 & 39.6 (CH_2NH rotamers), 27.4 & 27.2 ($C(CH_3)_3$) rotamers), 22.8 ($CH_3C=O$), 18.8 & 18.6 ($CHCH_3$ rotamers).

N-tert-Butyl-N-[N'-acetylglycyl-alanyl]-hydroxylamine. (10a)

A portion of FMOC-Ala-N(^{t}Bu)-O-Gly-Ac (**9a**) (42 mg, 87.2 μmol) was treated with a commercially available solution of dimethylamine in THF (2M, 436 μL, 0.87 mmol) and stirred at room temperature overnight. The yellow solution was evaporated and the yellow residue purified by chromatography [3:1 to 1:1 Hexane : EtOAc] to give the rearranged product **10a** as a colorless oil (17 mg, 75%).

^{1}H-NMR (500 MHz, CD_3OD, ppm) : δ 4.91 (q, 1H, J = 7.0, $CHCH_3$), 3.85 (dd, 2H, J = 29.3, 16.8, CH_2NH), 2.00 (s, 3H, $CH_3C=O$), 1.40 (s, 9H, $C(CH_3)_3$), 1.29 (d, 3H, $CHCH_3$).

^{13}C-NMR (125 MHz, APT, ppm) : δ 173.8 (C=O), 172.4 (C=O), 169.4 (C=O), 60.9 ($C(CH_3)_3$), 47.2 ($CHCH_3$), 42.1 (CH_2NH), 26.2 ($C(CH_3)_3$), 21.0 ($CH_3C=O$), 16.0 ($CHCH_3$).

N-tert-Butyl-N-([N'-benzyloxycarbonyl-L-leucyl]-glycyl-L-alanyl)-hydroxylamine. (10e)

A commercially available solution of dimethylamine in THF (167 μL, 0.34 mmol) was added to a stirred room temperature solution of FMOC-Ala-N(^{t}Bu)-O-Gly-Leu-N'Z (23 mg, 33.5 μmol) in THF (0.5 mL). Piperidine (6.6 mL, 67.0 μmol) was added to speed up the reaction. The very pale yellow solution was stirred at room temperature. TLC (1:1 Hexane : EtOAc) showed starting material remaining after 30 min. After running overnight, no starting material remained and a new spot at a low R_f appeared. This solution was evaporated to give the crude product, which was purified first by preparative TLC and then by repeated HPLC to give the ligated product as a colorless oil (4.5 mg, 24 %).

TLC : 1:1 hexane:ethyl acetate, UV, PMA, $FeCl_3$, Rf = 0.25.

^{1}H-NMR (250 MHz, $CDCl_3$, ppm) : δ 7.37 (br s, 5H, Ph CH), 5.77 (d, 1H, J = 8.0, NH), 5.08 (m, 2H, Z-CH_2), 4.33 (m, leu-α-CH), 4.05 (m, Ala-α-CH), 3.94 (d, 2H, J = 5.6, gly-CH_2), 1.69 - 1.54 (m, 3H, $CH_2CH(CH_3)_2$ & $CH_2CH(CH_3)_2$), 1.39 (s, 9H, ^{t}Bu CH_3), 1.33 (d, 3H, J = 6.7, Ala-CH_3), 0.92 (d, 6H, J = 6.3, leu-$(CH_3)_2$).