Supplementary Material (ESI) for Chemical Communications

Supporting Information

Phosphate-Assisted Peptide Ligation

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Table of contents

General materials and methods

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. A Waters Sunfire 5 μm, 2.1 x 150 mm column was used at a flow rate of 0.2 mL min^{-1} and results analysed with Waters Empower software. Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230 and 280 nm. A Waters Sunfire 5 μm, 19 x 150 mm column was used at a flow rate of 7 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Semi-preparative HPLC was performed on a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230 and 280 nm. A Grace Vydac "Protein and Peptide C18", 250 x 10 mm, 10-15 μM particle size column was used at a flow rate of 4 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B).

Ligation yields were determined by weighing on a high accuracy balance (Mettler Toledo XS105 Dual Range Balance) with weighing errors of ± 0.03 mg.

LC-MS was performed on a Thermo Separation Products: Spectra System consisting of P400 Pump and a UV6000LP Photodiode array detector on a Phenomenex Jupiter 5 μm, 2.1 x 150 mm column at a flow rate of 0.2 mL min^{-1} coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI) operating in positive mode. Separations involved a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B).

ESI mass spectrometry was performed on a Thermoquest Finnigan LCQ Deca mass spectrometer operating in positive mode.

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem. DCM and methanol were distilled from calcium hydride. DMF was obtained as peptide synthesis grade from Auspep or Labscan.

Synthesis of peptides and phosphopeptides (1-4) via the Fmoc strategy

Solid-phase peptide synthesis was carried out in syringes, equipped with teflon filters, purchased from Torviq.

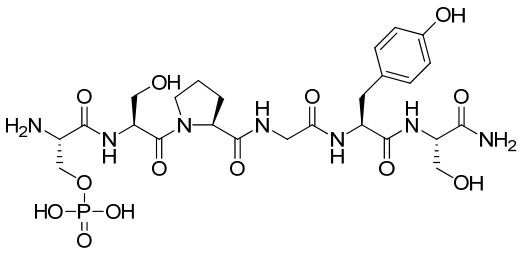
Preloading Rink Amide resin

Rink amide resin was initially washed with DCM (x 5) and DMF (x 5), followed by removal of the Fmoc group by treatment with 10% piperidine/DMF (2 x 5 min). The resin was washed DMF (x 5), DCM (x 5), DMF (x 5). PyBOP (4 eq.) and NMM (8 eq.) was added to a solution of Fmoc-Ser(OtBu)-OH (4 eq.) in DMF (final concentration 0.1 M). After 5 min of pre-activation, the mixture was added to the resin. After 2 h the resin was washed DMF (x 5), DCM (x 5), DMF (x 5), capped with acetic anhydride/pyridine (1:9 v/v) (2 x 5 min) and washed DMF (x 5), DCM (x 5) and DMF (x 5).

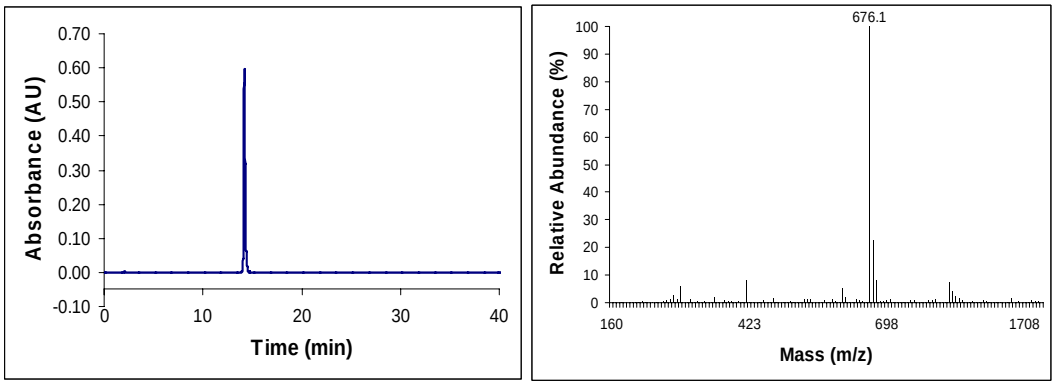
Iterative peptide assembly: *Deprotection:* The resin was treated with 10% piperidine/DMF (2 x 5 min) and washed with DMF (x 5), DCM (x 5) and DMF (x 5). *Amino acid coupling:* A preactivated solution of protected amino acid (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After 45 min, the resin was washed with DMF (x 5), DCM (x 5) and DMF (x 5). *Capping*: Acetic anhydride/pyridine (1:9 v/v) was added to the resin. After 5 min the resin was washed with DMF (x 5), DCM (x 5) and DMF (x 5). *Cleavage*: A mixture of TFA, thioanisole, triisopropylsilane (TIS) and water (17:1:1:1 v/v/v/v) was added to the resin. After 2 h, the resin was washed with TFA (3 x 2 mL) *Work-up*: The combined solutions were concentrated *in vacuo*. The residue was dissolved in water containing 0.1% TFA and purified by preparative HPLC (gradient 0 to 50% B over 40 min) and analyzed by LC-MS (0 to 50% B over 30 min) and ESI mass spectrometry.

Analytical data for peptides and phosphopeptides 1-4

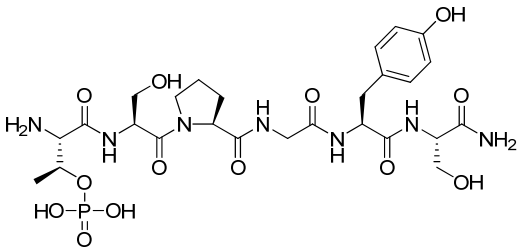
H-pSSPGYS-NH_2 (1)



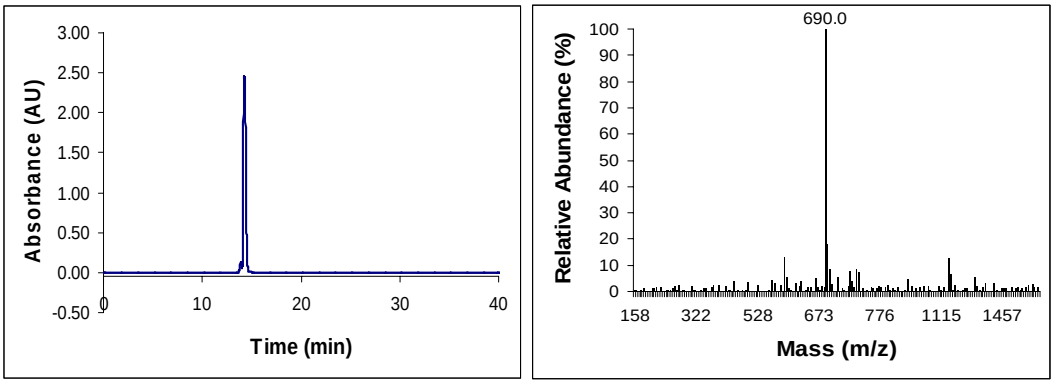
50 μM scale, Yield = 32 mg, 95%; ESI (m/z): Calculated Mass $[M+H]^+$: 676.2, Mass Found 676.1; HPLC: t_R: 14.2 min (Gradient 0 to 50% B over 40 min.).



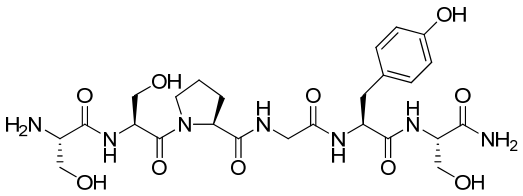
H-pTSPGYS-NH_2 (2)



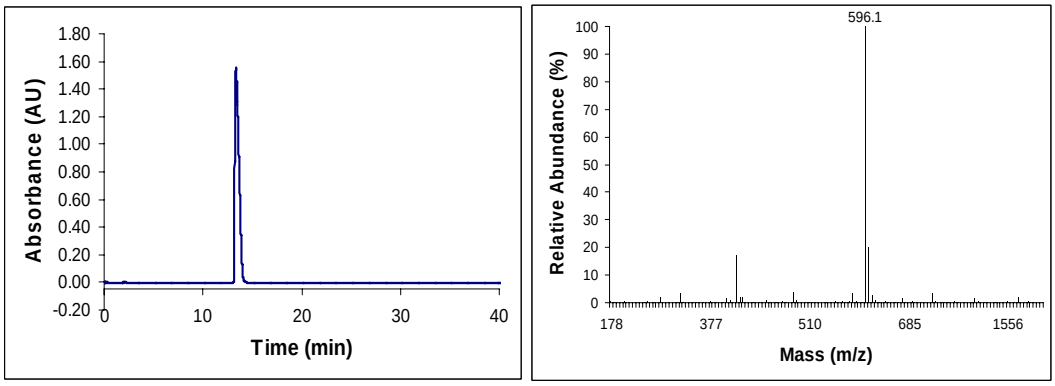
50 μM scale, Yield = 30 mg, 87%; ESI (m/z): Calculated Mass $[M+H]^+$: 690.3, Mass Found 690.0; HPLC: t_R: 14.3 min (Gradient 0 to 50% B over 40 min.).



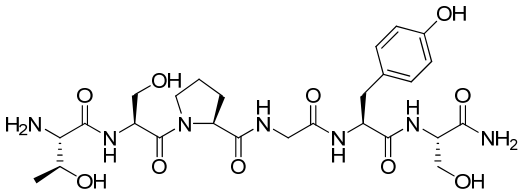
H-SSPGYS-NH_2 (3)



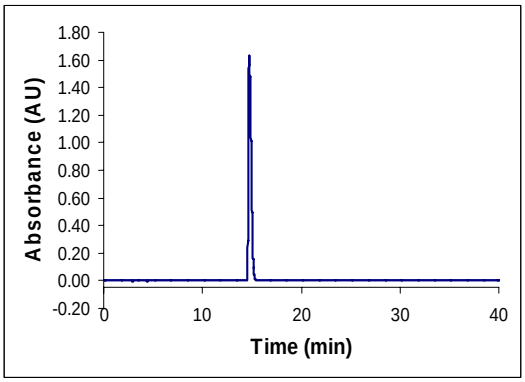
50 μM scale, Yield = 29 mg, 97%; ESI (m/z): Calculated Mass $[M+H]^+$: 596.3, Mass Found 596.1; HPLC: t_R: 13.3 min (Gradient 0 to 50% B over 40 min.).

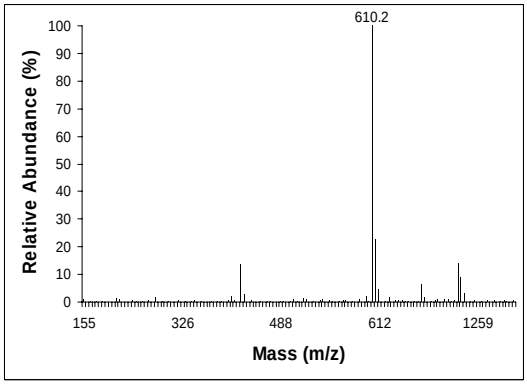


H-TSPGYS-NH_2 (4)



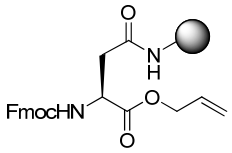
50 μM scale, Yield = 28 mg, 92%; Calculated Mass $[M+H]^+$: 610.3, Mass Found 610.2; HPLC: t_R: 14.7 min (Gradient 0 to 50% B over 40 min.).





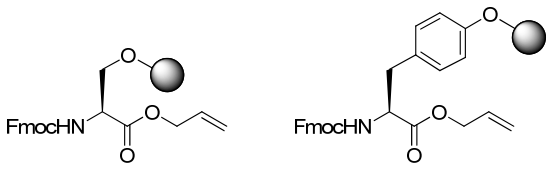
Synthesis of peptide thioesters (5-11) via the side chain anchoring strategy

Side chain anchoring of amino acids onto Rink Amide resin:



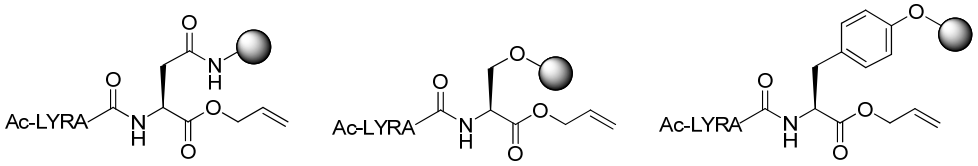
Rink Amide resin co. (100-200 mesh; 1% DVB) (290 mg, loading = 0.69 mmol/g, 200 µmol) was initially washed with DMF (x 5), DCM (x 5) and DMF (x 5), treated with DMF/piperidine (9:1) (2x 5 min) and washed with DMF (x 5), DCM (x 5) and DMF (x 5). A solution of Fmoc-Asp-OAllyl (800 µmol, 4 eq.) in dry DMF (4 ml) containing PyBOP (416 mg, 800 µmol, 4 eq.) and *N*-methylmorpholine (176 µl, 1600 µmol, 8 eq.) was preactivated for 4 min and then added to the resin. After two hours of shaking, the resin was washed with DMF (x 5), DCM (x 5) and DMF (x 5), treated with Ac_2O/Py (1:9 v/v) for 10 min and then washed with DMF (x 5), DCM (x 5) and DMF (x 5). Treatment of the resin with 10% piperidine/DMF (2 x 5 min) and measurement of the resulting fulvene-piperidine adduct at λ = 302 nm showed that the yield of the side chain anchoring was quantitative.

Side chain anchoring onto bromo-(4-methoxyphenyl)methylpolystyrene:



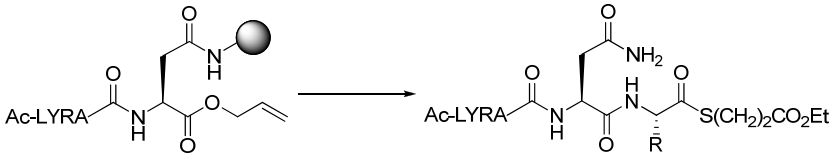
Bromo-(4-methoxyphenyl)methylpolystyrene resin (174 mg, loading = 2.3 mmol/g, 400 μmol) was initially washed with DMF (x 5) and DCM (x 10) and the resin swelled with DCM (5 mL) for 1 h with the exclusion of light. A solution of Fmoc-Ser-OAllyl[1] or Fmoc-Tyr-OAllyl[1] (1200 μmol, 3 eq.), DIEA (2400 μmol, 397 μL, 6 eq.) in DCM (4 mL) was added to the resin, which was shaken for 18-46 h at rt with the exclusion of light. The resin was washed with DCM (x 5) and DMF (x 10). Treatment of the resin with 10% piperidine/DMF (2x 5 min) and measurement of the resulting fulvene-piperidine adduct at $\lambda = 302$ nm was used to determine the loading. NB: The commercially available resin is sold with a very high loading, therefore preloadings of the resins were conducted for a length of time that allowed for the resin to be loaded no more than 1.4 mmol/g to prevent resin crowding and product aggregation. Final loadings: Fmoc-Ser-OAllyl (18 h): loading = 1.28 mmol/g; Fmoc-Tyr-OAllyl (46 h): loading = 1.02 mmol/g;

Solid phase peptide assembly



Peptide assembly was conducted as described for the iterative peptide synthesis above.

General procedure for the *C*-terminal introduction of amino acid thioesters:[1]



The resin (25 μmol) was swollen in dry DCM for 30 min, followed by the addition of a solution of $Pd(PPh_3)_4$ (25 mg, 22 μmol) and $PhSiH_3$ (123 μl, 108 mg, 1 mmol, 40 eq.) in dry DCM (2 mL). The resin was shaken for 1 h and the procedure was