Application of an Omonasteine Ligation Strategy for the Total Chemical Synthesis of the BRD7 Bromodomain
Supporting Information

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1 Structures and Sequences

Nonnative residues and modifications are shown in bold face. Standard Boc/Bzl SPPS protecting groups are indicated by an asterisk (see "reagents" for the exact nature of these protecting groups).

1.1 *t*Boc-Hcy(Trt)-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL-MBHA resin (1)

***t*Boc-Hcy(Trt)**-Ile-Ile-Lys(*)-His(*)-Pro-Met-Asp(*)-Phe-Ser(*)-Thr(*)-Met-Lys(*)-Glu(*)-Lys(*)-Ile-Lys(*)-Asn-Asn- Asp(*)-Tyr(*)-Gln-Ser(*)-Ile-Glu(*)-Glu(*)-Leu-Lys(*)-Asp(*)-Asn-Phe-Lys(*)-Leu-**mpa-Leu-NH-MBHA-resin**

1.2 Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL-MBHA resin (2)

Omo-Ile-Ile-Lys(*)-His(*)-Pro-Met-Asp(*)-Phe-Ser(*)-Thr(*)-Met-Lys(*)-Glu(*)-Lys(*)-Ile-Lys(*)-Asn-Asn- Asp(*)-Tyr(*)-Gln-Ser(*)-Ile-Glu(*)-Glu(*)-Leu-Lys(*)-Asp(*)-Asn-Phe-Lys(*)-Leu-**mpa-Leu-NH-MBHA-resin**

1.3 Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL (3)

Omo-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-**mpa-Leu-NH_2**

1.4 Hcy-[Cys(Acm)^{76}-Arg^{108}]-NH_2 (4)

Hcy-Cys(Acm)-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH_2

1.5 Ac-[Glu^{1}-Ser^{41}]-mpaL (5)

Ac-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-**mpa-Leu-NH_2**

1.6 6-TAMRA-Gly-[Glu^{1}-Ser^{41}]-mpaL (6)

6-TAMRA-Gly-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-**mpa-Leu-NH$_2$**

1.7 Hcy-[Ile^{43}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (7)

Hcy-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-**Hcy-Cys(Acm)**-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

1.8 Ac-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (8)

Ac-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-**Hcy**-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-**Hcy-Cys(Acm)**-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

1.9 6-TAMRA-Gly-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (9)

6-TAMRA-Gly-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-**Hcy**-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-**Hcy-Cys(Acm)**-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

1.10 Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (10)

Ac-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln- Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr- Ser-Met-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-Met-**Cys(Acm)**-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

1.11 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (11)

6-TAMRA-Gly-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-Met-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-Met-**Cys(Acm)**-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

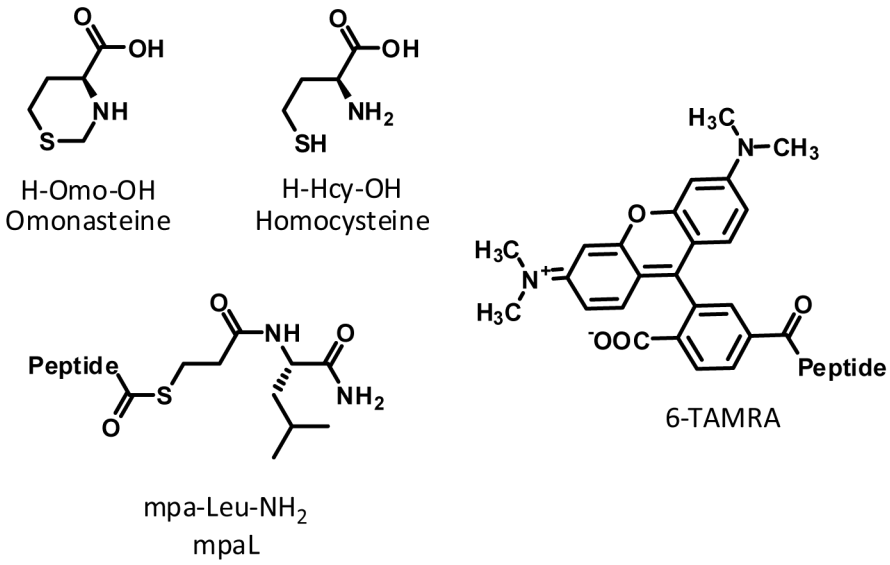

Figure 1: Structures of Omonasteine, Homocysteine, mpaL and 6-TAMRA

1.12 Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH$_2$ (12)

Ac-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln- Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr- Ser-Met-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-Met-Cys-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

1.13 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH$_2$ (13)

6-TAMRA-Gly-Glu-Glu-Val-Glu-Gln-Thr-Pro-Leu-Gln-Glu-Ala-Leu-Asn-Gln-Leu-Met-Arg-Gln-Leu-Gln-Arg-Lys-Asp-Pro-Ser-Ala-Phe-Phe-Ser-Phe-Pro-Val-Thr-Asp-Phe-Ile-Ala-Pro-Gly-Tyr-Ser-Met-Ile-Ile-Lys-His-Pro-Met-Asp-Phe-Ser-Thr-Met-Lys-Glu-Lys-Ile-Lys-Asn-Asn- Asp-Tyr-Gln-Ser-Ile-Glu-Glu-Leu-Lys-Asp-Asn-Phe-Lys-Leu-Met-Cys-Thr-Asn-Ala-Met- Ile-Tyr-Asn-Lys-Pro-Glu-Thr-Ile-Tyr-Tyr-Lys-Ala-Ala-Lys-Lys-Leu-Leu-His-Ser-Gly- Met-Lys-Ile-Leu-Ser-Gln-Glu-Arg-NH$_2$

2 Experimental

2.1 Reagents

2-(6-Chloro-1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was obtained from Peptides International. *t*Boc-Leu-OH, *t*Boc-Phe-OH, *t*Boc-Asp(OcHx)-OH, *t*Boc-Glu(OcHx)-OH, *t*Boc-Ile-OH.1/2H2O, *t*Boc-Ser(Bzl)-OH, *t*Boc-Met-OH, *t*Boc-Pro-OH, *t*Boc-Gly-OH, *t*Boc-Tyr(BrZ)-OH and *t*Boc-Ala-OH were obtained from Nova Biochem. *t*Boc-Lys(ClZ)-OH, *t*Boc-Asn(Xan)-OH, *t*Boc-His(DnP)-OH, *t*Boc-Arg(Tos)-OH.EtOAc and *t*Boc-Cys(Acm)-OH were purchased from Midwest Bio-tech Inc. *t*Boc-Gln(Xan)-OH, *t*Boc-Thr(Bzl)-OH, *t*Boc-Hcy(MBzl)-OH and *t*Boc-Hcy(Trt)-OH were obtained from Bachem. *S*-trityl-mercaptopropionic acid (mpa) and 1.0 meq/g MBHA-polystyrene resin were from Anaspec Inc. The 0.44 meq/g MBHA-polystyrene resin was obtained from Peninsula Laboratories Europe LTD. *N*,*N*-Diisopropylethylamine (DIPEA), *N*,*N*-dimethylformamide (DMF), dichloromethane, diethylether and acetonitrile were purchased from Biosolve. Methanol, hydrogen fluoride and sinapic acid were obtained from Fluka. Thiophenol, benzylmercaptan, α-cyano-4-hydroxycinnamic acid, triisopropylsilane, *p*-cresol, TPCK treated trypsin (from bovine pancreas) and formalin were from Sigma-Aldrich. Guanidinium hydrochloride was obtained from MP Biomedicals, LCC. Anhydrous acetic acid was obtained from Merck and trifluoroacetic acid (TFA) from Halocarbon Biograde. All solvents were analytical grade (*p.a.*) and were used as received.

2.2 HPLC Analysis and Purification

Analytical HPLC was performed using a Vydac C18 HPLC column (4.6 mm × 150 mm, 1 mL/min flow rate) connected to a Varian Prostar system consisting of two Varian Prostar 215 delivery modules and a Varian Prostar 320 UV/Vis detector (λ = 214 nm). A linear gradient of 0-67% buffer B in buffer A over 30 minutes was used, where buffer A = 0.1 *v*-% TFA in H_2O and buffer B = 0.1 *v*-% TFA, 10 *v*-% H_2O in CH_3CN.

Semi-preparative HPLC was performed using Vydac C18 HPLC columns (10 mm × 250 mm, 5 mL/min flow rate or 22 mm × 250 mm, 10 mL/min flow rate) connected to a Waters Deltaprep System consisting of a Waters Prep LC Controller and a Waters 2487 Dual wavelength Absorbance Detector (λ = 214 nm). Peptides were eluted using a shallow gradient of B in A, based on an exploratory analytical HPLC run (*vide supra*). Product containing fractions were analyzed by Electrospray Ionization Mass Spectrometry (ESI-MS) (*vide infra*), pooled, and lyophilized.

2.3 Mass Spectrometry

ESI-MS was performed on an Applied Biosystems SCIEX API 150 EX electrospray ionization quadrupole (ESI-Q) mass spectrometer. Peptide masses were calculated from the experimental mass to charge (m/z) ratios of all the protonation states observed in the ESI-MS spectrum of a peptide using Analyst 1.4.2 software (Sciex).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI TOF-MS) was performed on an Applied Biosystems 4800 MALDI TOF/TOF system in reflector or linear mode. α-cyano-4-hydroxycinnamic acid was used as a matrix. Monoisotopic and average theoretical masses of compounds were calculated using Instant JChem 5.5.0, 2011, ChemAxon (http://www.chemaxon.com).

2.4 Trypsinolysis

A solution of Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH_2 (**10**, 50 μg, 3.9 nanomole) and 2.5 μg of TPCK treated trypsin in 50 mM Tris.HCl buffer (pH 7.5, 100 μL) was allowed to react at 37 °C. After 1 h, a 1 μL sample was mixed with α-cyano-4-hydroxycinnamic acid matrix solution and analysed by MALDI-TOF MS in reflector mode.

2.5 Peptide Synthesis

All peptide fragments were synthesized by manual solid-phase peptide synthesis on a 0.3-0.4 mmol scale using the *in situ* neutralization/activation procedure for *t*Boc-/Bzl- peptide synthesis as previously described[4], but using HCTU instead of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as a coupling reagent. MBHA resin (1 meq/g) was used as the solid support. MpaLeu peptide thioesters were prepared as described before[1]. The peptides were deprotected and cleaved from the resin by acidolysis with anhydrous HF for 1 h at 0 °C, using 4 *v*-% p-cresol as a scavenger. Following cleavage, the peptides were precipitated with ice-cold diethylether, dissolved in aqueous buffer containing 6 M Gn.HCl, 0.1 M sodium acetate buffer (pH 4) and immediately purified by semi-preparative HPLC. Fractions containing the desired product were identified by ESI-MS, pooled and lyophilized.

2.5.1 Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL (3)

Fully protected, resin bound *t*Boc-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL was prepared on a 0.3 mmol scale as described above, resulting in 2.19 g of fully protected resin-bound peptide. A 382 mg aliquot ($\sim$ 0.05 mmol) was N^{α}-deprotected and reacted for 20 min with a preactivated mixture of *t*Boc-Hcy(Trt)-OH (259 mg, 0.54 mmol, 11 eq.), HCTU (0.9 mL of a 0.5 M solution in DMF, 9 eq.) and DIPEA (230 μ L), giving *t*Boc-Hcy(Trt)-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL-MBHA resin (**1**). The *t*Boc and Trt protecting groups were subsequently removed by treatment (2 × 5 s, 2 × 1 min.) with triisopropylsilane (2.5 *v*-%) and water (2.5 *v*-%) in TFA. The resin was washed extensively with DMF and treated (2 × 10 s, 2 × 20 min., DMF wash after each 20 min treatment) with a mixture composed of formalin (5 mL), DMF (22.5 mL) and 0.2 M $Na_xH_yPO_4$ buffer (pH 7.2, 32.5 mL), resulting in the formation of Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL-MBHA resin (**2**). After washing of the resin and drying in vacuo, the resin was triturated with cysteine (200 mg) and treated with *p*-cresol 4 *v*-% in HF as indicated above. After semi-preparative HPLC, product containing fractions were identified by ESI-MS, pooled and lyophilized to give 30.1 mg (6.9 μmole, 14%) of the title compound **3**. ESI-MS calculated 4391.11 (mono-isotopic) and 4394.04 (average), found 4394.04 ± 0.65.

note: To counter possible side reactions due to residual traces of formaldehyde, the resin was washed with a solution of cysteine.HCl in DMF, and cysteine was also added as an additional scavenger during HF cleavage[6]. Although the overall yield of the SPPS was quite low, as is apparent by the presence of many side-products in the analytical HPLC chromatogram, the homocysteine to omonasteine cyclization proceeded smoothly, with no detectable sideproducts, apart from some loss of histidine N_{τ}-2,4-dinitrophenyl (Dnp). The latter was found to happen during the quenching step with cysteine.HCl[5] and we found that omission of this step reduced DNP-loss without giving rise to additional side-products. Cysteine was also used as a formaldehyde scavenger during HF cleavage, but here no significant Dnp loss wass observed.

2.5.2 Hcy-[Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (4)

Resin bound and fully side-chain protected *t*Boc-[Cys(Acm)^{76}-Arg^{108}]-MBHA resin was prepared by manual SPPS on a 0.3 mmol scale, resulting in 2.68 g of resin bound peptide. A 500 mg aliquot was transferred to an SPPS reaction vial and, following *t*Boc removal by TFA treatment, *t*Boc-Hcy(MeBzl)-OH was coupled under standard amino acid coupling conditions (*vide supra*). The Dnp sidechain protecting group was removed by treatment with DIPEA 10 *v*-% and β-mercaptoethanol 20 *v*-% in DMF (2 × 30 seconds, 2 × 30 minutes). After removal of the *t*Boc protecting group, the peptide was cleaved from the resin and purified as described above. After purification and lyophilization, 78.6 mg (19.5 μmole, 30% yield) of **4** was obtained. ESI-MS calculated 4029.08 (mono-isotopic) and 4031.79 (average), found 4031.23 ± 0.20.

2.5.3 Ac-[Glu^{1}-Ser^{41}]-mpaL (5)

Resin bound *t*Boc-[Glu^{1}-Ser^{41}]-mpaL was synthesized on MBHA resin on a 0.3 mmol scale, as described above. 2.14 g of fully protected resin-bound peptide thioester was obtained and a 527 mg aliquot of this was N^{α}-deprotected by treatment with TFA and subsequently N^{α}-acetylated by treatment of with 0.25 M acetic anhydride, 0.25 M pyridine in DMF (5 mL, 2 × 2 min). After cleavage from the resin, purification and lyophilization, 33.9 mg of the title compound **5** was obtained (6.8 μmole, 9.2%). ESI-MS calculated 4968.45 (mono-isotopic) and 4971.58 (average), found 4971.32 ± 0.78.

2.5.4 6-TAMRA-Gly-[Glu^{1}-Ser^{41}]-mpaL (6)

A 250 mg (∼ 35 μmole) aliquot of the fully protected, resin bound peptide thioester *t*Boc-[Glu^{1}-Ser^{41}]-mpaL (*vide supra*) was *N*-terminally extended by the addition of a single *t*Boc-glycine residue. The N^{α}-Boc was removed by treatment with TFA and after washing with DMF, DIPEA 10 *v*-% in DMF and DMF, the resin was resuspended in DMF and transferred to a glass reaction vial. 6-TAMRA-OSu (8.2 mg, 15.5 μmole, 0.44 eq.) was added and the suspension was stirred at 37 °C for 1.5 h. The washing and coupling steps were repeated twice (with 5.5 mg and 6.2 mg of 6-TAMRA-OSu). Combined, 19.9 mg of 6-TAMRA-OSu was used (37.8 μmole, 1.1 eq.). After cleavage from the resin, purification and lyophilization (*vide supra*), 17 mg (3.2 μmole, 9%) of **6** was obtained. ESI-MS calculated 5395.60 (mono-isotopic) and 5399.03 (average), found 5398.64 ± 0.22.

2.5.5 Hcy-[Ile^{43}-Hcy^{75}-Cys(Acm)^{74}-Arg^{108}]-NH$_2$ (7)

A. Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL (**2**, 19.9 mg, 4.5 μmole) and Hcy-[Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (**4**, 26.2 mg, 6.6 μmole) were dissolved in 5 mL of a buffer containing 15 mM TCEP, 2 *v*-% thiophenol, 6.0 M Gn.HCl, 0.2 M $Na_xH_yPO_4$ buffer (pH ∼ 7.5). The reaction mixture was allowed to react at 37 °C for 24 h, during which the mixture was frequently homogenized. The reaction was followed by analytical HPLC and peaks were analyzed by ESI-MS. B. After 24 h, the reaction had proceeded to completion and MeONH$_2$.HCl (117 mg, 1.4 mmole, 0.28 M) was added. The mixture was allowed to react for an additional 5 h at 37 °C, diluted with 6.0 M Gn.HCl, 0.1 M sodium acetate buffer (pH 4.0), filtered and purified by preparative HPLC. Product containing fractions were identified by ESI-MS, pooled and lyophilized to give 20.3 mg (2.5 μmole, 55% yield) of **7**. ESI-MS calculated 8024.08 (mono-isotopic) and 8029.41 (average), found 8029.11 ± 0.80.

2.5.6 Ac-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (8)

Following part A of the procedure used for the synthesis of **7**, peptide **7** (19.3 mg, 2.4 μmole) and peptide **5** (21 mg, 4.2 μmole) were ligated and subsequently purified to give 10.5 mg (0.82 μmole, 34% yield) of the title compound **8**. ESI-MS calculated 12774.42 (mono-isotopic) and 12782.67 (average), found 12782.80 ± 0.96.

2.5.7 6-TAMRA-Gly-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (9)

Following the procedure sued for the synthesis of **7**, peptide **7** (7.5 mg, 0.93 μmole) and peptide **6** (6.5 mg, 1.2 μmole) were ligated, resulting in 5.4 mg (0.4 μmole, 44%) of the title compound **9**. ESI-MS calculated 13201.57 (mono-isotopic) and 13210.12 (average), found 13210.38 ± 1.31.

2.5.8 Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (10)

Peptide **8** (2.1 mg, 0.16 μmole) was dissolved in 1 mL of a N_2-flushed buffer containing 10 mM methionine, 4.3 mM EDTA, 6.0 M Gn.HCl, 0.5 M Tris.HCl buffer (pH 8.6). 10 μL of 1.0 M aqueous DTT was added and the resulting solution was allowed to stand for 30 min. Next, a mixture of 800 μL buffer containing 3.3 mM EDTA, 6.0 M Gn.HCl, 0.5 M Tris.HCl buffer (pH 8.6) and 200 μL of 0.5 M MeI in CH_3CN was added and the resulting solution was allowed to react for 10 minutes. The reaction was quenched by the addition of 300 μL 1.0 M DTT, diluted with water, filtered and purified by semi-preparative HPLC, giving 1.15 mg of the title compound **10** (0.09 μmole, 56%). To limit possible methionine oxidation of the product, 30 μmole of free methionine was added to the product prior to lyophilization. ESI-MS calculated 12802.45 (mono-isotopic) and 12810.72 (average), found 12811.60 ± 0.82.

2.5.9 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH$_2$ (11)

Following the procedure used for the synthesis of **10**, **9** (about 2.2 mg, 1.6 μmole) was *S*-methylated to give the 1.2 mg of the title compound **11** (0.9 μmole, 56%). ESI-MS calculated 13229.60 (mono-isotopic) and 13238.17 (average), found 13239.23 ± 1.98.

2.5.10 Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH$_2$, (12)

Peptide **10** (1.15 mg, 0.09 μmole, containing methionine) was dissolved in N_2-bubbled H_2O/CH_3CN/TFA (5 mL, 50:50:0.1, *v*/*v*/*v*) and 0.2 mL of a solution of aqueous $Hg(OAc)_2$ solution (1 mg/mL) was added. After 2.5 h, MALDI-MS analysis of the reaction mixture indicated complete removal of the *S*-Acm protecting group and 0.3 mL of 1.0 M DTT in water was added. After 30 more min, the reaction mixture was diluted with 6.0 M Gn.HCl, 0.1 M sodium acetate buffer (pH 4.0), filtered and purified by preparative HPLC to give 910 μg of the title compound **12** (0.07 μmole, 79% yield). ESI-MS calculated 12731.41 (mono-isotopic) and 12739.64 (average), found 12739.34 ± 1.66.

*note:*The conversion of *S*-Acm into the corresponding free thiol is typically done using either silver trifluoromethanesulfonate or mercury(II) salts[2], and Saporito and coworkers used a modification of the former method[3]. However, when we tried to use this procedure for the deprotection of the BRD7 bromodomain, the protein rapidly precipitated from solution and no deprotected peptide could be detected in the precipitate. The use of mercury(II) gave much better results: upon

reacting the peptides **8** and **9** (17 μM) with $Hg(OAc)_2$ (120 μM), the deprotection reaction was complete in 2.5 hours at room temperature.

2.5.11 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH_2 (13)

Following the procedure for the synthesis of **12**, peptide **11** (1.2 mg, 0.9 μmole) was *S*-deprotected to give 0.76 mg (0.58 μmole, 64%) of the title compound **13**. ESI-MS calculated 13158.57 (monoisotopic) and 13167.09 (average), found 13169.06 $\pm$ 2.94.

3 Analytical Data - ESI-MS and HPLC data of purified compounds

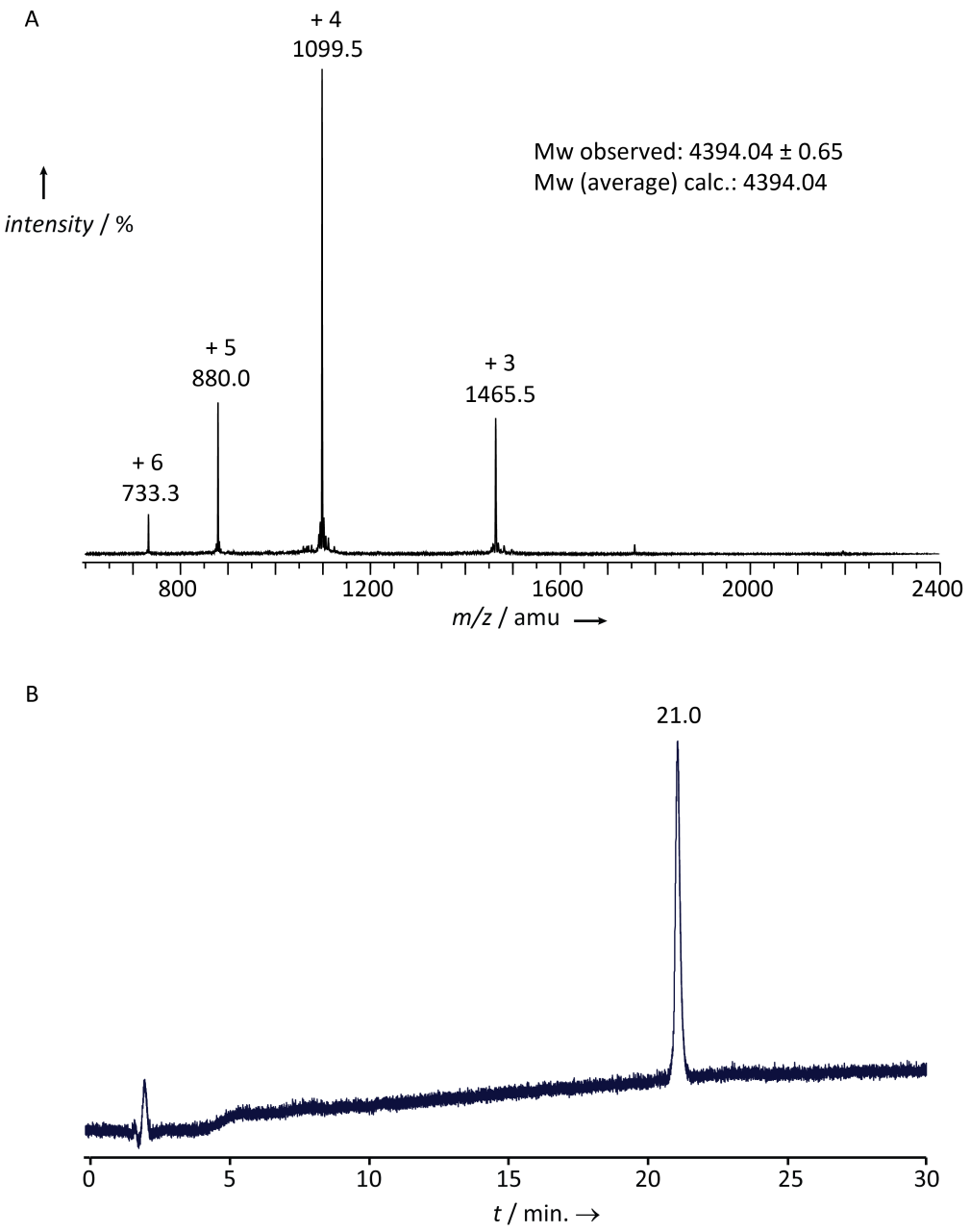

Figure 2: Omo-[Ile^{43}-His(Dnp)^{46}-Leu^{74}]-mpaL (**3**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

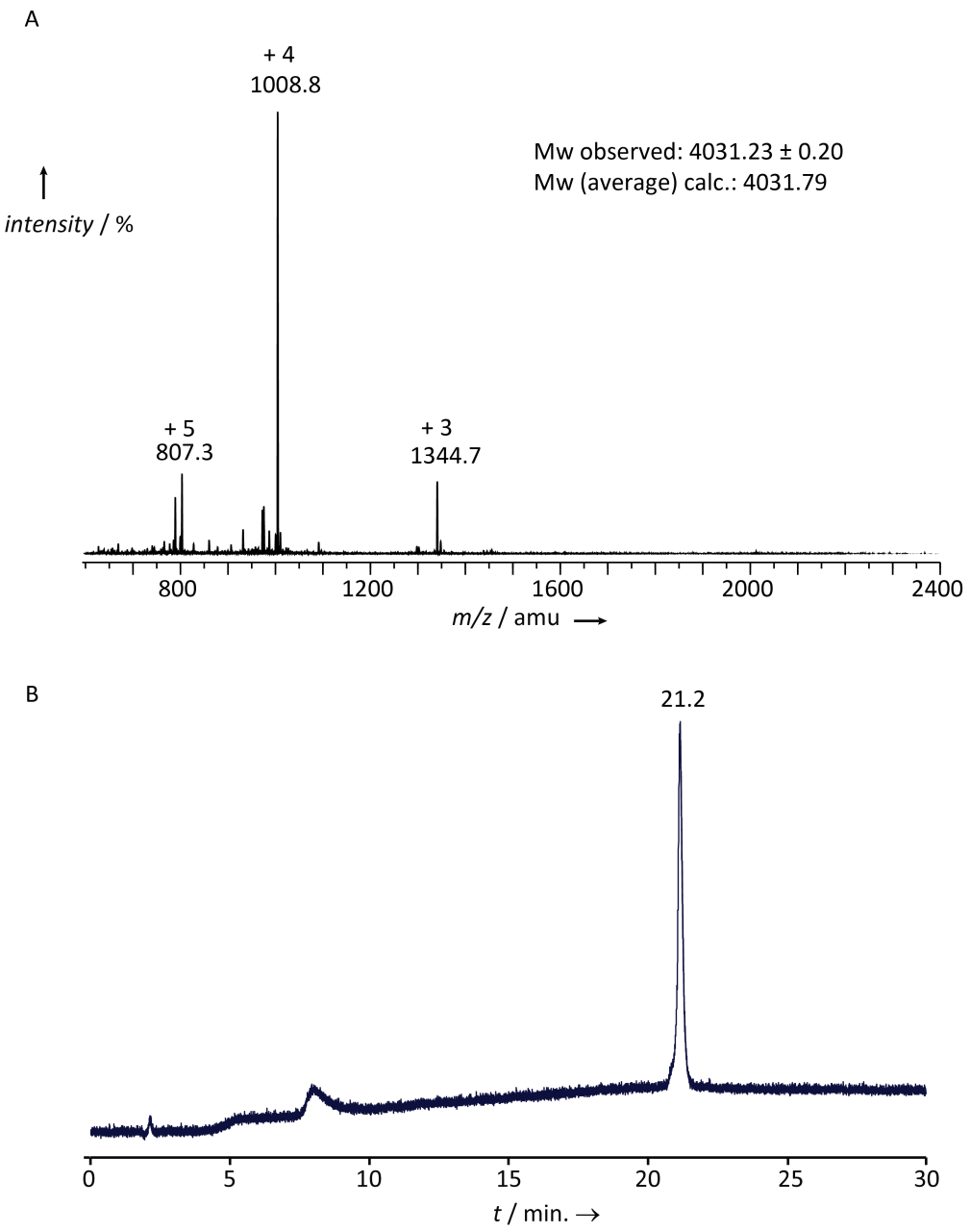

Figure 3: Hcy-[Cys(Acm)^{76}-Arg^{108}]-NH_4 (**1**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

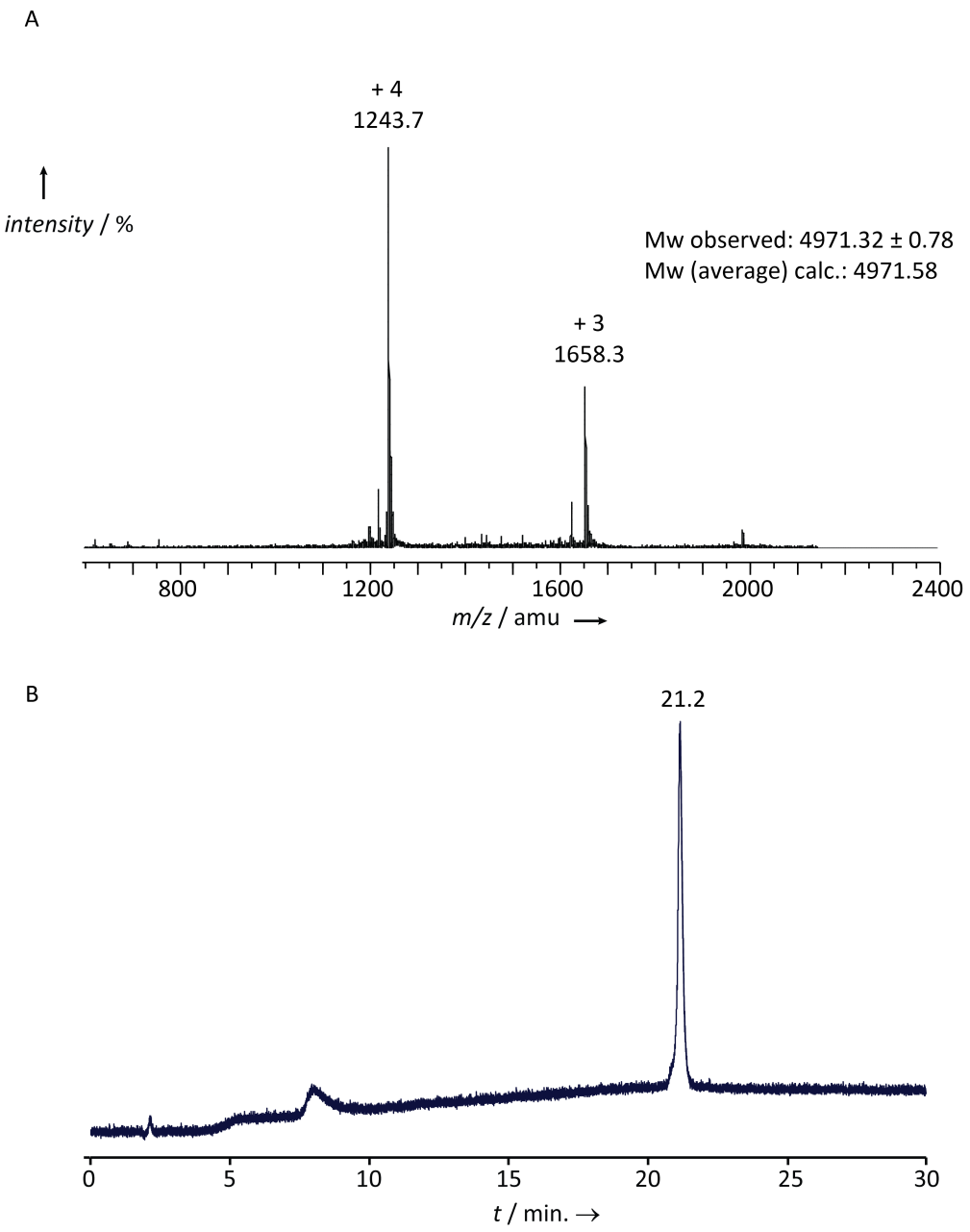

Figure 4: Ac-[Glu^{1}-Ser^{41}]-mpaL (**5**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

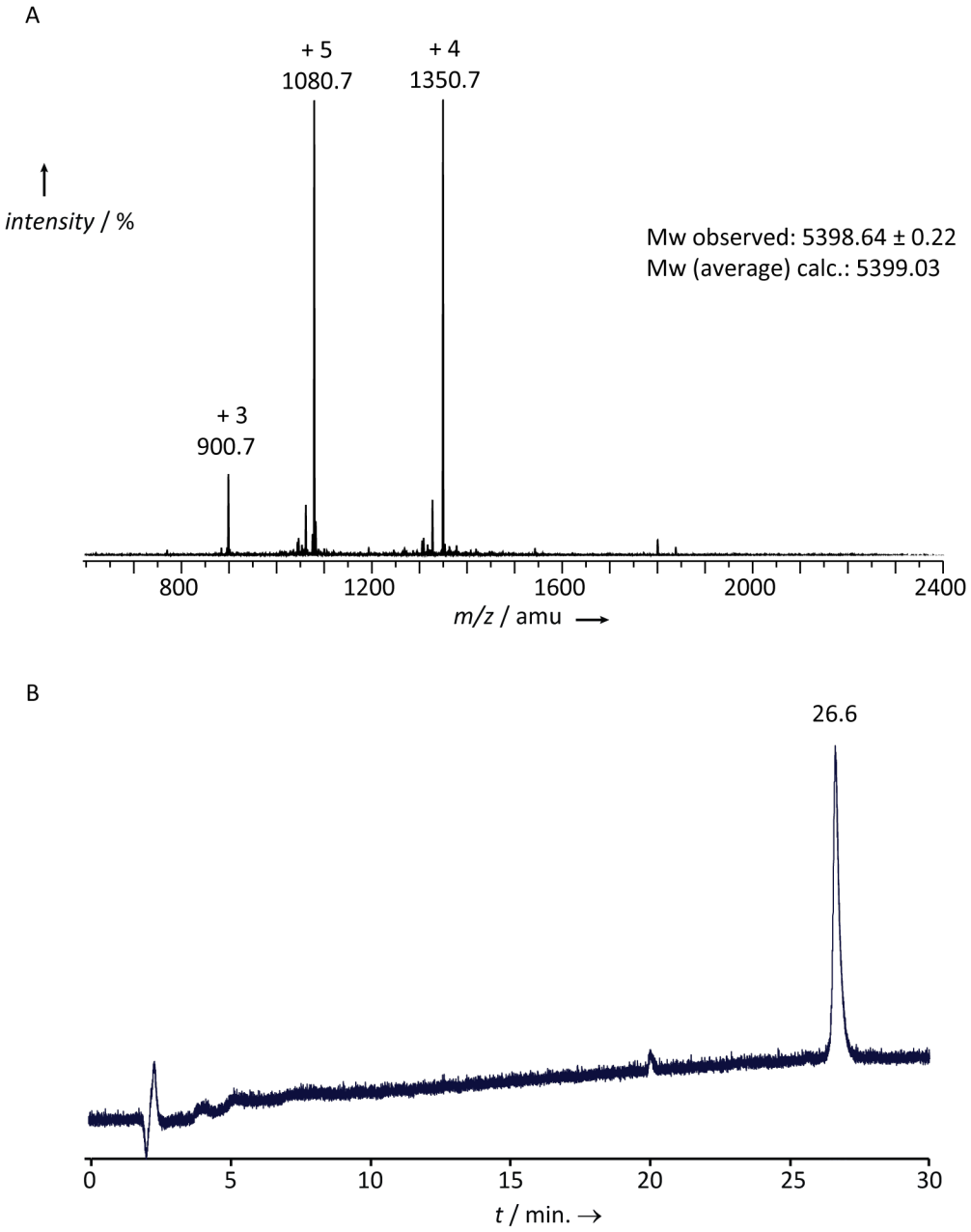

Figure 5: 6-TAMRA-Gly-[Glu^{1}-Ser^{41}]-mpaL (**6**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

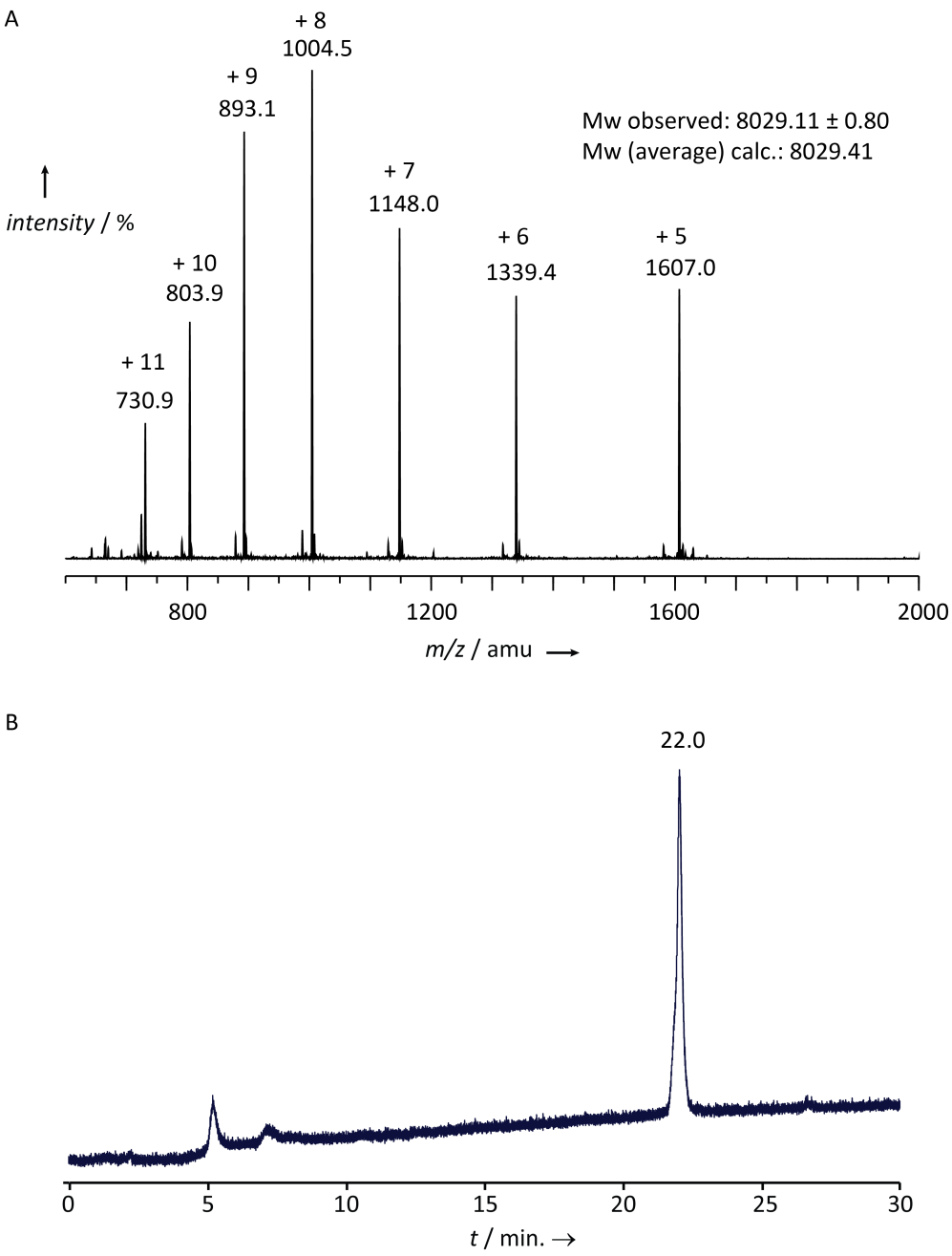

Figure 6: Hcy-[Ile^{43}-Hcy^{75}-Cys(Acm)^{74}-Arg^{108}]-NH_2 (**7**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

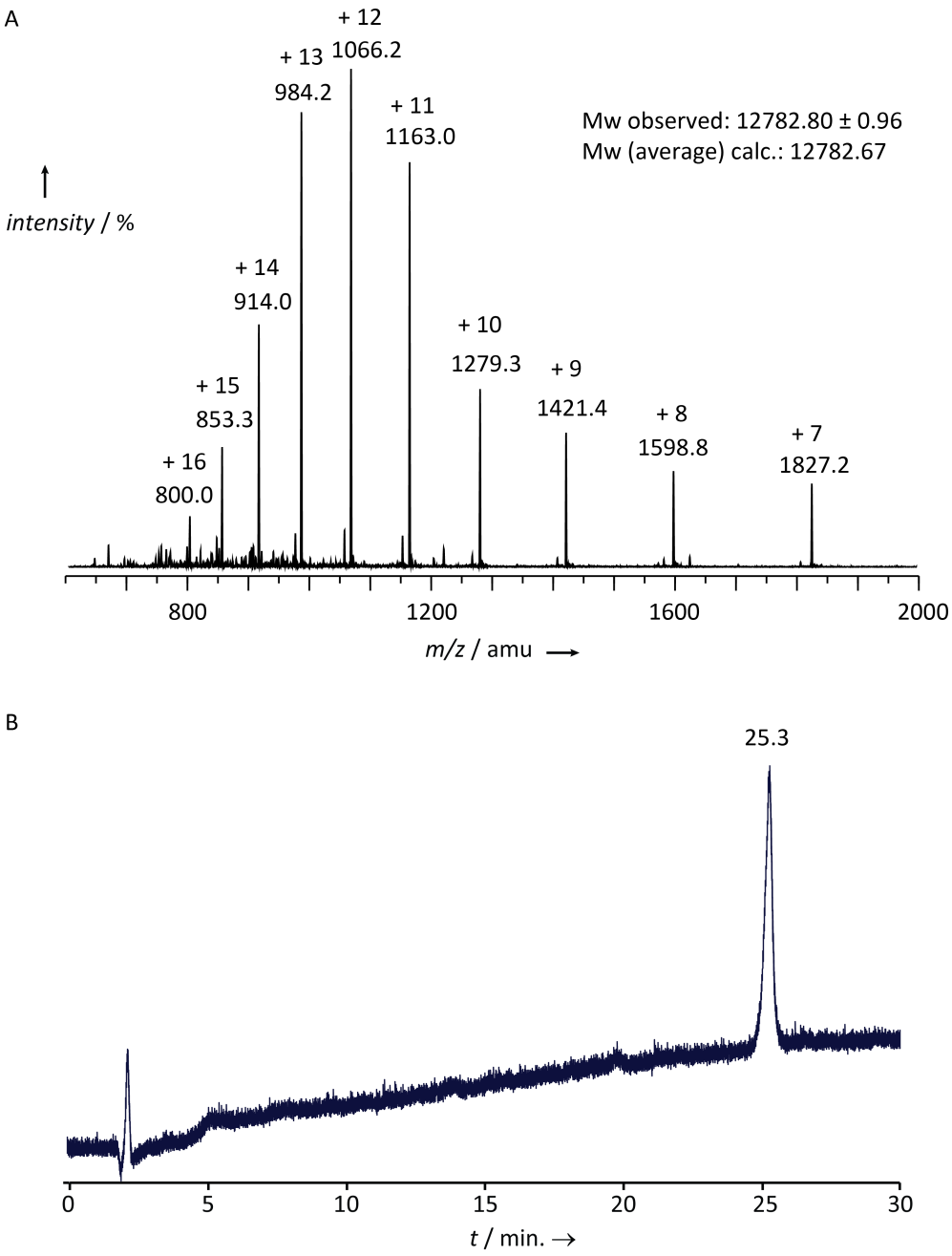

Figure 7: Ac-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}-Arg^{108}]-NH_2 (**8**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

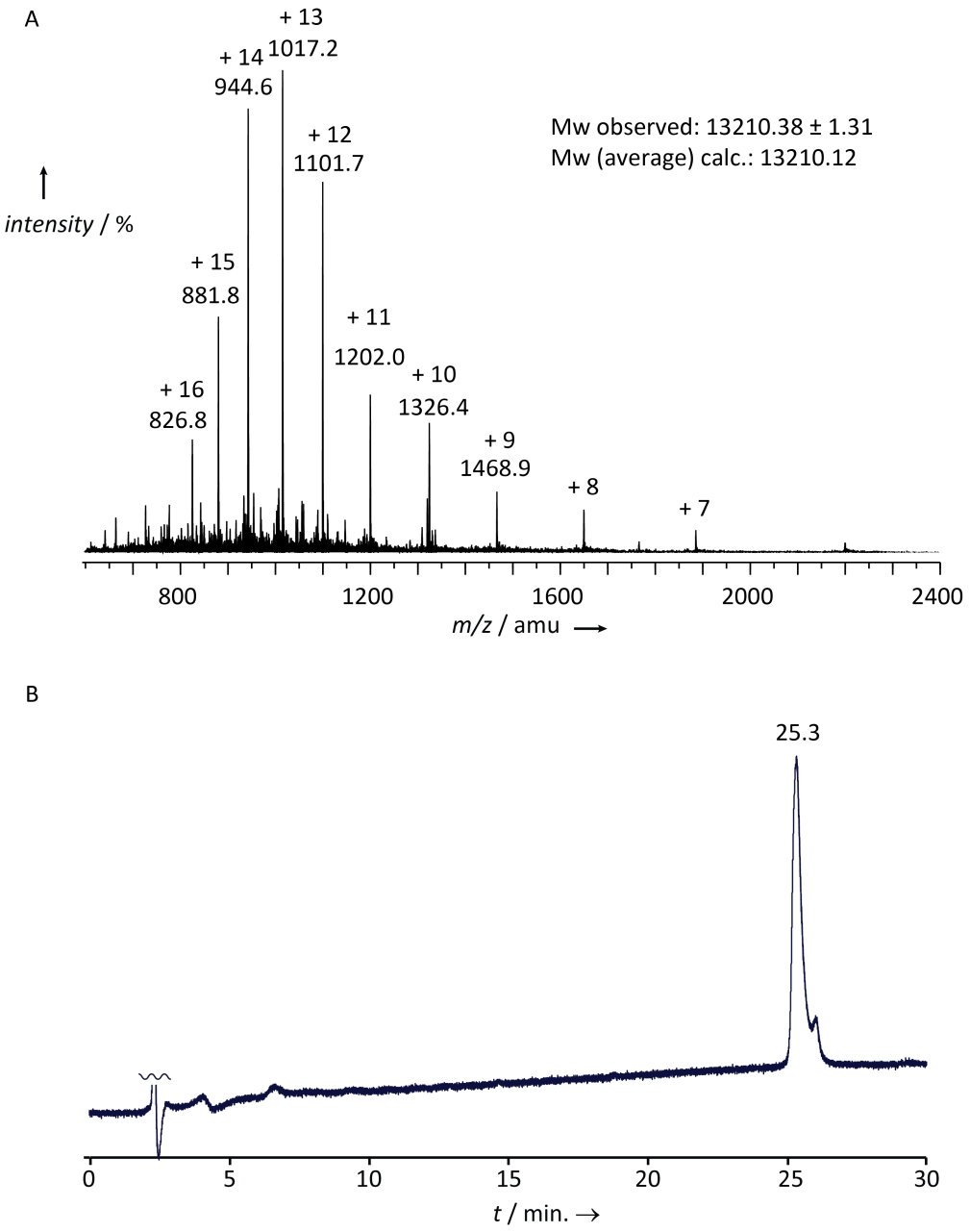

Figure 8: 6-TAMRA-Gly-[Glu^{1}-Hcy^{42}-Hcy^{75}-Cys(Acm)^{76}- Arg^{108}]-NH_2 (**9**), purified. **A**: ESI-MS spectrum. **B**: analytical HPLC chromatogram

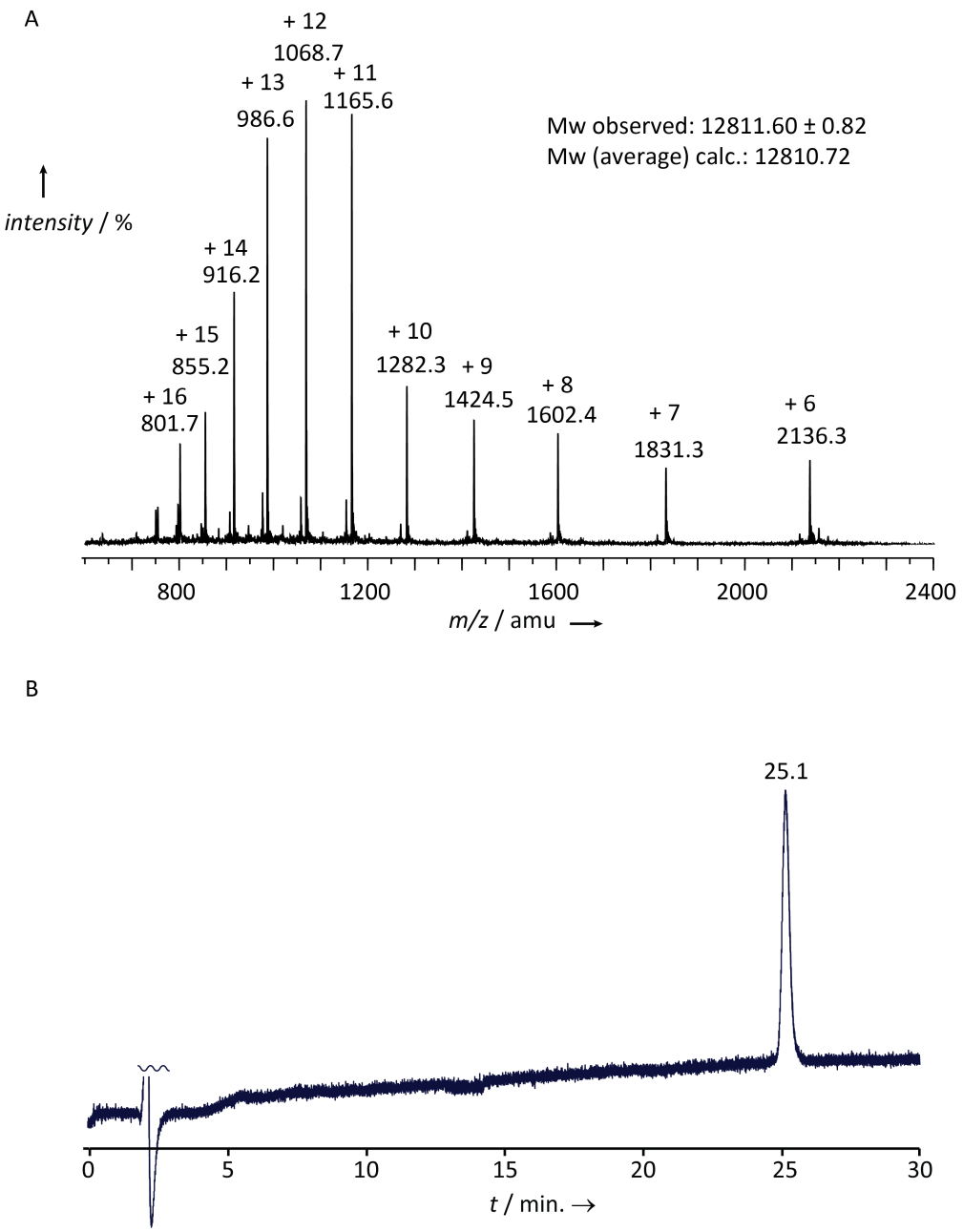

Figure 9: Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH_2 (**10**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

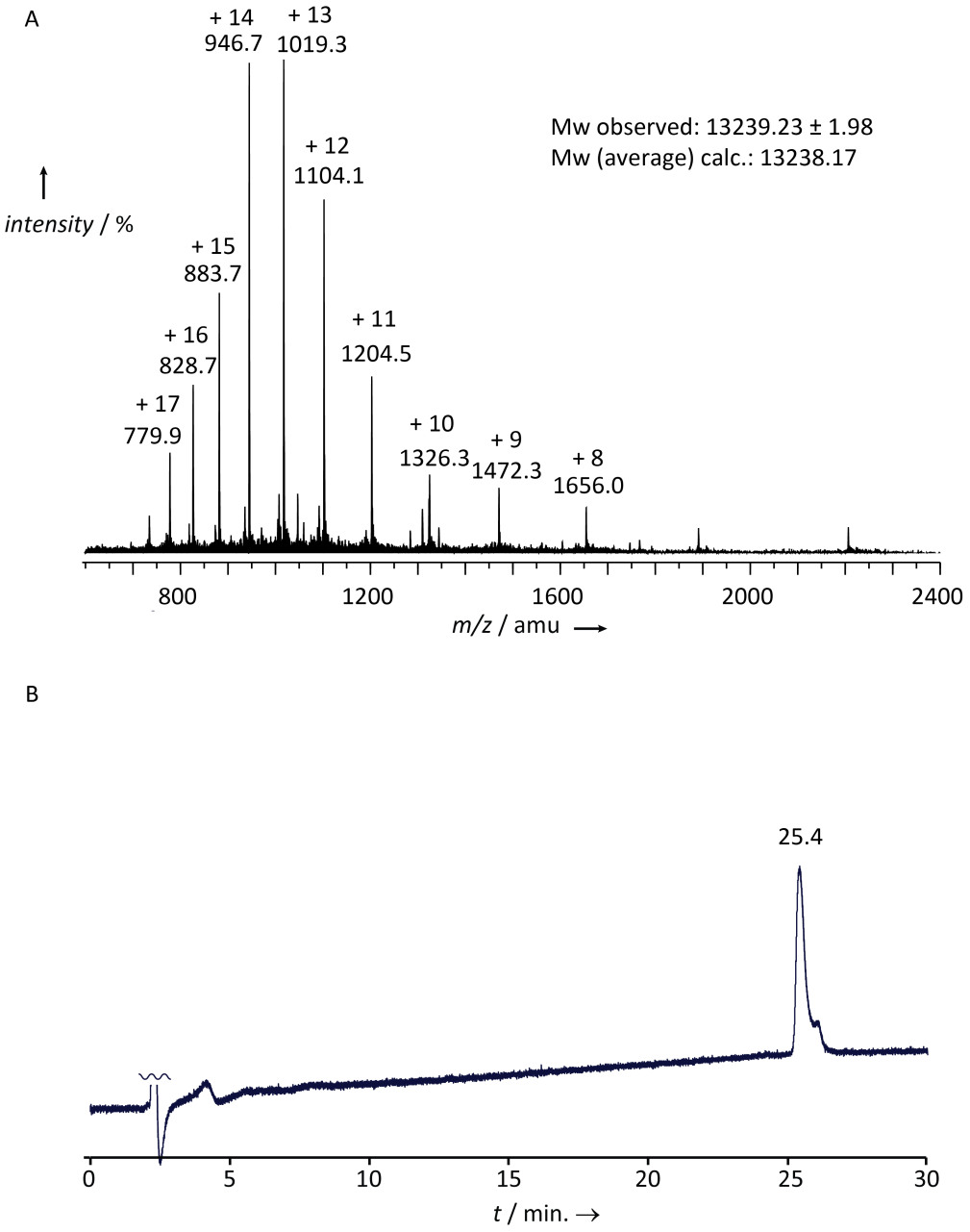

Figure 10: 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys(Acm)^{76}-Arg^{108}]-NH_2 (**11**), purified. **A**: ESI-MS spectrum. **B**: analytical HPLC chromatogram

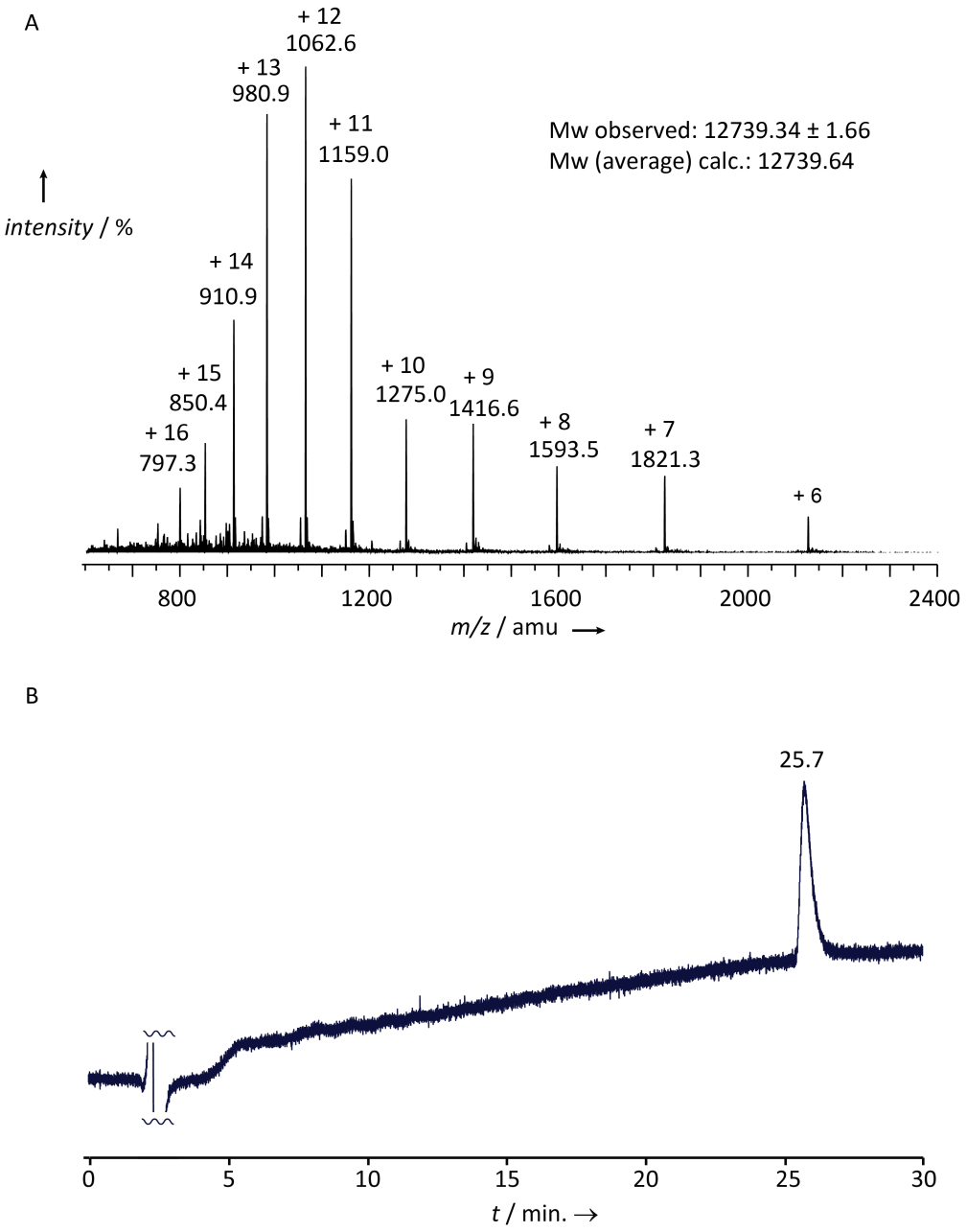

Figure 11: Ac-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH_2 (**12**), purified.
A: ESI-MS spectrum. **B**: analytical HPLC chromatogram

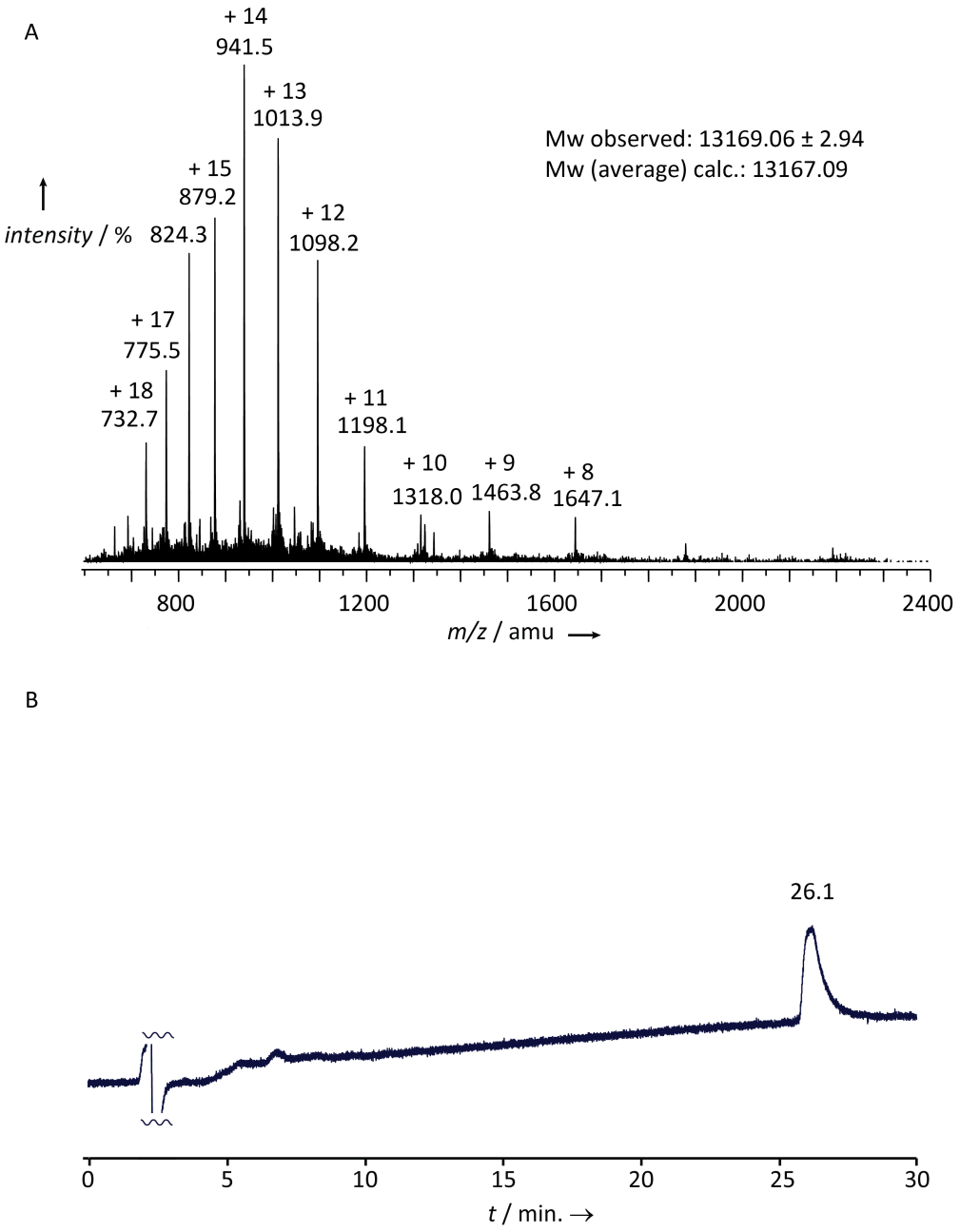

Figure 12: 6-TAMRA-Gly-[Glu^{1}-Met^{42}-Met^{75}-Cys^{76}-Arg^{108}]-NH_2 (**13**), purified. **A**: ESI-MS spectrum. **B**: analytical HPLC chromatogram

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