Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Peptide Ligation from Alkoxyamine Based Radical Addition

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Materials

Fluorenylmethoxycarbonyl (Fmoc) amide resin (0.51 mmol g^{-1}) and coupling reagents were purchased from Iris Biotech GmbH (Germany). Fmoc amino acids were from Novabiochem (Switzerland). BlocBuilder MA was kindly provided by Arkema (France). Further chemicals were purchased from Acros Organics, Sigma-Aldrich or Merck.

Peptide synthesis

The peptides GGGWIKVAV, GGG, GGGK and RGDK were synthesized by the solid-phase method[1] using an automated synthesizer (Model 433A, Applied Biosystems). The peptide chains were assembled stepwise on 0.25 mmol Fmoc amide resin (1% cross-linked, 0.51 mequiv of amino group/g) using 1 mmol of Fmoc amino acid derivatives. Side-chain protecting groups used for trifunctional residues were as follows: *tert*-butyloxycarbonyl (Boc) for tryptophan and pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine. Lysine of the GGGWIKVAV peptide was side chain protected with Boc and that of the RGDK peptide was side chain protected with monomethoxytrityl (Mmt). *N*-α-amino groups were deprotected by treatment with 18% and 20% piperidine/N-methylpyrrolidone (NMP) for 3 and 8 min, respectively. The Fmoc-amino acid derivatives were coupled (20 min) as their hydroxybenzotriazole active ester in *N*-methylpyrrolidone (4-fold excess). The 2-(1H-

benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate / hydroxybenzotriazole (HBTU/HOBt) coupling agents in combination with diisopropylethylamine (DIPEA) were used.[2]

Chain-end functionalization of the peptides with SG1 and vinyl moieties

The N-terminus SG1-functionalized resin peptide (SG1-MAMA-GGGWIKVAV, **5**) was synthesized as previously described[3] and kept on the resin for further conjugation reactions. The N-terminus vinyl functionalized peptides (=GGG, **6**; =RGDK, **7**) were obtained by coupling acrylic acid (4 fold excess) on the α-amine of the tyrosine residue as the last step of the SPPS, using HOBt/HBTU coupling agents. The *C*-terminus vinyl functionalized peptide (GGGK=, **12**) was obtained by coupling ε-amine of lysine with acryloyl chloride during the SPPS. More precisely, after incorporation of the last amino acid (Fmoc-Gly-OH) on the peptide sequence, the Mmt protecting group on the lysine ε-amine was selectively removed using AcOH/TFE/DCM (1:2:7),[4] and the ε-amine was allowed to react with acryloyl chloride (4 fold excess) in the presence of a base DIEPA in NMP. After Fmoc deprotection, the peptide was cleaved from resin with TFA.

Determination of dissociation rate constant of SG1-GGGWIKVAV resin peptide (5)

Electron spin resonance (ESR) experiments were performed on a Bruker EMX 300 spectrometer. The appearance of the nitroxide was followed in either *tert*-butylbenzene or DMF as solvent (0.5 mL) containing around 2 mg of alkoxyamines functionalized resin. The time evolution of the doubly integrated ESR signal of the SG1 nitroxide radical was followed by ESR spectroscopy at 50-85 °C for 2-4 hours. A first order fit of the signal allows the determination of the k_d value. The final signal was compared to a standard of TEMPO at a known concentration to determine the concentration of the released SG1.

It has to be noted that the resin not being soluble in organic media, measurements were performed in heterogeneous conditions. O_2 was used as radical scavenger. The activation energy *E*a was calculated from k_d as $k_d = Ae^{-\frac{E_a}{RT}}$ with A = 2.4 x 10^{14} s^{-1}.[5]

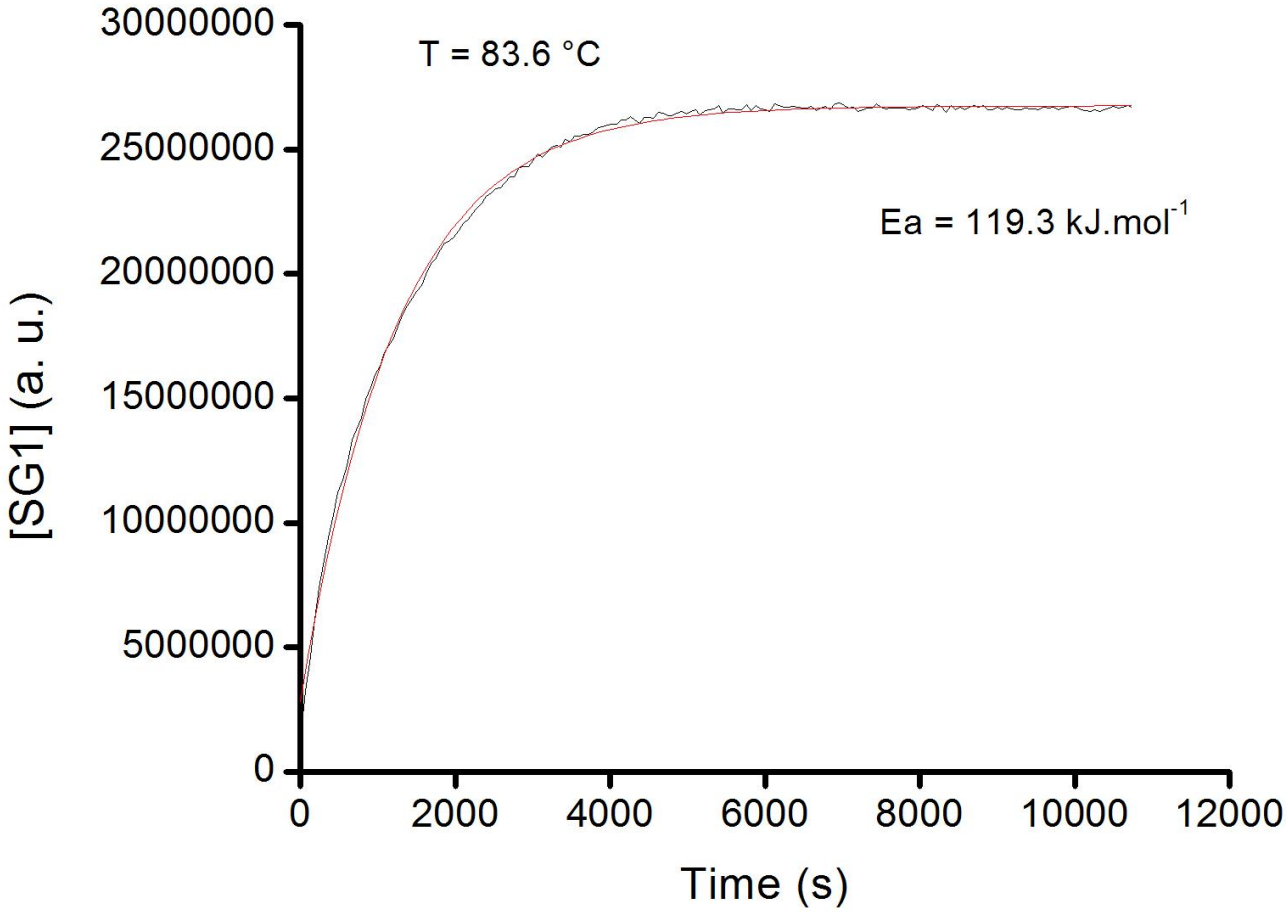


Figure S1. Time dependence of the SG1 concentration during thermolysis of the alkoxyamine-functionalized resin in *tert*-butylbenzene with O_2 as radical scavenger.

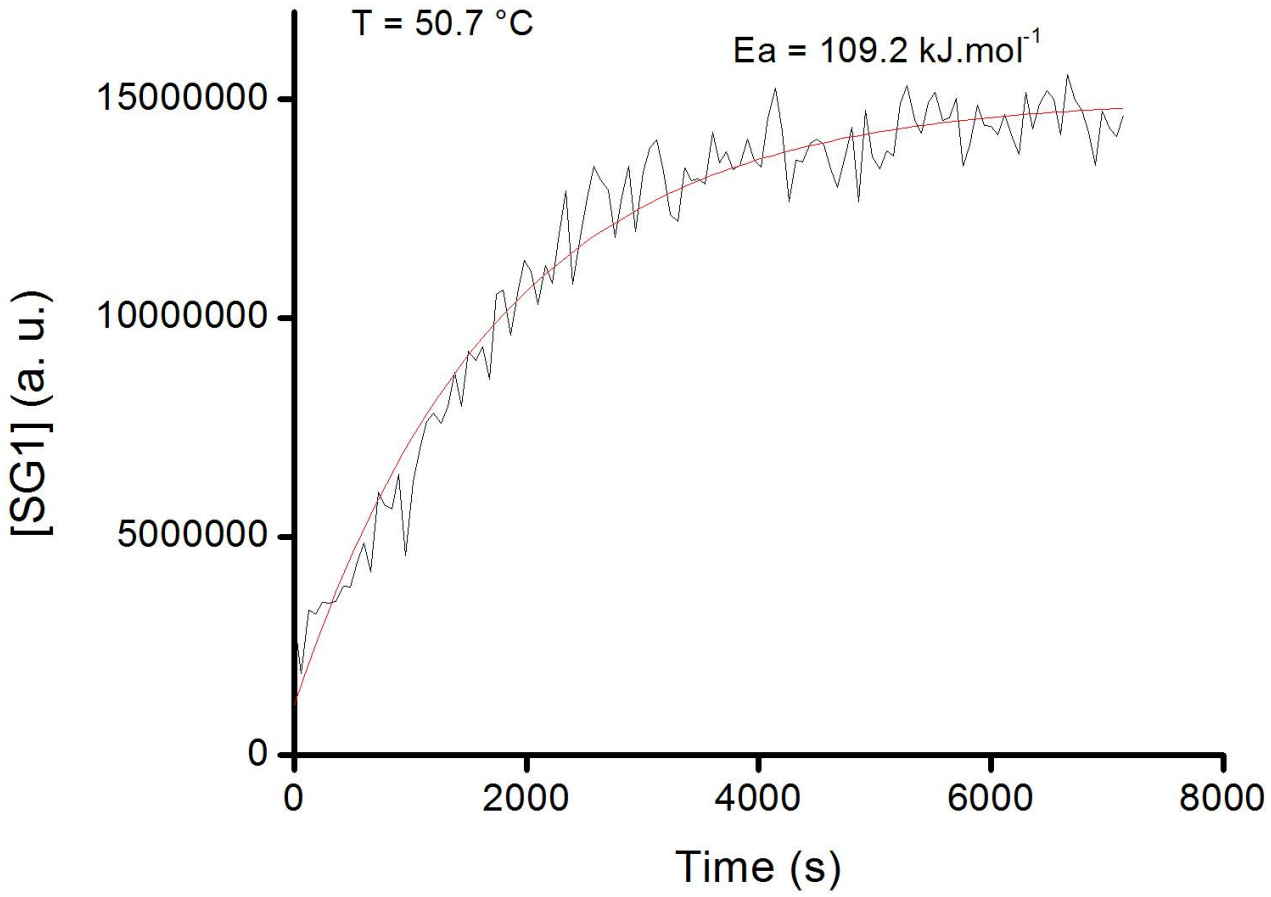


Figure S2. Time dependence of the SG1 concentration during thermolysis of the alkoxyamine-functionalized resin in DMF with O_2 as radical scavenger.

Intermolecular Radical 1,2-Addition (IRA) conjugation reaction

Typically, the SG1 resin peptide SG1-MAMA-GGGWIKVAV **5** (127 mg, 0.03 mmol), N-terminus vinyl peptide =GGG **6** (8.7 mg, 0.036 mmol) and DMF (2 mL) were introduced in a round-bottom flask, which was fitted with a septum. The mixture was deoxygenated for 30 minutes argon bubbling at room temperature, and the flask was immersed in an oil bath at 70°C (90°C in some minor cases). After stirring for a predetermined time under argon, the mixture was allowed to cool overnight. The resin was then filtered, washed with DMF, then dichloromethane, and stirred in a mixture TFA/H2O/EDT (92.5/5/2.5 v%) during 110 minutes. The resin was then filtered and the

filtrate precipitated in diethyl ether. The crude peptide product was recovered by centrifugation (5000 rpm, 15 min. at 4°C), washed with diethyl ether and dried under vacuum, and further purified by HPLC (35% yield, 15 mg). IRAs of the SG1 resin peptide SG1-MAMA-GGGWIKVAV on N-terminal vinyl peptide =RGDK (**7**) and C-terminal vinyl peptide GGGK= (**12**) were performed in the same manner. (30% and 15% yield after peptide purification, respectively).

Peptide/peptide conjugate characterization and isolation

HPLC-UV/MS analysis of the peptides was performed with a Shimadzu LCMS-2010 EV system, on a C18 column (100 Å, 5 µm), using a linear gradient of acetonitrile 0.1 % (B) TFA in 0.1% TFA in water (A) (20-70% of B in 50 min, UV detection at 214 nm).

The peptide adducts were also analyzed on a Bruker ESI MicroTofQ coupled to an Agilent RS200 UPLC system. The peptides were separated on a 100 x 2 mm Nucleodur C18 Pyramid, 1.8µm, Nacherey Nagel by a linear gradient of acetonitrile 0.1 % TFA in 0.1% TFA in water (20 at 70% in 50 min, temperature of column 40°C,flow 0.3 ml/min). The mass spectrometer was calibrated in external mode with a Tunemix solution from Bruker.

Crude peptides were purified to homogeneity by semipreparative reversed-phase HPLC (Knauer, C18 Eurosil Bioselect 16 mm × 120 mm) using a linear gradient (60 min) from 0 to 60% of buffer B at a flow rate of 4 ml/min. Detection was monitored at 214 nm. The homogeneity and identity of peptide was assessed by analytical HPLC, mass determination by LC-MS.

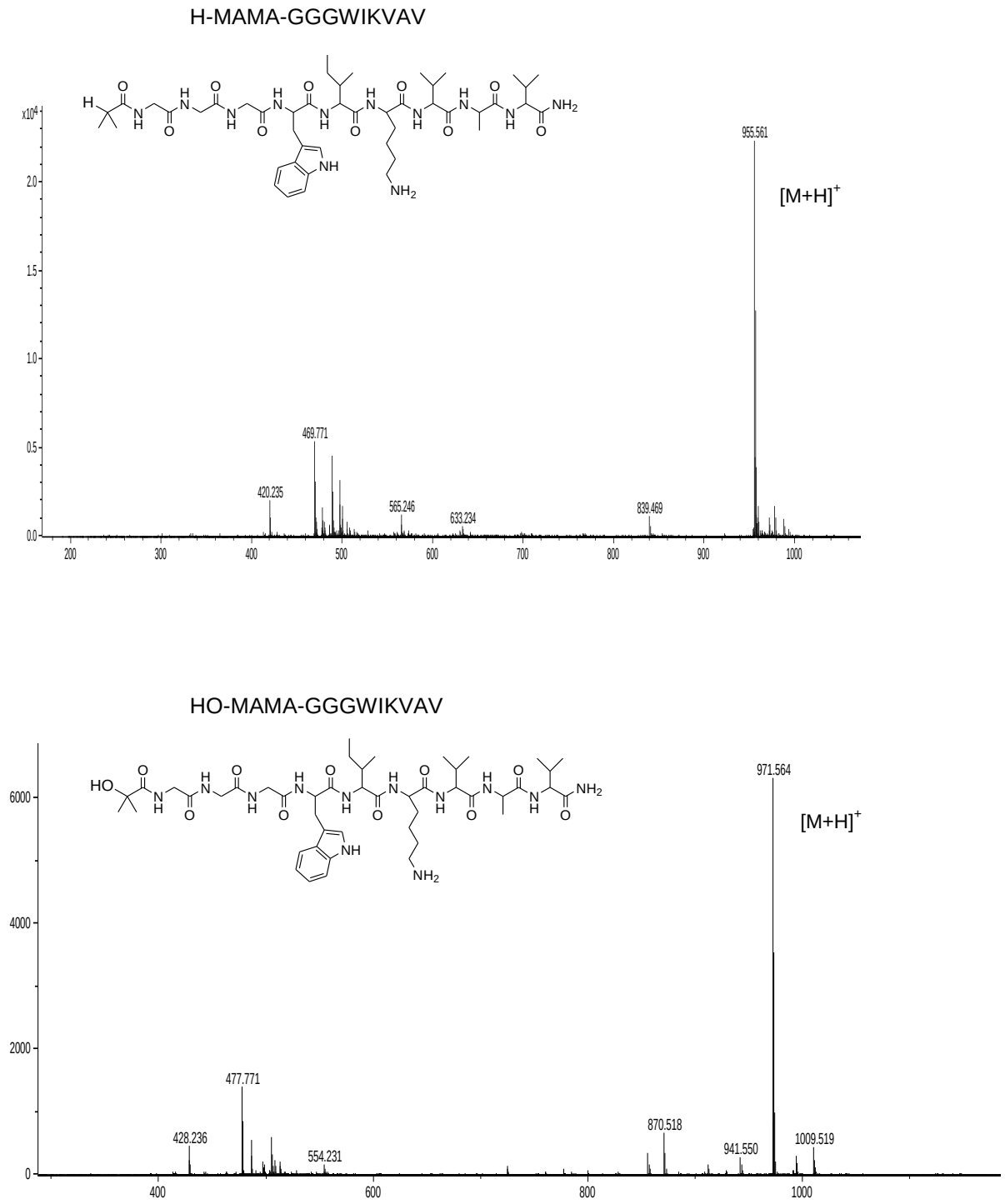
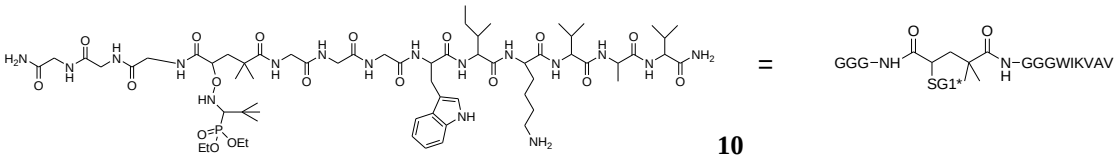


Figure S3. MS analysis of the side products H-MAMA-GGGWIKVAV (M=954.57) and HO-MAMA-GGGWIKVAV (M=970.56) obtained in IRA reaction conditions.



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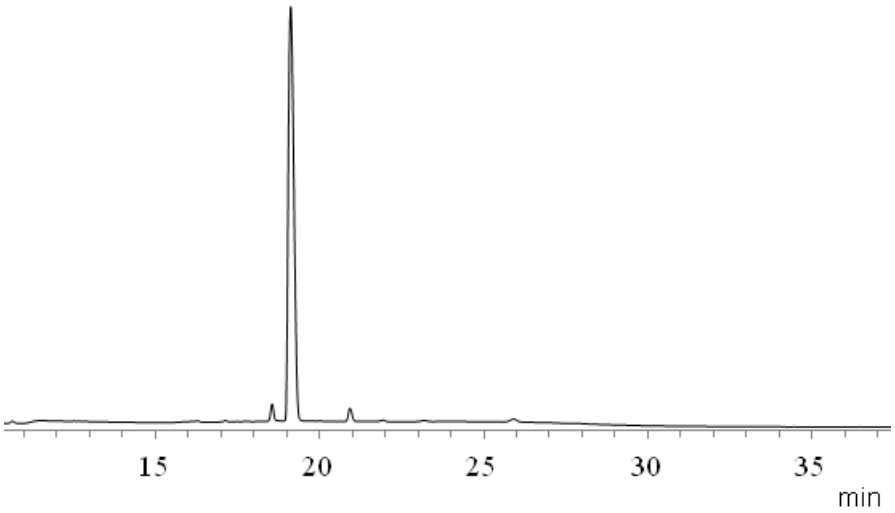


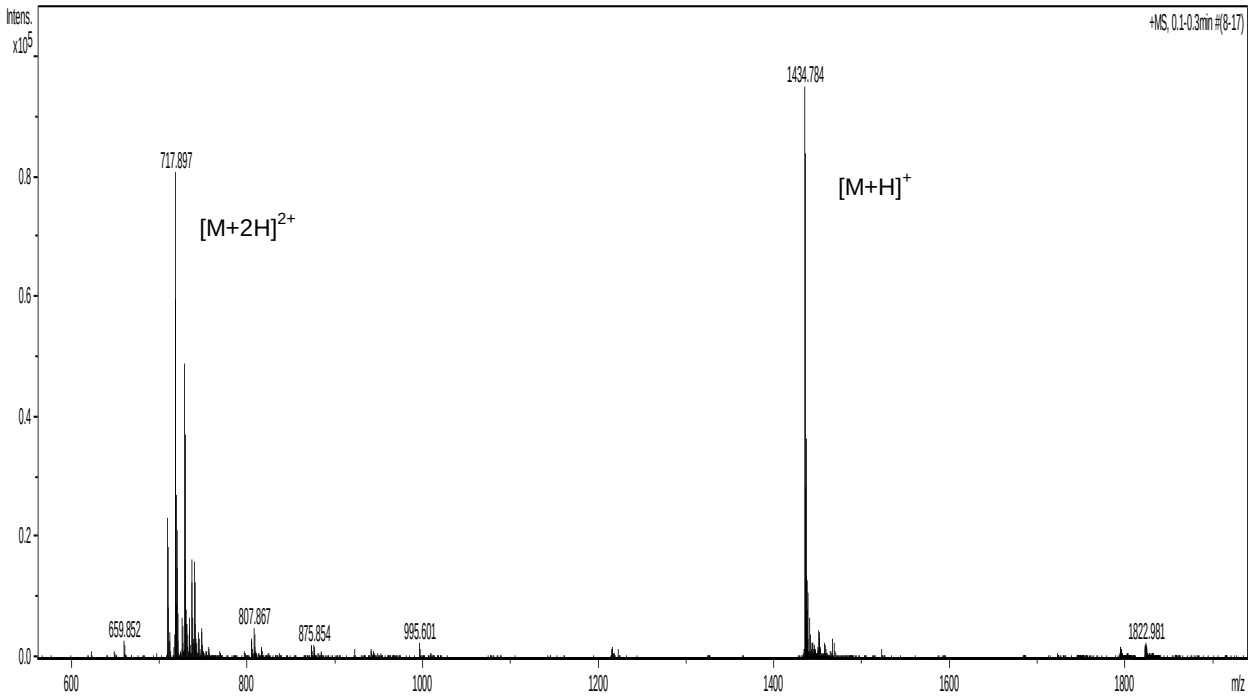


Figure S4. HPLC and MS analysis of the purified peptide adduct **10** (M=1433.78) obtained by IRA from the N-terminus vinyl functionalized peptide =GGG (**6**).

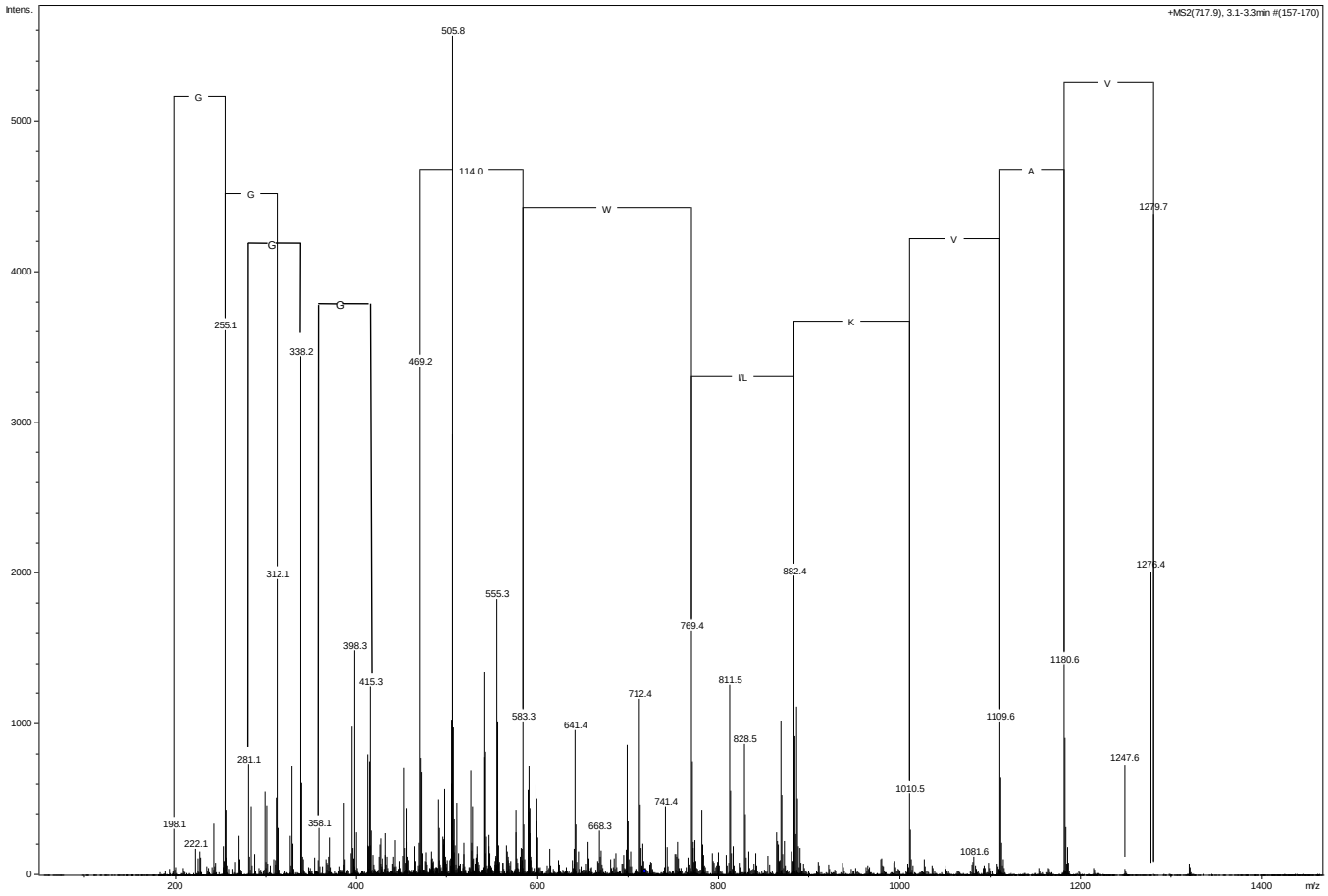
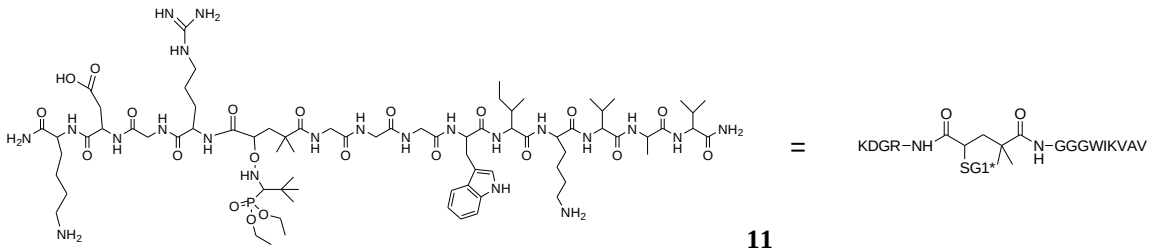


Figure S5. MS/MS analysis of the peptide adduct **10** obtained by IRA from the N-terminus vinyl functionalized peptide =GGG (**6**).



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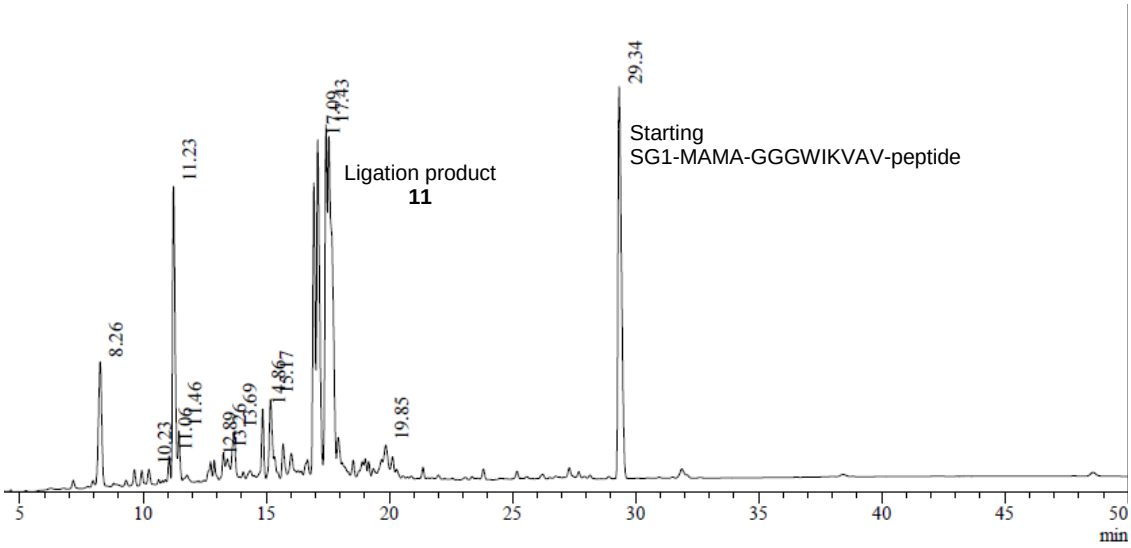
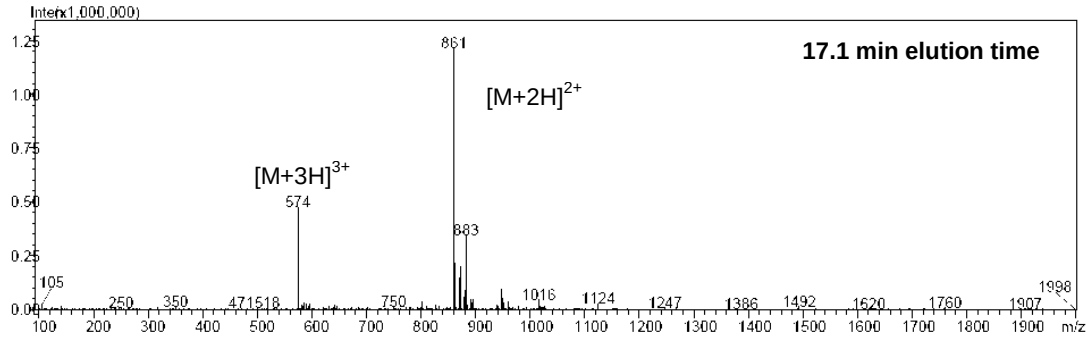




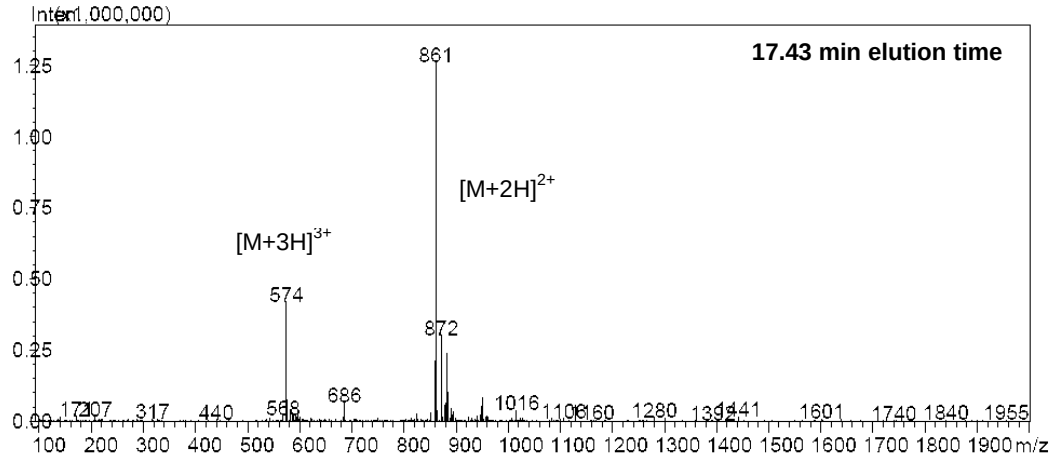
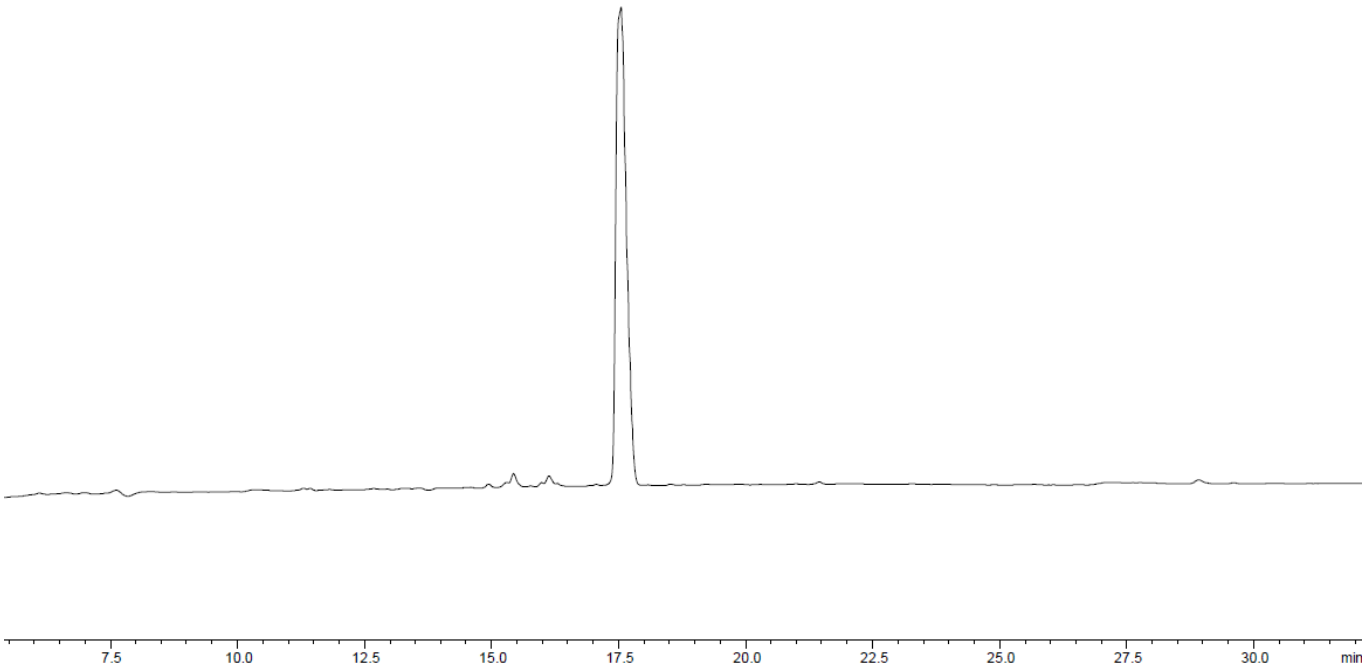


Figure S6. HPLC and MS analysis of the peptide adduct **11** (M=1719.0) obtained by IRA (90°, 3 h) from the N-terminus vinyl functionalized peptide =RGDK (**7**) (crude reaction mixture).



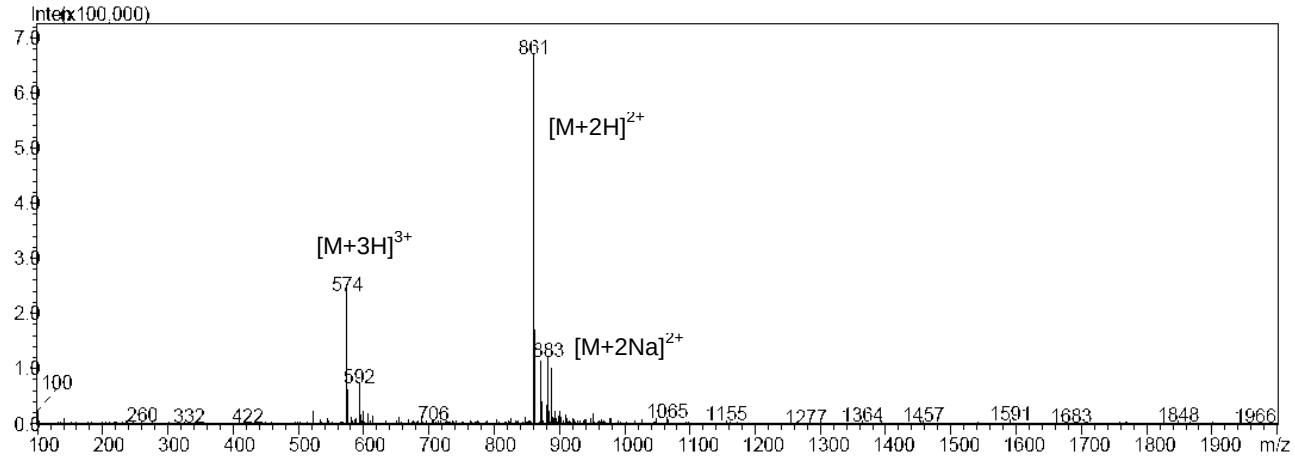
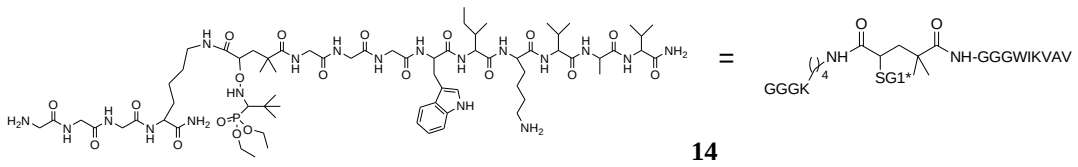


Figure S7. HPLC and MS analysis of the purified peptide adduct **11** (M=1719.0) obtained by IRA from the N-terminus vinyl functionalized peptide =RGDK (**7**).



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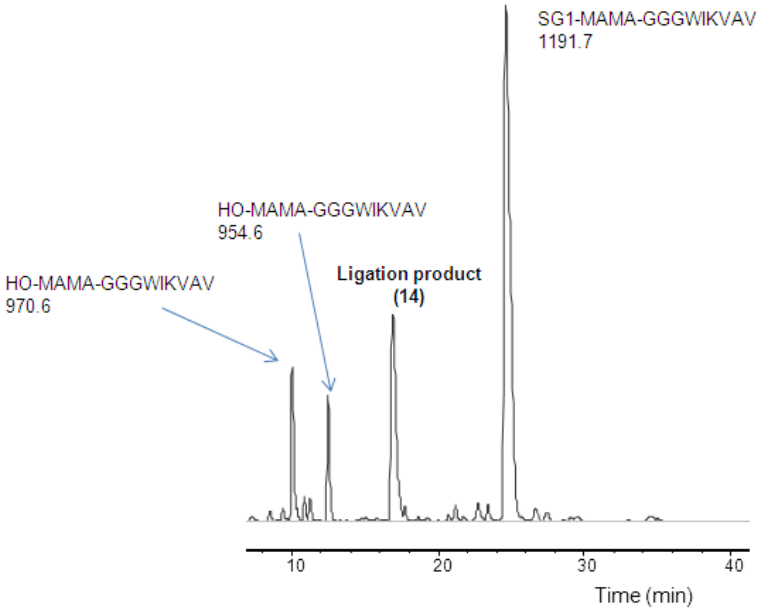

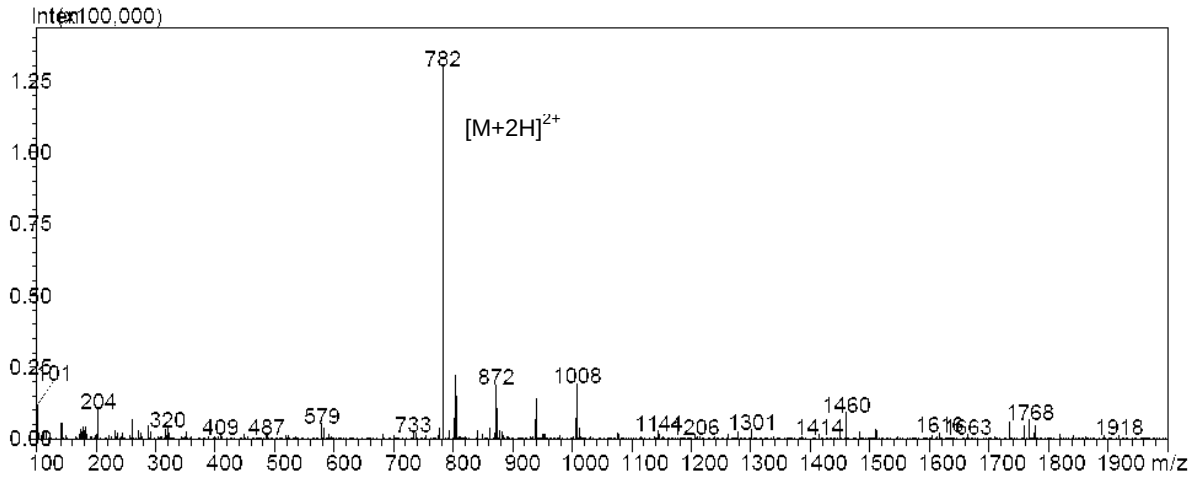

Figure S8. HPLC and MS analysis of the peptide adduct **14** (M=1561.9) obtained by IRA from the C-terminus vinyl functionalized peptide GGGK= (**12**) (crude reaction mixture).

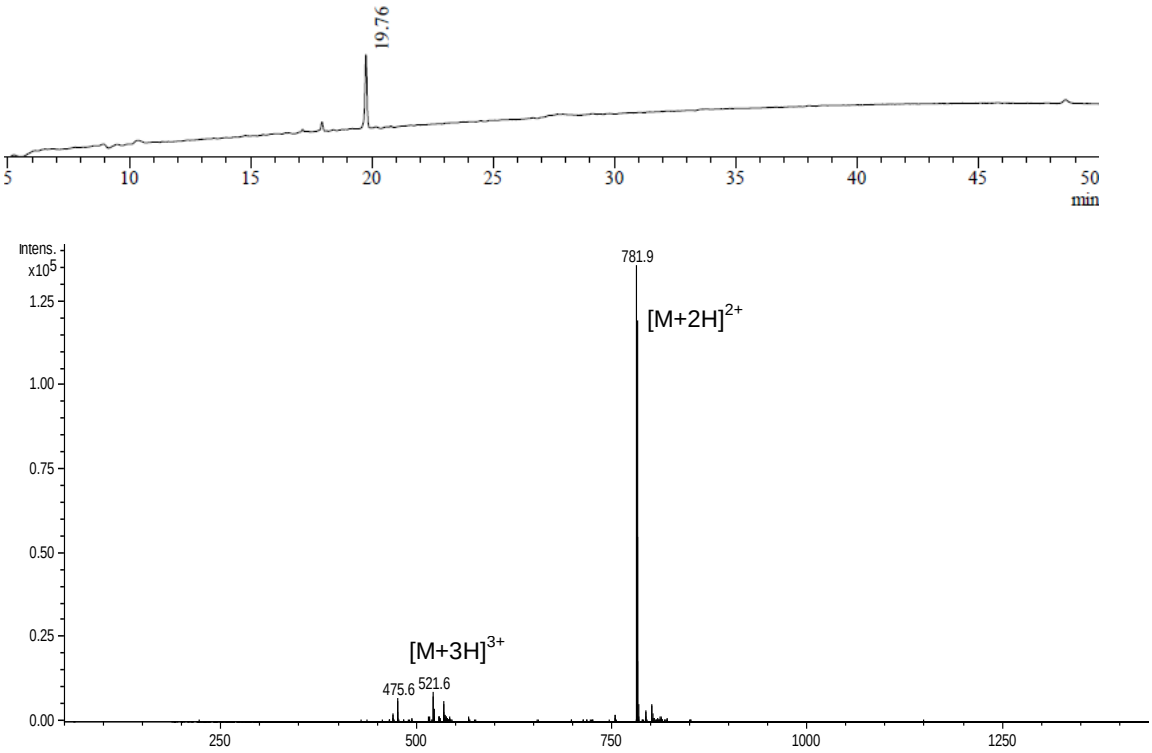

Figure S9. HPLC and MS analysis of the purified peptide adduct **14** (M=1561.9) obtained by IRA from the C-terminus vinyl functionalized peptide GGGK= (**12**).

References

(1) Merrifield, B. *Science* **1986**, *232*, 341.

(2) Mabrouk, K.; Ram, N.; Boisseau, S.; Strappazzon, F.; Rehaim, A.; Sadoul, R.; Darbon, H.; Ronjat, M.; De Waard, M. *Biochim. Biophys. Acta* **2007**, *1768*, 2528.

(3) Trimaille, T.; Mabrouk, K.; Monnier, V.; Charles, L.; Bertin, D.; Gigmes, D. *Macromolecules* **2010**, *43*, 4864.

(4) Matysiak, S.; Böldicke, T.; Tegge, W.; Frank, R. *Tetrahedron lett.* **1998**, *39*, 1733.

(5) Bertin, D.; Gigmes, D.; Marque, S. R. A.; Tordo, P. *Chem. Soc. Rev*. **2011**, 40, 2189-2198.