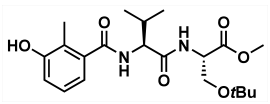
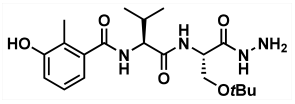
Synthesis and analytical data for compounds

General: All reagents were commercial grade and were used as received unless indicated otherwise. Toluene (Tol.)(purum), ethyl acetate (EtOAc) (puriss.), diethyl ether (Et_2O) and light petroleum ether (PetEt) (puriss.) were obtained from Riedel-de Haën. Dichloromethane (DCM), dimethyl formamide (DMF) and dioxane (Biosolve) were stored on 4Å molecular sieves. Methanol (MeOH) was obtained from Biosolve. Reactions were monitored by TLC-analysis using DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254 nm), spraying with 20% H_2SO_4 in ethanol followed by charring at ~150 °C, by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C or spraying with an aqueous solution of $KMnO_4$ (20%) and K_2CO_3 (10%). Column chromatography was performed on Screening Divices Silica gel (0.040 – 0.063 nm). LC/MS analysis was performed on a LCQ Adventage Max (Thermo Finnigan) equipped with a Gemini C18 column (Phenomenex). The applied buffers were A: H_2O, B: MeCN and C: 1.0 % aq. TFA. HRMS were recorded on a LTQ Orbitrap (Thermo Finnigan). ^{1}H- and ^{13}C-APT-NMR spectra were recorded on a Jeol JNM-FX-200 (200/50), Bruker DPX-300 (300/75 MHz), Bruker AV-400 (400/100 MHz) equipped with a pulsed field gradient accessory, a Bruker AV-500 (500/125 MHz) or a Bruker DMX-600 (600/150 MHz) with cryoprobe. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants are given in Hz. All presented ^{13}C-APT spectra are proton decoupled. Optical rotations were measured on a Propol automatic polarimeter (sodium D line, λ = 589 nm)(1). Boc-Leu-ve (2), Boc-Leu-ek(3) and Boc-Leu-mvs, Boc-Leu-pvs, Boc-Phe-mvs (4) were synthesised as described in literature. They were coupled to the left-handed peptide fragments via azido coupling exactly as described (5).

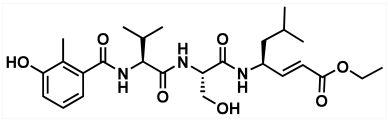
Synthesis of Hmb-VSL-ve (route 1).



(Val-Ser(*t*Bu)-OMe)-3-hydroxy-2-methylbenzamide. DBU (0.15 ml, 1 equiv.) was added to a solution of Fmoc-Val-Ser(*t*Bu)-OMe (0.5 g, 1 mmol) in DMF and stirred for 5 min., before HOBt (0.61 g, 4.5 mmol, 4.5 equiv.) was added. After 1 min. 3-hydroxy-2-methyl benzoic acid (0.15 g, 1 mmol, 1 equiv.), BOP (0.49 g, 1.1 mmol, 1.1 equiv.) and DiPEA (0.66 ml, 4 mmol, 4 equiv.) were added and the reaction mixture was stirred for 2 hr. The reaction mixture was washed with 0.5 M HCl (aq.) and sat. aq. $NaHCO_3$, separated and dried over $MgSO_4$. Purification by flash column chromatography (PetEt → 50% EtOAc in PetEt) yielded the title compound as a white solid (0.27 g, 0.67 mmol, 67%). ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm 7.06 (t, J = 7.8 Hz, 1H), 6.84 (d, J = 7.8 Hz, 2H), 4.64 (t, J = 4.0 Hz, 1H), 4.45 (d, J = 7.6 Hz, 1H), 3.83 (dd, J_1 = 9.3, J_2 = 4.2 Hz, 1H), 3.74 (s, 3H), 3.65 (dd, J_1 = 9.3, J_2 = 3.8 Hz, 1H), 2.30-2.06 (m, 4H), 1.17 (s, 9H), 1.04 (t, J = 7.0 Hz, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ ppm 171.40, 171.13, 170.23, 155.00, 137.20, 125.79, 121.65, 117.30, 115.64, 73.13, 61.11, 58.36, 52.74, 51.49, 30.44, 26.30, 18.42, 17.28, 11.61.



(Val-Ser(*t*Bu)-hydrazinyl)-3-hydroxy-2-methylbenzamide (Hmb-VS(tBu)-hydrazide). (Val-Ser(*t*Bu)-OMe)-3-hydroxy-2-methylbenzamide (0.27 g, 0.67 mmol) was dissolved in MeOH. Hydrazine monohydrate (1.95 ml, 40.2 mmol, 60 equiv.) was added and the reaction mixture was refluxed for 15 hr., before being co-evaporated with Tol. (3×). Column chromatography (DCM → 7.5% MeOH in DCM) gave the pure title compound (0.24 g, 0.59 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.06 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.8 Hz, 2H), 4.49 (dd, J_1 = 5.8, J_2 = 4.7 Hz, 1H), 4.41 (d, J = 6.9 Hz, 1H), 3.70 (dd, J_1 = 9.0, J_2 = 4.5 Hz, 1H), 3.56 (dd, J_1 = 9.0, J_2 = 6.2 Hz, 1H), 2.25 (s, 3H), 2.23-2.14 (m, 1H), 1.19 (s, 9H), 1.03 (dd, J_1 = 13.1, J_2 = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 171.53, 171.35, 169.92, 155.11, 137.01, 125.92, 121.82, 117.48, 115.87, 73.49, 60.91, 58.88, 52.06, 30.18, 26.51, 18.61, 17.44, 11.80.



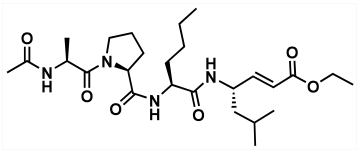
(Val-Ser-Leu-vinyl ethyl ester)-3-hydroxy-2-methylbenzamide (Hmb-VSL-ve). Following the general procedure for azide coupling the title compound was obtained from Boc-LeuVE (49.7 mg, 0.17 mmol, 1.1 equiv.) and (Val-Ser(*t*Bu)-hydrazinyl)-3-hydroxy-2-methylbenzamide (**Hmb-VS(tBu)L-hydrazide**, 61.3 mg, 0.15 mmol). Column chromatography (n-hexane → 30% acetone in n-hexane) gave **Hmb-VS(tBu)L-ve** (69 mg, 0.12 mmol, 82%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.05 (t, J = 7.8 Hz, 1H), 6.90-6.82 (m, 3H), 6.01 (dd, J_1 = 15.7, J_2 = 1.7 Hz, 1H), 4.68-4.58 (m, 1H), 4.52 (dd, J_1 = 6.5, J_2 = 3.9 Hz, 1H), 4.34 (d, J = 7.2 Hz, 1H), 4.20-4.13 (m, 2H), 3.73 (dd, J_1 = 8.6, J_2 = 3.9 Hz, 1H), 3.60 (dd, J_1 = 8.6, J_2 = 6.7 Hz, 1H), 2.22 (s, 3H), 2.21-2.12 (m, 1H), 1.76-1.61 (m, 1H), 1.52 (ddd, J_1 = 15.2, J_2 = 10.7, J_3 = 4.7 Hz, 1H), 1.37 (ddd, J_1 = 13.9, J_2 = 9.6, J_3 = 4.6 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H), 1.21 (s, 9H), 1.03 (dd, J_1 = 6.7, J_2 = 3.6 Hz, 6H), 0.87 (dd, J_1 = 11.5, J_2 = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, MeOD): δ ppm 173.98, 173.34, 171.93, 168.07, 157.10, 149.94, 139.19, 127.46, 123.33, 121.36, 119.15, 117.12, 74.84, 62.80, 61.52, 61.41, 55.04, 49.87, 43.66, 31.44, 27.77, 25.79, 23.48, 21.88, 19.84, 19.08, 14.59, 13.10. **Hmb-VS(tBu)L-ve** (69 mg, 0.12 mmol) was dissolved in TFA/DCM 1/1 (v/v) and stirred for 30 min., before being co-evaporated with Tol. (3×). Column chromatography (DCM → 3.5% MeOH in DCM) gave **Hmb-VSL-ve** (54.2 mg, 0.11 mmol, 89%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.05 (t, J = 7.8 Hz, 1H), 6.90-6.81 (m, 3H), 5.99 (dd, J_1 = 15.7, J_2 = 1.6 Hz, 1H), 4.66-4.59 (m, 1H), 4.50 (t, J = 5.7 Hz, 1H), 4.37 (d, J = 7.3 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.83 (dd, J_1 = 10.7, J_2 = 5.3 Hz, 1H), 3.78 (dd, J_1 = 10.8, J_2 = 6.1 Hz, 1H), 2.21 (s, 3H), 2.20-2.12 (m, 1H), 1.75-1.62 (m, 1H), 1.51 (ddd, J_1 = 15.1, J_2 = 10.1, J_3 = 5.2 Hz, 1H), 1.40 (ddd, J_1 = 13.9, J_2 = 9.0, J_3 = 5.2 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 6.9 Hz, 6H), 0.90 (d, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, MeOD): δ ppm 173.75, 173.58, 171.83, 168.12, 157.04, 149.79, 139.24, 127.47, 123.34, 121.52, 119.13, 117.09, 62.92, 61.59, 61.16, 56.61, 49.86, 43.92, 31.70, 25.85, 23.39, 22.09, 19.90, 19.06, 14.58, 13.00. HRMS: calcd. for $[C_{26}H_{39}N_3O_7H]^+$ 506.28608, found 506.28592. This spectral data is identical to published in literature (2).

Synthesis of Hmb-VSL-ve (route 2).

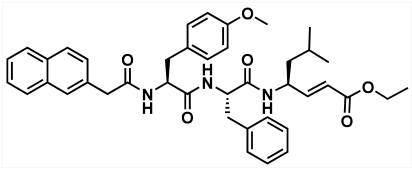
This route used solid phase synthesis of Hmb-Val-Ser(tBu)-OH and its traditional EDC coupling to Boc-Leu-ve. Polystyrene HMPB MBHA resin (2 mmol) was suspended in 1,4-dioxane and

evaporated to dryness (3x). This resin was loaded using FmocSer(tBu)OH (5 mmol, 2.5 equiv.), DIC (5 mmol, 2.5 equiv.) and DMAP (0.25 mmol, 0.12 equiv.) in DCM for 2 hr. The resin was washed with DCM (2x), MeOH (2x) and DCM (2x) and the coupling and washing was repeated twice. The loading of the resin was determined to be 1.2 mmol/gr. This resin was elongated stepwise. For deprotection, 20% piperidine in NMP, 10 min was used. Washing was done with NMP (5x) and coupling was performed with the appropriate carboxylic acid (first FmocValOH then Hmba, 2.5 equiv.), HCTU (2.5 equiv.), DiPEA (5 equiv.) in NMP, 2.5 hr. The tripeptide was cleaved from the resin using 1% TFA in DCM, 30 min, 6x) and the combined DCM fractions were co-evaporated with toluene (3x). BocLeuVE was stirred in 1:1 TFA:DCM for 30 minutes before being co-evaporated with toluene (3x). This crude TFA salt was coupled to the crude tripeptide using EDC and DiPEA in DMF. This mixture was diluted with EtOAc and extracted with 1M HCl, sat. aq. $NaHCO_3$ and brine and dried with Na_2SO_4. The resulting residue was deprotected with TFA for 40 minutes and coevaporated with toluene (3x). The residue was purified and enantiomers separated on a isomeerscheiding prep HPLC phenomenex Gemini V18 column with 0.2% TFA in water / MeCN gradient followed by lyophilization. This procedure yieldd HMB-Val-Ser-Leu-VE with NMR and mass data identical to the compound synthesized via route 1 (see above) and published in literature (2).

Synthesis of other vinyl esters used on Fig. 1



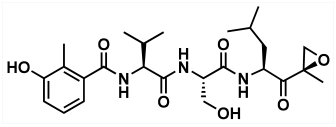
Ac-Ala-Pro-Nle-Leu-ve (NC-001-ve). Following the general procedure for azide coupling the title compound was obtained from Boc-Leu-ve (49.7 mg, 0.17 mmol, 1.1 equiv.) and Ac-Ala-Pro-Nle-hydrazide ((5), 53.3 mg, 0.15 mmol). Purification by flash column chromatography (DCM → 4% MeOH in DCM) gave **NC-001-ve** as white solid (53.1 mg, 0.1 mmol, 70%). ^{1}H NMR (500 MHz, DMSO, T = 353K): δ ppm 7.54 (d, J = 6.1 Hz, 1H), 6.79 (dd, J_1 = 15.7, J_2 = 5.5 Hz, 1H), 5.84 (d, J = 15.6 Hz, 1H), 4.60-4.53 (m, 1H), 4.53-4.45 (m, 1H), 4.41-4.32 (m, 1H), 4.21-4.15 (m, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.70-3.60 (m, 1H), 3.59-3.52 (m, 1H), 2.15-1.99 (m, 1H), 1.95-1.85 (m, 3H), 1.84 (s, 3H), 1.76-1.67 (m, 1H), 1.66-1.53 (m, 2H), 1.51-1.42 (m, 1H), 1.42-1.35 (m, 1H), 1.32-1.25 (m, 4H), 1.24-1.19 (m, 6H), 0.93-0.82 (m, 9H). HRMS: calcd. for $[C_{26}H_{44}N_4O_6H]^+$ 509.33336, found 509.33315.



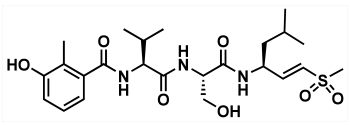
(Tyr(Me)-Phe-Leu-vinyl ethyl ester)-2-(naphthalen-2-yl)-acetamide (NC-005-ve). Following the general procedure for azide coupling the title compound was obtained from Boc-Leu-ve (49.7 mg, 0.17 mmol, 1.1 equiv.) and (Tyr(Me)-Phe-hydrazinyl)-2-(naphthalen-2-yl)-acetamide ((5), 78.7 mg, 0.15 mmol). Upon washing the reaction mixture with EtOAc white precipitate forms. The crude product was filtered and redissolved in DCM. Crystallization with EtOAc gave the title compound as a white solid (70 mg, 0.13 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.92-7.86 (m, 1H), 7.85-7.79 (m, 2H), 7.60-7.53 (m, 2H), 7.52 (s, 1H), 7.30-7.24 (m, 3H), 7.11 (dd, J_1 = 8.4, J_2 = 1.6 Hz, 1H), 7.01 (dd, J_1 = 7.3, J_2 = 1.9 Hz, 2H), 6.70 (dd, J_1 = 15.7, J_2 = 5.7 Hz, 1H), 6.63 (d, J = 8.6 Hz, 2H), 6.45 (d, J = 8.6 Hz, 2H), 6.24 (d, J = 8.1 Hz, 1H), 6.19 (d, J = 8.3 Hz, 1H), 5.79 (dd, J_1 = 15.7, J_2 = 1.5 Hz, 1H), 5.76 (d, J = 7.1 Hz, 1H), 4.68-4.54 (m, 2H), 4.42

(q, J = 6.2 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 3.68 (s, 3H), 3.61 (d, J = 16.3 Hz, 1H), 3.44 (d, J = 16.2 Hz, 1H), 3.22 (dd, J_1 = 13.8, J_2 = 5.7 Hz, 1H), 2.89-2.78 (m, 3H), 1.50-1.33 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H), 1.31-1.23 (m, 1H), 0.88 (t, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 171.71, 170.19, 169.65, 166.37, 158.60, 147.52, 136.19, 133.52, 132.58, 131.23, 129.85, 129.34, 129.14, 128.74, 128.37, 127.79, 127.64, 127.12, 127.07, 126.95, 126.70, 126.38, 120.84, 114.06, 60.35, 55.14, 54.86, 53.84, 48.56, 43.53, 42.83, 37.20, 35.86, 24.59, 22.78, 21.96, 14.27. HRMS: calcd. for $[C_{41}H_{47}N_3O_6H]^+$ 678.35376, found 678.35406.

Synthesis of non-ester derivatives of Hmb-VSL-ve

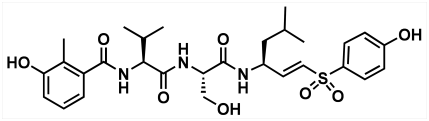


(Val-Ser-Leu-epoxyketone)-3-hydroxy-2-methylbenzamide (Hmb-VSL-ek). Following the general procedure for azide coupling the title compound was obtained from Boc-Leu-ek (47.4 mg, 0.17 mmol, 1.1 equiv.) and (Val-Ser(*t*Bu)-hydrazinyl)-3-hydroxy-2-methylbenzamide (Hmb-VS(tBu)L-hydrazide, 61.3 mg, 0.15 mmol). Column chromatography (n-hexane → 25% acetone in n-hexane) gave **Hmb-VS(tBu)L-ek** (73 mg, 0.13 mmol, 89%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.04 (t, J = 7.8 Hz, 1H), 6.87-6.81 (m, 2H), 4.63 (dd, J_1 = 10.5, J_2 = 3.2 Hz, 1H), 4.51 (t, J = 5.1 Hz, 1H), 4.39 (d, J = 7.3 Hz, 1H), 3.69 (dd, J_1 = 8.9, J_2 = 4.7 Hz, 1H), 3.57 (dd, J_1 = 8.9, J_2 = 5.7 Hz, 1H), 3.25 (d, J = 5.0 Hz, 1H), 2.93 (d, J = 5.1 Hz, 1H), 2.21 (s, 3H), 2.20-2.14 (m, 1H), 1.76-1.63 (m, 1H), 1.52-1.42 (m, 4H), 1.42-1.32 (m, 1H), 1.18 (s, 9H), 1.04 (dd, J_1 = 10.5, J_2 = 6.8 Hz, 6H), 0.89 (dd, J_1 = 11.0, J_2 = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, MeOD): δ ppm 209.18, 173.70, 173.38, 172.03, 157.06, 139.30, 127.43, 123.28, 119.09, 117.06, 74.79, 62.84, 61.06, 59.99, 54.84, 52.94, 51.49, 40.67, 31.54, 27.73, 26.15, 23.74, 21.58, 19.93, 18.97, 17.02, 13.06. **Hmb-VS(tBu)L-ek** (73 mg, 0.13 mmol) was dissolved in TFA/DCM 1/1 (v/v) and stirred for 30 min., before being co-evaporated with Tol. (3×). Column chromatography (DCM → 3.5% MeOH in DCM) gave the title compound **Hmb-VSL-ek** (43.8 mg, 89 μmol, 69%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.05 (t, J = 7.8 Hz, 1H), 6.88-6.80 (m, 2H), 4.60 (dd, J_1 = 10.6, J_2 = 3.1 Hz, 1H), 4.51 (t, J = 5.6 Hz, 1H), 4.39 (d, J = 7.4 Hz, 1H), 3.77 (d, J = 5.6 Hz, 2H), 3.26 (d, J = 5.0 Hz, 1H), 2.93 (d, J = 5.1 Hz, 1H), 2.21 (s, 3H), 2.20-2.11 (m, 1H), 1.78-1.64 (m, 1H), 1.55-1.48 (m, 1H), 1.47 (s, 3H), 1.41-1.29 (m, 1H), 1.03 (dd, J_1 = 10.4, J_2 = 6.8 Hz, 6H), 0.92 (d, J = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, MeOD): δ ppm 209.60, 173.65, 173.58, 172.07, 157.03, 139.32, 127.46, 123.33, 119.11, 117.05, 63.14, 60.89, 60.11, 56.39, 53.12, 51.93, 40.49, 31.83, 26.27, 23.77, 21.56, 19.94, 19.00, 17.07, 12.95. HRMS: calcd. for $[C_{25}H_{37}N_3O_7H]^+$ 492.27043, found 492.27033.



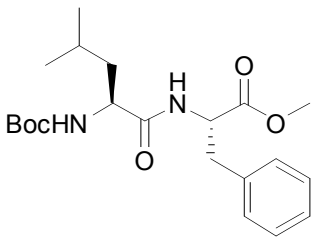
((Val-Ser-Leu-methyl vinylsulfone)-3-hydroxy-2-methylbenzamide (Hmb-VSL-mvs). Following the general procedure for azide coupling the title compound was obtained from Boc-Leu-mvs (50.7 mg, 0.17 mmol, 1.1 equiv.) and (Val-Ser(*t*Bu)-hydrazinyl)-3-hydroxy-2-methylbenzamide (Hmb-VS(tBu)L-hydrazide**,** 61.3 mg, 0.15 mmol). Column chromatography (n-hexane → 35% acetone in n-hexane) gave **Hmb-VS(tBu)L-mvs** (63.5 mg, 0.11 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ ppm 7.48-7.40 (m, 2H), 7.35-7.28 (m, 1H), 7.05 (t, J = 7.8, 7.8 Hz, 1H), 6.91-6.86 (m, 2H), 6.83 (dd, J_1 = 15.3, J_2 = 4.1 Hz, 1H), 6.62 (d, J = 15.1 Hz, 1H), 4.79-4.64 (m, 1H), 4.51-4.44 (m, 1H), 4.35-4.27 (m, 1H), 3.89-3.80 (m, 1H), 3.54 (dd, J_1 = 8.8, J_2 = 5.5 Hz, 1H), 2.96 (s, 3H), 2.25-2.21 (m, 1H), 2.19 (s,

3H), 1.70-1.60 (m, 1H), 1.59-1.49 (m, 1H), 1.44-1.31 (m, 1H), 1.19 (s, 9H), 1.05 (dd, J_1 = 15.7, J_2 = 6.8 Hz, 6H), 0.85 (dd, J_1 = 23.7, J_2 = 6.5 Hz, 6H). **Hmb-VS(tBu)L-mvs** (18 mg, 31.7 µmol) was dissolved in TFA/DCM 1/1 (v/v) and stirred for 30 min., before being co-evaporated with Tol. (3×). Column chromatography (DCM → 4% MeOH in DCM) gave the title compound (10.4 mg, 20.3 µmol, 64%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.06 (t, J = 7.8 Hz, 1H), 6.89-6.73 (m, 4H), 4.77-4.66 (m, 1H), 4.47 (t, J = 5.5 Hz, 1H), 4.33 (d, J = 7.1 Hz, 1H), 3.87 (dd, J_1 = 10.6, J_2 = 4.9 Hz, 1H), 3.79 (dd, J_1 = 10.6, J_2 = 6.5 Hz, 1H), 2.96 (s, 3H), 2.21 (s, 3H), 2.20-2.12 (m, 1H), 1.78-1.66 (m, 1H), 1.61-1.51 (m, 1H), 1.49-1.40 (m, 1H), 1.04 (t, J = 6.0 Hz, 6H), 0.90 (d, J = 6.5 Hz, 6H). HRMS: calcd. for $[C_{24}H_{37}N_3O_7SH]^+$ 512.24250, found 512.24232.



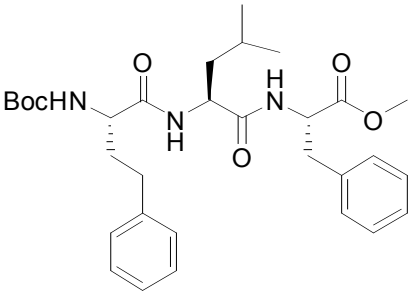
(Val-Ser-Leu-4-hydroxyphenyl-vinylsulfone)-3-hydroxy-2-methylbenzamide (Hmb-VS(tBu)L-pvs). Following the general procedure for azide coupling the title compound was obtained from Boc-Leu-pvs (61 mg, 0.17 mmol, 1.1 equiv.) and (Val-Ser(*t*Bu)-hydrazinyl)-3-hydroxy-2-methylbenzamide (Hmb-VS(tBu)L-hydrazide, 61.3 mg, 0.15 mmol). Crystallization from EtOAc with Et_2O gave **Hmb-VS(tBu)L-pvs** as a white solid (61.1 mg, 95 µmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.00 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 7.7 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.03 (t, J = 7.8, 7.8 Hz, 1H), 6.91 (d, J = 8.8 Hz, 2H), 6.86-6.79 (m, 3H), 6.60 (dd, J = 15.0, 1.7 Hz, 1H), 4.74-4.61 (m, 1H), 4.49-4.43 (m, 1H), 4.24 (d, J = 7.1 Hz, 1H), 3.69 (dd, J_1 = 8.8, J_2 = 3.7 Hz, 1H), 3.54 (dd, J_1 = 8.6, J_2 = 6.6 Hz, 1H), 2.19 (s, 3H), 2.18-2.10 (m, 1H), 1.73-1.60 (m, 1H), 1.58-1.47 (m, 1H), 1.43-1.32 (m, 1H), 1.13 (s, 9H), 1.01 (d, J = 6.8 Hz, 6H), 0.85 (dd, J = 12.0, 6.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 173.94, 173.44, 173.36, 172.07, 172.05, 163.78, 157.02, 146.69, 139.04, 131.85, 131.48, 131.19, 127.42, 123.30, 119.14, 117.13, 116.93, 74.81, 62.60, 61.45, 55.23, 49.45, 43.31, 31.32, 27.77, 25.72, 23.49, 21.72, 19.82, 19.06, 13.14. **Hmb-VS(tBu)L-pvs** (61.1 mg, 95 µmol) was dissolved in TFA/DCM 1/1 (v/v) and stirred for 30 min., before being co-evaporated with Tol. (3×). Column chromatography (DCM → 5% MeOH in DCM) gave the title compound Hmb-VSL-pvs (48.8 mg, 83 µmol, 87%). ^{1}H NMR (400 MHz, MeOD): δ ppm 7.69 (d, J = 8.8 Hz, 2H), 7.04 (t, J = 7.8 Hz, 1H), 6.91 (d, J = 8.8 Hz, 2H), 6.86-6.82 (m, 2H), 6.80 (dd, J_1 = 15.2, J_2 = 4.0 Hz, 1H), 6.72 (dd, J_1 = 15.1, J_2 = 1.3 Hz, 1H), 4.71-4.65 (m, 1H), 4.42 (dd, J_1 = 6.2, J_2 = 5.1 Hz, 1H), 4.28 (d, J = 7.2 Hz, 1H), 3.81 (dd, J_1 = 10.6, J_2 = 5.0 Hz, 1H), 3.72 (dd, J_1 = 10.6, J_2 = 6.4 Hz, 1H), 2.19 (s, 3H), 2.18-2.09 (m, 1H), 1.74-1.61 (m, 1H), 1.52 (ddd, J_1 = 15.2, J_2 = 10.3, J_3 = 5.0 Hz, 1H), 1.42 (ddd, J_1 = 13.9, J_2 = 9.2, J_3 = 4.9 Hz, 1H), 1.00 (dd, J_1 = 6.8, J_2 = 2.9 Hz, 6H), 0.88 (d, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, MeOD): δ ppm 173.81, 173.58, 172.03, 163.84, 157.03, 146.21, 139.20, 132.26, 131.56, 131.12, 127.47, 123.34, 119.13, 117.09, 116.98, 62.76, 61.24, 56.59, 49.43, 43.42, 31.59, 25.87, 23.39, 21.88, 19.86, 19.02, 12.99. HRMS: calcd. for $[C_{29}H_{39}N_3O_8SH]^+$ 590.25306, found 590.25312.

Synthesis of YU-101, PR-171, YU-101-mvs and PR-171-mvs.

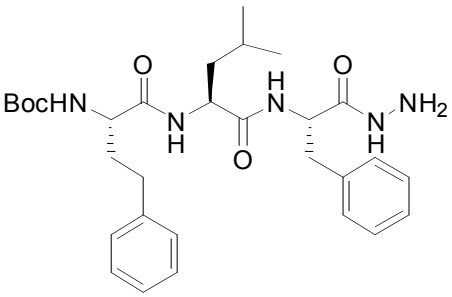


Boc-Leu-Leu-Phe-OMe Boc-Leu-OH·H_2O was co-evaporated with Tol. (3x), before dissolved in DCM. After addition of HCTU (2.487 g, 6 mmol, 1.2 equiv.), HCl.HPhe-OMe (1.77 g, 5 mmol, 1 equiv.) and DiPEA (2.89 ml, 17.5 mmol, 3.5 equiv.). After addition of 10 ml of

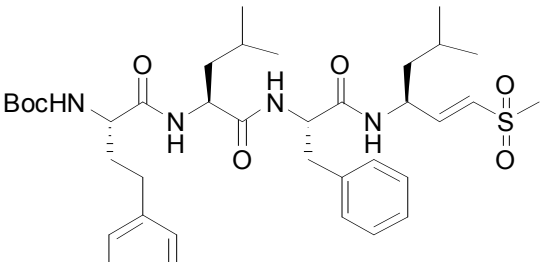
DCM and DiPEA (1.2 ml, 15 mmol, 1.5 equiv.), the reaction mixture was stirred overnight. TLC analysis (25% EtOAc in PetEt) showed complete consumption of the starting materials and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc, before being washed with 1 M HCl (3x), sat. aq. $NaHCO_3$ (2x) and Brine (2x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10% EtOAc in PetEt → 25% EtOAc in PetEt) yielded the title compound (2.0 g, 5 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.31-7.07 (m, 5H), 6.89 (d, *J* = 7.15 Hz, 1H), 5.21 (d, *J* = 7.89 Hz, 1H), 4.91-4.77 (m, 1H), 4.22-4.13 (m, 1H), 3.67 (s, 3H), 3.17-3.01 (m, 2H), 1.74-1.52 (m, 2H), 1.46-1.41 (m, 1H), 1.43 (s, 9H), 0.90 (t, *J* = 6.40 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 172.21, 171.53, 155.34, 135.66, 129.07, 128.25, 126.78, 79.55, 53.03, 52.82, 51.98, 41.04, 37.65, 28.08, 24.41, 22.65, 21.77.



Boc-hPhe-Leu-Phe-OMe. Boc-Leu-Phe-OMe (0.784 g, 2 mmol, 1.1 equiv.) was dissolved in TFA/DCM 1/1 (6 ml). The reaction mixture was stirred for 15 min., before being co-evaporated with Tol. (3x). The crude TFA salt was dissolved in DMF and a solution of Boc-hPhe-OH (0.5 g, 1.79 mmol), HCTU (0.827 g, 2 mmol, 1.1 equiv.) and DiPEA (1.04 ml, 6.27 mmol, 3.5 equiv.) in DCM (20 ml) was added. The reaction mixture was stirred for 2 hr., before it was diluted with DCM. The reaction mixture was washed with 1M HCl (3x), sat. aq. $NaHCO_3$ (2x) and Brine (2x), before being dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10% EtOAc in PetEt → 35% EtOAc in PetEt) yielded the title compound (0.818 g, 1.48 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.28-7.03 (m, 10H), 7.01 (d, *J* = 7.72 Hz, 1H), 5.54 (d, *J* = 8.19 Hz, 1H), 4.81 (dd, *J1* = 14.07, J2 = 6.38 Hz, 1H), 4.60-4.50 (m, 1H), 4.26-4.17 (m, 1H), 3.63 (s, 3H), 3.11-2.98 (m, 2H), 2.72-2.56 (m, 2H), 2.10-1.97 (m, 1H), 1.95-1.84 (m, 1H), 1.69-1.57 (m, 2H), 1.56-1.47 (m, 1H), 1.43 (s, 9H), 0.86 (dd, *J* = 9.70, 6.00 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 172.01, 171.61, 171.55, 155.63, 140.88, 135.68, 129.03, 128.31, 128.26, 126.84, 125.89, 79.74, 53.88, 53.27, 52.05, 51.55, 41.02, 37.75, 34.07, 31.75, 28.18, 24.44, 22.61, 22.04.

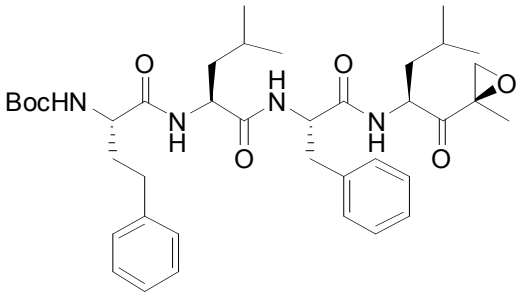


Boc-hPhe-Leu-Phe-hydrazide. After Boc-hPhe-Leu-Phe-OMe (0.819 g, 1.48 mmol) was dissolved in MeOH (50 ml), hydrazine monohydrate (2.16 ml, 44.4 mmol, 30 equiv.) was added. The reaction mixture was refluxed for 3 hr., before being co-evaporated with Tol. (3x). Purification by column chromatography (EtOAc → 20% MeOH and 0.1% TEA in EtOAc) yielded the title compound (0.632 g, 1.15 mmol, 77%). ^{1}H NMR (400 MHz, CD_3OD): δ ppm 7.32-7.10 (m, 10H), 4.53 (t, *J* = 7.42, 7.42 Hz, 1H), 4.38 (dd, *J* = 9.41, 5.37 Hz, 1H), 4.04 (dd, *J* = 8.29, 4.95 Hz, 1H), 3.10 (dd, *J* = 13.80, 6.84 Hz, 1H), 2.95 (dd, *J* = 13.71, 8.12 Hz, 1H), 2.75-2.57 (m, 2H), 2.05-1.93 (m, 1H), 1.93-1.81 (m, 1H), 1.69-1.58 (m, 1H), 1.48 (s, 11H), 0.93 (d, *J* = 6.52 Hz, 3H), 0.89 (d, *J* = 6.42 Hz, 3H).

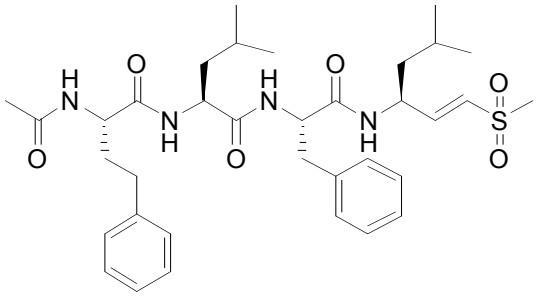


Boc-hPhe-Leu-Phe-Leu-mvs Prepared according to the general procedure for azide couplings using Boc-

Leu-mvs (0.128 g, 0.44 mmol, 1.1 equiv.) and Boc-hPhe-Leu-Phe-hydrazide (0.218 g, 0.4 mmol) Purification by column chromatography (DCM → 7% MeOH in DCM) yielded the title compound (0.221 g, 0.31 mmol, 77.5%). ^{11}H NMR (400 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)) δ ppm 7.31-7.13 (m, 10H), 6.68 (dd, *J* = 15.06, 4.22 Hz, 1H), 6.10 (d, *J* = 15.17 Hz, 1H), 4.66-4.57 (m, 2H), 4.35-4.26 (m, 1H), 3.99 (dd, *J* = 8.69, 5.35 Hz, 1H), 3.21-3.11 (m, 1H), 3.00-2.93 (m, 1H), 2.90 (s, 3H), 2.76-2.59 (m, 2H), 2.07-1.94 (m, 1H), 1.94-1.82 (m, 1H), 1.68-1.55 (m, 2H), 1.47 (s, 11H), 1.39-1.26 (m, 1H), 0.95-0.83 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 178.43, 174.83, 173.89, 172.22, 148.19, 141.82, 137.62, 129.88, 129.78, 129.36, 129.23, 129.18, 127.76, 126.82, 80.77, 55.67, 55.50, 53.38, 48.72, 43.13, 42.85, 41.35, 38.20, 34.43, 32.69, 28.71, 25.41, 25.29, 23.34, 23.19, 22.11, 21.81.

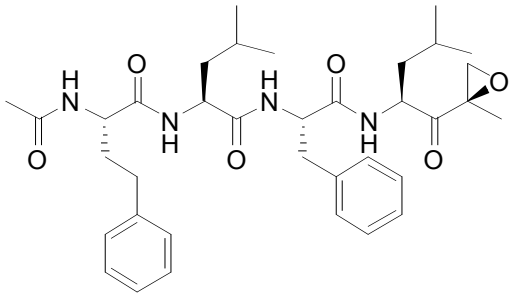


Boc-hPhe-Leu-Phe-Leu-ek. Prepared according to the general procedure for azide couplings using Boc-Leu-ek (0.14 g, 0.55 mmol, 1.1 equiv.) and Boc-hPhe-Leu-Phe-hydrazide (0.218 g, 0.4 mmol). Purification by column chromatography (DCM → 7% MeOH in DCM) yielded the title compound (0.318 g, 0.46 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.33 (s, 1H), 7.25-6.99 (m, 11H), 6.92 (s, 1H), 5.46 (s, 1H), 4.89-4.77 (m, 1H), 4.62-4.52 (m, 2H), 4.20 (s, 1H), 3.24 (d, *J* = 4.71 Hz, 1H), 3.08-2.98 (m, 1H), 2.98-2.89 (m, 1H), 2.82 (d, *J* = 4.85 Hz, 1H), 2.70-2.53 (m, 2H), 2.08-1.86 (m, 2H), 1.63-1.39 (m, 17H), 1.32-1.20 (m, 1H), 0.89-0.80 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 207.86, 172.16, 171.58, 170.70, 155.92, 140.87, 136.39, 129.11, 128.41, 128.31, 126.65, 126.03, 80.01, 58.81, 54.32, 53.83, 52.22, 51.84, 49.67, 41.26, 40.00, 38.06, 34.02, 31.91, 28.32, 25.01, 24.66, 23.19, 22.72, 22.07, 21.43, 16.56.

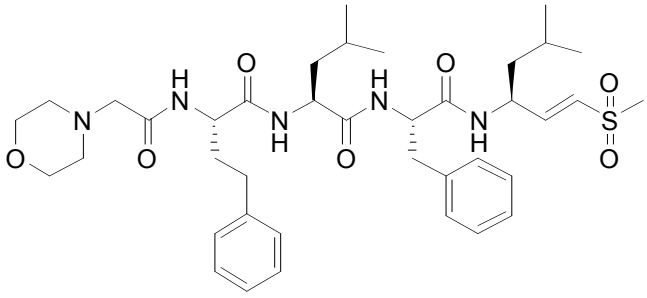


Ac-hPhe-Leu-Phe-Leu-mvs (YU-101-mvs). Boc-hPhe-Leu-Phe-Leu-mvs (35.6 mg, 0.05 mmol) was dissolved in TFA/DCM 1/1 (2 ml) and stirred until TLC analysis (10% MeOH in DCM) showed complete consumption of the starting material. The reaction mixture was co-evaporated with Tol.(3x) yielded the crude hPhe-Leu-Phe-Leu-mvs TFA salt, which was dissolved in DCM (5 ml) and cooled to 0°C. DiPEA (17 µl, 0.105 mmol, 2.1 equiv.) and Ac_2O (5 µl, 0.055 mmol, 1.1 equiv.) were added and the reaction mixture was stirred for 2 hr. The reaction mixture was then washed with H_2O (3x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (DCM → 7% MeOH in DCM) yielded the title compound (29.5 mg, 0.045 mmol, 90%). $[\alpha]_D^{20}$ = 30.5° (5.9 mg/ml, MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 7.31-7.16 (m, 10H), 6.73 (dd, *J* = 15.13, 4.49 Hz, 1H), 6.17 (dd, *J* = 15.14, 1.70 Hz, 1H), 4.64-4.56 (m, 2H), 4.28-4.18 (m, 2H), 3.23-3.14 (m, 1H), 3.06-2.98 (m, 1H), 2.92 (s, 3H), 2.76-2.60 (m, 2H), 2.05 (s, 3H), 2.03-1.91 (m, 2H), 1.69-1.25 (m, 6H), 0.95-0.79 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 172.82, 172.50, 171.91, 170.87, 146.89, 140.16, 136.29, 128.48, 128.23, 127.91, 127.84, 127.76, 126.32, 125.53, 54.45, 53.39, 52.16, 47.32, 41.72, 41.61, 39.51, 36.62, 32.62, 31.27, 24.07, 23.93, 22.01, 21.86, 21.42, 20.68, 20.58. LCMS: Rt 8.73 min (linear

gradient 10-90% MeOH in H_2O, 0.1% TFA, 15 min). HRMS: calcd. for $[C_{35}H_{50}N_4O_6SH]^+$ 655.35238, found 655.35184.

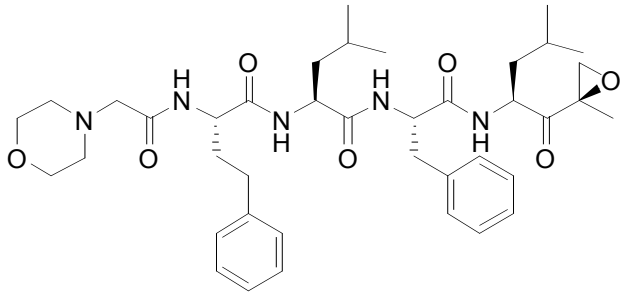


Ac-hPhe-Leu-Phe-Leu-ek (YU-101). Boc-hPhe-Leu-Phe-Leu-ek (0.0346 g, 0.05 mmol) was dissolved in TFA/DCM 1/1 (2 ml) and stirred until TLC showed complete Boc de-protection. The reaction mixture was co-evaporated with Tol.(3x) yielded the crude hPhe-Leu-Phe-Leu-ek TFA salt, which was dissolved in DCM (5 ml), put under argon atmosphere and cooled to 0°C. DiPEA (17 µl, 0.105 mmol, 2.1 equiv.) and Ac_2O (5 µl,0.055 mmol, 1.1 equiv.) were added and the reaction mixture was stirred for 2 hr. More Ac_2O (5 µl, 0.055 mmol, 1.1 equiv.) was added and the mixture was stirred until TLC showed complete consumption of the starting material. The reaction mixture was then washed with H_2O (3x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (DCM → 3% MeOH in DCM) yielded the title compound (11.3 mg, 0.0178 mmol, 35.6%). $[\alpha]_D^{20}$ = 4.5° (2.2 mg/ml, MeOH). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (1/5 v/v)): δ ppm 7.33-7.07 (m, 10H), 4.62 (dd, *J* = 8.51, 5.70 Hz, 1H), 4.52 (dd, *J* = 10.41, 3.29 Hz, 1H), 4.33 (t, *J* = 7.47 Hz, 1H), 4.27 (dd, *J* = 8.60, 5.52 Hz, 1H), 3.20 (d, *J* = 4.83 Hz, 1H), 3.14 (dd, *J* = 14.01, 5.64 Hz, 1H), 2.91 (dd, *J* = 14.03, 8.65 Hz, 1H), 2.86 (d, *J* = 5.01 Hz, 1H), 2.72-2.55 (m, 2H), 2.01 (s, 3H), 1.97-1.84 (m, 2H), 1.48 (s, 3H), 1.70-1.52 (m, 2H), 1.44-1.12 (m, 4H), 0.95-0.83 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (1/5 v/v)): δ ppm 207.51, 172.19, 172.00, 171.49, 170.94, 140.30, 136.03, 128.43, 127.64, 127.51, 125.89, 125.29, 58.22, 53.30, 52.80, 51.43, 51.33, 49.53, 48.41, 39.78, 38.58, 36.86, 32.76, 31.24, 24.27, 23.92, 22.18, 21.78, 21.11, 20.43, 20.02, 15.49. LC-MS: Rt 9.45 min (linear gradient 10-90% MeOH in H_2O, 0.1% TFA 15 min). HRMS: calcd. for $[C_{36}H_{50}N_4O_6H]$+ 635.38031, found 635.37973.



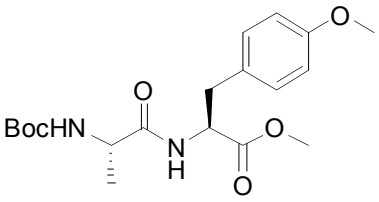
MorpholinoAc-hPe-Leu-Phe-Leu-mvs (PR-171-mvs). Boc-hPhe-Leu-Phe-Leu-mvs (35.6 mg, 0.05 mmol) was dissolved in TFA/DCM 1/1 (2 ml) and stirred for 25 min. The reaction mixture was coevaporated with Tol. (3x), before being dissolved in DCM (6.5 ml) and put under argon atmosphere. Morpholinoacetic acid TFA salt (8.8 mg, 0.06 mmol, 1.2 equiv.), HBTU (22.8 mg, 0.06 mmol, 1.2 equiv.) and DiPEA (0.038 ml, 0.225 mmol, 4.5 equiv.) were dissolved in DCM (5 ml) and this solution was added to the reaction mixture. The reaction mixture was stirred for 2 hr., before being washed with sat. aq. $NaHCO_3$ (3x), dried with Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatograhpy (DCM → 3% MeOH in DCM) yielded the title compound (25.7 mg, 0.0347 mmol, 69.4%). $[\alpha]_D^{20}$ = 1.9° (5.14 mg/ml, MeOH). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)) δ ppm 7.34-7.16 (m, 10H), 6.67 (dd, *J* = 15.17, 4.68 Hz, 1H), 6.06 (dd, *J* =15.18, 1.69 Hz, 1H), 4.66-4.58 (m, 2H), 4.48-4.37 (m, 2H), 3.80-3.70 (m, 4H), 3.09 (d, *J* =2.28 Hz, 2H), 3.17-3.09 (m, 1H), 3.06-2.98 (m, 1H), 2.92 (s, 3H), 2.73-2.63 (m, 2H), 2.60-2.52 (m, 4H), 2.16-1.92 (m, 2H), 1.72-1.24 (m, 6H), 0.99-0.85 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 174.19, 174.00, 172.76, 172.53, 148.38,

142.37, 138.13, 130.39, 129.71, 129.52, 128.11, 127.15, 67.88, 62.47, 56.33, 54.77, 54.14, 53.42, 49.09, 43.42, 42.88, 41.81, 38.65, 35.37, 33.07, 25.82, 25.62, 23.50, 23.32, 22.13, 21.83. LC-MS: Rt 6.91 min (linear gradient 10-90% MeOH in H_2O, 0.1% TFA, 15 min). HRMS: calcd. for $[C_{39}H_{57}N_5O_7SH]^+$ 740.40515, found 740.40474.



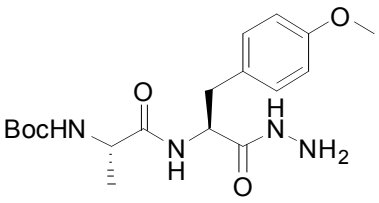
MorpholinoAc-hPhe-Leu-Phe-Leu-epoxyketone (PR-171). Boc-hPhe-Leu-Phe-Leu-ek (0.0346 g, 0.05 mmol) was dissolved in TFA/DCM 1/1 (2 ml) and stirred for 25 min. The reaction mixture was co-evaporated with Tol. (3x), before being dissolved in DCM (6.5 ml) and put under argon atmosphere. Morpholinoacetic acid TFA salt (8.8 mg, 0.06 mmol, 1.2 equiv.), HBTU (22.8 mg, 0.06 mmol, 1.2 equiv.) and DiPEA (38 µl, 0.225 mmol, 4.5 equiv.) were dissolved in DCM (5 ml) and this solution was added to the reaction mixture. The reaction mixture was stirred for 2 hr., before being washed with sat. aq. $NaHCO_3$ (4x), dried with Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatograhpy (DCM → 3% MeOH in DCM) yielded the title compound (0.0086 g, 0.0119 mmol, 24%). $[\alpha]_D^{20} = 0°$ (1.7 mg/ml, MeOH). $_1$H NMR (400 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 7.32-7.08 (m, 10H), 4.61 (dd, *J* = 8.10, 5.90 Hz, 1H), 4.52 (dd, *J* = 10.46, 3.27 Hz, 1H), 4.42 (dd, *J* = 8.27, 5.32 Hz, 1H), 4.34 (t, *J* = 7.44, 7.44 Hz, 1H), 3.80-3.71 (m, 4H), 3.19 (d, *J* = 4.99 Hz, 1H), 3.12 (dd, *J* = 13.99, 5.89 Hz, 1H), 3.02 (d, *J* = 2.74 Hz, 2H), 2.92 (dd, *J* = 14.05, 8.17 Hz, 1H), 2.86 (d, *J* = 4.99 Hz, 1H), 2.64-2.48 (m, 6H), 2.11-2.00 (m, 1H), 1.98-1.85 (m, 1H), 1.69-1.41 (m, 7H), 1.38-1.24 (m, 2H), 0.96-0.84 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ (1/10 v/v)): δ ppm 172.42, 172.00, 171.35, 170.76, 140.84, 136.48, 129.12, 128.33, 128.25, 128.17, 126.58, 125.99, 66.75, 61.35, 53.87, 53.51, 52.43, 52.01, 51.95, 50.06, 40.55, 39.34, 37.57, 34.16, 31.81, 24.92, 24.56, 22.90, 22.47, 21.18, 20.76, 16.21. LCMS: Rt 7.58 min (linear gradient 10-90% MeOH in H_2O, 0.1% TFA, 15 min). HRMS: calcd. for $[C_{40}H_{57}N_5O_7H]^+$ 720.43308, found 720.43265.

Synthesis of PR-957 and PR-957-mvs

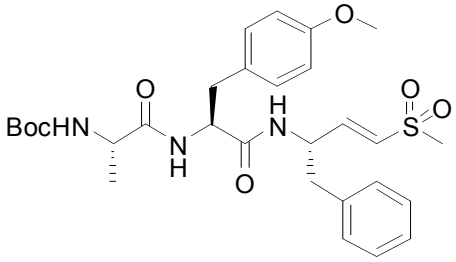


Boc-Ala-Tyr(Me)-OMe: BocAlaOH (0.34 g, 1.8 mmol, 1.2 equiv.), HBTU (0.74 g, 1.8 mmol, 1.2 equiv.) and HCl·H-Tyr(Me)-OH (0.369 g, 1.5 mmol) were dissolved in DCM (10 mL). The reaction was initiated by addition of DiPEA (0.87 mL, 5.25 mmol, 3.5 equiv.) to the stirred solution. After 18 hr. the reaction mixture was concentrated, the concentrate was dissolved in EtOAc and the solution was washed with 1M aq. HCl (3x), saturated aq. $NaHCO_3$ (5x), Brine, dried over $MgSO_4$ and concentrated. This yielded Boc-Ala-Tyr(Me)-OMe (0.564 g, 1.48 mmol, 98.8%) as a viscous light-brown oil. TLC-analysis (eluent: 40% EtOAc / 60% PetEt) showed product at *Rf* 0.26, which was used without further purification. $[\alpha]_D^{23} = +34.85°$ (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.03 (d, *J* = 8.56 Hz, 2H), 6.89 (d, *J* = 6.39 Hz, 1H), 6.80 (d, *J* = 8.58 Hz, 2H), 5.38 (d, *J* = 6.34 Hz, 1H), 4.79 (dd, *J* = 13.22, 6.08 Hz, 1H), 4.21 (s, 1H), 3.75 (s, 3H), 3.69 (s, 3H), 3.04 (dq, *J* = 13.96, 6.10 Hz, 2H), 1.43 (s, 9H), 1.31 (d, *J* = 7.07 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 172.32, 171.64, 158.36, 155.14, 130.03, 127.55,

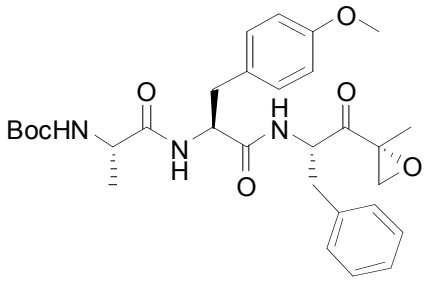
113.67, 79.59, 54.87, 53.20, 52.01, 49.78, 36.75, 28.06, 18.16. LC-MS: Rt 7.32 min (linear gradient 10-90% MeCN in H_2O, 0.1% TFA, 15 min). HRMS: Calculated for $C_{19}H_{28}N_2O_6Na^+$: 403.18396; found: 403.18352.



Boc-Ala-Tyr(Me)-hydrazide: To a solution of Boc-Ala-Tyr(Me)-OMe (0.508 g, 1.34 mmol) in MeOH (22.5 mL) was added hydrazine monohydrate (1.95 mL of 64% solution, 40.05 mmol, 30 equiv.). The reaction mixture was refluxed at 70°C. TLC-analysis showed complete conversion of **Boc-Ala-Tyr(Me)-OMe** after 2.5 hr. and the reaction mixture was co-evaporated with toluene (3x) to yield Boc-Ala-Tyr(Me)-hydrazide (0.475 g, 1.25 mmol, 93.4%) as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 7.12 (d, *J* = 8.54 Hz, 2H), 6.83 (d, *J* = 8.69 Hz, 2H), 4.50 (t, *J* = 7.18 Hz, 1H), 3.96 (q, *J* = 7.18 Hz, 1H), 3.75 (s, 3H), 2.97 (ddd, *J* = 21.78, 13.65, 7.12 Hz, 2H), 1.43 (s, 9H), 1.20 (d, *J* = 7.21 Hz, 3H). LC-MS: Rt 5.31 min (linear gradient 10-90% MeCN in H_2O, 0.1% TFA, 15 min). HRMS: Calculated for $C_{18}H_{28}N_4O_5H^+$: 381.21325; found: 381.21300.

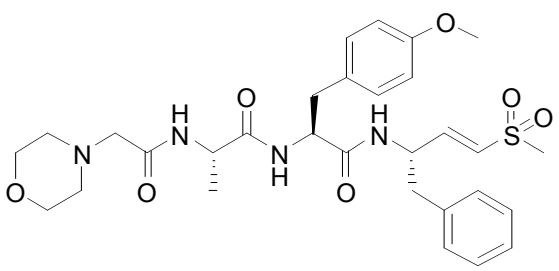


Boc-Ala-Tyr(Me)-Phe-mvs: Prepared according to the general procedure for azide couplings using Boc-Phe-mvs (0.181 g, 0.55 mmol, 1.1 equiv.) and Boc-Ala-Tyr(Me)--hydrazide (0.191 g, 0.5 mmol, 1 equiv.). Purification by column chromatography (50% EtOAc in PetEt → 80% EtOAc in PetEt) yielded Boc-Ala-Tyr(Me)-Phe-mvs (0.186 g, 0.321 mmol, 64.3%) as a white solid. $[\alpha]_D^{23}$ = +29.57° (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.31-7.13 (m, 5H), 7.06 (d, *J* = 4.2 Hz, 2H), 6.94 (d, *J* = 7.2 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 2H), 6.78-6.73 (m, 1H), 6.66 (d, *J* = 7.2 Hz, 1H), 6.19 (d, *J* = 14.8 Hz, 1H), 5.02 (m, 1H), 4.97 (m, 1H), 4.61 (q, *J* = 6.4 Hz, 1H), 4.03 (m, 1H), 3.76 (s, 3H), 3.14-3.10 (m, 2H), 3.00-2.89 (m, 2H), 2.80 (s, 3H), 1.37 (s, 9H), 1.31 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 172.65, 170.40, 158.69, 156.07, 145.75, 136.03, 130.31, 130.22, 128.74, 128.47, 127.75, 127.11, 114.26, 80.87, 55.16, 54.28, 51.32, 50.40, 42.58, 40.15, 36.45, 28.25, 17.79. LCMS: Rt 8.09 min (linear gradient 10-90% MeCN in H_2O, 0.1% TFA, 15 min). HRMS: Calculated for $C_{29}H_{39}N_3O_7SH^+$: 574.25815; found: 574.25755.

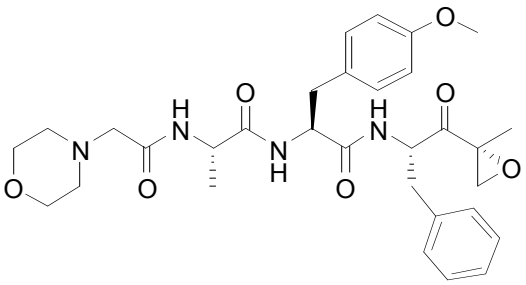


Boc-Ala-Tyr(Me)-Phe-ek: Prepared according to the general procedure for azide couplings using Boc-Phe-ek (84 mg, 0.28 mmol, 1.1 equiv.) and Boc-Ala-Tyr(Me)-hydrazide (94.7 mg, 0.25 mmol, 1 equiv.). Purification by column chromatography (PetEt → 60% EtOAc in PetEt) yielded Boc-Ala-Tyr(Me)-Phe-ek (0.100 g, 0.180 mmol, 72.1%). $[\alpha]_D^{23}$ = +25.77° (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.28-7.17 (m, 3H, 7.08 (d, *J* = 8.57 Hz, 2H), 7.04-6.99 (m, 2H), 6.78 (d, *J* = 8.63 Hz, 2H), 6.74 (d, *J* = 7.74 Hz, 1H), 6.44 (s, 1H), 5.09 (d, *J* = 7.22 Hz, 1H), 4.74) dd, *J* = 13.09, 7.41 Hz, 1H), 4.54 (dd, *J* = 13.94, 7.31 Hz, 1H), 4.12 (dd, *J* = 14.21, 7.09 Hz, 1H), 3.76 (s, 3H), 3.26-2.63 (m, 6H), 1.44 (s, 3H), 1.42 (s, 9H), 1.26 (d, *J* =

7.07 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 206.95, 172.36, 170.30, 158.56, 155.35, 135.45, 130.32, 129.17, 128.43, 128.19, 126.99, 113.94, 80.08, 59.04, 55.11, 54.13, 52.30, 52.26, 50.15, 37.17, 28.23, 18.28, 16.31. LC-MS: Rt 8.92 min (linear gradient 10-90% MeCN in H_2O, 0.1% TFA, 15 min). HRMS: Calculated for $C_{30}H_{39}N_3O_7H^+$: 554.28608; found: 554.28539.



MorholinoAc-Ala-Tyr(Me)-Phe-mvs (PR-957-mvs): Boc-Ala-Tyr(Me)-Phe-mvs (0.106 g, 0.184 mmol, 1.0 equiv.) was dissolved in DCM and TFA (2:1 ratio). The reaction was monitored by TLC-analysis. After 1.5 hr. the reaction mixture was co-evaporated with toluene. (3x). and the concentrate was dissolved in DCM at standard concentrations. To this solution was added morpholinoacetic acid TFA salt (54 mg, 0.203 mmol, 1.1 equiv.), HBTU (84 mg, 0.203 mmol, 1.1 equiv.) and DiPEA (0.137 mL, 0.829 mmol, 4.5 equiv.). The reaction was followed by LCMS. After 3.5 hr. the reaction mixture was concentrated and the concentrate was dissolved in EtOAc. The organic solution was subsequently washed with sat. aq. $NaHCO_3$ (5x), Brine (1x), dried and concentrated. Further purification by column chromatography (DCM → 4% MeOH in DCM) yielded **PR-957-mvs** (90 mg, 0.150 mmol, 81.4%). $[\alpha]_D^{23}$ = -19.63° (c=1, MeOH). ^{1}H NMR (400 MHz, DMSO-d6): δ ppm 8.26 (d, *J* = 8.25 Hz, 1H), 8.10 (d, *J* = 8.21 Hz, 1H), 7.78 (d, *J* = 7.62 Hz, 1H), 7.32-7.15 (m, 5H), 7.10 (d, *J* = 8.57 Hz, 2H), 6.81 (d, *J* = 8.59 Hz, 2H), 6.67 (dd, *J* = 15.25, 4.86 Hz, 1H), 6.30 (dd, *J* = 15.26, 1.48 Hz, 1H), 4.74-4.65 (m, 1H), 4.44 (dd, *J* = 14.90, 8.16 Hz, 1H), 4.27 (p, *J* = 7.10 Hz, 1H), 3.70 (s, 3H), 3.59-3.54 (m, 4H), 3.04-2.65 (m, 9H), 2.40-2.37 (m, 4H), 1.12 (d, *J* = 6.96 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d6): δ ppm 171.56, 170.25, 168.37, 157.64, 145.33, 137.27, 130.08, 129.87, 129.11, 128.10, 127.99, 126.27, 113.38, 66.04, 61.16, 54.75, 54.19, 53.07, 50.26, 47.38, 42.08, 38.73, 36.82, 18.65. LC-MS: Rt 5.92 min (linear gradient 10-90% MeCN in H_2O, 0.1% NH_4OAc, 15 min). HRMS: Calculated for $C_{30}H_{40}N_4O_7SH^+$: 601.26905; found: 601.26838.



MorpholinoAc-Ala-Tyr(Me)-Phe-ek (PR-957): A solution of Boc-Ala-Tyr(Me)-Phe-EK (62.8 mg, 0.113 mmol, 1.0 equiv.) in DCM : TFA (2:1) was made at standard concentrations. The reaction progress was monitored by TLC-analysis. The reaction mixture was co-evaporated with toluene (3x) after 1 hr. to yield the TFA salt, which was then dissolved in DCM at standard concentration. To the solution were added morpholinoacetic acid TFA salt (33 mg, 0.125 mmol, 1.1 equiv.), HBTU (52 mg, 0.125 mmol, 1.1 equiv.) and DiPEA (84 µl, 0.510 mmol, 4.5 equiv.). The reaction was monitored by means of LCMS analysis. After 18 hr. the reaction mixture was concentrated and the concentrate dissolved in EtOAc. The organic solution was washed with sat. aq. $NaHCO_3$ (5x), Brine (1x), dried and concentrated. Purification by column chromatography (DCM → 3% MeOH in DCM) afforded the title compound **PR-957** (44.3 mg, 0.0764 mmol, 67.4%). $[\alpha]_D^{23}$ = +24.33° (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.44 (d, *J* = 7.58 Hz, 1H), 7.29-7.20 (m, 3H), 7.08 (d, *J* = 8.63 Hz, 2H), 7.02 (dd, *J* = 7.62, 1.56 Hz,2H), 6.78 (d, *J* = 8.65 Hz, 2H), 6.69 (d, *J* = 7.55 Hz, 1H), 6.31 (d, *J* = 7.37 Hz, 1H),

4.74 (dt, J = 7.87, 5.01 Hz, 1H), 4.49 (q, J = 7.00 Hz, 1H), 4.37 (p, J = 7.10 Hz, 1H), 3.77 (s, 3H), 3.69 (t, J = 4.58 Hz, 4H), 3.26 (d, J = 4.92 Hz, 1H), 3.11-2.65 (m, 7H), 2.48-2.43 (m, 4H), 1.48 (s, 3H), 1.29 (d, J = 7.04 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 206.91, 171.79, 170.26, 170.09, 158.55, 135.53, 130.33, 129.19, 128.48, 128.22, 127.06, 113.96, 66.86, 61.58, 59.14, 55.17, 54.23, 53.70, 52.56, 52.41, 48.22, 37.03, 36.73, 17.65, 16.45. LC-MS: Rt 6.72 min (linear gradient 10-90% MeCNMeCN in H_2O, 0.1% NH_4OAc, 15 min). HRMS: Calculated for $C_{31}H_{40}N_4O_7H^+$: 581.29698; found: 581.29664.

References

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Abbreviations:

Ac, acetyl
Ac_2O, acetic anhydride
APT, attached proton test
Aq,. aqueous
Boc, *tert*-butyloxycarbonyl
BOP, benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphonate
calcd., calculated
cat., catalytic amount
δ, chemical shift
d, doublet
DBU, diazabicyclo[5.4.0]undec-7-ene
DCM, dichloromethane
dd, double doublet
ddd, double double doublet
DiPEA, Diisopropylethylamine
DMAP, 4-(dimethylamino)pyridine
DMF, *N*,*N*-dimethylformamide
DMSO, dimethylsulfoxide
dt, double triplet
dq, double quartet
EDC, 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide
ek, α',β'-epoxyketone
Et_3N triethyl amine

Et_2O, diethyl ether
EtOAc, ethyl acetate
equiv., molar equivalent
Fmoc, (9*H*-fluoren-9-yl)methoxycarbonyl
HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU, (2-(6-Chloro-1H-benzotriazole- 1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate)
HMB, 3-hydroxy-2-methylbenzamide
HMPB, 4-(4-Hydroxymethyl-3-methoxyphenoxy)butyric acid
HOBt, *N*-hydroxybenzotriazole
HRMS, high resolution mass spectrometry
Hz, Hertz
J, coupling constant
LC/MS, liquid chromatography/ mass spectrometry
M, multiplet
MBHA, para-methybenzylhydryl amine
Me, methyl
MeCN, acetonitrile
MeOD, CD_3OD
MeOH, methanol
MS (ESI), mass spectrometry (electrospray ionization)
mvs, methyl vinyl sulfone
NMP, N-methyl-2-pyrrolidone
m/z, mass-to-charge ratio
o/n, overnight
OAc, acetate
PetEt, petroleum ether
Ph, phenyl
ppm, parts per million
pvs 4-hydroxyphenyl vinyl sulfone
PyBOP benzotriazol-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphonate
q quartet
quant. quantitative
rt, room temperature
Rt, retention time
s, singlet
sat. saturated
t, triplet
t, tert tertiary
T, temperature
*t*Bu, *tert*-butyl
*t*BuONO, *tert*-butyl nitrite
td, triple doublet
TEA, triethyl amine
TFA, trifluoroacetic acid
TLC, thin layer chromatography
Tol. toluene
ve, vinyl ethyl ester