Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Palladium mediated deallylation in fully aqueous conditions for native chemical ligation at aspartic and glutamic acid sites

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Experimental Section

Materials and methods

SPPS was carried out by using an automated peptide synthesizer (CS336X, CSBIO). Analytical grade N, N-Dimethylformamide (DMF), dichloromethane (DCM), and Trifluoroacetic acid (TFA) were purchased from Biolab. Diethyl ether and HPLC-grade acetonitrile (ACN) was purchased from Avantor. triisopropyl silane (TIS) was purchased from Sigma-Aldrich. Commercial reagents were used without further purification. Resins were purchased from Creosalus and all protected amino acids were purchased from GL Biochem. The activating reagents [(2-(1Hbenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), hydroxybenzotriazole (HOBt),[(6-chlorobenzotriazol-1-yl)oxy(dimethylamino)methylidene]- dimethyl-azanium hexafluorophosphate (HCTU), were purchased from Luxembourg Bio Technologies. Unless otherwise mentioned, all reactions were carried out at room temperature. 2-chlorotrityl chloride resin (100-200 mesh, 1.07 mmol/g) was purchased from Chem-Impex. Rink amide resin (100-200 mesh, 0.26 mmol/g) was purchased from CreoSalus. $PdCl_2$ was purchased from Across organics. $[Pd(allyl)Cl]_2$, was purchased from Strem chemicals.

List of the protected amino acids used in peptides synthesis:

Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, FmocHis(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Leu-Thr(ψMe,MePro)-OH, Fmoc-Asp(OtBu)-(Dmb)Gly-OH, Fmoc-Gly-Thr(ψMe,MePro)-OH, Fmoc-Ser(tBu)-Ser(ψMe,MePro)-OH, Fmoc-Asp(Oallyl)-OH, Fmoc-Glu(Oallyl)-OH.

General method for coupling amino acids:

Coupling the amino acids was achieved using automated peptide synthesizer in presence of 4 equiv. of AA, 8 equiv. of DIEA and 4 equiv. of HCTU of the initial loading of the resin.

Cleavage of the peptide from resin:

The resin was washed with (3x5ml) DMF, (3x5ml) MeOH, and (3x5ml) DCM and dried then cleavage cocktail of 95% (v/v) TFA, 2.5% (v/v) H_2O, and 2.5% (v/v) TIS was added to the resin and the reaction mixture was shaken for 1.5 hr. The resin was removed by filtration and was washed with additional TFA (2 × 1 mL). To precipitate the peptide, the combined filtrate was added dropwise to cold ether followed by centrifugation, decanting of ether and by dissolution of residue in acetonitrile-water for freeze-drying in the lyophilizer.

Peptide synthesis:

Synthesis of LYRAGLYRAD(Oallyl)-$NHNH_2$:

The synthesis of this peptide was carried out on hydrazide resin. The first amino acid Fmoc-Asp(Oallyl)-OH was coupled manually using 4 equiv. of the amino acid, 4 equiv. of HOBt, 4 equiv. of HBTU and 4 equiv. of DIEA to the initial loading of the resin, for 1 hour. The remaining amino acids were coupled using peptide synthesizer as described above. Finally, the peptide was cleaved from the resin with the cleavage cocktail as mentioned above for and purified using preparative HPLC C18 column with a gradient of 10-50%B over 40 min. Overall yield 60%.

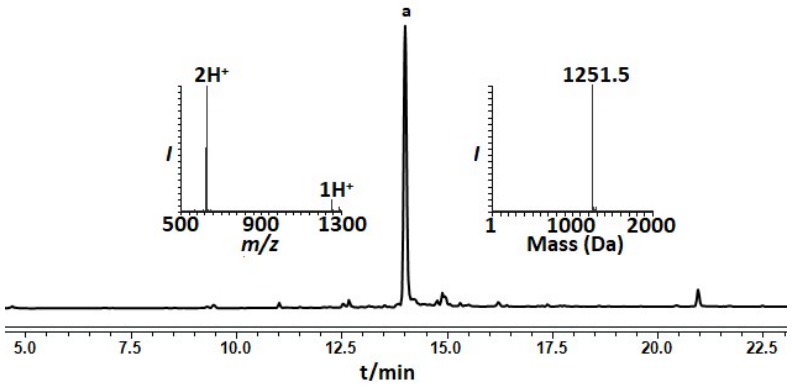

Figure S1: Synthesis of LYRAGLYRAD(Oallyl)-$NHNH_2$: Analytical HPLC and mass analysis of crude LYRAGLYRAD(Oallyl)-$NHNH_2$. peak a corresponds to LYRAGLYRAD(Oallyl)-$NHNH_2$ with the observed mass 1251.5 ± 0.2 Da; calcd 1251.3 Da.

Synthesis of LYRAGLYRAE(Oallyl)-$NHNH_2$:

The synthesis of the peptide was carried out on hydrazide resin, the first amino acid Fmoc-Glu(Oallyl)-OH was coupled manually using 4 equiv of the amino acid, 4 equiv of HOBt, 4 equiv of HBTU and 4 equiv of DIEA to the initial loading of the resin, for 1 hour. The remaining amino acids were coupled using peptide synthesizer as described above. Finally, the peptide was cleaved from the resin with the cleavage cocktail as mentioned above for and purified using preparative HPLC C18 column with a gradient of 10-50%B over 40 min. Overall yield 65%.

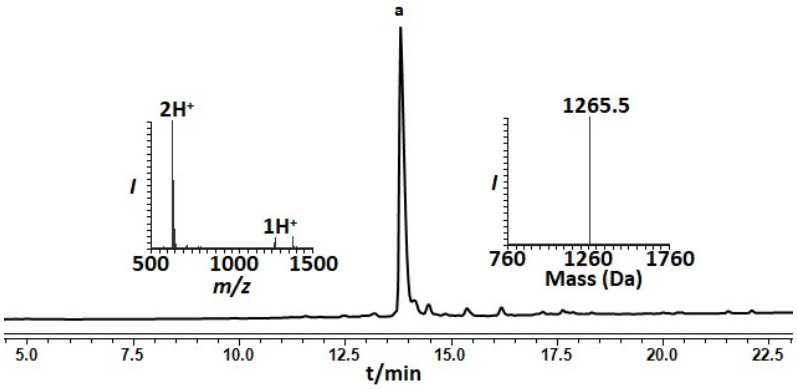

Figure S2: Synthesis of LYRAGLYRAE(Oallyl)-$NHNH_2$: Analytical HPLC and mass analysis of crude LYRAGLYRAD(Oallyl)-$NHNH_2$ peak a corresponds to LYRAGLYRAE(Oallyl)-$NHNH_2$ with observed mass 1265.5 ± 0.1 Da; calcd 1266.3 Da.

Synthesis of CLYRAGLYRAG:

The synthesis of this peptide was carried out on Rink amide resin. The remaining amino acids were coupled using peptide synthesizer as described above and purified using preparative HPLC C18 column with a gradient of 10-50%B over 40 min. Overall yield 50 %.

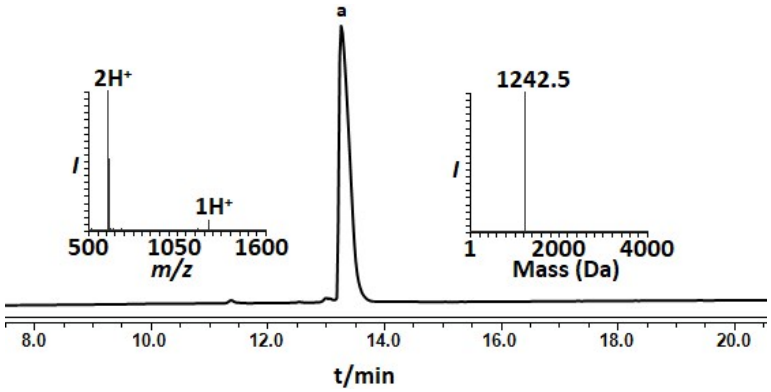

Figure S3: Synthesis of CLYRAGLYRAGNH_2: A) Analytical HPLC mass analysis of the purified CLYRAGLYRAG with the observed mass 1242.5 ± 0.1 Da; calcd 1241.4 Da

Ligation of LYRAGLYRAD(Oallyl)-$NHNH_2$ and CLYRAGLYRAG:

2 mg of LYRAGLYRAD(Oallyl)-$NHNH_2$ fragment was dissolved in 300 µl of 6 M Gnd·HCl, 200 mM Na_2HPO_4 buffer pH~3, followed by the addition of 15 equiv. of $NaNO_2$ in H_2O. The reaction was kept in ice-salt bath at -15^{o}c for 15-20 min. Then a solution of 30 equiv. of MPAA and 20 equiv. TCEP in 6 M Gnd·HCl, 200 mM Na_2HPO_4 buffer at pH 7 was added. Finally, the reaction mixture was added to the dissolved CLYRAGLYRAG fragment (2 mg, ~1 equiv.) solution in 6 M Gnd·HCl, 200 mM Na_2HPO_4 buffer, the reaction pH was adjusted to ~6.7 and kept at room temperature. The reaction progress was monitored by HPLC and ESI mass analysis. The ligation product was purified via semipreparative HPLC C4 column using a gradient of 20-60% B over 40 min to provide the desired product in ~72% yield.

Ligation of LYRAGLYRAE(Oallyl)-$NHNH_2$ and CLYRAGLYRAG:

The ligation reaction was performed as described above. The ligation product was purified via semipreparative HPLC C4 column in a gradient of 20-60% B over 40 min to provide the desired product in ~75% yield.

Deallylation of LYRAGLYRAD(Oallyl)CLYRAGLYRAG:

A stock solution of 25 equiv. $[Pd(allyl)Cl]_2$ and 25 equiv. GSH was prepared in 6 M Gn·HCl, 200 mM Na_2HPO_4 buffer, pH~7. Then the palladium/GSH solution was added to the pre-dissolved 1 mg LYRAGLYRAD(Oallyl)CLYRAGLYRAG in 6 M Gn·HCl, 200 mM Na_2HPO_4 buffer, Ph~7.2 (200 μL, 2 mM), the final pH was adjusted to ~8 and incubated at 37 °C for 10 min. The progress of the reaction was monitored by analytical HPLC using C4 analytical column with a gradient of 0-60% buffer B over 30 min, after quenching the reaction with DTT. After completion of the reaction, 40 equiv. of DTT was added to quench and precipitate the free Pd from the reaction mixture. After centrifuge the reaction, the supernatant solution was collected purified using semi-preparative HPLC, C4 column and gradient of 20-60%B over 40 min to provide the desired product in ~60% yield.

Deallylation of LYRAGLYRAE(Oallyl)CLYRAGLYRAG:

The deallylation reaction was performed as described above. The HPLC analysis was carried out on a C4 analytical column using a gradient of 0-60% B over 30 min by quenching the reaction with DTT. After completion of the reaction, 40 equiv. of DTT was added to quench and precipitate the free Pd from the reaction mixture. After centrifuge the reaction, the supernatant solution was collected purified using semi-preparative HPLC, C4 column and gradient of 20-60%B over 40 min to provide the desired product in ~62% yield.

Ono-pot ligation and deallylation of LYRAGLYRAD(Oallyl)-$NHNH_2$ and CLYRAGLYRAG:

The ligation reaction followed by the deallylation reaction was performed as described above. The HPLC analysis was carried out on a C4 analytical column using a gradient of 0-60% B over 30 min.

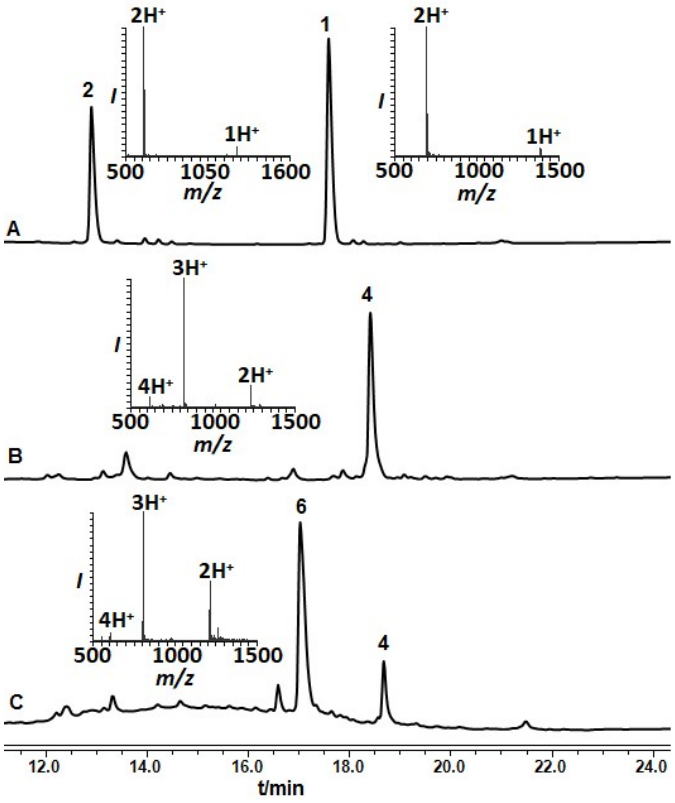

Figure S4: One-pot NCL and deallylation at Asp sit: Analytical HPLC and mass traces of A) ligation at time zero for peptides 1-MPAA thioester and peptide 2 with the observed mass 1387.6 ± 1.3 Da (calcd 1388 Da) and 1442.5 ± 0.1 Da, (calcd 1441 Da), respectively. B) Ligation product 4 with the observed mass 2462 ± 0.5 Da, (calcd 2461 Da). D) the deallylated product **6** (>85%) with the observed mass 2421.8 ± 0.6 Da, (calcd 2422 Da), **4** is the ligation product with the observed mass 2462 ± 0.5 Da.

Ono-pot ligation and deallylation of LYRAGLYRAE(Oallyl)-NHNH$_2$ and CLYRAGLYRAG:

The ligation reaction followed by the deallylation reaction was performed as described above. The HPLC analysis was carried out on a C4 analytical column using a gradient of 0-60% B over 30 min.

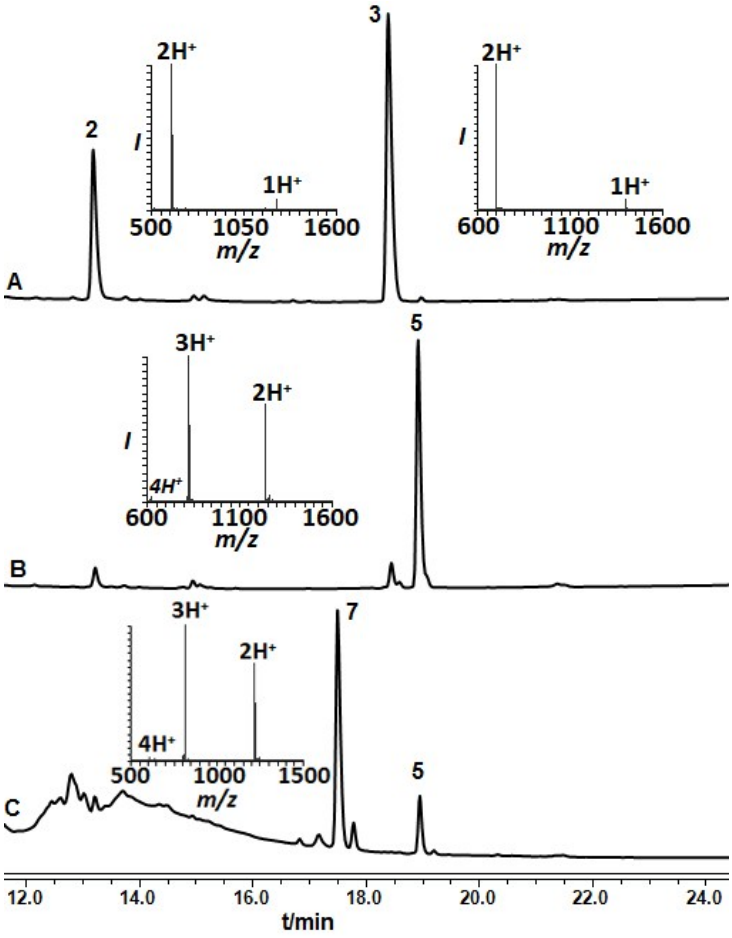

Figure S5: One-pot NCL and deallylation at Asp site: Analytical HPLC and mass traces of A) ligation at time zero for peptides 3-MPAA thioester and peptide 2 with the observed mass 1401.6 ± 1.1 Da, calcd 1402 Da and 1442.5 ± 0.1 Da, (calcd 1441 Da) respectively. B) Ligation product 5 with the observed mass 2475.9 ± 0.7 Da, (calcd 2477 Da). C) the deallylated product **7** (>85%) with the observed mass 2435.9 ± 0.3 Da, (calcd 2436 Da), **5** is the ligation product with the observed mass 2475.9 ± 0.7 Da.

Role of GSH in deallylation:

The mechanism by which the deallylation reaction proceeds involves trapping the allyl group by the GSH molecule.

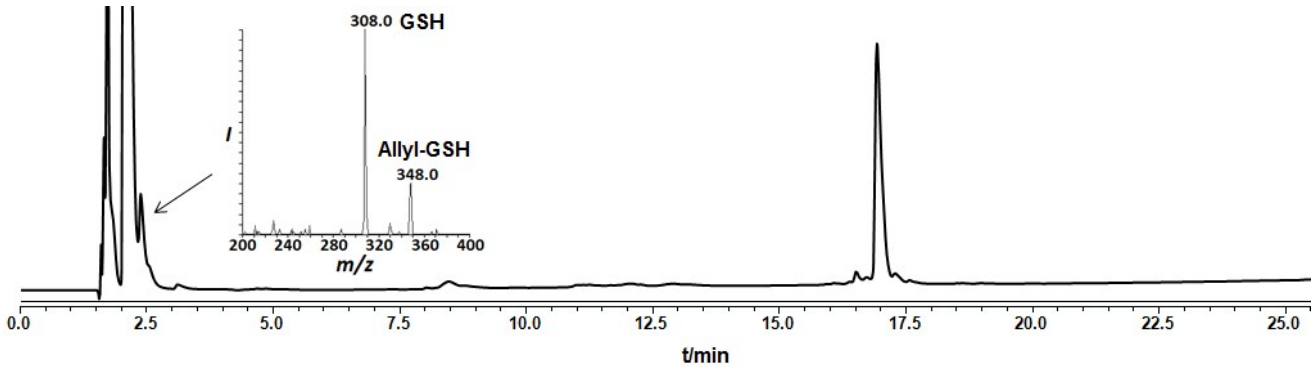

Figure S6: Mass analysis of the injection peak of the deallylation reaction, with a mass that corresponds to the mass of GSH and GSH with the addition of allyl.