A Reagent for the Convenient, Solid Phase Synthesis of *N*-Terminal Peptide Hydroxylamines for Chemoselective Ligations

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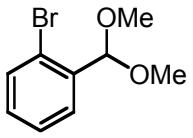
Supporting Information

1. General Methods

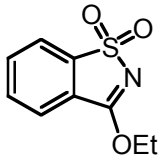
Reagents: All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry Argon. CH_2Cl_2 was distilled over CaH_2. THF was distilled from Na/benzophenone. CH_3OH and DMF were dried by passage over molecular sieves under Ar atmosphere. *N,N*-Diisopropylethylamine (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 F254, Art 5715, 0.25 mm) and was visualized by fluorescence quenching under UV light or by staining with phosphomolybdic acid. Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates prepared from Merck Kieselgel 60 PF254 (Art 7747). Column chromatography was performed on E. Merck Silica Gel 60 (230–400 Mesh) using a forced flow of 0.5–1.0 bar.

Instruments: ^{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; 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 were measured on a Jasco P–2000 polarimeter operating at the sodium D line with a 100 mm path length cell, and are reported as follows: $[\alpha]_D^T$ (concentration (g/100 ml), solvent). UV-vis spectra were recorded with a JASCO V-570 spectrometer. Analytical HPLC was performed using a C18 column (Shiseido CAPCELL PAK C18 UG120, S-5 μm, 4.6 mm I.D. × 250 mm) or (YMC R-ODS-10A, S-10 μm, 4.6 mm I.D. × 250 mm). All separations involved a gradient of CH_3CN and Millipure water containing 0.1 % trifluoroacetic acid. Standard separation condition: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min. Preparative HPLC was performed using a C18 column (YMC-Pack R&D ODS/D, S-10 μm, 20 mm I.D. × 250 mm). Standard separation condition: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 20.0 ml/min. SFC analysis was performed using Daicel Chiralpak AD-H (4.6 mm I.D. ×250 mm) column and a gradient of ^{i}PrOH (IPA) and super critical CO_2 ($scCO_2$). Standard separation condition; gradient 5–80% $scCO_2$/IPA over 7.5 min at a flow rate of 2.0 ml/min, oven temperature 50 °C, detection 254 nm.

2. Preparation of Reagents



2-Bromobenzaldehyde dimethyl acetal (3)[1]. A solution of 2-bromobenzaldehyde **2** (20.0 g, 108 mmol) in MeOH (50 ml) was refluxed in the presence of NH_4Cl (286 mg, 5.35 mmol) overnight. After cooling, sat aq $NaHCO_3$ was added. The reaction mixture was extracted with Et_2O, washed with sat aq NaCl solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by distillation under reduced pressure to give the acetal **3** (22.1 g, 95%) as a colorless oil. R_f=0.50 (hexanes/EtOAc 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.39 (s, 6H), 5.57 (s, 1H), 7.20 (t, J = 7.5 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 54.2 (2C), 103.3, 123.3, 127.5, 128.6, 130.4, 133.2, 137.1; IR (thin film): ν = 2990, 2933, 2905, 2828, 1570, 1468, 1434, 1364, 1267, 1204, 1190, 1124, 1103, 1074, 1056, 1025, 980, 909, 754, 688, 653 cm^{-1}. The analytical data was consistent with the literature.

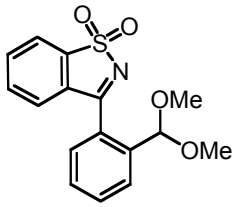


3-Ethoxy-l,2-benzisothiazole 1,1-dioxide (4)[2]. Saccharin (52.0 g, 0.284 mol) and PCl_5 (71.8 g, 0.345 mol) were gently heated until the reaction mixture became an oil and the temperature was raised to 175 °C for additional 1.5 h. The byproduct $POC1_3$ was removed under reduced pressure, and the crude 3-chloro-1,2-benzisothiazole 1,l-dioxide was treated with absolute EtOH (400 ml). The reaction mixture was refluxed for 1 h and cooled to room temperature. The precipitated product was collected by filtration and washed with ice-cold EtOH. The mother liquor was cooled in an ice bath to obtain more precipitate. The solids were combined and dried to give *O*-ethylsaccharin **4** (23.7 g, 33%) as a colorless fine-crystalline material. m.p.: 216.0–219.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.54 (t, J = 7.0 Hz, 3H), 4.67 (q, J = 7.0 Hz, 2H), 7.68–7.72 (m, 1H), 7.74–7.78 (m, 2H), 7.88 (d, J = 7.5 Hz, 1H);

(1) Martin, C.; Mailliet, P.; Maddaluno, J.; *J. Org. Chem*. **2001**, *66*, 3797–3805.

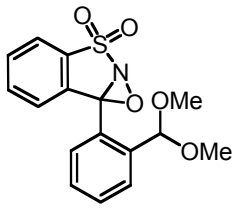
(2) Davis, F. A.; Towson, J. C.; Vashi, D. B.; ThimmaReddy, R.; McCauley, J. P., Jr.; Harakal, M. E.; Gosciniak, D. J.; *J. Org. Chem*. **1990**, *55*, 1254–1261.

^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.4, 68.6, 122.2, 123.6, 127.5, 133.7, 134.3, 143.9, 169.5. The analytical data was consistent with the literature.



3-(2-(Dimethoxymethyl)-phenyl)-1,2-benzisothiazole 1,1-dioxide (5).

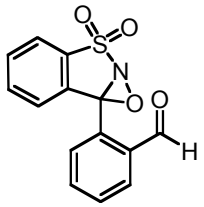
To a solution of 2-bromobenzaldehyde dimethylacetal **3** (5.02 g, 21.7 mmol, 1.1 equiv) in THF (50 ml) was added *n*-BuLi in hexanes (2.5 M, 8.7 ml, 21.7 mmol, 1.1 equiv) at –78 °C, and the mixture was stirred for 30 min. A solution of 3-ethoxy-l,2-benzisothiazole 1,1-dioxide **4** (4.10 g, 19.4 mmol, 1.0 equiv) in THF (50 ml) was added via cannula. The reaction temperature was increased to rt over 2 h. The reaction was quenched with sat aq NH_4Cl, extracted with EtOAc, washed with sat aq NaCl solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (hexanes/EtOAc 1:1) to give the product **5** (5.97 g, 97%) as a colorless solid. R_f=0.50 (hexanes/EtOAc 1:1); m.p.: 95–97 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.22 (s, 6H), 5.69 (s,1H), 7.37 (d, J = 7.6 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.73–7.78 (m, 2H), 8.00 (d, J = 7.5 Hz, 1H)); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 54.0 (2C), 101.5, 122.6, 126.1, 127.9, 128.6, 128.8, 129.3, 131.3, 132.5, 133.5, 134.0, 138.2, 139.7, 173.7; IR (thin film): ν = 2937, 1607, 1550, 1450, 1338, 1302, 1287, 1200, 1175, 1116, 1091, 1057, 982, 968, 909, 794, 782, 768, 749, 721, 699, 674, 672 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{16}H_{15}NNaO_4S$: 340.0619; found: 340.0605 [M+Na]$^+$.



3-(2-(Dimethoxymethyl)-phenyl)-1,2-benzisothiazole 1,1-dioxide oxide (6).

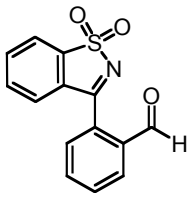
To a solution of the 3-substituted 1,2-benzisothiazole 1,1-dioxide **5** (1.12 g, 3.21 mmol, 1.0 equiv) in CH_2Cl_2 (30 ml) and sat aq K_2CO_3 (30 ml) was added 70% *m*CPBA (1.16 g, 4.72 mmol, 1.5 equiv) at

0 °C, and the suspension was stirred vigorously for 3 h at 0 °C. The progress of the reaction was monitored by TLC. The R_f value of the oxaziridine **6** was higher than the imine **5**. Upon completion, H_2O was added. The reaction mixture was extracted with CH_2Cl_2, washed with sat aq $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (hexanes/EtOAc 7:3) to give the product **6** (959 mg, 90%) as a colorless solid. R_f=0.60 (hexanes/EtOAc 1:1); m.p. 133.5–135.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.03 (s, 3H), 3.23 (s, 3H), 5.41 (s, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 7.57 (t, J = 7.5 Hz, 2H), 7.62 (t, J = 7.5 Hz, 1H), 7.69 (t, J = 8.0 Hz, 2H), 7.84 (d, J = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 53.2, 54.2, 86.1 (oxaziridine C), 101.2, 123.8, 127.0, 127.1, 127.7, 128.8, 129.1, 131.0, 132.4, 132.7, 133.9, 137.0, 137.7; IR (thin film): ν = 2937, 2833, 1704, 1452, 1362, 1329, 1202, 1186, 1163, 1112, 1087, 1058, 945, 765, 737, 700 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{16}H_{15}NNaO_5S$: 356.0569; found: 356.0575 [M+Na]$^+$.



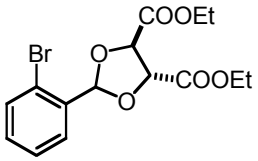
3-(2-formylphenyl)-l,2-benzisothiazole 1,l-dioxide oxide (±)-1.

A solution of the acetal **5** (544.0 mg, 1.632 mmol) in THF (20 ml), 1 M HCl (10 ml) was stirred at rt for 2 h. The reaction mixture was extracted with CH_2Cl_2, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (hexanes/EtOAc 1:1) to give the aldehyde **(±)-1** (441 mg, 94%) as a colorless solid. R_f=0.30 (hexanes/EtOAc 1:1); m.p. 140.0–141.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.10 (d, J = 7.5 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.76–7.78 (m, 1H), 7.80–7.82 (m, 2H), 7.86 (d, J = 7.5 Hz, 1H), 7.99–8.99 (m, 1H), 9.97 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 85.8 (oxaziridine C), 124.2, 125.7, 129.2, 129.8, 131.8, 132.5, 133.2, 134.1, 134.2, 134.4, 134.7, 136.4, 190.8; IR (thin film): ν = 2834, 2750, 1703, 1578, 1452, 1361, 1327, 1306, 1240, 1204, 1186, 1163, 946, 863, 765, 737, 728, 699, 604 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{14}H_9NNaO_4S$: 310.0150; found: 310.0135 [M+Na]$^+$.



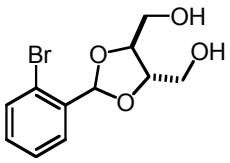
3-(2-Formylphenyl)-l,2-benzisothiazole 1,1-dioxide (S1).

A solution of the acetal **5** (103.1 mg, 0.325 mmol) in AcOH (1 ml), H_2O (1 ml) and EtOAc (1 ml) was stirred at rt overnight. The reaction mixture was extracted with CH_2Cl_2, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was filtrated and washed by (hexanes/EtOAc 1:1) to give the aldehyde **S1** (33.0 mg, 37%) as a colorless solid. R_f=0.20 (hexanes/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.27 (d, J = 8.0 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.70–7.72 (m, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.84–7.85 (m, 2H), 8.02 (d, J = 8.0 Hz, 1H), 8.12–8.14 (m, 1H), 10.14 (s, 1H); ^{13}C NMR (125 MHz, DMSO): δ = 123.5, 127.1, 130.5, 130.6, 131.8, 133.1, 134.3, 134.8, 135.4, 135.5, 136.0, 138.9, 174.4, 193.3; IR (thin film): ν = 3086, 2857, 1689, 1606, 1571, 1550, 1330, 1297, 1258, 1200, 1170, 1129, 969, 857, 769, 751, 721, 675, 659, 633 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{14}H_9NNaO_3S$: 294.0201; found: 294.0208 [M+Na]$^+$.



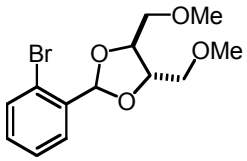
(*4R,5R*)-Diethyl 2-(2-bromophenyl)-1,3-dioxolane-4,5-dicarboxylate (S2).

A solution of (+)-diethyl-L-tartrate (14.19 g, 68.82 mmol, 1.0 equiv), 2-bromobenzaldehyde (12.73 g, 68.80 mmol, 1.0 equiv) and TsOH (129.1 mg, 0.68 mmol, 1 mol%) in toluene (50 ml) was refluxed with a Dean Stark condenser. After 12 h, the solvent was removed under reduced pressure to give the acetal **S2** (22.1 g, 86%) as a colorless oil. The oil became a solid upon standing at –20 °C. R_f=0.20 (hexanes/EtOAc 1:1); $[\alpha]_D^{30}$ –0.65 (c = 0.38, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.30 (t, J = 7.0 Hz, 3H), 1.37 (t, J = 7.0 Hz, 3H), 4.27 (q, J = 7.0 Hz, 2H), 4.31–4.36 (m, 2H), 4.86 (d, J = 4.0 Hz, 1H), 4.97 (d. J = 4.0 Hz, 1H), 6.48 (s, 1H), 7.25 (t, J = 8.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3, 14.4, 62.3, 62.4, 77.4, 78.0, 105.5, 123.4, 127.8, 129.1, 131.5, 133.0, 134.7, 169.3, 169.7; IR (thin film): ν = 2983, 1755, 1475, 1444, 1393, 1370, 1338, 1275, 1213, 1161, 1127, 1107, 1046, 1026, 963, 950, 857, 759, 680 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{15}H_{17}BrO_6$: 373.0287; found: 373.0282 [M+H]$^+$.



((*4S,5S*)-2-(*o*-Bromophenyl)-1,3-dioxolane-4,5-diyl)dimethanol (S3).

To a solution of the ester **S2** (18.89 g, 49.8 mmol, 1.0 equiv) in THF (250 ml) was added slowly $LiAlH_4$ (3.78 g, 99.6 mmol, 2.0 equiv) at 0 °C. The suspension was stirred for 2 h at rt before $Na_2SO_4 \cdot 10H_2O$ was added slowly at 0 °C. The mixture was dried over anhydrous Na_2SO_4. The suspension was filtrated through Celite and washed with THF. The crude mixture was purified by distillation under reduced pressure to give the diol **S3** (7.92 g, 55%) as a colorless oil. R_f=0.20 (hexanes/EtOAc 1:4); $[\alpha]_D^{30}$ –0.65 (*c* = 0.38, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.69 (br, 2H), 3.74–3.87 (m, 4H), 4.12–4.17 (m, 2H), 6.22 (s, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 62.4, 62.6, 78.8, 79.8, 102.9, 123.1, 127.9, 128.5, 131.3, 133.3, 136.2; IR (thin film): ν = 3389, 2924, 1571, 1474, 1442, 1403, 1379, 1272, 1212, 1126, 1102, 1042, 1024, 950, 862, 759, 733, 677, 627 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{11}H_{12}BrO_4$: 286.9919; found: 286.9879 $[M+H]^+$.

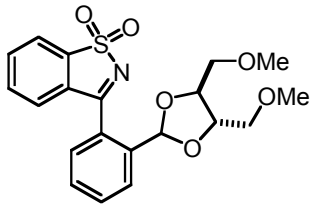


(*4S,5S*)-2-(2-Bromophenyl)-4,5-bis(methoxymethyl)-1,3-dioxolane (S4)[3].

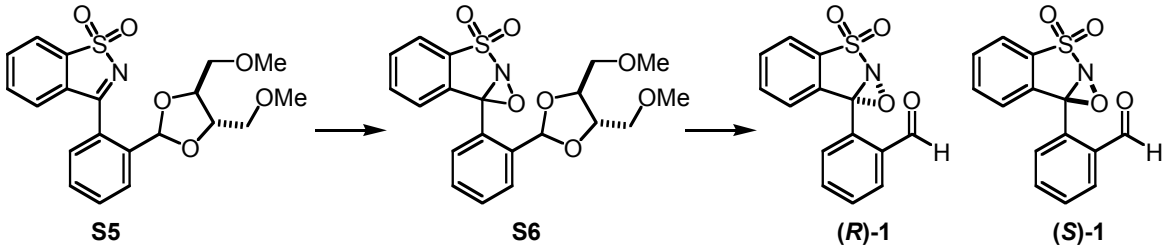
Sodium hydride (60%) in mineral oil (3.28 g, 82.0 mmol, 3.0 equiv) was washed with dry hexanes, DMF (150 ml) was added, and the mixture was cooled to 0 °C. To the mixture was added diol **S3** (7.92 g, 27.4 mmol, 1.0 equiv) in DMF (10 ml) over 1 h. To the resulting mixture was added dropwise MeI (5.0 ml, 80.3 mmol, 3.0 equiv) and stirred 2 h at rt. The reaction was quenched by addition of H_2O. The reaction mixture was extracted with EtOAc, washed with sat aq NaCl solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to provide the diether **S4** (8.13 g, 94%) as a colorless oil. R_f=0.70 (hexanes/EtOAc 1:1); $[\alpha]_D^{30}$ +7.4 (*c* = 1.64, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.42 (s, 3H), 3.45 (s, 3H), 3.59–3.67 (m, 4H), 4.16–4.23 (m, 2H), 6.24 (s, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H); ^{13}C

(3) M. Commerçon, P. Mangeney, T. Tejero, A. Alexaks, *Tetrahedron: Asymmetry*, **1990**, *1*, 287-290

NMR (125 MHz, $CDCl_3$): δ = 59.8, 59.9, 73.2 (2C), 78.1, 78.5, 103.0, 123.3, 127.8, 128.8, 131.0, 133.1, 136.4; IR (thin film): ν = 2924, 2889, 1470, 1443, 1378, 1272, 1199, 1128, 1087, 1044, 1024, 984, 957, 759 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{13}H_{17}BrNaO_4$: 339.2028; found: 339.0195 $[M+Na]^+$.

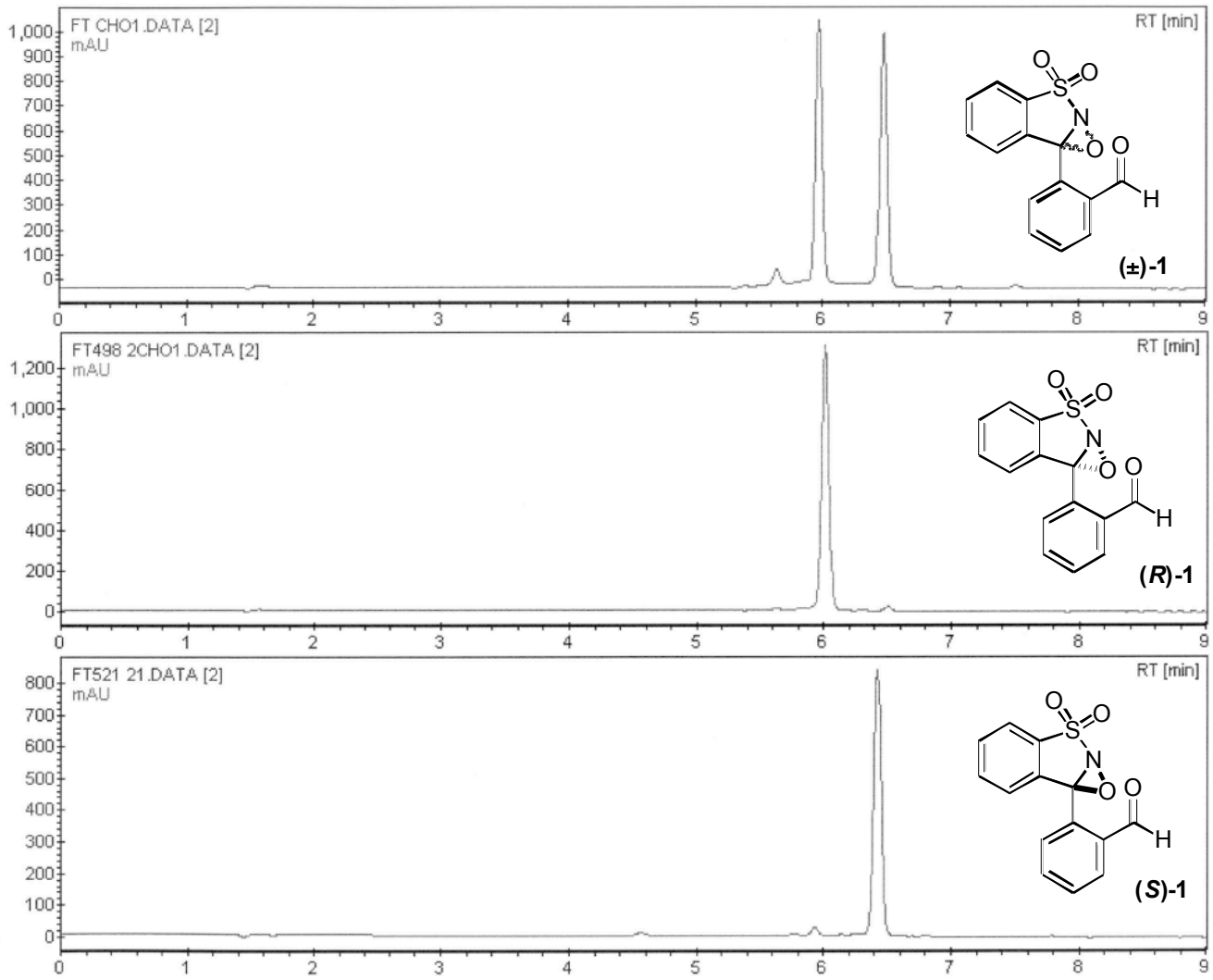


3-(2-((*4S,5S*)-4,5-Bis(methoxymethyl)-1,3-dioxolan-2-yl)phenyl)-1,2-benzisothiazole 1,1-dioxide (S5). An oven-dried flask was charged with the bromide **S4** (4.00 g, 12.6 mmol, 1.1 equiv) and purged with N_2. Dry THF (50 ml) was added and cooled to –78 °C before *n*-BuLi (2.5 M, 5.0 ml, 12.5 mmol, 1.1 eq) was added dropwise and the mixture stirred for 30 min at –78 °C. A THF suspension (100 ml) of 3-ethoxy-l,2-benzisothiazole 1,1-dioxide **3** (2.42 g, 11.5 mmol, 1.0 equiv) was added via cannula at –78 °C. The reaction mixture was warmed to room temperature over 2 h and the reaction was quenched by the addition of sat aq NH_4Cl solution. The mixture was extracted with EtOAc, washed with sat aq NaCl solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (hexanes/EtOAc 1:1) to provide the product **S5** (3.49 g, 75%) as a colorless solid. R_f=0.20 (hexanes/EtOAc 1:1); $[\alpha]_D^{30}$ –4.7 (*c* = 1.23, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$): δ = 3.28 (s, 3H), 3.29 (s, 3H), 3.38–3.45 (m, 4H), 3.84–3.88 (m, 1H), 4.07–4.11 (m, 1H), 6.15 (s, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.98 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 59.5, 59.6, 72.6, 72.7, 77.8, 78.5, 102.0, 122.5, 126.8, 128.6, 129.0, 129.3, 129.6, 131.4, 132.8, 133.5, 134.0, 136.8, 139.8, 173.1; IR (thin film): ν = 2926, 2888, 1551, 1452, 1338, 1302, 1283, 1191, 1175, 1128, 1080, 984, 968, 961, 781, 768, 752, 741, 698, 673, 643 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{20}H_{22}NO_6S$: 404.1167; found: 404.1178 $[M+H]^+$.



Oxidation and Separation of Oxaziridine diastereomer

To a mixture of the 3-substituted 1,2-benzisothiazole 1,1-dioxide **S5** (261.0 mg, 0.647 mmol, 1.0 equiv) in CH_2Cl_2 (5 ml) and sat aq K_2CO_3 (5 ml) was added slowly 70% *m*CPBA (242.6 mg, 0.984 mmol, 1.5 equiv) at 0 °C and the suspension stirred vigorously for 1 h at rt. Water (30 ml) was added and the reaction mixture was extracted with CH_2Cl_2, washed with sat aq $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to provide the crude oxaziridine **S6** as a (1:0.7) diastereomer ratio (determined by ^{1}H-NMR). The diastereomers were separated by silica gel chromatography (hexanes/EtOAc 3:2). R_f=0.50 (hexane/EtOAc 1:1), ΔR_f=0.08. The acetal **S6** was removed immediately from corresponding diastereomerically pure oxaziridine. To the acetal protected oxaziridine **S6** in THF (1 ml) was added 6 M HCl solution (0.5 ml) at rt and the mixture stirred 12 h. The reaction mixture was extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by PTLC (CH_2Cl_2/MeOH 99:1) to give each enantiomers (**(*R*)-1**, 51.5 mg) and (**(*S*)-1**, 36.7 mg) as colorless solid. The enantiopurity was confirmed by chiral SFC analysis. **(*R*)-1**: $[\alpha]_D^{30}$ +97.6 (*c* = 1.05, $CHCl_3$), **(*S*)-1**: $[\alpha]_D^{30}$ –98.8 (*c* = 1.13, $CHCl_3$). Other spectral data was identified to that of **(±)-1**.



SFC analysis: gradient 5–80% $scCO_2$/IPA over 7.5 min at a flow rate of 2.0 ml/min, 254 nm, CHIRALPAK AD-H. Retention time: 5.97 min **(*R*)-1**, 6.48 min **(*S*)-1**.

Determination of the absolute configuration by x-ray analysis.

The absolute configuration of **(*S*)-1** was determined from the Flack parameter[4], which is 0.03(7). See the CIF files for further information.

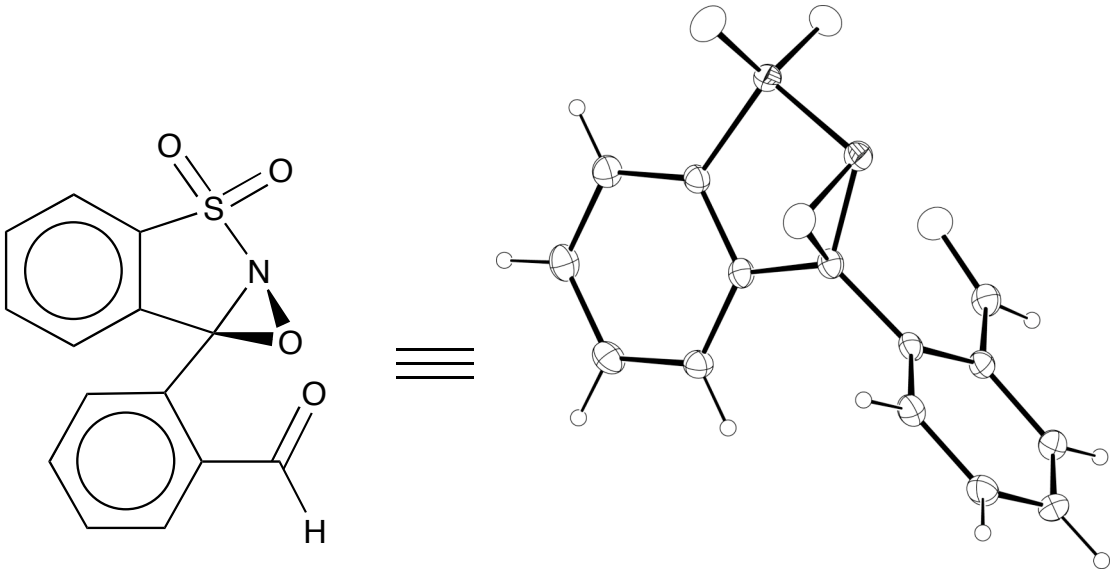


Figure 1. ORTEP drawing of the title compound

X-ray intensity data were collected on a Rigaku Mercury CCD area detector employing graphite-monochromated Mo-Ka radiation (λ=0.71073 Å) at a temperature of 153K. Preliminary indexing was performed from a series of twelve 0.5° rotation images with exposures of 30 seconds. A total of 360 rotation images were collected with a crystal to detector distance of 35 mm, a 2θ swing angle of -12°, rotation widths of 0.5° and exposures of 60 seconds: scan no. 1 was a ϕ-scan from 135° to 315° at ω = 0° and χ = -30°. Rotation images were processed using CrystalClear[5], producing a listing of unaveraged F^2 and $\sigma(F^2)$ values which were then passed to the CrystalStructure[6] program package for further processing and structure solution on a Dell Pentium III computer. A total of 5822 reflections were measured over the ranges 5.18 ≤ 2q £ 50.02 °, -8 ≤ h ≤ 8, -10 ≤ k ≤ 12, -18 ≤ l ≤ 15 yielding 2237 unique reflections (R_{int} = 0.0155). The intensity data were corrected for Lorentz and polarization effects and for absorption using REQAB[7] (minimum and maximum transmission 0.870, 1.000).

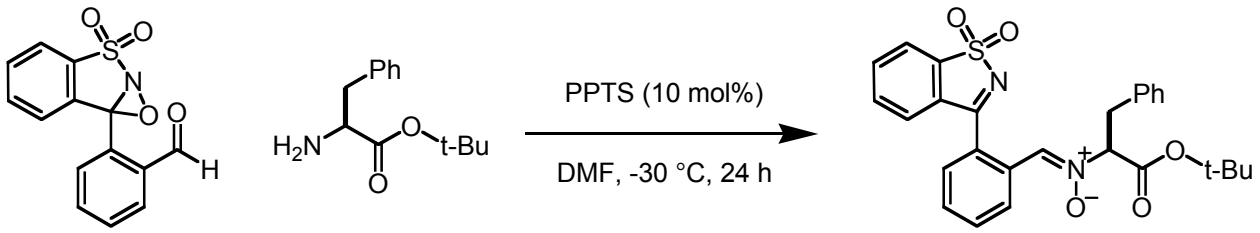
(4) Flack, H. D., Acta Cryst. A39 (1983) 876-881.
(5) CrystalClear: Rigaku Corporation, 1999.
(6) CrystalStructure: Crystal Structure Analysis Package, Rigaku Corp. Rigaku/MSC (2002).
(7) REQAB4: R.A. Jacobsen, (1994).

The structure was solved by direct methods (SIR97[8]). Refinement was by full-matrix least squares based on F^2 using SHELXL-97[9]. All reflections were used during refinement (F^2's that were experimentally negative were replaced by $F^2 = 0$). The weighting scheme used was $w=1/[\sigma^2(F_o^2)+ 0.0377P^2 + 0.2459P]$ where $P = (F_o^2 + 2F_c^2)/3$. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using a "riding" model. Refinement converged to R1=0.0284 and wR2=0.0684 for 2202 reflections for which $F > 4\sigma(F)$ and R_1=0.0290, wR_2=0.0692 and GOF = 1.123 for all 2237 unique, non-zero reflections and 182 variables[10]. The maximum Δ/σ in the final cycle of least squares was 0.000 and the two most prominent peaks in the final difference Fourier were +0.137 and -0.294 e/Å^3.

(8) SIR97: Altomare, A., M. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A. Moliterni, G. Polidori & R. Spagna (1999). *J. Appl. Cryst.*, **32**, 115-119.
(9) SHELXL-97: Program for the Refinement of Crystal Structures, Sheldrick, G.M. (1997), University of Göttingen, Germany.
(10) $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$
$wR_2 = \{ \sum w (F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2 \}^{1/2}$
$GOF = \{ \sum w (F_o^2 - F_c^2)^2 / (n - p) \}^{1/2}$
where n = the number of reflections and p = the number of parameters refined.

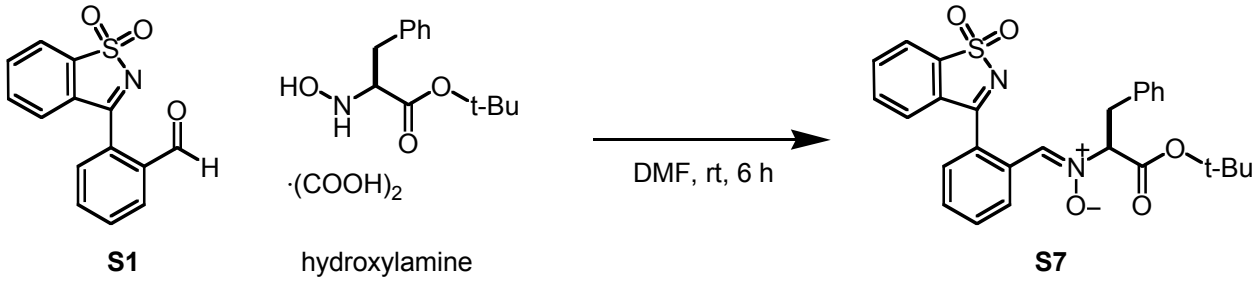
3. Solution Phase Nitrone Synthesis and Conformation of Structure

Method A: from amine



(*S*)-Phenylalanine *tert*-butyl ester HCl salt (161.4 mg, 0.626 mmol, 1.2 equiv) was extracted with aq $NaHCO_3$ solution and CH_2Cl_2, dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The resulting phenylalanine *tert*-butyl ester was dissolved in DMF (3 ml) and pyridinium *p*-toluenesulfonate (13.5 mg, 0.0537 mmol, 10 mol%) was added. The mixture was cooled to –30 °C and the reagent **(±)-1** (149.4 mg, 0.520 mmol) was added and the mixture stirred 24 h at –30 °C. The excess DMF was removed under reduced pressure, the reaction mixture was poured into H_2O, and aqueous layer extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (hexanes/EtOAc 1:1) to provide the nitrone **S7** (102.5 mg, 40%) as a yellow solid.

Method B: from hydroxylamine

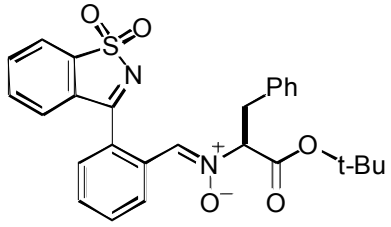


(*S*)-Phenylalanine *tert*-butyl ester hydroxylamine was prepared from the corresponding phenylalanine *tert*-butyl ester by using the method of Fukuyama.[11]

To the hydroxylamine (24.5 mg, 0.0748 mmol, 1.0 equiv) in DMF (0.5 ml) was added the aldehyde **S1** (20.6 mg, 0.0759 mmol, 1.0 equiv) for 6 h at rt. Upon completion, H_2O was added to the mixture, and the aqueous layer was extracted with EtOAc. The organic layer was washed with sat aq NaCl solution

(11) Tokuyama, H.; Kuboyama, T.; Fukuyama, T.; *Org. Synth*. **2003**, *80*, 207–218.

and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by PTLC (hexane/EtOAc 1:1) to provide the nitrone **S7** (15.0 mg, 41%) as a yellow solid.



Phenylalanine *tert*-butyl ester *N*-oxide Nitrone (S7). R_f=0.20 (hexanes/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.40 (s, 9H), 3.28 (dd, J = 7.0, 14.0 Hz, 1H), 3.54 (dd, J = 8.5, 14.0 Hz, 1H), 4.61 (dd, J = 7.0, 8.5 Hz, 1H), 7.02 (t, J = 7.0 Hz, 1H), 7.16 (t, J = 8.0 Hz, 2H), 7.19 (t, J = 7.5 Hz, 2H), 7.52 (d, J = 7.5 Hz, 1H), 7.55–7.58 (m, 2H), 7.68 (t, J = 8.0 Hz, 2H), 7.67 (s, 1H), 7.78 (t, J = 7.5 Hz, 1H), 8.00 (d, J = 7.5 Hz, 1H), 8.75 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.1 (3C), 36.2, 80.5, 83.7, 123.0, 126.6, 127.2, 128.8 (2C), 128.9, 129.1, 129.2, 129.4 (2C), 130.0, 130.1, 131.7, 132.2, 132.4 (2C), 133.7, 134.1, 136.2, 140.5, 166.2; IR (thin film): ν = 2978, 2922, 2849, 1736, 1698, 1604, 1546, 1454, 1393, 1369, 1338 s, 1306, 1262, 1206, 1175 s, 1083, 1051, 995, 968, 842, 766, 741, 700 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{27}H_{26}N_2NaO_5S$: 513.1460; found: 513.1472 $[M+Na]^+$.

4. Solid-Phase Peptide Synthesis

Peptides were prepared by standard Fmoc manual solid-phase synthesis protocols using Rink amide MBHA resin (substitution: 0.7 mmol/g). The following Fmoc amino acids from NovaBiochem were employed: Fmoc-Ala-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH. Coupling on the resin was carried out with Fmoc amino acids (3 equiv), HBTU (3equiv), HOBt (3 equiv) and DIPEA (6 equiv) in DMF for 3 h. Fmoc deprotections were conducted by treatment with 20% piperidine in DMF for 30 minutes.

The Fmoc loading of the resin was calculated by following method[12].

1. Two quartz UV cells were used.
2. A dry Fmoc amino acid resin (5 μmol) was weight carefully into a vial. To the vial was added 2% DBU in DMF (2 ml) and stirred gently for 30 min (solution A). The solution A was filtered into a 10 ml volumetric flask and diluted to the mark with CH_3CN (solution B). The solution B (2 ml) was taken into 25 ml volumetric flask and diluted to the mark with CH_3CN (solution C).
3. A reference solution was prepared as in step 2, without addition of the resin (solution D).
4. The sample solution (solution C) and the reference solution (solution D) were added to cells.
5. Two cells were placed in a spectrophotometer and recorded a density at 304 nm.
6. An estimate of the residue attachment was obtained from equation below.

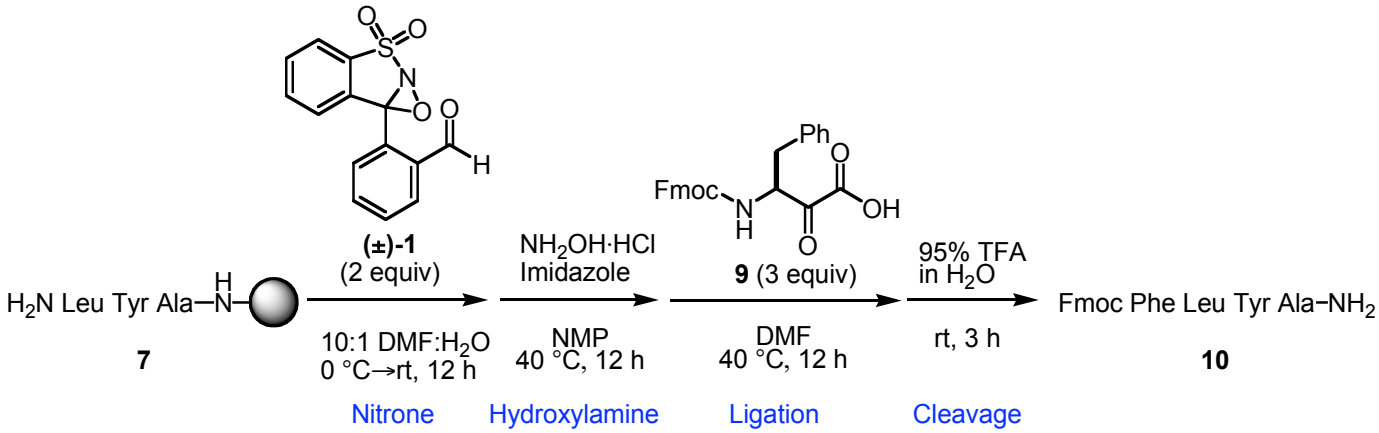
Fmoc loading: mmol/g = $(Abs_{sample} - Abs_{reference}) \times 16.4$ / (mg of the resin)

Table 1. Fmoc loading of resins

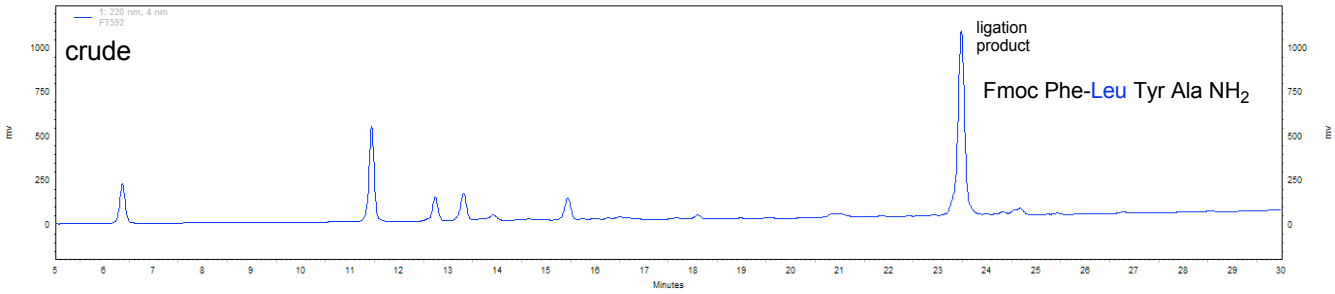
Peptide on Rink amide MBHA resin		Fmoc Loading of the resin
Fmoc Leu Tyr Ala—NH–	**7**	0.54 mmol/g
Fmoc Ser Asp Tyr Lys Ala—NH–	**12a**	0.46 mmol/g
Fmoc Glu Asp Tyr Lys Ala—NH–	**12b**	0.41 mmol/g
Fmoc Phe Asp Tyr Lys Ala—NH–	**12c**	0.45 mmol/g
Fmoc Ala Asp Tyr Lys Ala—NH–	**12d**	0.47 mmol/g
Fmoc Leu Lys Ser Glu Tyr—NH–	**12e**	0.44 mmol/g

(12) Novabiochem® Peptide Synthesis 2008/2009 Catalog

5. General Procedure for Amine Oxidation Followed by Ligation on Solid Support



To the resin bound peptide 7 (0.54 mmol/g, 78.5 mg) in DMF/H_2O (10:1, 0.5 ml) was added **(±)-1** (24.4 mg, 0.0849 mmol, 2 equiv) in DMF/H_2O (10:1, 0.5 ml) at 0 °C, the reaction mixture was stirred slowly, and warmed to rt over 12 h. The resin was filtered, and washed with DMF and CH_2Cl_2. The resin was mixed with (NH_2OH/imidazole/NMP 1.25 g:0.918 g:5 ml)[13] (1 ml), warmed up to 40 °C and N_2 gas was bubbled via pipet for 12 h at 40 °C. The resin was filtered, and washed with DMF and CH_2Cl_2. The resin was mixed with Fmoc phenylalaine ketoacid **9**[14] in DMF (0.5 ml) and N_2 gas was bubbled via pipet for 12 h at 40 °C. The resin was filtered, and washed with DMF and CH_2Cl_2. The peptide residue was cleaved from the resin by 95% TFA in H_2O at room temperature for 3 h. The solvent was removed under reduced pressure and the purity was cheked by HPLC analysis. The crude mixture was purified by preparative TLC to give the desired ligation product **10** (6.4 mg). HRMS (ESI): *m/z*: calcd for $C_{42}H_{47}N_5NaO_7$: 756.3373; found: 756.3378 [*M*+Na]$^+$.



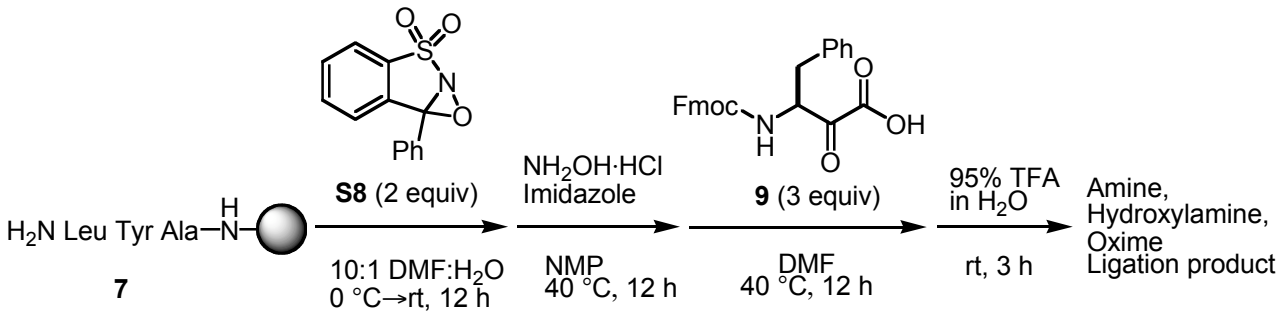
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm).

(13) Juan Jose Díaz-Mochón, J. J.; Bialy, L.; Bradley, M.; *Org Lett.* **2004**, *6*, 1127–1129.

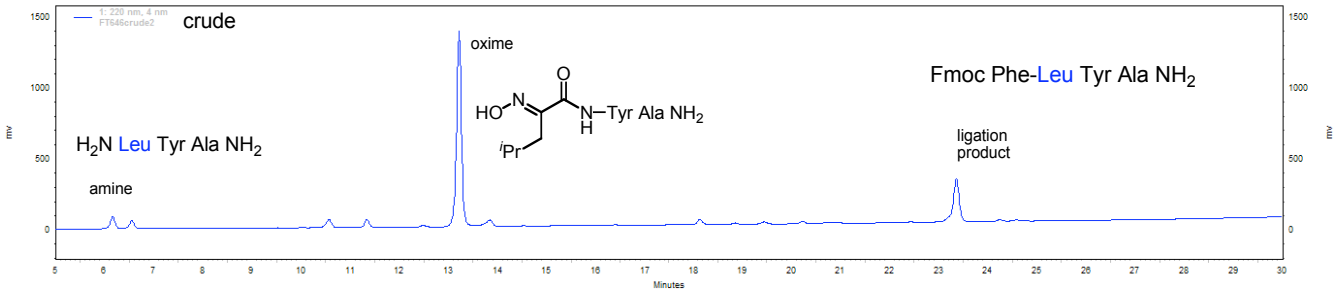
(14) Ju, L.; Lippert, A. R.; Bode, J. W.; *J. Am. Chem. Soc*, **2008**, *130*, 4253–4255.

6. Control Reactions for Oxidations of N-Terminal Peptides Amines

Control experiment using oxadiridine S8[15], or using oxadiridine S8 and benzaldehyde

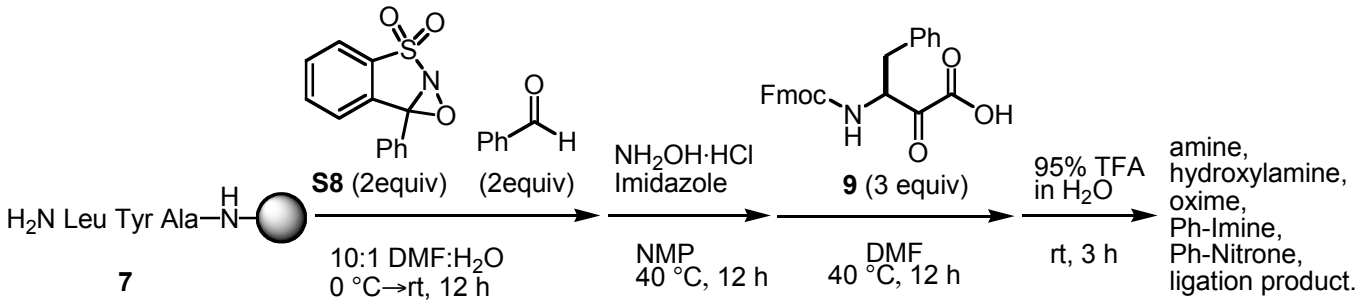


The resin **7** (0.54 mmol/g, 19.5 mg) and oxadiridine **S8** (5.6 mg) were used according to the general procedure.

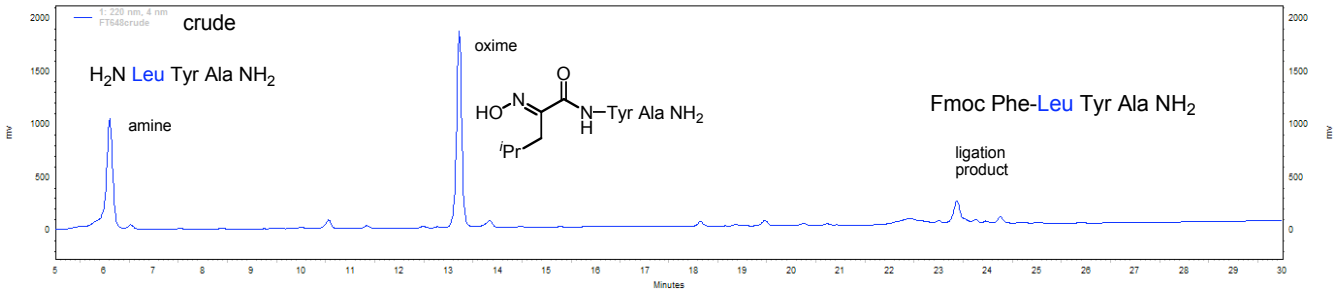


HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Peak area% on 220 nm: peptide amine (6.1 min, 4.64%), peptide oxime (13.2 min, 74.70%), ligation product (23.4 min, 20.65).

(15) Davis, F. A.; Towson, J. C.; Vashi, D. B.; ThimmaReddy, R.; McCauley, J. P., Jr.; Harakal, M. E.; Gosciniak, D. J.; *J. Org. Chem*. **1990**, *55*, 1254–1261.

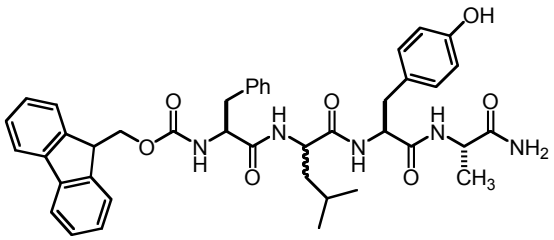


The resin **7** (0.54 mmol/g, 20.0 mg), oxadiridine **S8** (5.8 mg) and benzaldehyde (2.5 µl) were used according to the general procedure.

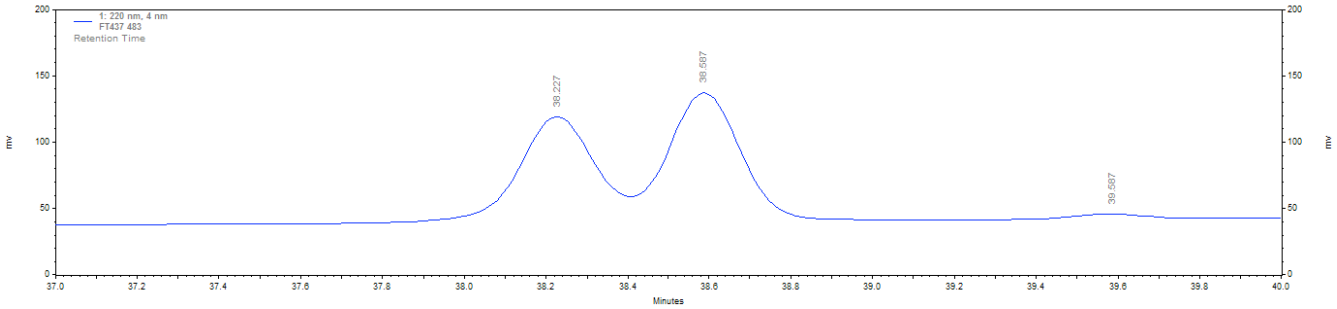


HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 µm). Peak area% on 220 nm: peptide amine: (6.1 min, 40.52%), peptide oxime: (13.2 min, 48.65%), ligation product: (23.4 min, 10.83%).

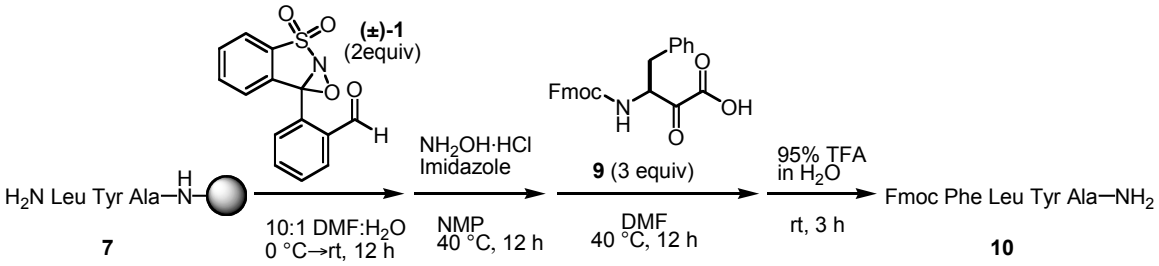
7. Establishment of Stereoretention at N-Terminus Following Oxidation

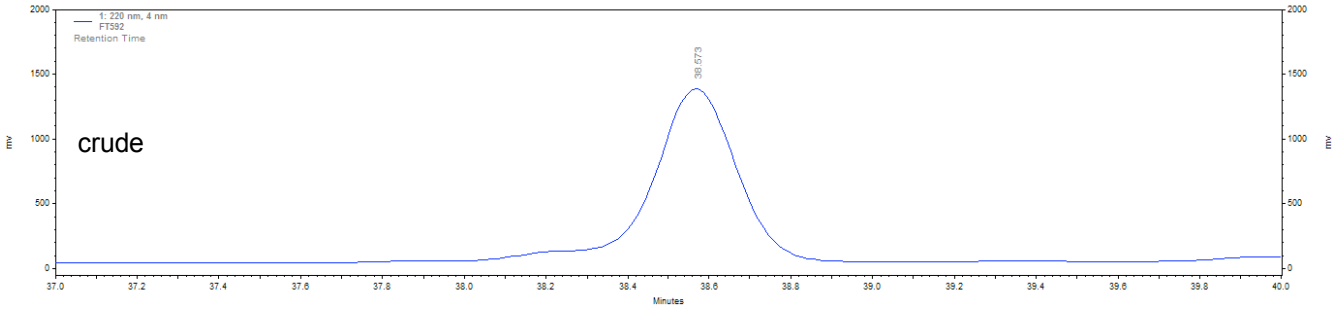


Standard sample (D/L) mixture



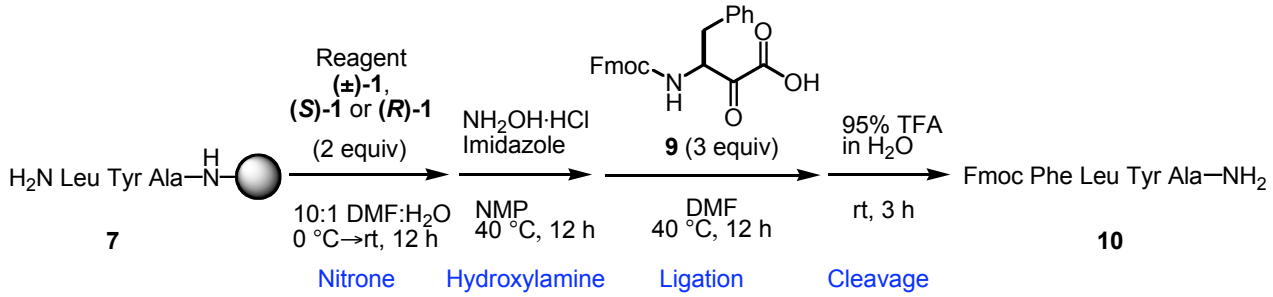
The standard sample was made using D/L-Leu, according to standard Fmoc synthesis procedures. HPLC analysis: gradient 10–90% CH_3CN/H_2O over 60 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 38.2 min (D-Leu), 38.6 min (L-Leu).

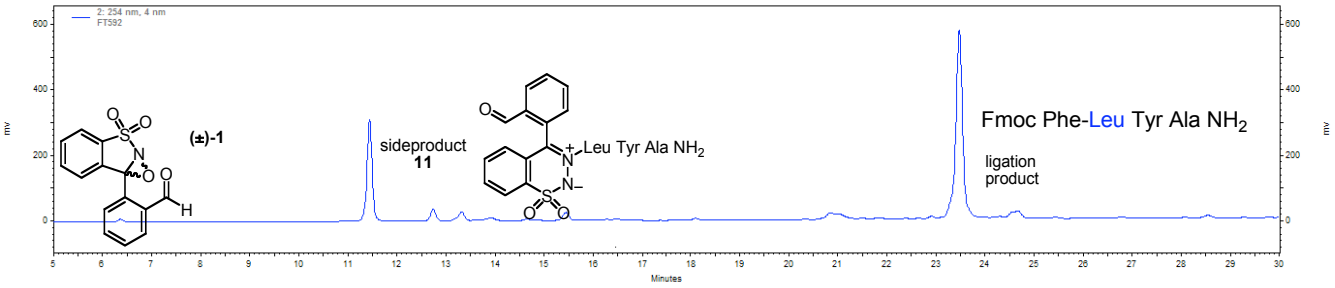


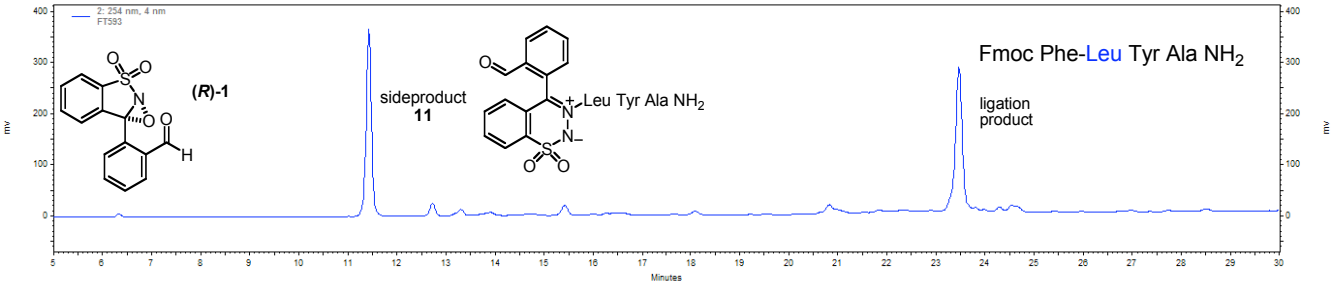


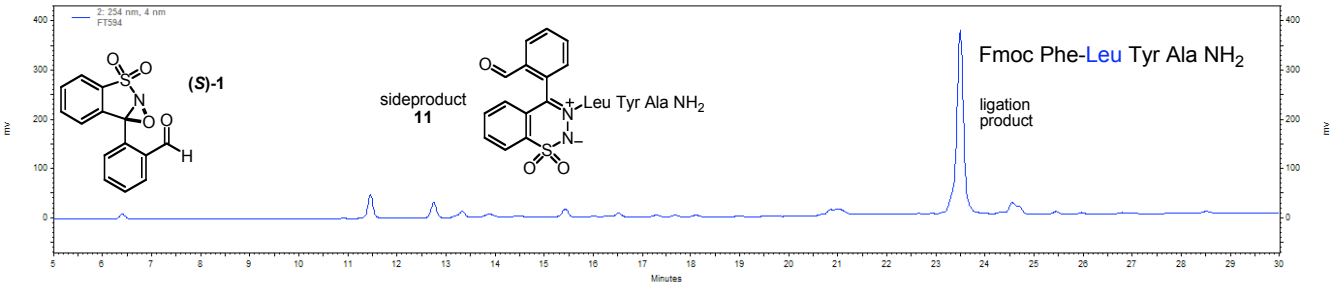
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 60 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 38.6 min.

8. Tentative Structure of Reaction Side Product and Effect of Reagent Stereochemistry



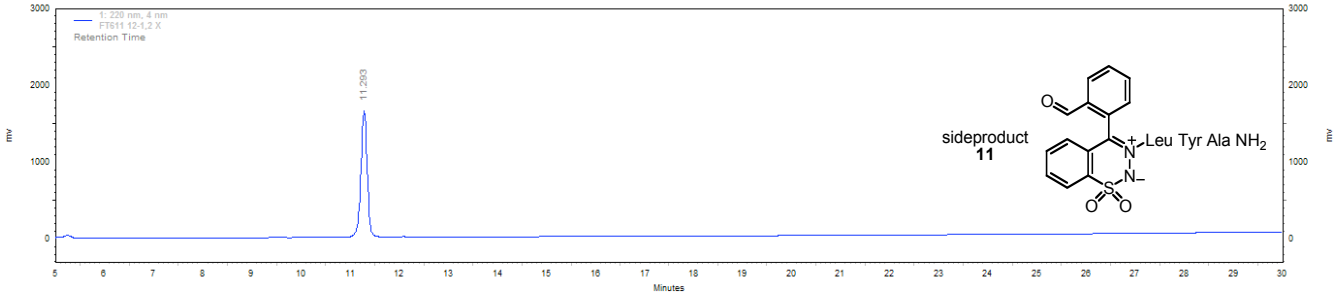






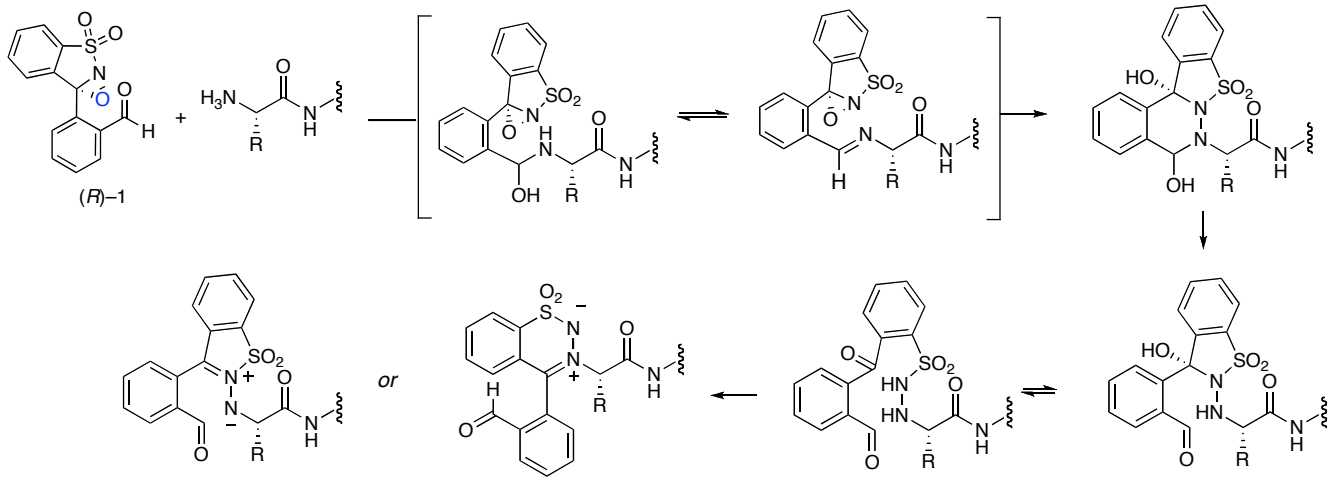
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 254 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Ligation product **10**: 23.5 min. Ligation product peak area% on 254 nm: from **(±)-1** (54%), from **(*R*)-1** (39%), from **(*S*)-1** (63%).

The side product was assigned tentatively as azomethineylide. This product has the identical mass as the expected nitrone intermediate.

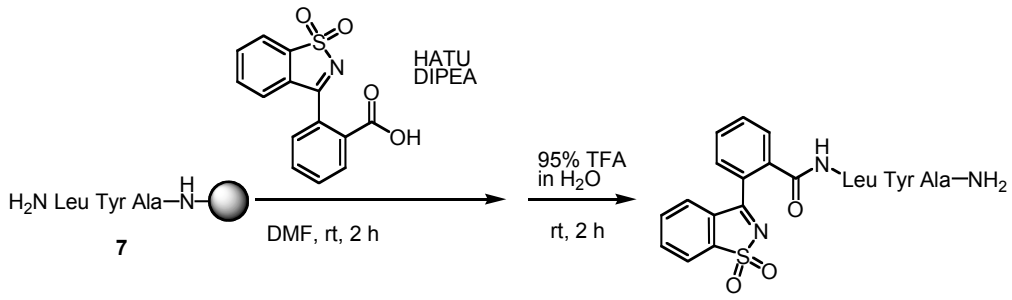


HPLC analysis: gradient 10-90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 11.3 min.. HRMS (ESI): *m/z*: calcd for $C_{32}H_{35}N_5NaO_7S$: 656.2155; found: 656.2164 $[M+Na]^+$. This compound is an approximately 2:1 mixture of atropisomers in the ^1H NMR and a copy of this NMR is included in the spectral data. Although it has a strong UV absorption, only a small amount of this product is obtained following preparative HPLC and we have not been able to obtain sufficient material for further characterization.

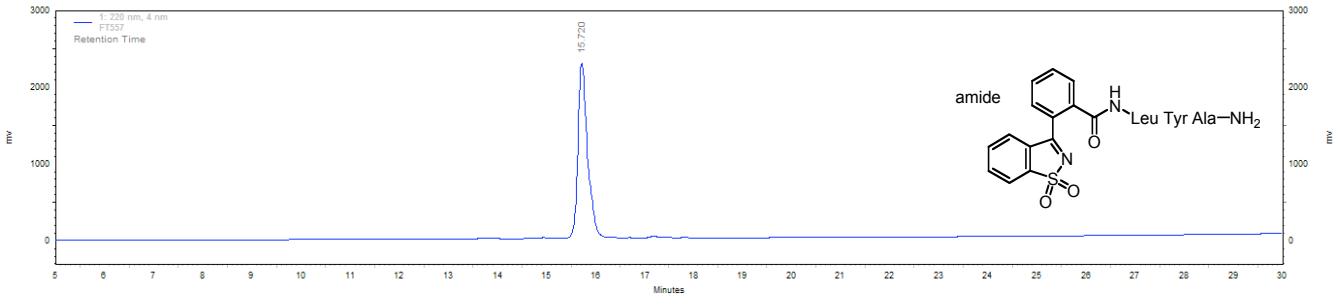
The postulated reaction pathway for its formation is:



Another possible structure for this side product is the reagent linked to the peptide via an amide bond. This possibility was excluded by independent synthesis of this possible side product:



To the resin bound peptide **7** (0.54 mmol/g, 21.3 mg) in DMF was carboxylic acid (6.4 mg, 0.0225 mmol, 2 equiv), HATU (8.9 mg, 0.0234 mmol, 2 equiv) and DIPEA (0.01 ml, 0.0574 mmol, 5 equiv) in DMF 0.5 ml at rt, the reaction mixture was shaken over 2 h. The resin was filtered, and washed with DMF and CH_2Cl_2. The peptide residue was cleaved from the resin by 95% TFA in H_2O at room temperature for 2 h. The solvent was removed under reduced pressure. The crude mixture was purified by preparative HPLC to give the desired amide product (2.0 mg). HRMS (ESI): *m/z*: calcd for $C_{32}H_{35}N_5NaO_7S$: 656.2155; found: 656.2137 $[M+Na]^+$. The ^1H NMR spectrum of this compound is included in the Spectral Data.

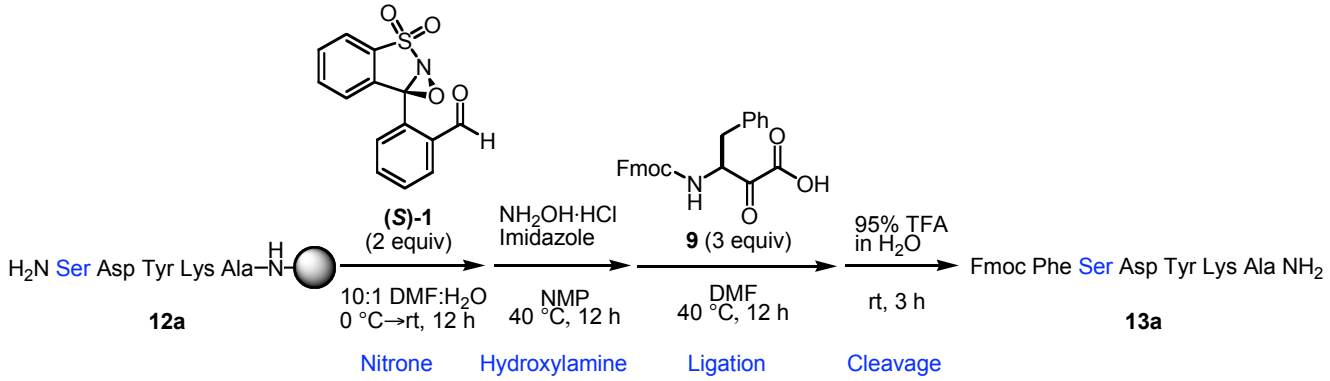


HPLC analysis: gradient 10-90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 mL/min, 220 nm, C18 column (shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 15.7 min.

9. Charactrization Data and HPLC Traces for Peptides

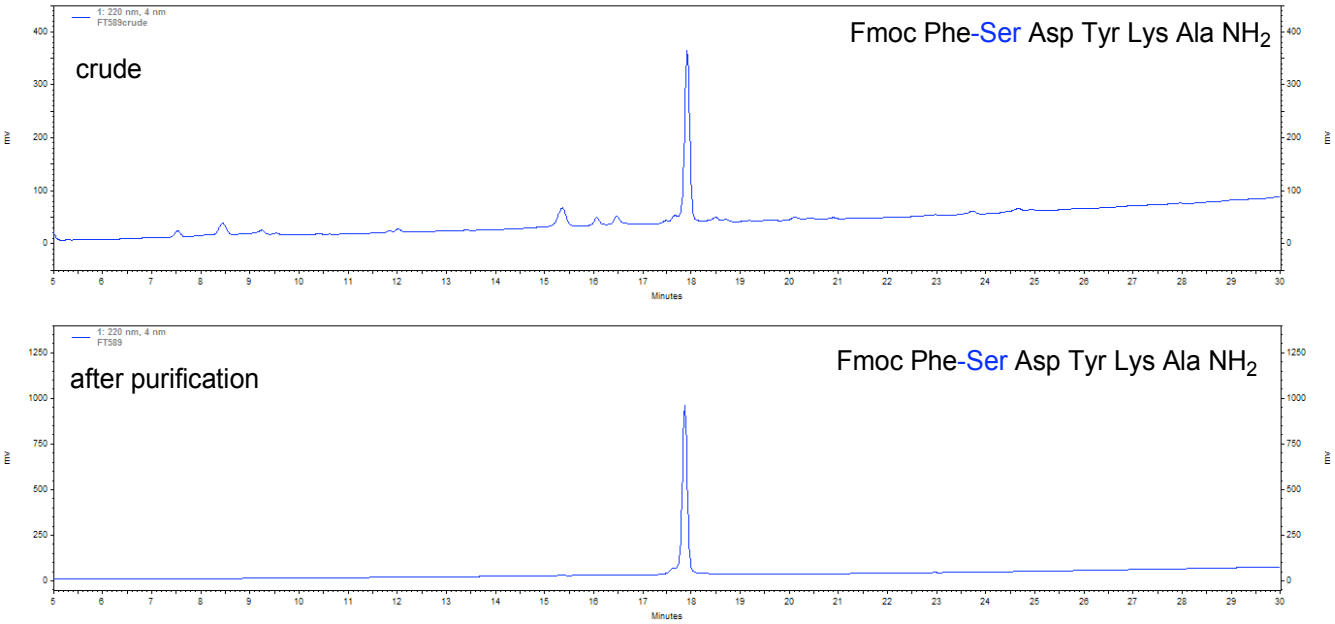
HPLC Traces for Peptides

(table 1, entry 1)



The resin **12a** (0.46 mmol/g, 49.4 mg) and **(*S*)-1** (13.5 mg) were used according to general procedure. The crude peptide was purified by preparative HPLC. The solvent was removed by (freeze-dryer) to give the ligation product **13a** (4.8 mg), HRMS (ESI): *m/z*: calcd for $C_{49}H_{59}N_8O_{12}$: 951.4252; found: 951.4247 $[M+H]^+$.

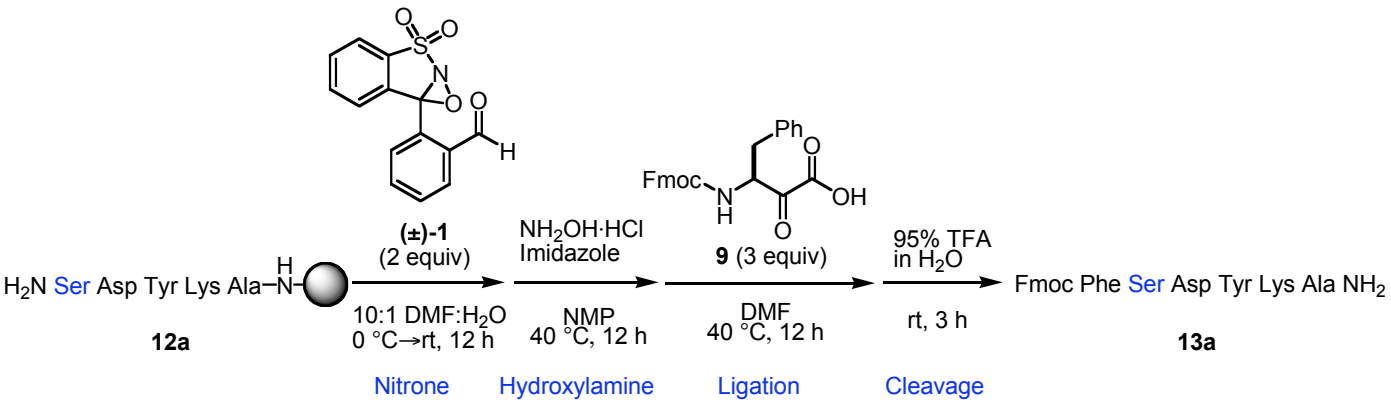
Ligation product peak area% on 220 nm (55%)



HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18

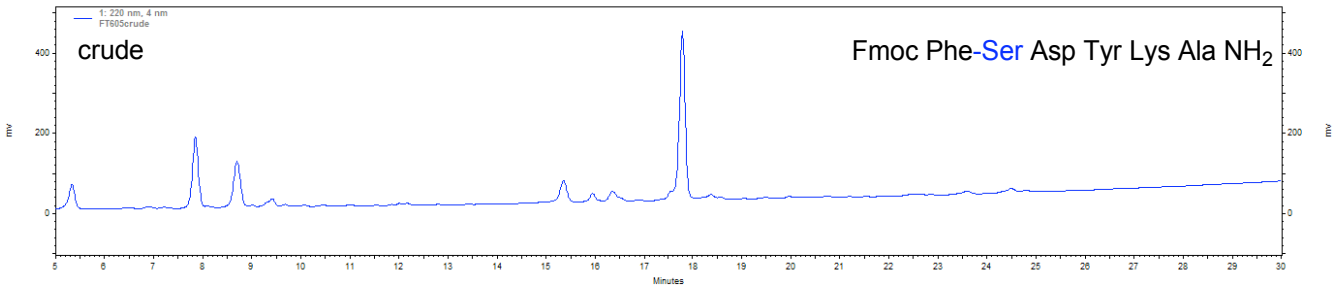
column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 17.9 min (ligation product **13a**).

(table 1, entry 2)



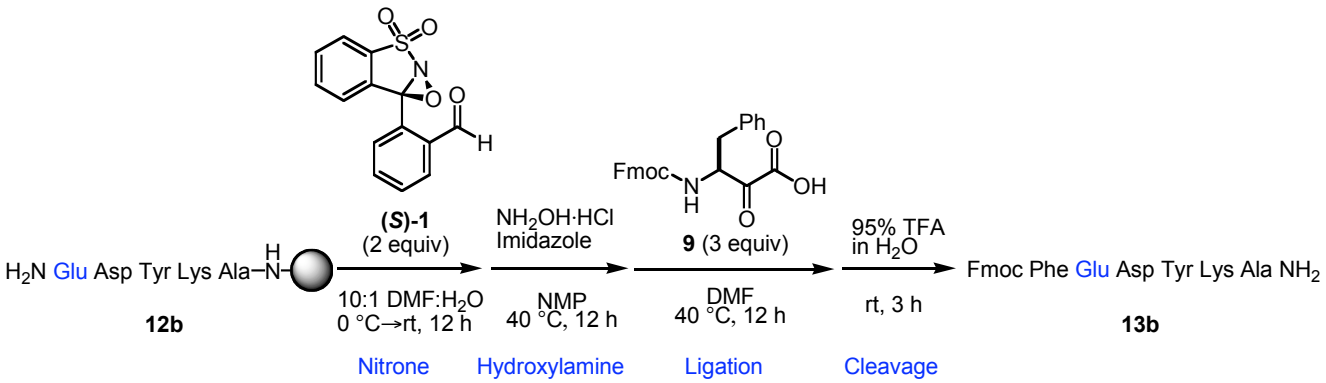
The resin **12a** (0.46 mmol/g, 17.4 mg) and **(±)-1** (4.6 mg) were used according to the general procedure.

Ligation product peak area% on 220 nm (37%)



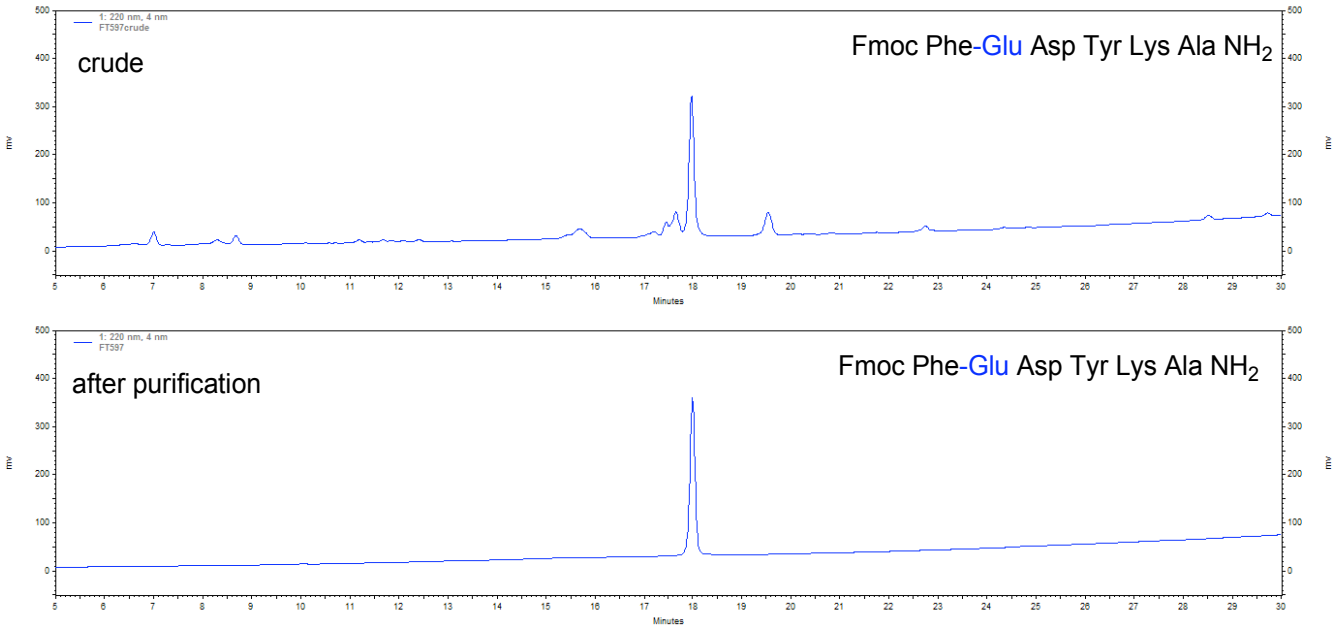
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 17.9 min (ligation product **13a**).

(table 1, entry 3)



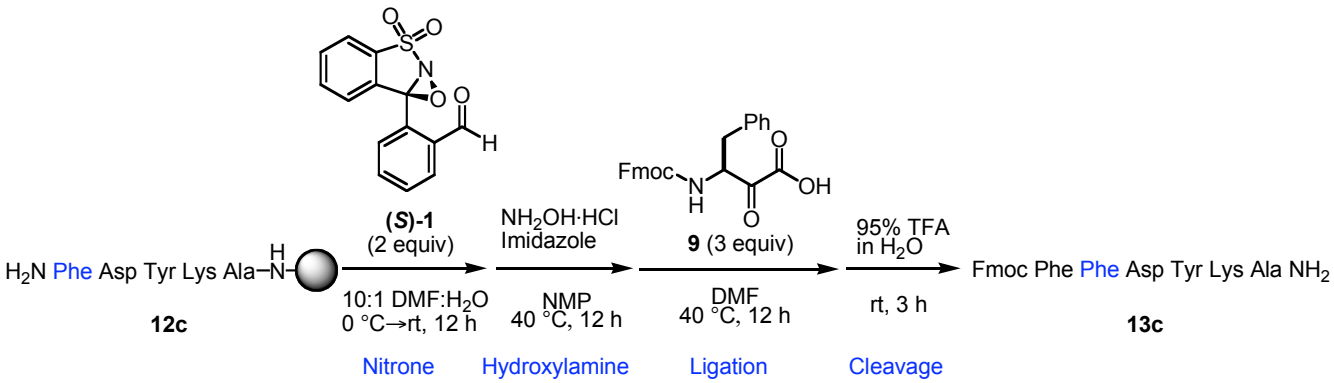
The resin **12b** (0.41 mmol/g, 66.2 mg) and **(*S*)-1** (15.2 mg) were used according to the general procedure. The crude peptide was purified by preparative HPLC. The solvent was removed by (freeze-dryer) to give the ligation product **13b** (6.7 mg), HRMS (ESI): *m/z*: calcd for $C_{51}H_{60}N_8NaO_{13}$: 1015.4178; found: 1015.4187 $[M+H]^+$.

Ligation product peak area% on 220 nm (41%)



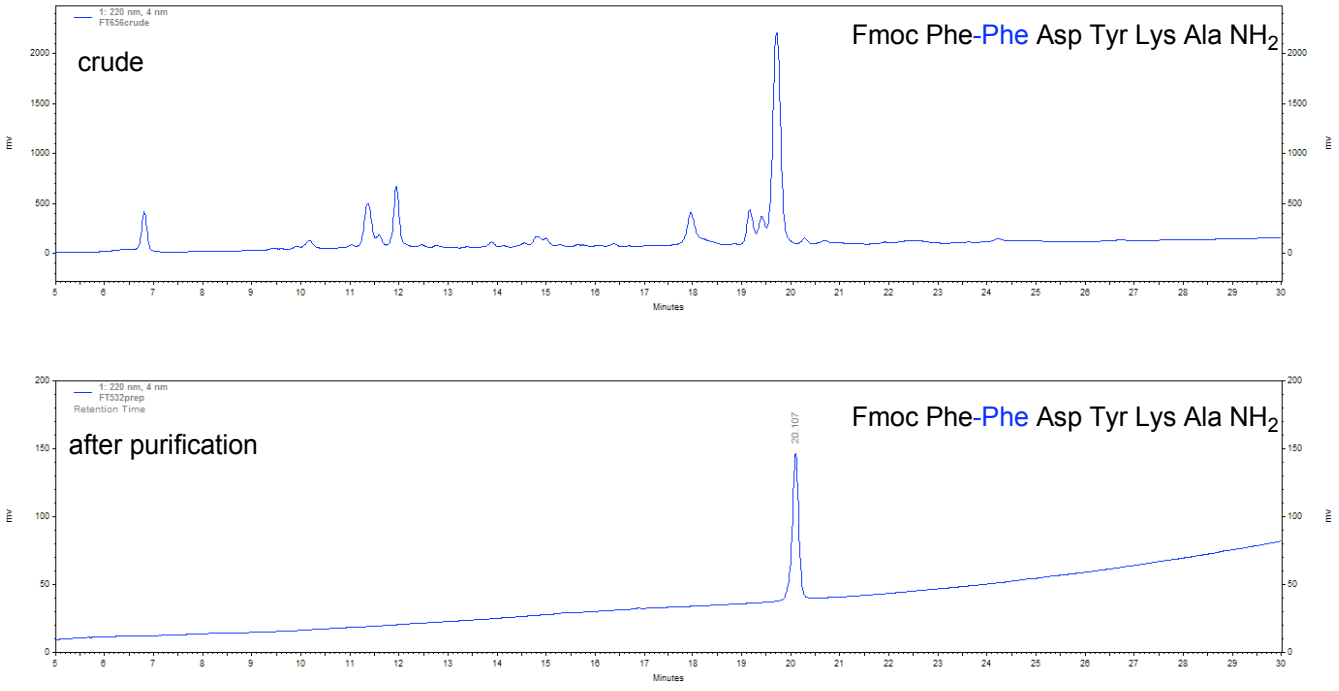
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 18.0 min (ligation product).

(table 1, entry 4)



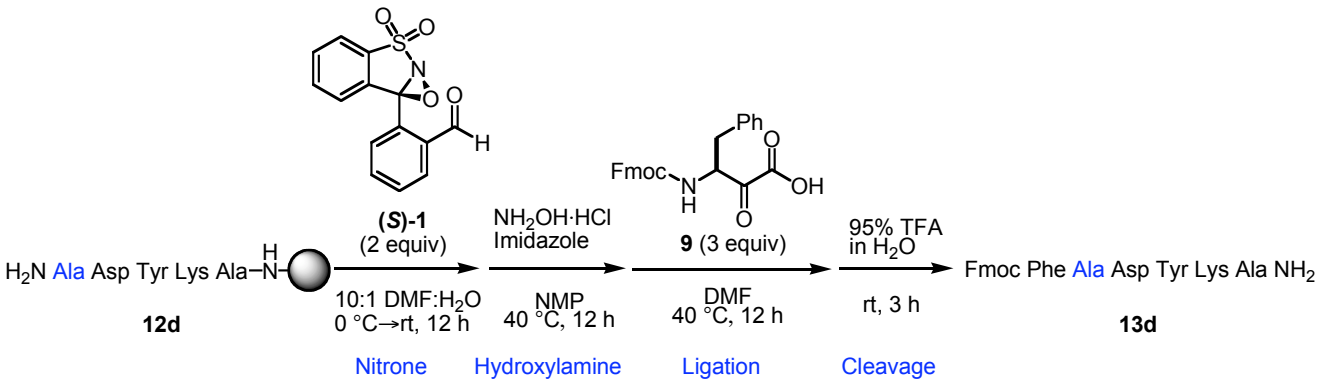
The resin **12c** (0.45 mmol/g, 64.6 mg) and **(*S*)-1** (17.0 mg) were used according to the general procedure. The crude peptide was purified by preparative HPLC. The solvent was removed by (freeze-dryer) to give the ligation product **13c** (5.1 mg), HRMS (ESI): *m/z*: calcd for $C_{55}H_{63}N_8O_{11}$: 1011.4616; found: 1011.4635 $[M+H]^+$.

Ligation product peak area% on 220 nm (42%)



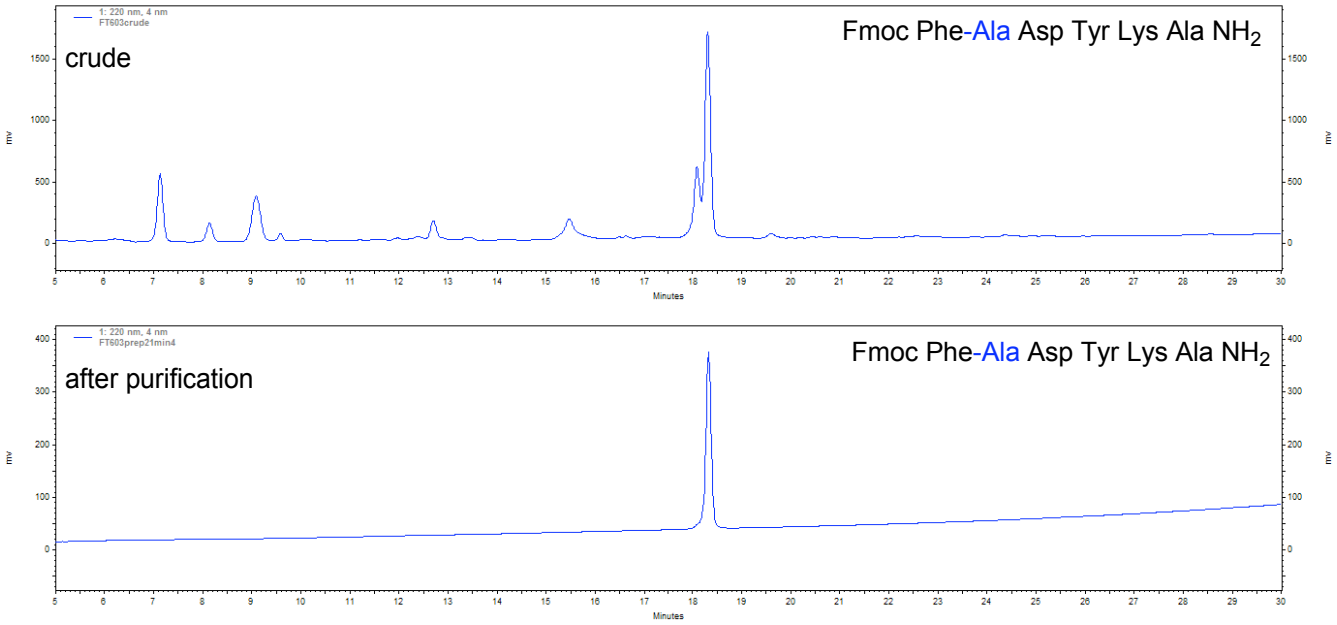
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 20.1 min (ligation product **13c**).

(table 1, entry 5)



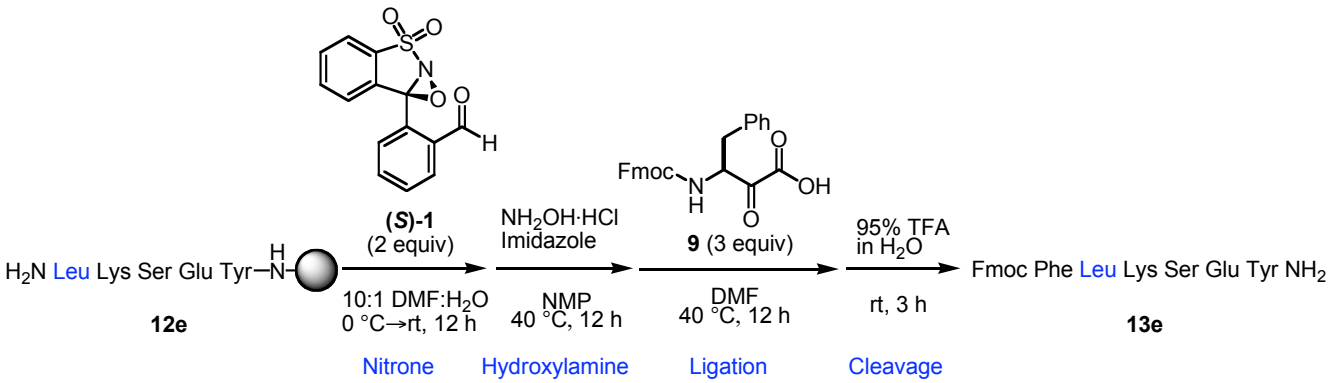
The resin **12d** (0.47 mmol/g, 47.3 mg) and **(*S*)-1** (12.8 mg) were used according to the general procedure. The crude peptide was purified by preparative HPLC. The solvent was removed by (freeze-dryer) to give the ligation product **13d** (3.8 mg), HRMS (ESI): *m/z*: calcd for $C_{49}H_{59}N_8O_{12}$: 935.4303; found: 935.4335 $[M+H]^+$.

Ligation product peak area% on 220 nm (47%)



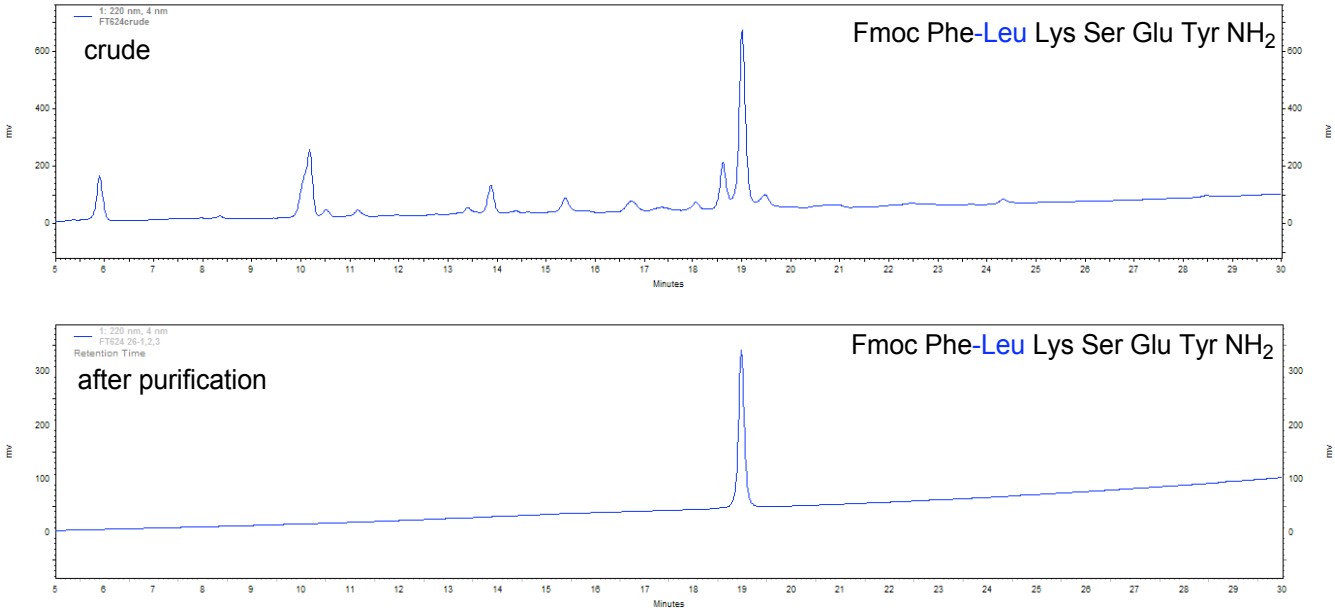
HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 18.3 min (ligation product **13d**).

(table 1, entry 6)



The resin **12e** (0.44 mmol/g, 38.7 mg) and **(*S*)-1** (9.6 mg) were used according to the general procedure. The crude peptide was purified by preparative HPLC. The solvent was removed by (freeze-dryer) to give the ligation product **13e** (3.4 mg), HRMS (ESI): *m/z*: calcd for $C_{53}H_{67}N_8O_{12}$: 1006.4878; found: 1007.4899 $[M+H]^+$.

Ligation product peak area% on 220 nm (31%)



HPLC analysis: gradient 10–90% CH_3CN/H_2O over 30 min at a flow rate of 1.0 ml/min, 220 nm, C18 column (Shiseido UG120, 4.6 mm × 250 mm, 5 μm). Retention time: 19.0 min (ligation product **13e**).