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Section 1. General

Reagents and Solvents

All reagents were purchased in the highest purity available and used as supplied. Amino acid derivatives and coupling reagents were obtained from GL Biochem (Shanghai, China), Iris Biotech (Marktredwitz, Germany), and Roth (Karlsruhe, Germany). Resins for SPPS were acquired from Novabiochem (Darmstadt, Germany) and Sigma Aldrich (St. Louis, USA). All other Chemicals were obtained from varying commercial sources. Solvents for reactions were of the grade pro analysis (p.a.) or absolute. Technical grade solvents for flash chromatography were distilled prior to use. Solvents for HPLC and spectroscopic applications were of HPLC grade. Demineralized water was further purified using an arium mini lab water system by Sartorius (Göttingen, Germany) to obtain ultrapure water.

Reactions

All air and moisture sensitive reactions were carried out in argon atmosphere using dry solvents. The glassware used in such reactions was dried by heating with a heat gun and the interior of the flask was purged using the Schlenk technique. All light sensitive compounds were protected from light with aluminium foil.

Lyophilization

Aqueous or dioxane solutions were frozen in liquid nitrogen and freeze-dried using a Christ Alpha 2-4 LD plus lyophilizer (Osterode in Harz, Germany) connected to a high vacuum pump. In fractions collected from the HPLC the organic content was reduced to a minimum amount with a flow of nitrogen and they were likewise freeze-dried. For small volumes (< 2 mL) a Christ RVC 2-18 CD plus vacuum centrifuge (Osterode in Harz, Germany) connected to the lyophilizer was used.

Storage

Chemicals and resins for SPPS were stored according to suppliers' recommendations at -18 °C, 2 °C or room temperature and under argon when appropriate. Synthesized compounds and cleaved and purified peptides were stored at -18 °C while on-resin-peptides and compounds meant for the determination of extinction coefficients were kept in a desiccator. Light-sensitive compounds were protected with aluminium

Thin Layer Chromatography (TLC)

The progression of reactions was monitored by thin layer chromatography on *Merck* (Darmstadt, Germany) silica gel 60 F254 coated aluminium sheets. Spots were detected by fluorescence quenching at 254 nm or fluorescence at 366 nm or visualized by TLC staining solutions and heating with a heat gun. Amines were identified with ninhydrin solution (1.5 g ninhydrin and 3 mL acetic acid in 100 mL n-butanol).

Flash Column Chromatography

Purification by flash chromatography was performed using *Merck* (Darmstadt, Germany) silica gel type 60 (particle size 40-63 µm) and a pressure of 0.1-1 bar. Silica gel was suspended in the eluent of choice and packed in an appropriately sized glass column equipped with a glass frit. Crude samples were loaded in a thin layer preabsorbed to the fivefold amount by weight of silica gel.

Reversed Phase High Performance Liquid Chromatography (RP-HPLC)

Semi-preparative HPLC purification was carried out on a *JASCO* (Tokyo, Japan) system consisting of two pump modules (*PU 2080 Plus*), degasser (*DG-2080-53*) and a multi wavelength detector (*MD-2010 Plus*). If needed, a fraction collector (*CHF122SC*) from *Advantec* (Multiplas, USA) was coupled to the *JASCO* (Tokyo, Japan) system by a connector module (*FC-2088-30*). The purification was conducted at room temperature.

The following columns were used for compound retention:

Column 1:	ACE Excel 2 C18	100A-2-C18 100 mm x 2.1 mm, 2 μ m	UPLC
Column 2:	MN Nucleodur RP-C18-ec	300-5-C18, 250 mm x 10 mm, 5 μ m	semi-prep.

Applying a flow rate of 0.30 mL/min (UPLC) or 3 mL/min (semipreparative) a linear gradient of solvent H₂O + 0.1% TFA to MeCN + 0.1% TFA from the eluent systems listed below was conducted.

Peptides constructed only from proteinogenic amino acids were detected at 215 nm, 254 nm, and 280 nm. Chromatograms of coumarin derivatives were recorded at 390 nm. UPLC analysis was conducted at 50 °C.

Prior to injection the analytes were dissolved in a minimal amount of a solvent mixture corresponding to the starting conditions of the HPLC run. The solutions were filtrated through a *Chromafil* syringe filter (0.45 μ m pore size) by *Macherey Nagel* (Düren, Germany).

Section 2. Characterization

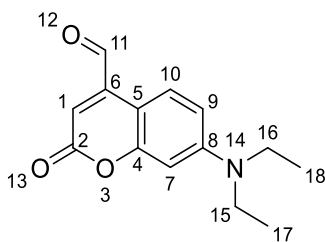
Mass Spectrometry

All compounds were characterized by electron spray ionization mass spectrometry (ESI-MS) and high resolution ESI (HR-MS) on a maXis or MicroTOF spectrometer from Bruker (Bremen Germany). Values are given in m/z .

Nuclear Magnetic Resonance (NMR) Spectroscopy

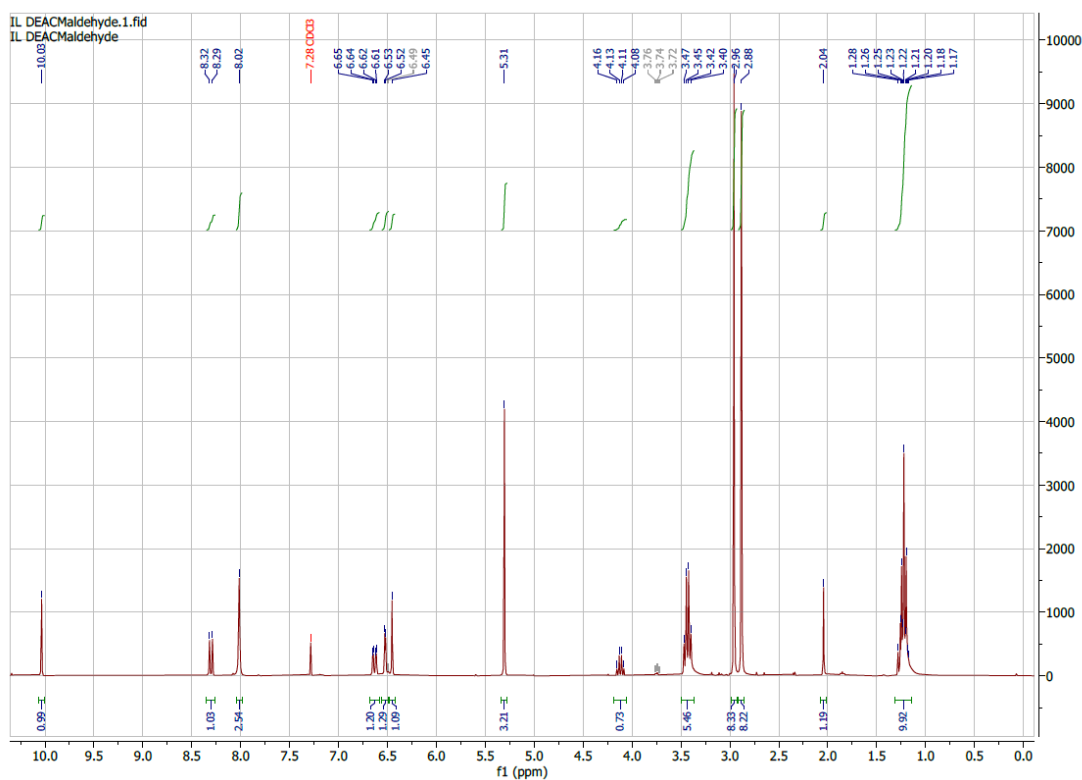
^1H - and ^{13}C -NMR spectra were recorded on a Varian (Palo Alto, USA) instrument (*Mercury-VX 300*, *VNMRS-300* or *INOVA-500*). The measurement frequency and the utilized deuterated solvent are indicated with each compound. Residual proton signals of the solvents served as an internal standard. Chemical shifts δ are given in parts per million (ppm) and coupling constants nJ are given in Hertz (Hz), where n indicates the order of coupling. Multiplicities are described with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Signals were partially attributed with the help of COSY- (Correlated Spectroscopy), HSQC- (Heteronuclear Single Quantum Coherence) and HMBC- (Heteronuclear Multiple Bond Correlation) spectra.

7-(Diethylamino)-4-formylcoumarin (3)

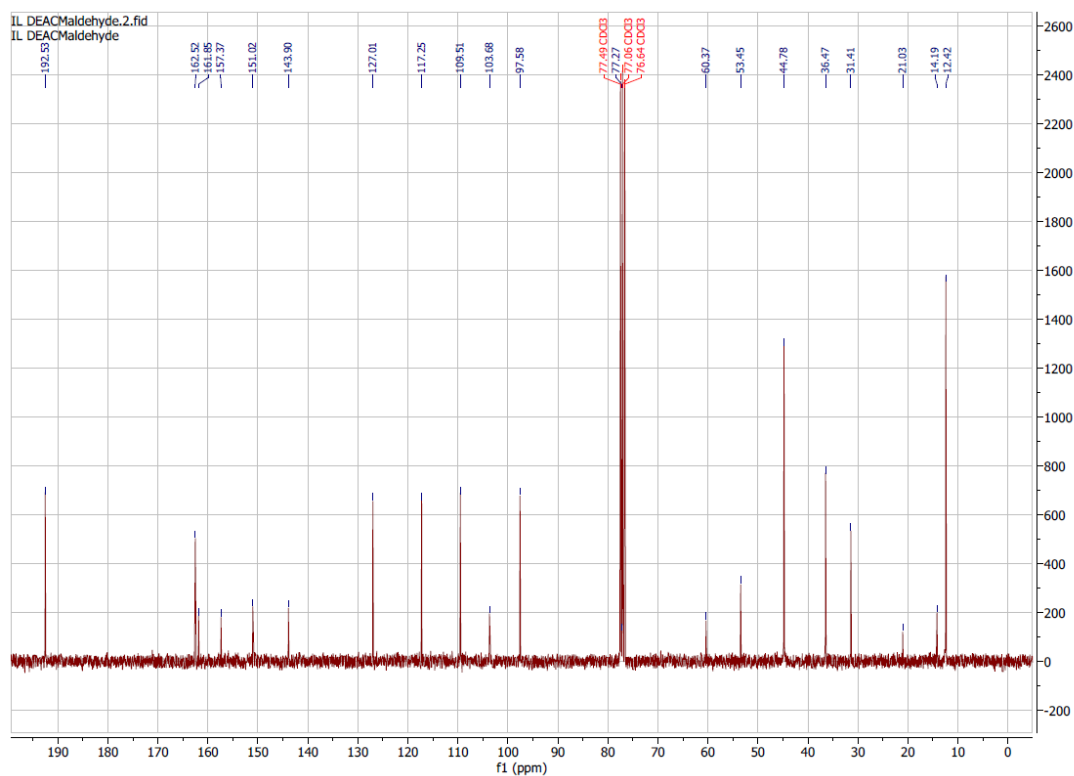


$C_{14}H_{15}NO_3$
245.3 g/mol
3

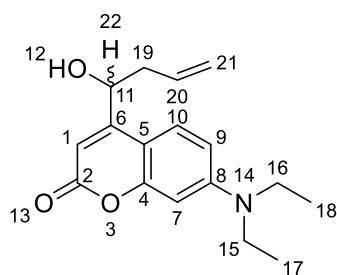
1H -NMR (400 MHz, $CDCl_3$): δ (ppm) = 10.0 (s, 1 H, 11-CHO), 8.28 (d, $^3J_{HH} = 9.2$ Hz, 1 H, 10-CH), 6.61 (dd, $^3J_{HH} = 9.2$ Hz, $^4J_{HH} = 2.6$ Hz, 1 H, 9-CH), 6.50 (d, $^4J_{HH} = 2.6$ Hz, 1 H, 7-CH), 6.43 (s, 1 H, 1-CH), 3.41 (q, $^3J_{HH} = 7.2$ Hz, 4 H, 15, 16- CH_2), 1.21 (t, $^3J_{HH} = 7.1$ Hz, 6 H, 17, 18- CH_3).



^{13}C -NMR (75 MHz, CDCl_3): δ (ppm) = 192.4 (11-CHO), 161.8 (5-C), 157.3 (2-C), 151.0 (8-C), 143.8 (7-C), 128.8 (4-C), 117.2 (1-C), 109.5 (6-C), 103.6 (10-C), 97.5 (3-C), 44.7 (15, 16- CH_2), 12.40 (17, 18- CH_2).



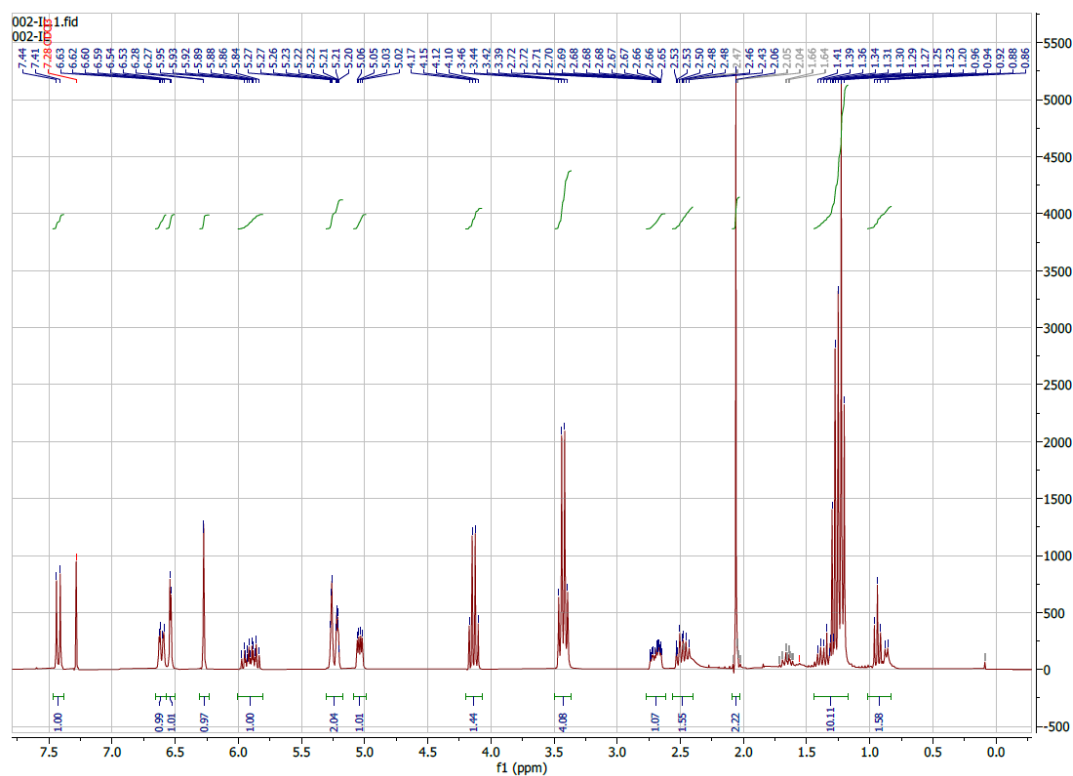
7-(Diethylamino)-4-(1-hydroxybut-3-en-1-yl)-coumarin (4a)



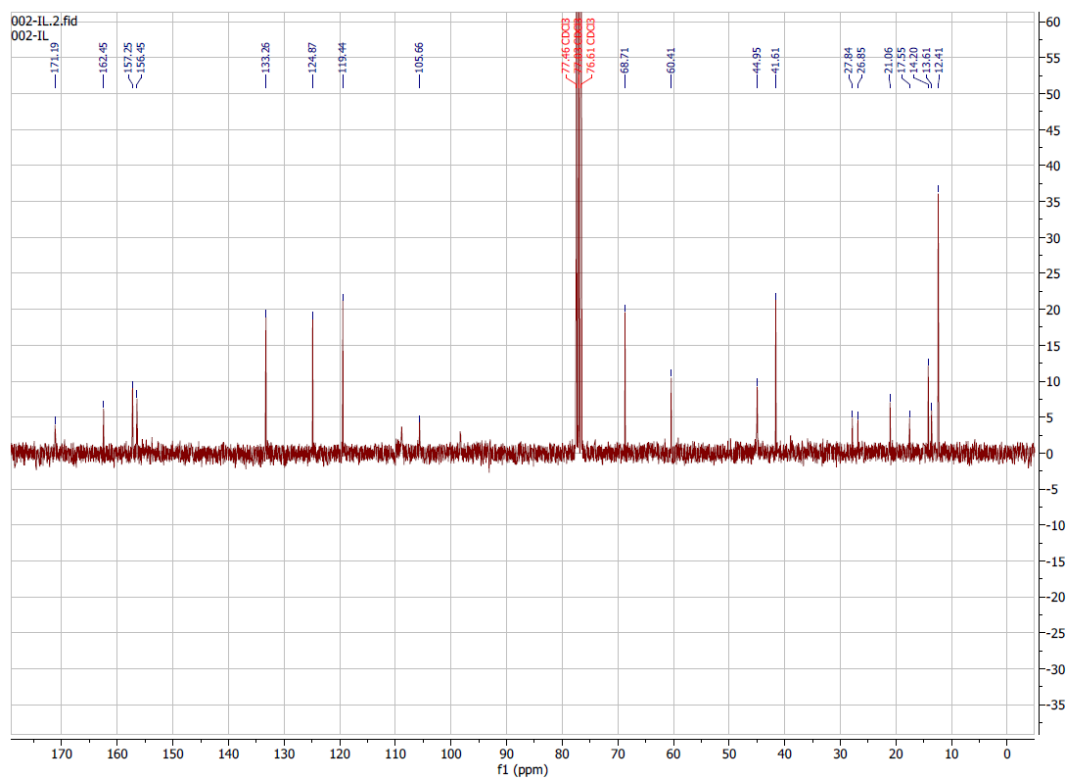
$\text{C}_{17}\text{H}_{21}\text{NO}_3$
287.2 g/mol

4a

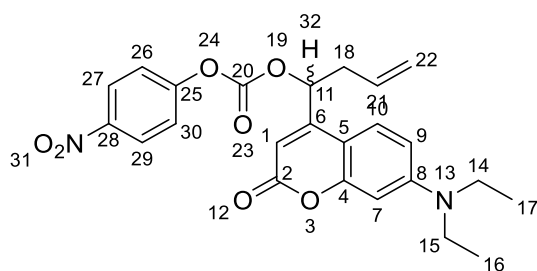
$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) = 7.37 (d, $^3J_{\text{HH}} = 9.1$ Hz, 1 H, 10-CH), 6.57 (dd, $^3J_{\text{HH}} = 9.1$ Hz, $^4J_{\text{HH}} = 2.6$ Hz, 1 H, 9-CH), 6.48 (d, $^4J_{\text{HH}} = 2.6$ Hz, 1 H, 7-CH), 6.24 (t, $^4J_{\text{HH}} = 1.3$ Hz, 1 H, 1-CH), 5.95-5.80 (m, 1 H, 20-CH_{allyl}), 5.24-5.15 (m, 2 H, 21-CH_{2,sp2,allyl}), 5.01 (dd, $^3J_{\text{HH}} = 7.89$ Hz, $^3J_{\text{HH}} = 3.7$ Hz, 22-CHOH_{coum}), 3.39 (q, $J = 7.1$ Hz, 4 H, 15, 16-CH₂), 2.71-2.38 (m, 2 H, 19-CH_{2,sp3,allyl}), 1.19 (t, $J = 7.1$ Hz, 6 H, 17, 18-CH₃).



^{13}C -NMR (75 MHz, CDCl_3): δ (ppm) = 162.4 (2-C), 157.3 (8-C), 156.3 (5-C), 150.2 (7-C), 133.2 (20- CH_{allyl}), 124.8 (4-C), 119.1, 21- $\text{CH}_{2,\text{sp}^2,\text{allyl}}$, 108.5 (10-C), 106.1 (6-C), 105.4 (1-C), 98.0 (3-C), 68.8 (11- $\text{CHOH}_{\text{coum}}$), 44.7 (15, 16- CH_2), 12.5 (17,18- CH_3).



1-(7-Diethylaminocoumarin-4-yl)but-3-en-1-yl (4-nitrophenyl) carbonate (6a)

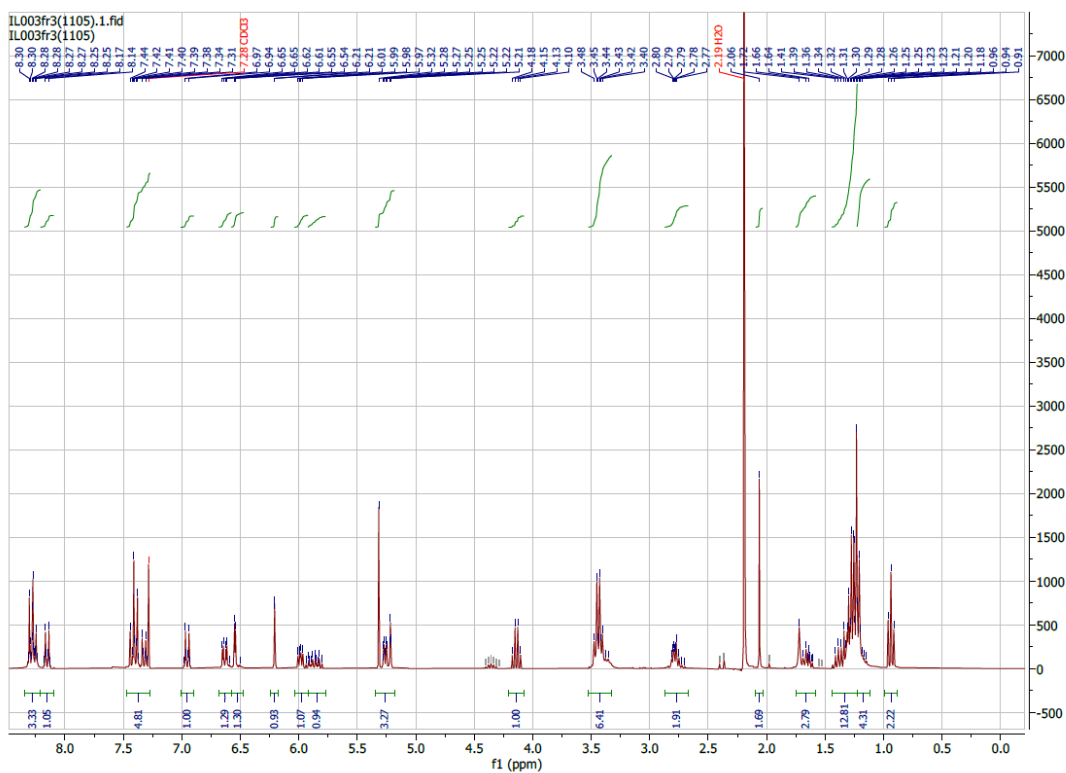


$C_{24}H_{24}N_2O_7$

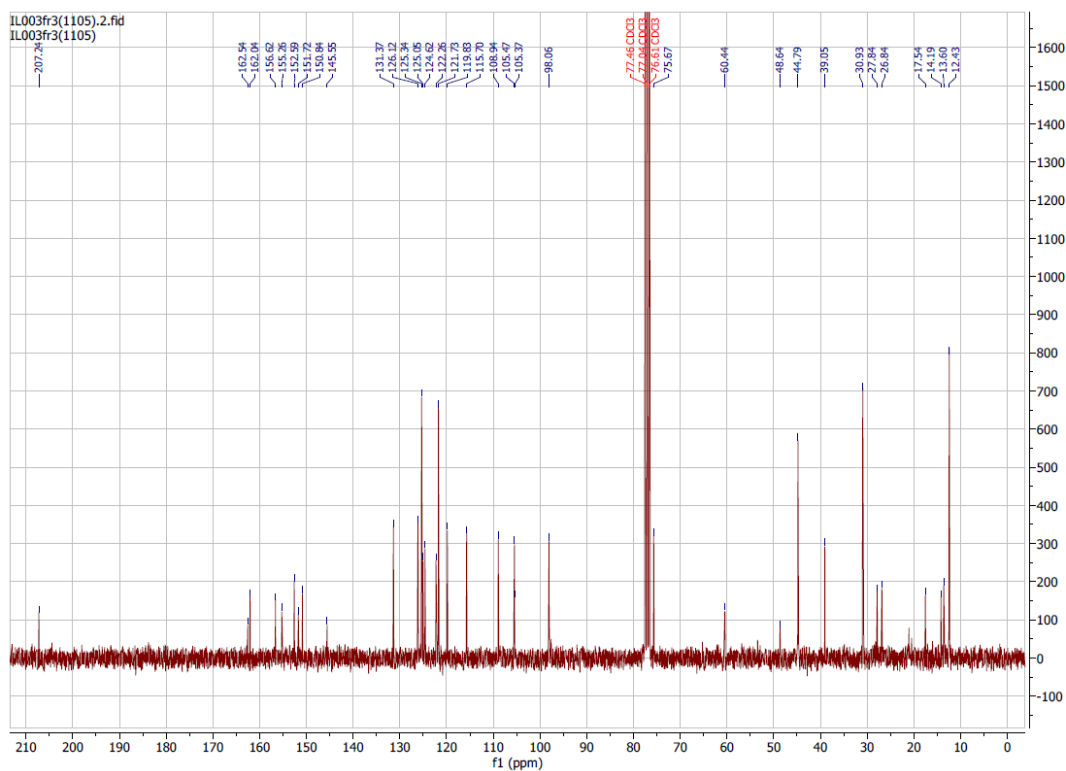
452.5 g/mol

6a

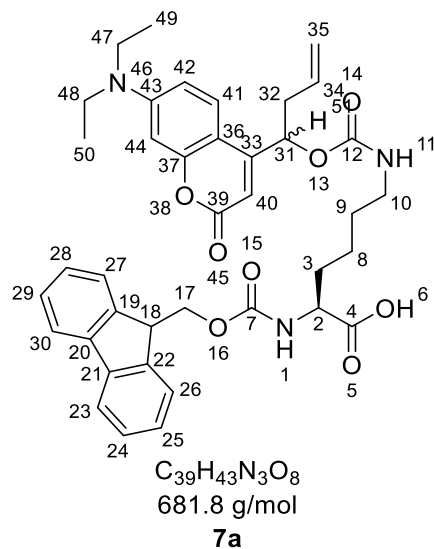
$^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) = 8.30 (dt, $^3J_{\text{HH}} = 9.2$ Hz, $^4J_{\text{HH}} = 2.2$ Hz, 2 H, 26, 30-CH), 7.42 (d, $^3J_{\text{HH}} = 9.1$ Hz, 1 H, 10-CH), 7.38 (dt, $^3J_{\text{HH}} = 9.2$ Hz, $^5J_{\text{HH}} = 2.2$ Hz, 2 H, 27, 29-CH), 6.60 (dd, $^3J_{\text{HH}} = 9.1$ Hz, $^4J_{\text{HH}} = 2.6$ Hz, 1 H, 9-CH), 6.51 (d, $^4J_{\text{HH}} = 2.6$ Hz, 1 H, 7-CH), 6.17 (s, 1 H, 1-CH), 5.95 (dd, $^3J_{\text{HH}} = 7.6$ Hz, $^3J_{\text{HH}} = 4.8$ Hz, 1 H, 32-CHO_{coum}), 5.88-5.78 (m, 1 H, 21-CH_{sp2,allyl}), 5.24-5.17 (m, 2 H, 22-CH_{2,sp2,allyl}), 3.40 (q, $^3J_{\text{HH}} = 7.1$ Hz, 4 H, 14, 15-CH₂), 2.80-2.67 (m, 2 H, 18-CH_{2,sp3,allyl}), 1.19 (t, $^3J_{\text{HH}} = 7.1$ Hz, 6 H, 16,17-CH₃).



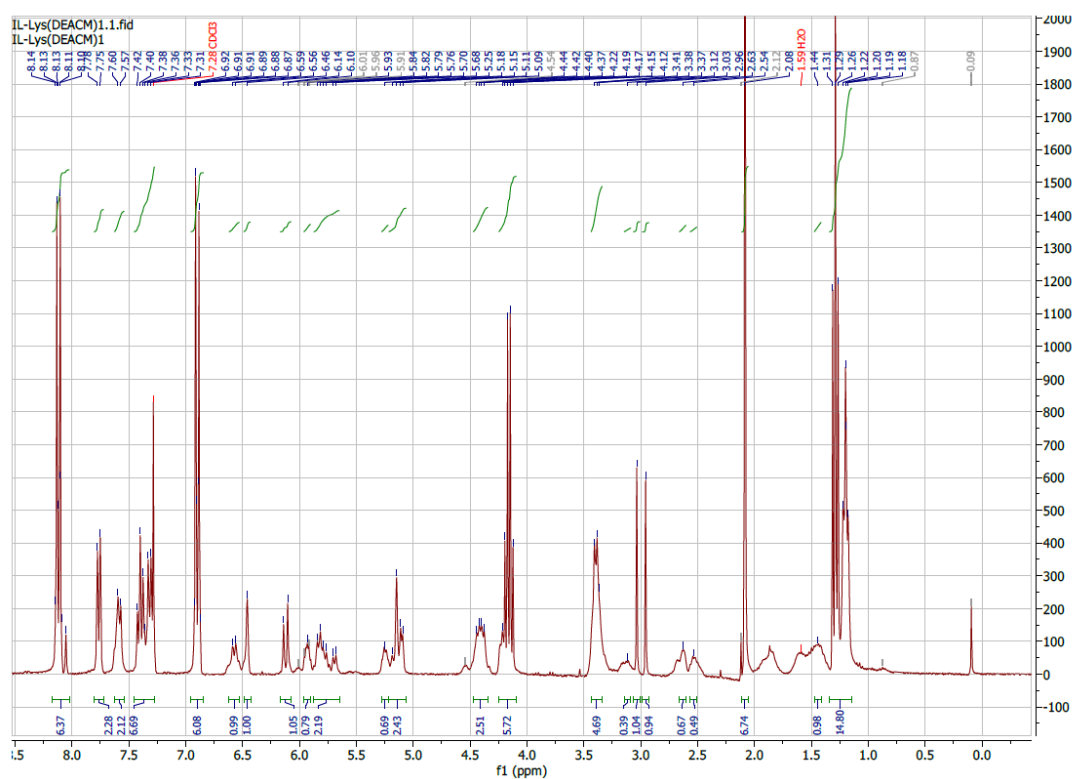
^{13}C -NMR (125 MHz, CDCl_3): δ (ppm) = 161.8 (2-C), 156.6 (8-C), 155.2 (28-C), 152.4 (5-C), 151.7 (20- $\text{CO}_{\text{carbonate}}$), 150.7 (7-C), 145.5 (25-C), 131.6 (21-CH), 125.3 (26, 30-C), 124.6 (4-C), 121.7 (27, 29-C), 119.8 (22- $\text{CH}_{2,\text{sp}2,\text{allyl}}$), 108.9 (10-C), 105.6 (1-C), 105.4 (6-C), 98.1 (3-C), 75.6 (11- CHO_{coum}), 44.9 (14, 15- CH_2), 39.0 (18- $\text{CH}_{2,\text{sp}3,\text{allyl}}$), 12.4 (16,17- CH_3).



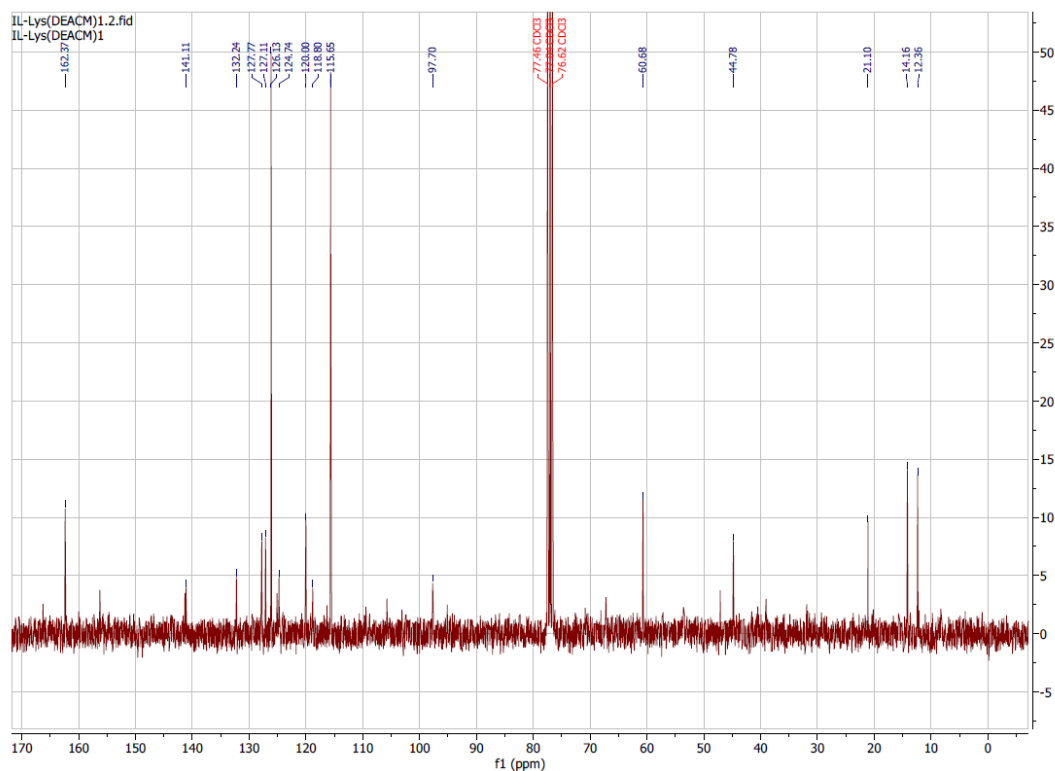
Fmoc-L-Lys(DEACM^{allyl})-OH (7a)



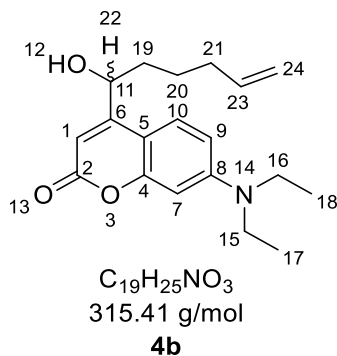
¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 7.72 (d, ³J_{HH} = 7.5 Hz, 2 H, 26, 27-CH_{ar,Fmoc}), 7.62-7.52 (m, 2 H, 25, 28-CH_{ar,Fmoc}), 7.41-7.32 (m, 3 H, 41-CH_{coum}, 24, 29-CH_{ar,Fmoc}), 7.31-7.24 (t, ³J_{HH} = 7.5 Hz, 2 H, 23, 30-CH_{ar,Fmoc}), 6.64-6.52 (m, 1 H, 42-CH_{coum}), 6.52-6.47 (m, 1 H, 44-CH_{coum}), 6.10 (s, 1 H, 40-CH_{coum}), 5.95-5.87 (m, 1 H, 51-CHO_{coum}), 5.79-5.72 (m, 1 H, 34-CH_{sp2,allyl}), 5.14-5.03 (m, 2 H, 35-CH_{2,sp2,allyl}), 4.44-4.29 (m, 3 H, 2-CH, 17-CH_{2,Fmoc}), 4.21-4.14 (m, 1 H, 18-CH_{sp3,Fmoc}), 3.38 (q, ³J_{HH} = 7.0 Hz, 4 H, 47, 48-CH₂), 3.30-2.96 (m, 2 H, 8-CH₂), 2.70-2.40 (m, 2 H, 32-CH_{2,sp3,allyl}), 1.96-1.71 (m, 2 H, 3-CH₂), 1.64-1.27 (m, 4 H, 9-CH₂, 10-CH₂), 1.16 (t, ³J_{HH} = 7.2 Hz, 6 H, 49, 50-CH₃).



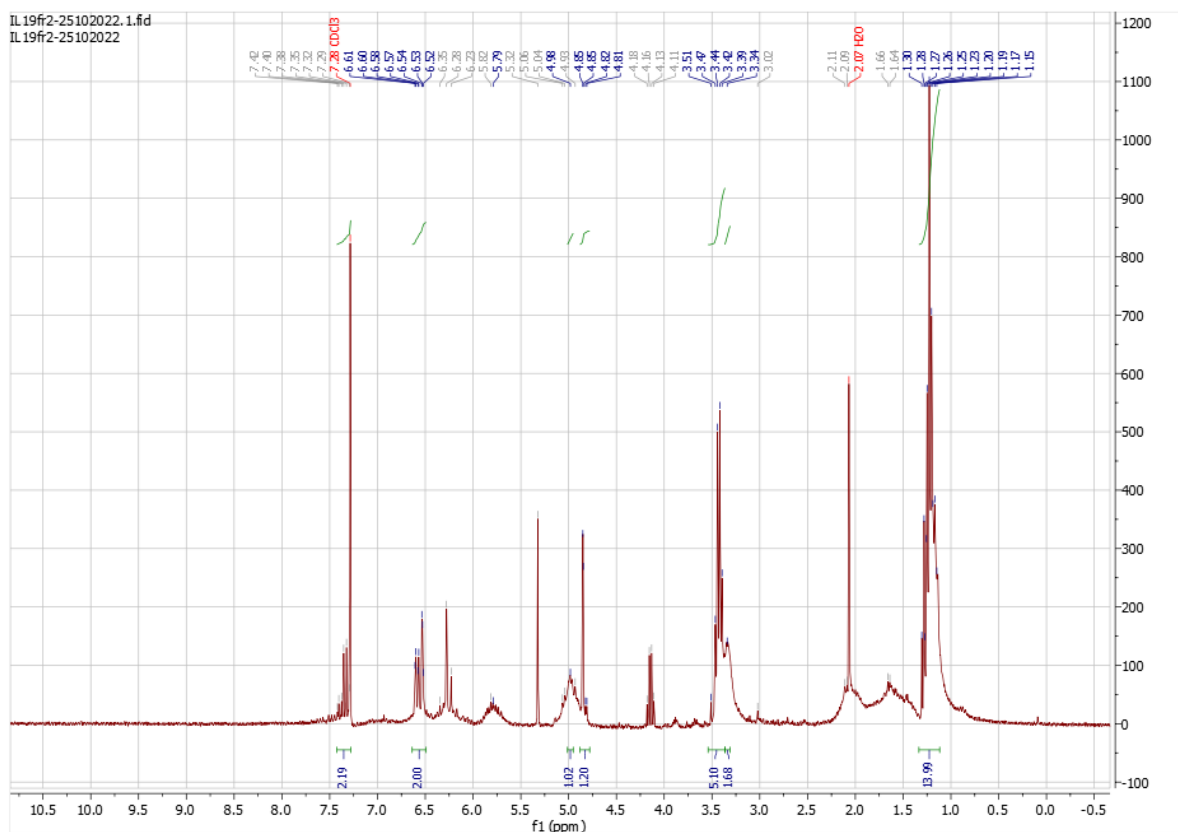
^{13}C -NMR (100 MHz, CDCl_3): δ (ppm) = 173.7 (4- CO_2H), 163.9 (39- CH_{coum}), 156.4 (12- CO_{coum} -carbamate), 155.2 (43- C_{coum}), 155.9 (7- CO_{Fmoc} -carbamate), 155.3 (44- CH_{coum}), 150.6 (36- CH_{coum}), 143.8 (19, 22- $\text{C}_{\text{q,Fmoc}}$), 141.3 (20,21- $\text{C}_{\text{q,Fmoc}}$), 132.4 (35- $\text{CH}_{\text{sp2,allyl}}$), 127.7 (23, 30- $\text{CH}_{\text{ar,Fmoc}}$), 127.1 (24, 29- $\text{CH}_{\text{ar,Fmoc}}$), 125.2 (37- CH_{coum}), 124.8 (25, 28- $\text{CH}_{\text{ar,Fmoc}}$), 119.9 (26, 27- $\text{CH}_{\text{ar,Fmoc}}$), 118.5 (35- $\text{CH}_{2,\text{sp2,allyl}}$), 109.2 (40- CH_{coum}), 106.1 (42- CH_{coum}), 104.0 (33- CH_{coum}), 98.2 (44- CH_{coum}), 70.4 (31- CHO_{coum}), 67.0 (17- $\text{CH}_{2,\text{Fmoc}}$), 53.6 (2- CH), 47.1 (18- CH), 44.9 (47, 48- CH_2), 40.4 (32- CH_2), 39.0 (10- CH_2), 32.0 (3- CH_2), 28.9 (8- CH_2), 21.6 (9- CH_2), 12.6 (49, 50- CH_3).



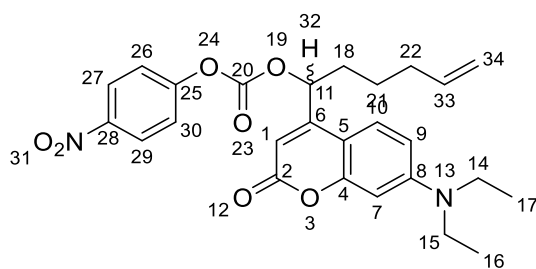
7-(diethylamino)-4-(1-hydroxyhex-5-en-1-yl)-2H-chromen-2-one (4b)



1H -NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.45 (d, $^3J_{HH} = 9.1$ Hz, 1 H, 10-CH), 6.75 (dd, $^3J_{HH} = 9.1$ Hz, $^4J_{HH} = 2.6$ Hz, 1 H, 9-CH), 6.54 (d, $^4J_{HH} = 2.6$ Hz, 1 H, 7-CH), 6.23 (t, $^4J_{HH} = 1.3$ Hz, 1 H, 1-CH), 5.93-5.62 (m, 4 H, 20, 21- CH_{allyl}), 5.01-4.88 (m, 2 H, 24- $CH_{2,sp2,allyl}$), 4.57 (dd, $^3J_{HH} = 7.89$ Hz, $^3J_{HH} = 3.7$ Hz, 22- $CHOH_{coum}$), 3.40 (q, $J = 7.1$ Hz, 4 H, 15, 16- CH_2), 3.39-3.25 (m, 2 H, 19- $CH_{2,sp3,allyl}$), 1.17 (t, $J = 7.1$ Hz, 6 H, 17, 18- CH_3).



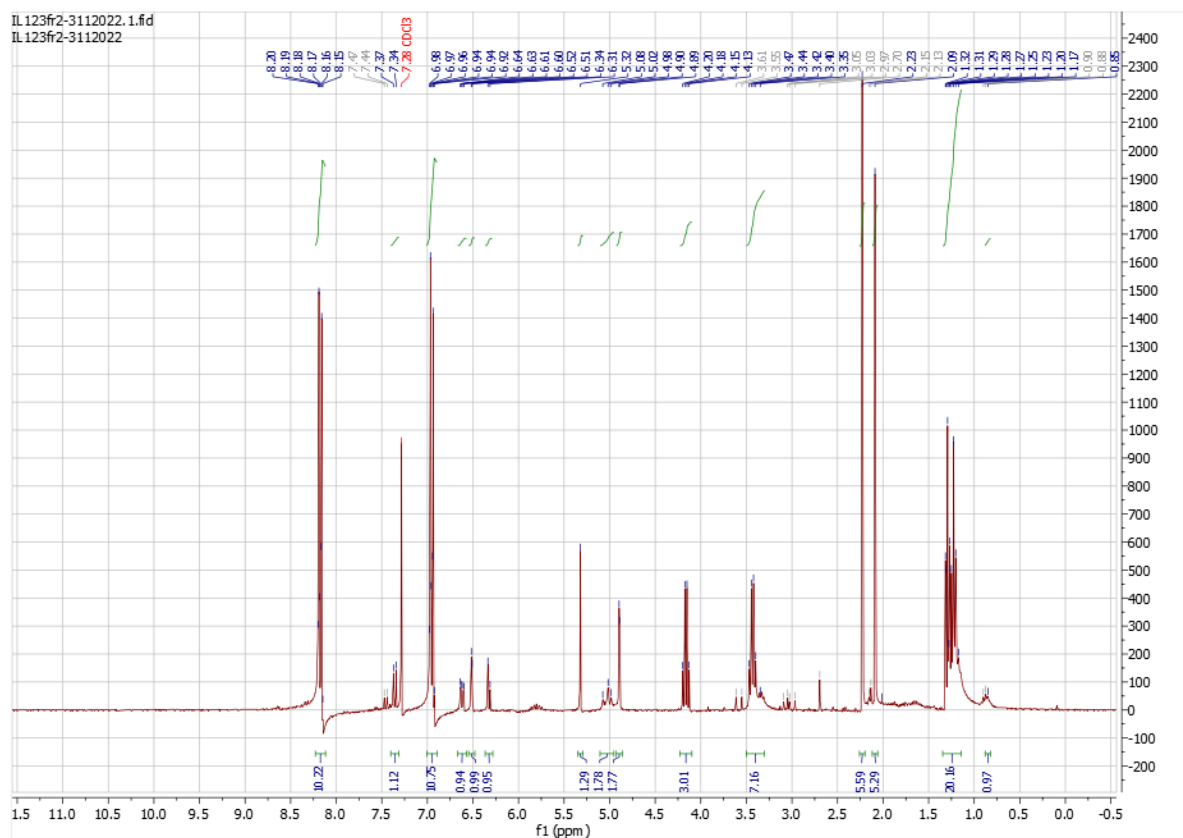
1-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)hex-5-en-1-yl (4-nitrophenyl) carbonate (6b)



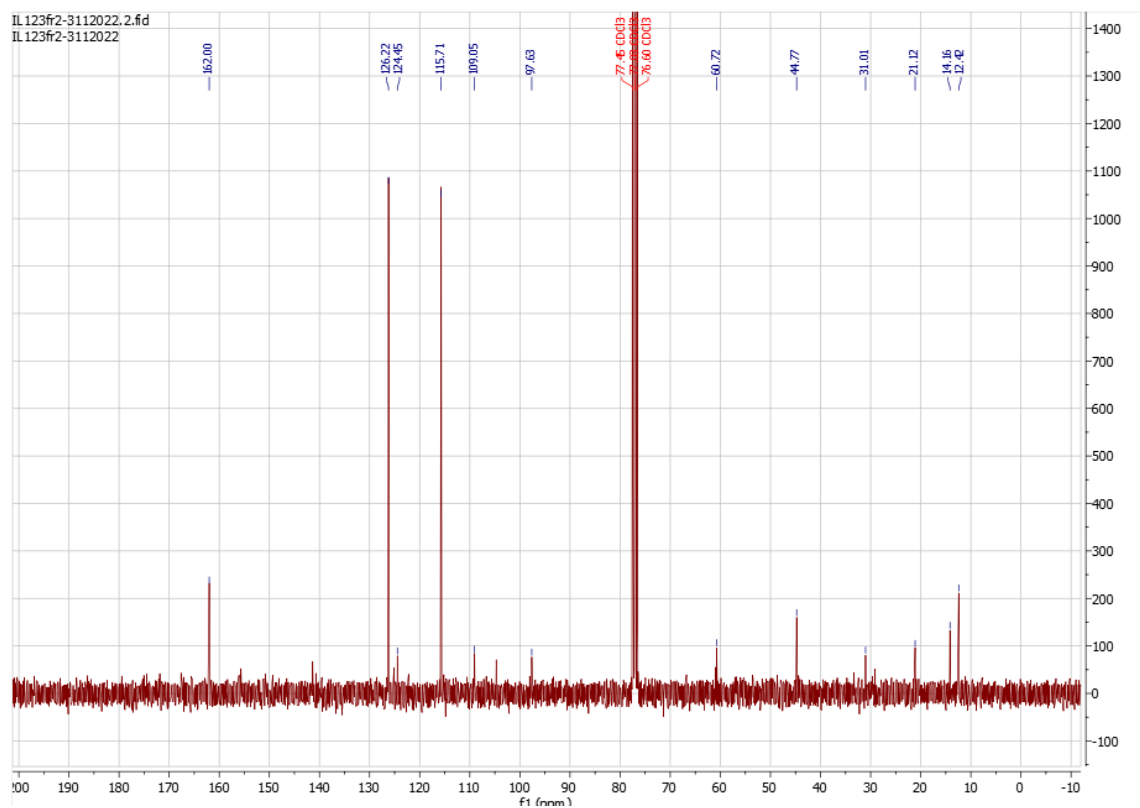
$C_{26}H_{28}N_2O_7$
480.52 g/mol

6b

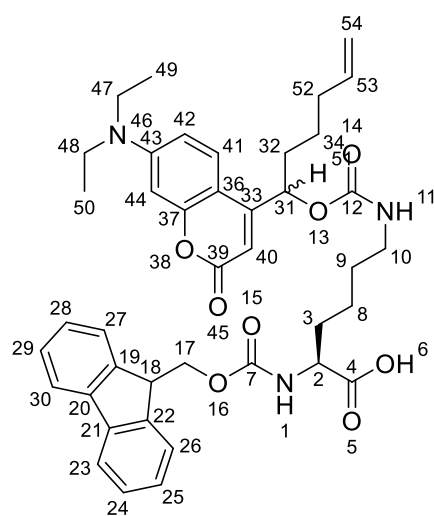
1H -NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.27 (dt, $^3J_{HH} = 9.2$ Hz, $^4J_{HH} = 2.2$ Hz, 2 H, 26, 30-CH), 8.13 (d, $^3J_{HH} = 9.1$ Hz, 1 H, 10-CH), 7.38 (dt, $^3J_{HH} = 9.2$ Hz, $^5J_{HH} = 2.2$ Hz, 2 H, 27, 29-CH), 6.62 (dd, $^3J_{HH} = 9.1$ Hz, $^4J_{HH} = 2.6$ Hz, 1 H, 9-CH), 6.52 (d, $^4J_{HH} = 2.6$ Hz, 1 H, 7-CH), 6.14 (s, 1 H, 1-CH), 5.87 (dd, $^3J_{HH} = 7.6$ Hz, $^3J_{HH} = 4.8$ Hz, 1 H, 32- CHO_{coum}), 5.69-5.56 (m, 1 H, 21- $CH_{sp2,allyl}$), 5.08-4.83 (m, 4 H, 22,23- $CH_{2,sp2,allyl}$), 3.40 (q, $^3J_{HH} = 7.1$ Hz, 4 H, 14, 15- CH_2), 2.86-2.77 (m, 2 H, 18- $CH_{2,sp3,allyl}$), 1.16 (t, $^3J_{HH} = 7.1$ Hz, 6 H, 16,17- CH_3).



^{13}C -NMR (75 MHz, CDCl_3): δ (ppm) 162.0 (2-C), 126.2 (26, 30-C), 124.5 (4-C), 115.7 (22- $\text{CH}_{2,\text{sp}2,\text{allyl}}$), 109.1 (10-C), 97.6 (3-C), 60.7 (11- CHO_{coum}), 44.7 (14, 15- CH_2), 31.0 (18- $\text{CH}_{2,\text{sp}3,\text{allyl}}$), 21.1 (22-C), 14.2 (21-C), 12.4 (16,17- CH_3).

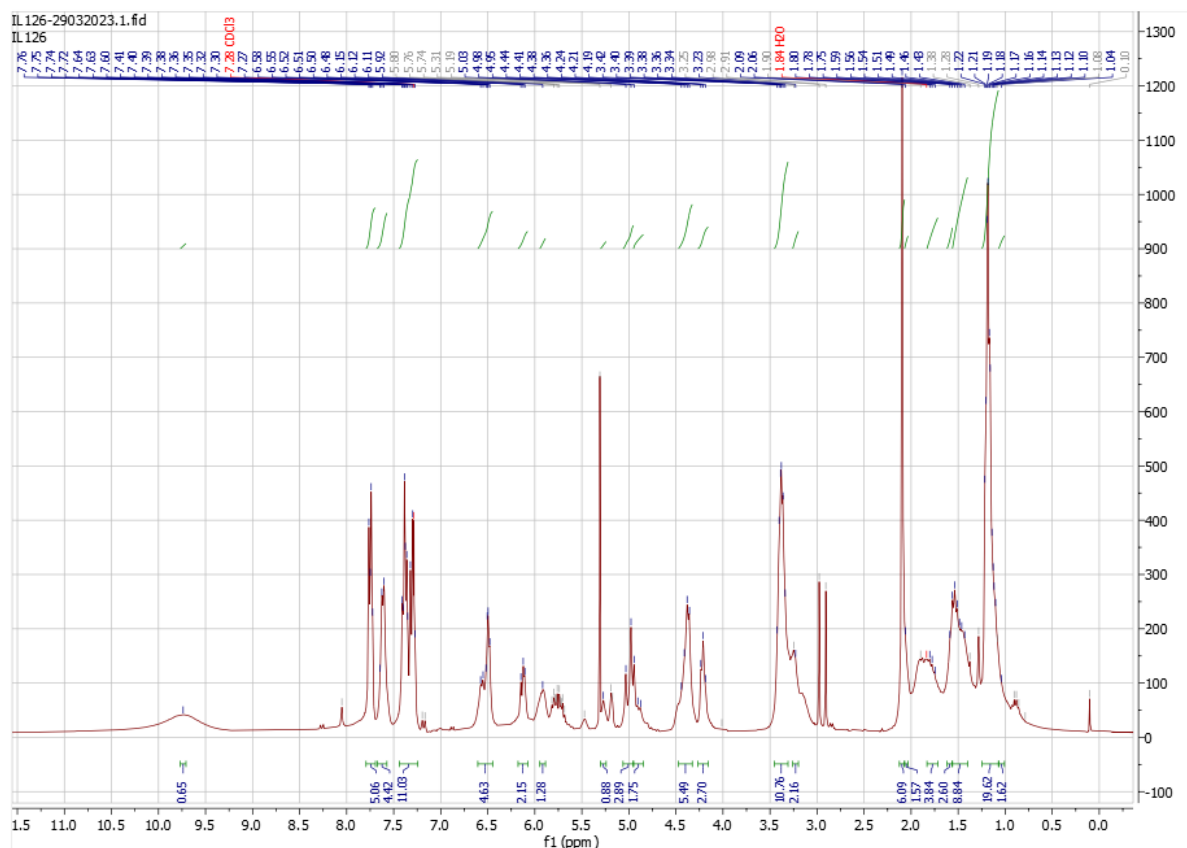


Fmoc-L-Lys(DEACM^{pentyl})-OH (7b)

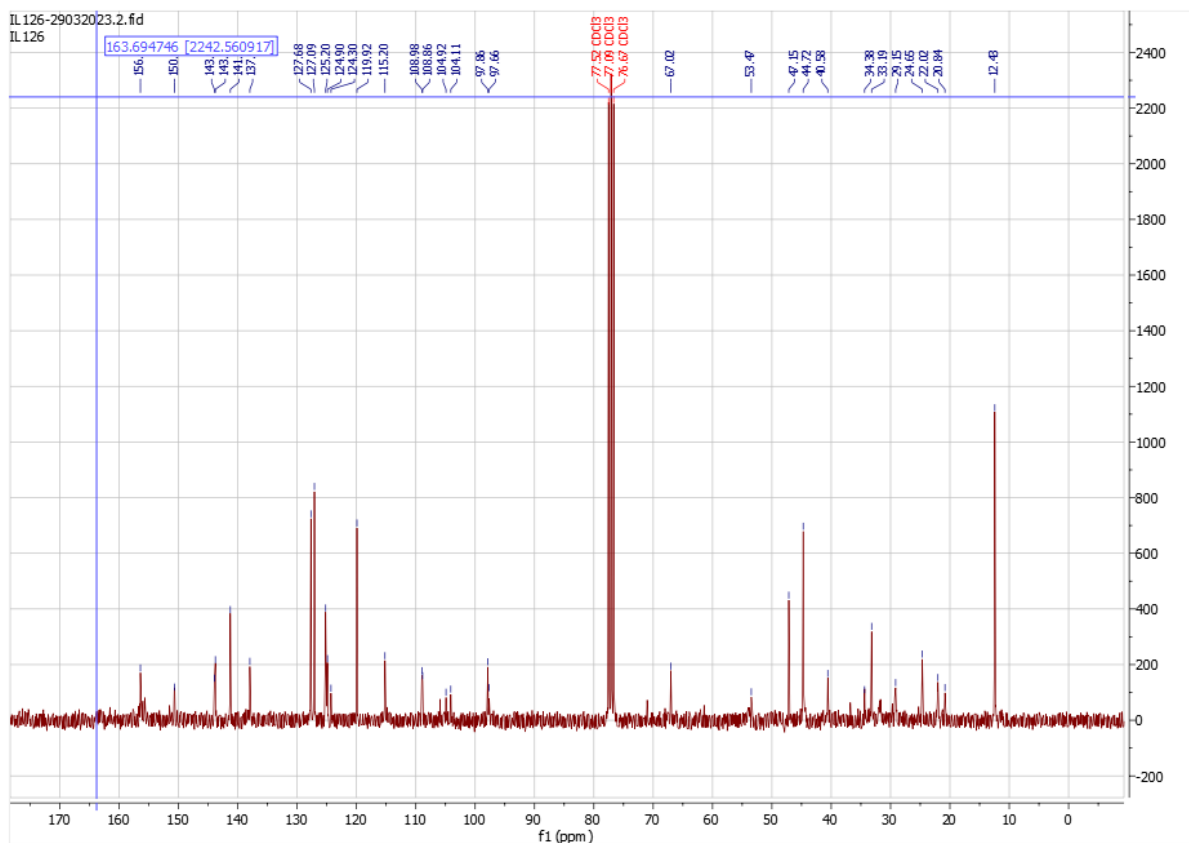


$\text{C}_{41}\text{H}_{47}\text{N}_3\text{O}_8$
709.84 g/mol
7b

¹H-NMR (300 MHz, CDCl₃): δ (ppm) = 9.74 (s, 1H, 6-OH), 7.76 (d, ³J_{HH} = 7.5 Hz, 2 H, 26, 27-CH_{ar},Fmoc), 7.64-7.59 (m, 2 H, 25, 28-CH_{ar},Fmoc), 7.44-7.35 (m, 3 H, 41-CH_{coum}, 24, 29-CH_{ar},Fmoc), 7.35-7.29 (t, ³J_{HH} = 7.5 Hz, 2 H, 23, 30-CH_{ar},Fmoc), 6.59-6.53 (m, 1 H, 42-CH_{coum}), 6.53-6.47 (m, 1 H, 44-CH_{coum}), 6.12 (s, 1 H, 40-CH_{coum}), 5.94-5.82 (m, 1 H, 51-CHO_{coum}), 5.82-5.69 (m, 1 H, 34-CH_{sp2,allyl}), 5.06-4.94 (m, 2 H, 35-CH_{2,sp2,allyl}), 4.55-4.29 (m, 3 H, 2-CH, 17-CH₂,Fmoc), 4.27-4.17 (m, 1 H, 18-CH_{sp3},Fmoc), 3.42 (q, ³J_{HH} = 7.0 Hz, 4 H, 47, 48-CH₂), 3.33-3.08 (m, 2 H, 8-CH₂), 2.02-1.75 (m, 2 H, 32-CH_{2,sp3,allyl}), 2.07 (m, 2 H, 3-CH₂), 1.67-1.32 (m, 4 H, 9-CH₂, 10-CH₂), 1.26 (t, ³J_{HH} = 7.2 Hz, 6 H, 49, 50-CH₃).



^{13}C -NMR (100 MHz, CDCl_3): δ (ppm) = 156.4 (12- $\text{CO}_{\text{coum-carbamate}}$), 150.7 (43- C_{coum}), 144.0 (7- $\text{CO}_{\text{Fmoc-carbamate}}$), 143.8 (44- CH_{coum}), 141.3 (36- CH_{coum}), 137.9 (35- $\text{CH}_{\text{sp}^2,\text{allyl}}$), 127.7 (23, 30- $\text{CH}_{\text{ar,Fmoc}}$), 127.1 (24, 29- $\text{CH}_{\text{ar,Fmoc}}$), 125.2 (37- CH_{coum}), 124.9 (25, 28- $\text{CH}_{\text{ar,Fmoc}}$), 119.9 (26, 27- $\text{CH}_{\text{ar,Fmoc}}$), 115.2 (35- $\text{CH}_{2,\text{sp}^2,\text{allyl}}$), 109.0 (40- CH_{coum}), 108.9 (42- CH_{coum}), 104.9 (33- CH_{coum}), 97.9 (44- CH_{coum}), 97.7 (31- CHO_{coum}), 67.0 (17- $\text{CH}_{2,\text{Fmoc}}$), 53.5 (2- CH), 47.2 (18- CH), 44.7 (47, 48- CH_2), 40.6 (32- CH_2), 34.4 (10- CH_2), 33.2 (3- CH_2), 29.2 (8- CH_2), 24.7 (52- CH_2), 22.0 (9- CH_2), 20.8 (34- CH_2), 12.4 (49, 50- CH_3).



Section 3. Peptide Synthesis

Solid Phase Peptide Synthesis (SPPS)

N-Terminal Acylation

SOP1: To obtain an uncharged *N*-terminus the resin bound peptide was treated with the acylation cocktail (10% Ac₂O, 5% DIPEA in DMF) for 10 min at room temperature. The process was repeated twice.

On Resin Metathesis

SOP2: Macrocyclization of the *N*-terminally protected or acylated peptides was performed on resin. After SPPS the resin was washed successively with DMF (5 x), MeOH (5 x), and DCM (5 x) and dried in a desiccator. The resin was placed in a BD syringe and the catalyst (0.25 mg/ mg of resin) was added. The syringe was immediately afterwards quenched with argon. Degassed DCE (0.04 mL/ mg resin) was added and the mixture was shaken at 50 °C for 2 h. The resin was filtered off and the process was repeated with fresh catalyst and solvent to complete the reaction. After filtration and thorough washing of the resin with DCE (10 x) and DMF (10 x).

Cleavage

SOP3: After synthesis, the resin was filtered off, washed successively with DMF (5 x), MeOH (5 x) and DCM (10 x) and dried *in vacuo*. Acidic cleavage from the resin was achieved by treatment with a mixture of a) trifluoroacetic acid (TFA) / triisopropylsilane / water (95:2.5:2.5, 4 mL, 2 h, cleavage) for sequences without cysteine or b) trifluoroacetic acid (TFA) / triisopropylsilane / ethanedithiol / water (94:2.5:2.5:1, 4 mL, 2 h) for cysteine containing peptides. The resin was extracted with additional TFA (4 mL) and the combined extracts were concentrated to 2 mL under a flow of nitrogen. The crude peptide was then precipitated in cold diethyl ether (10 mL) and isolated by centrifugation and decantation of the supernatant. The precipitate was washed twice with ice-cold diethyl ether and subsequently lyophilized.

For test cleavage usually a small spatula-tipfull of resin was placed in a 2 mL BD syringe and treated as described above only with adjusted volumes (1 mL cleavage cocktail, 1.5 mL diethyl ether). After lyophilization the peptide was analyzed by UPLC and mass spectrometry.

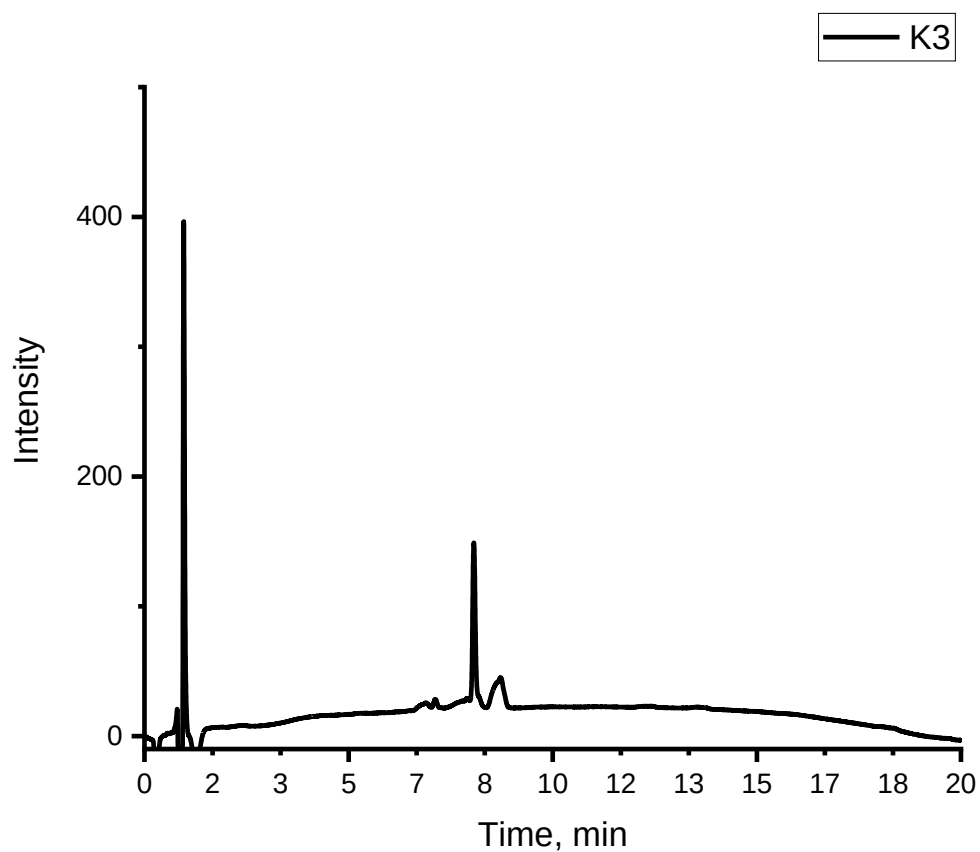
Synthesized Peptides

K3 (8)



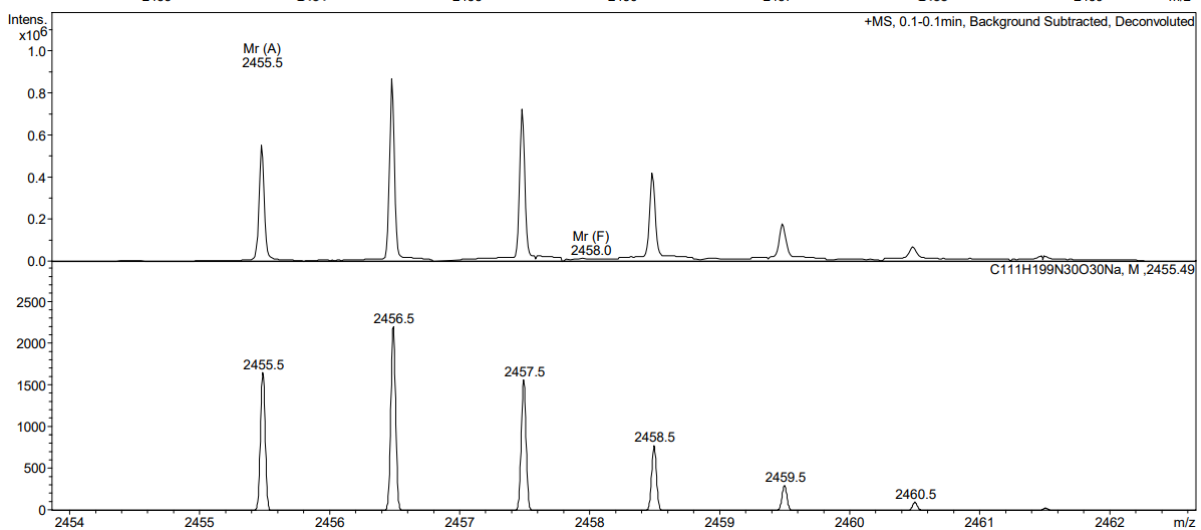
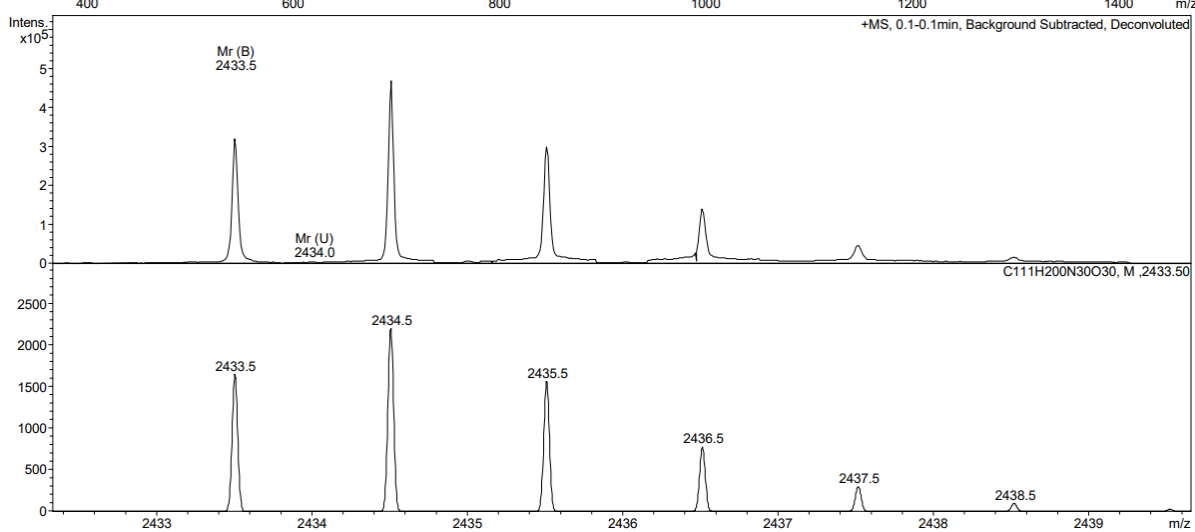
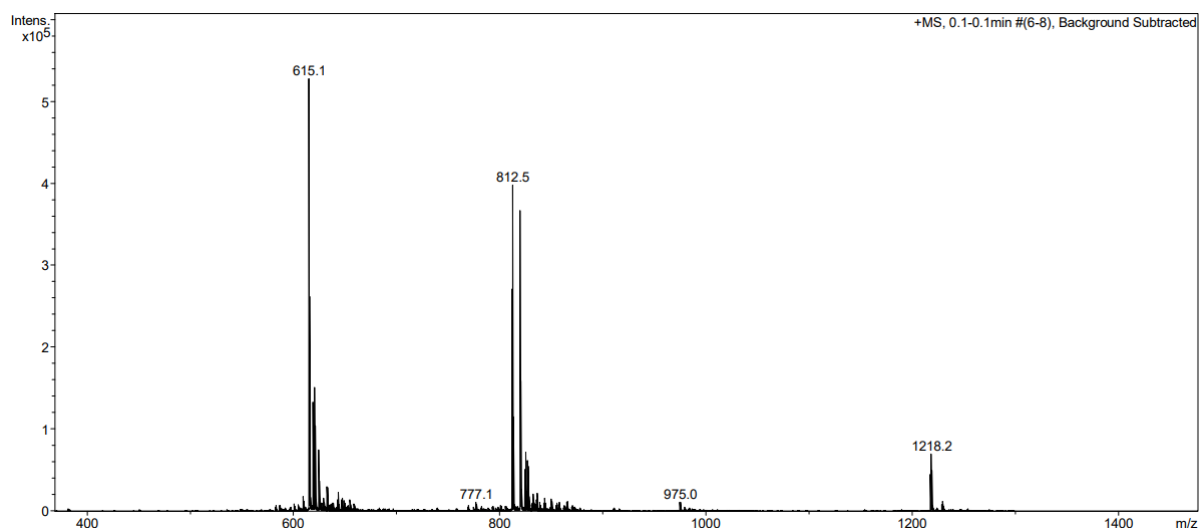
The peptide was synthesized at a 50 μmolar scale on Rink Amide resin by automated SPPS following the protocol previously described. The peptide was then capped using **SOP6**. After cleavage (**SOP7**) and lyophilization the crude product was purified by semi-preparative HPLC utilizing column 2 with a linear gradient of (40-60% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 13% yield.

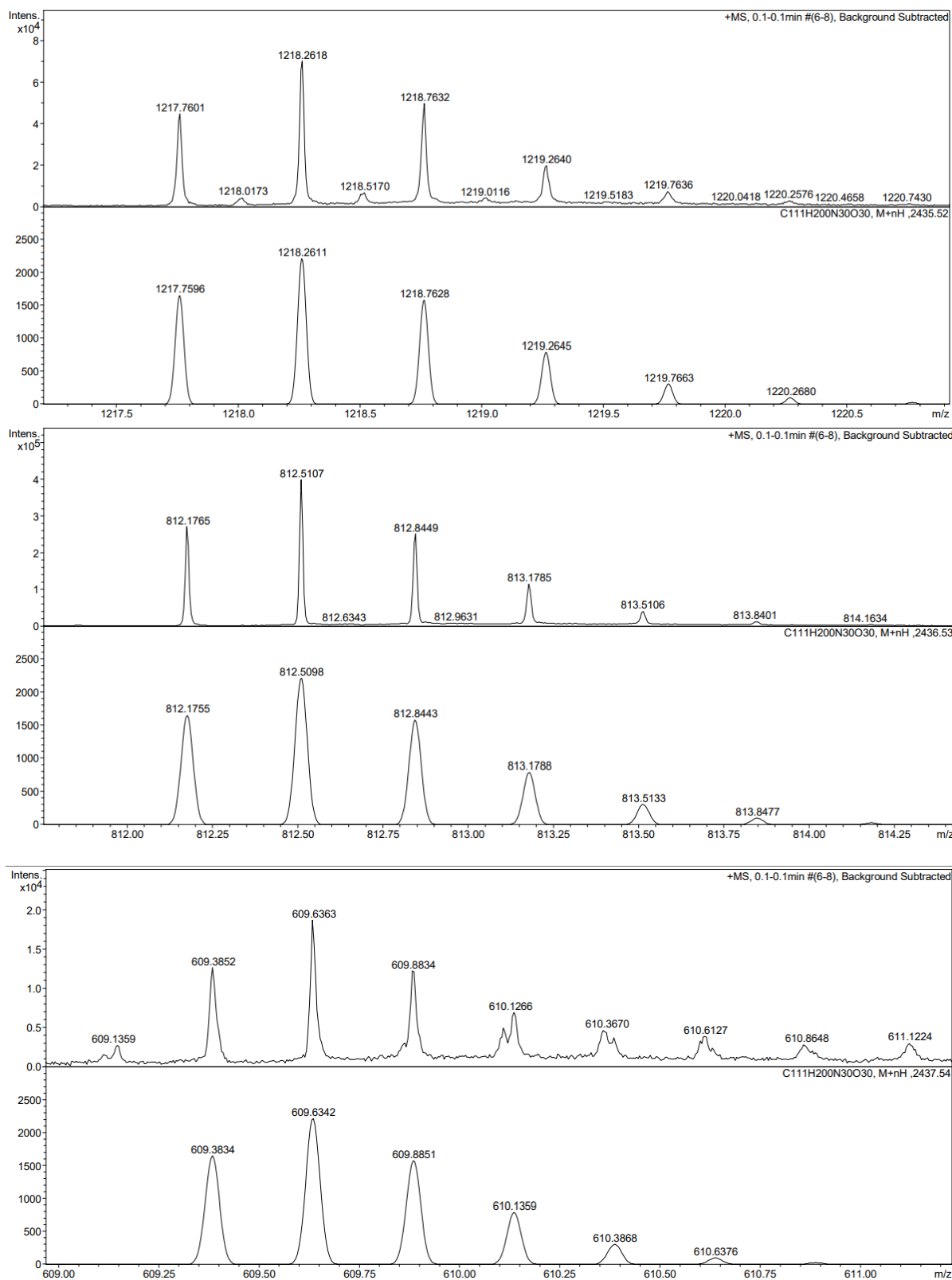
UPLC (column 1, 20-80% in 15 min, detection at 215 nm) : R_t = 8.07 min.



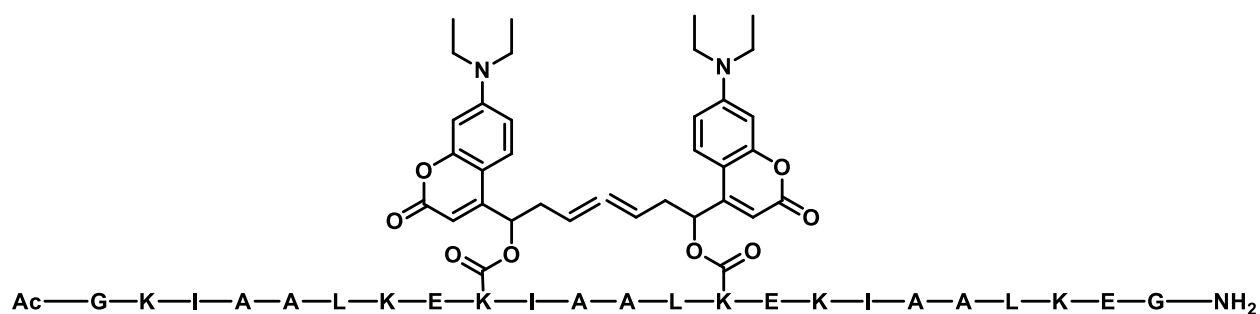
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.



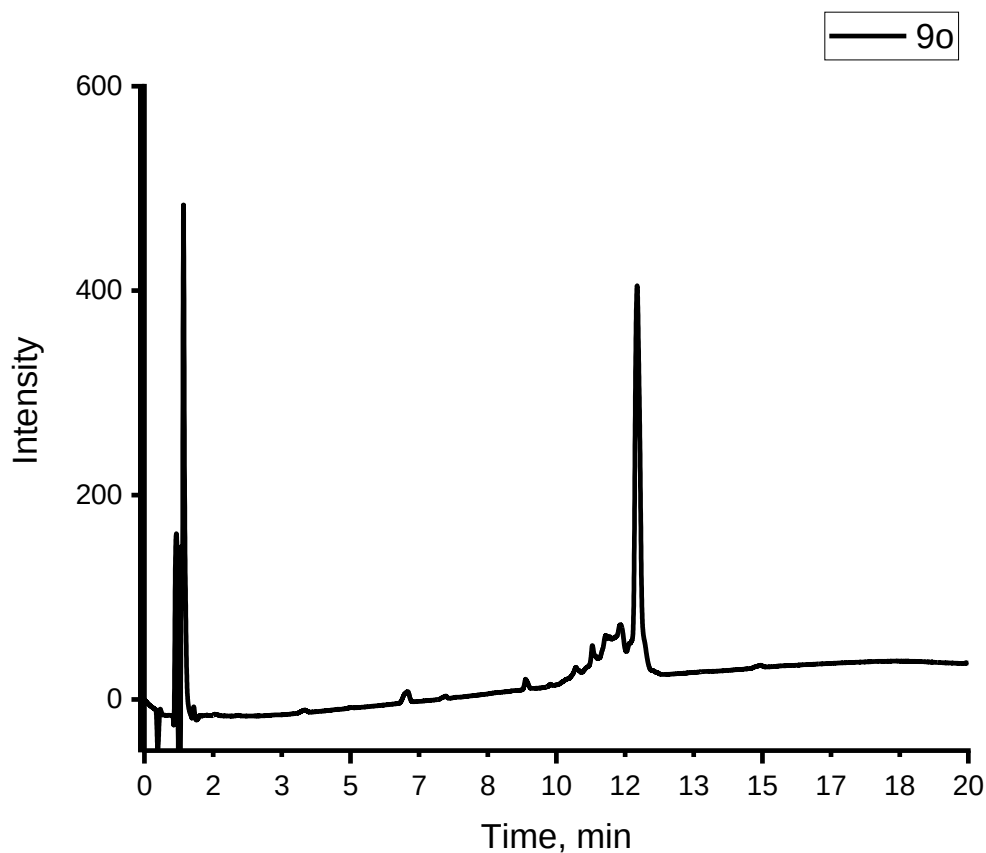


9o



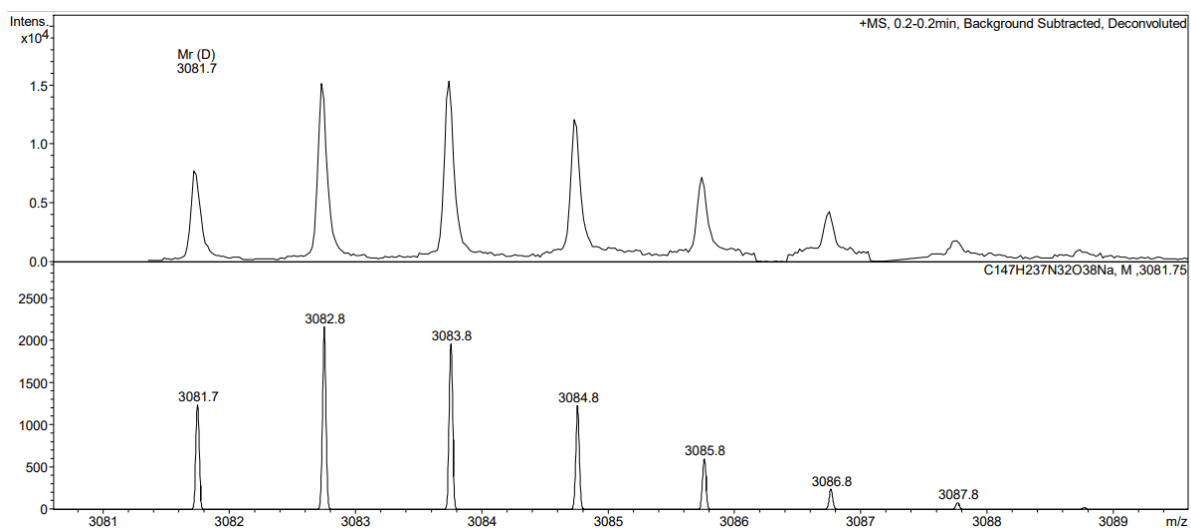
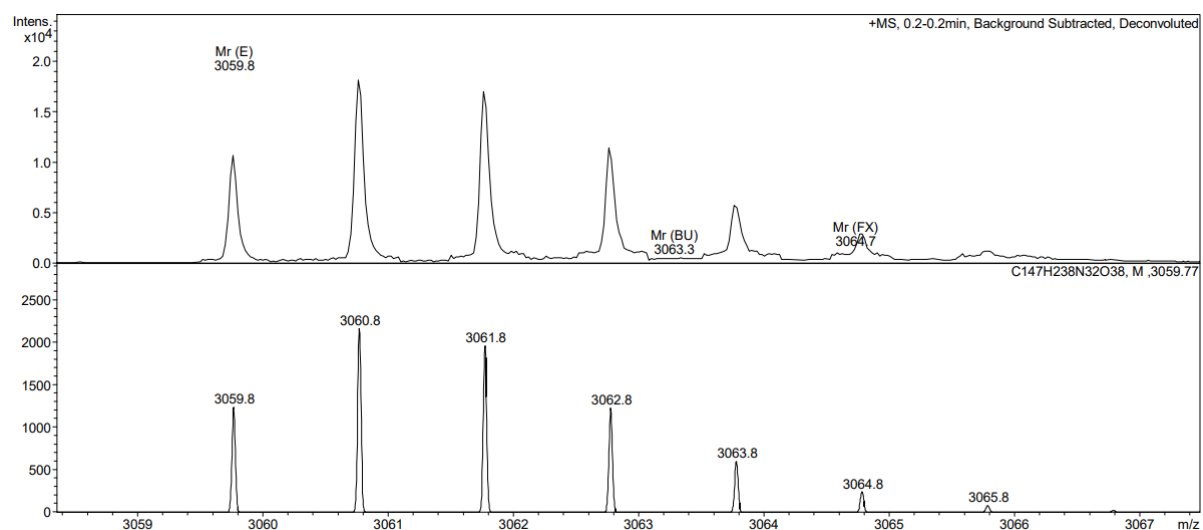
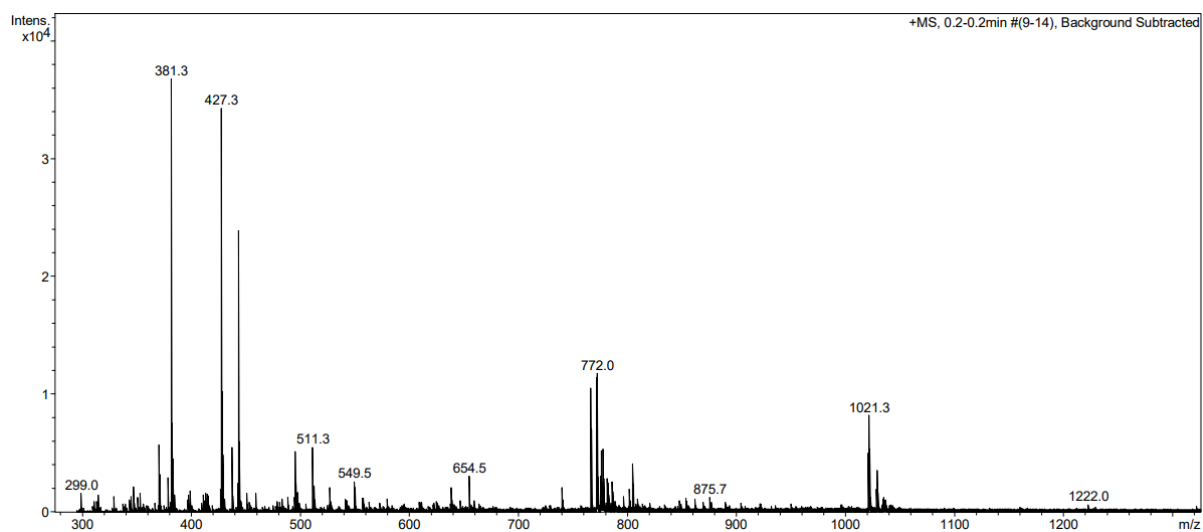
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines (**7a**) were coupled by manual SPPS (**SOP3**). The coupling of coumarin modified Lysines (**7a**) was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP5**). The peptide was cleaved (**SOP7**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient of eluent system (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 22% yield.

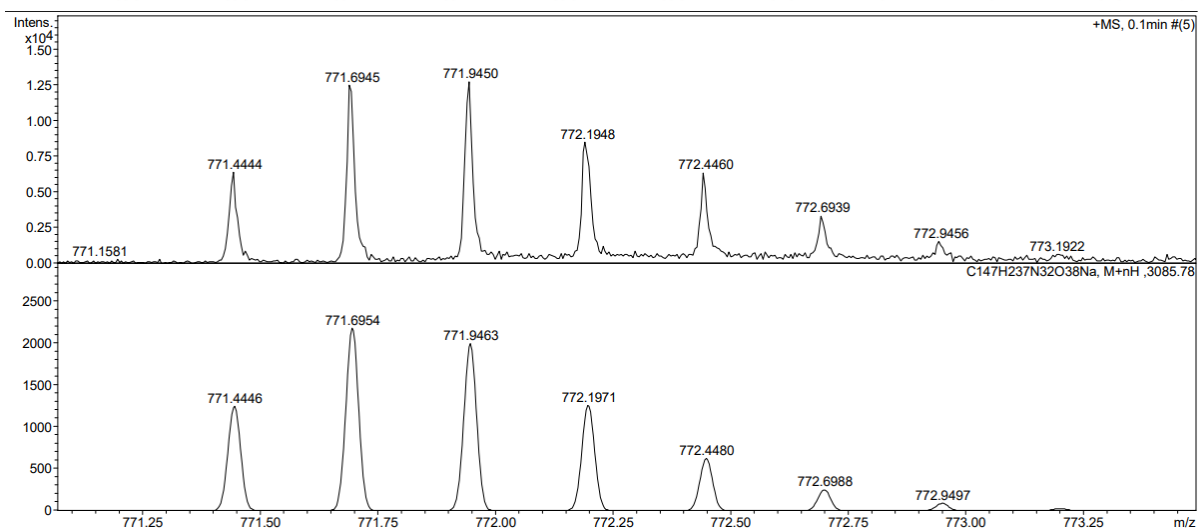
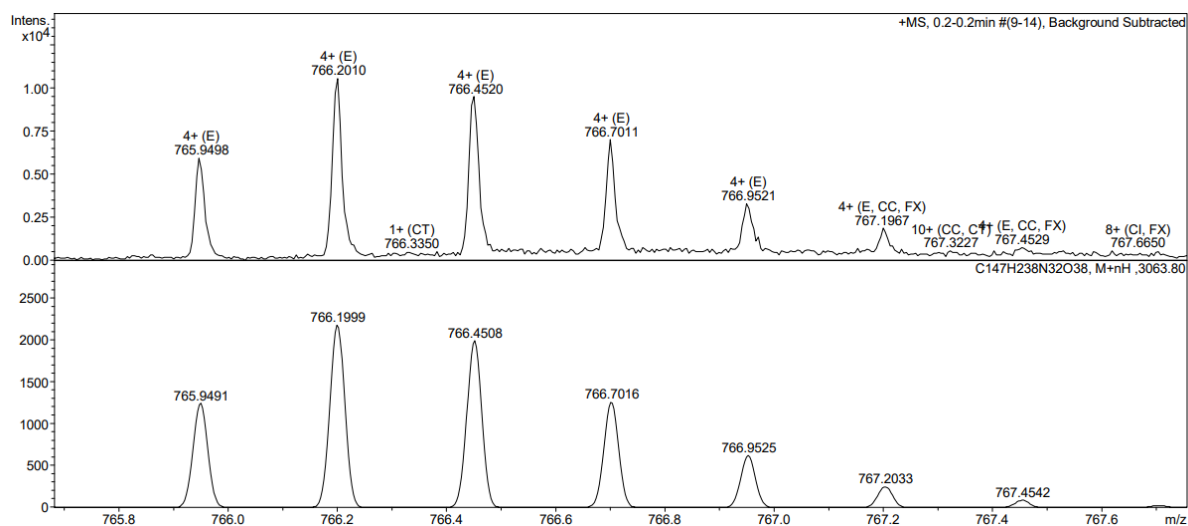
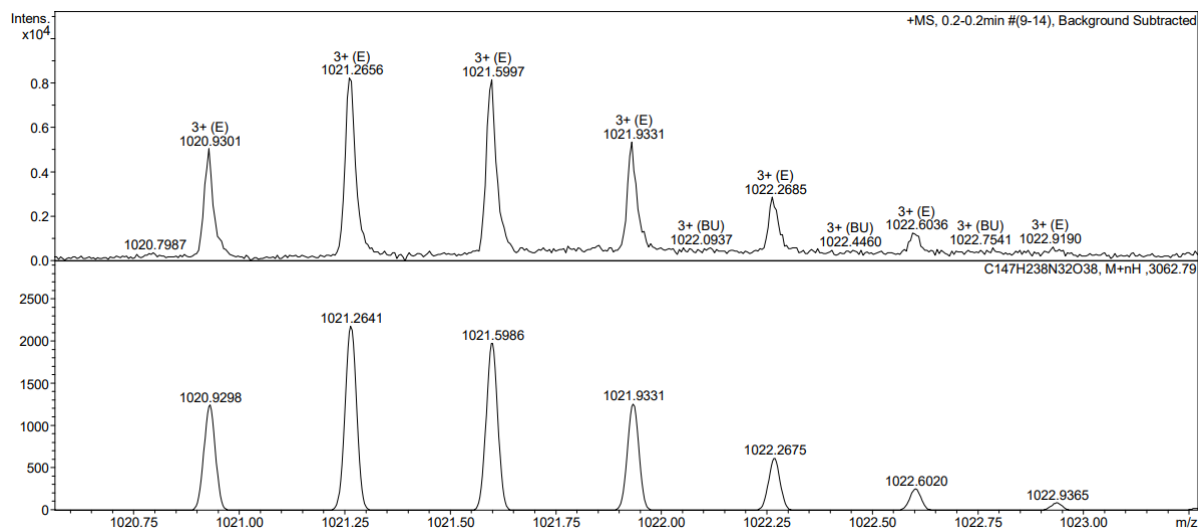
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 12.83 min.

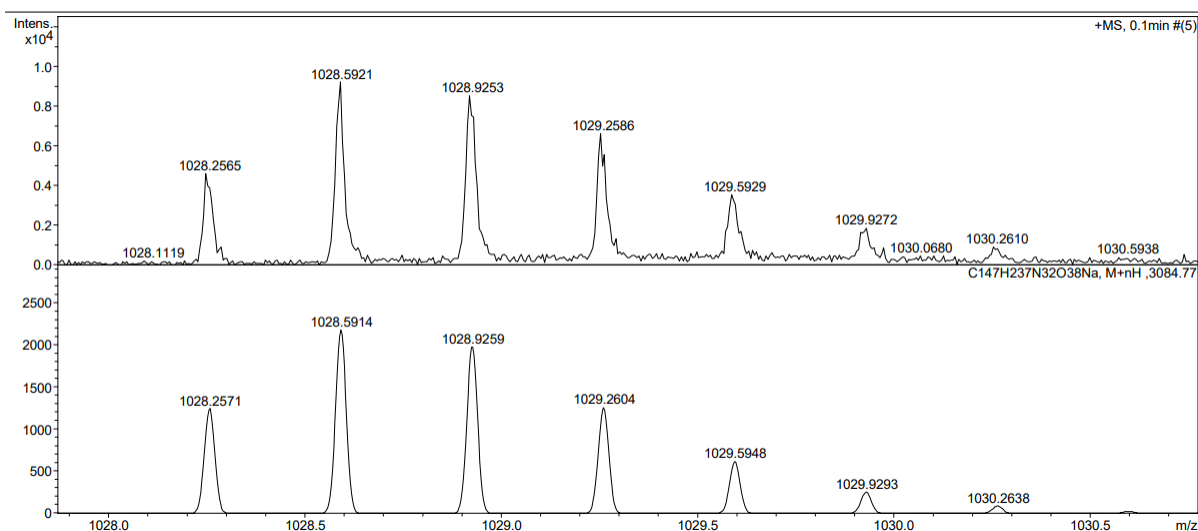


ESI-MS m/z : 3060.8 $[M+H]^+$.

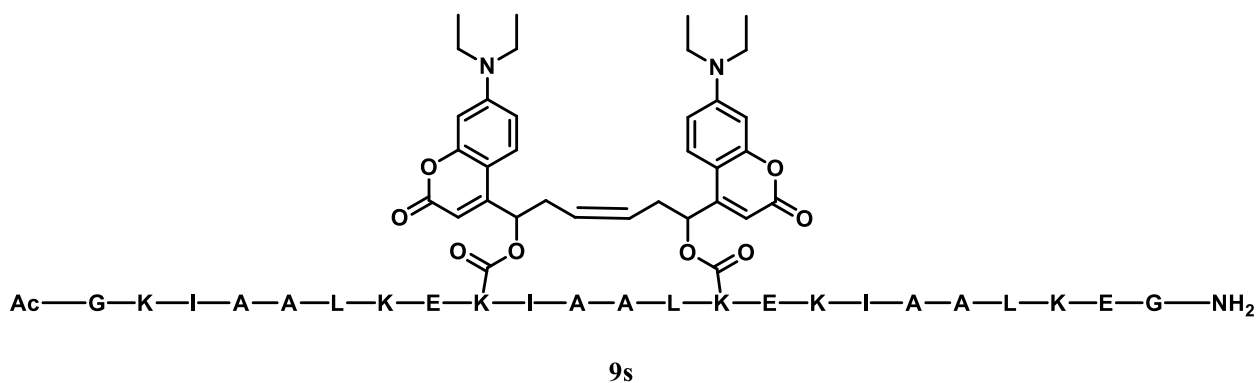
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9478.





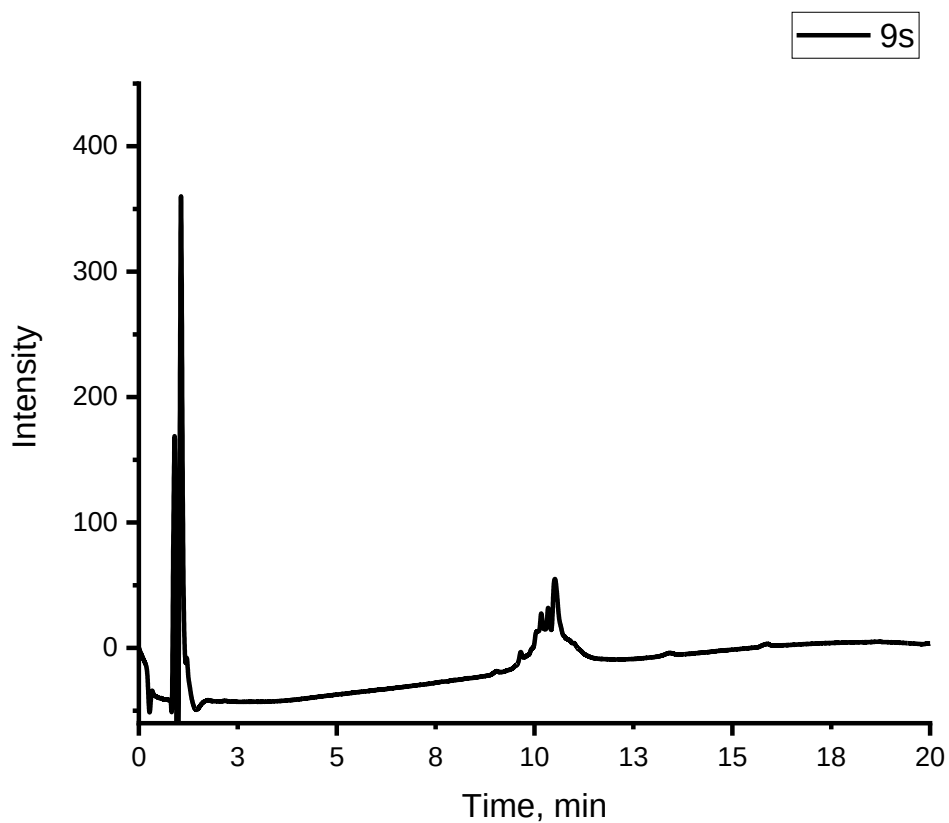


9s



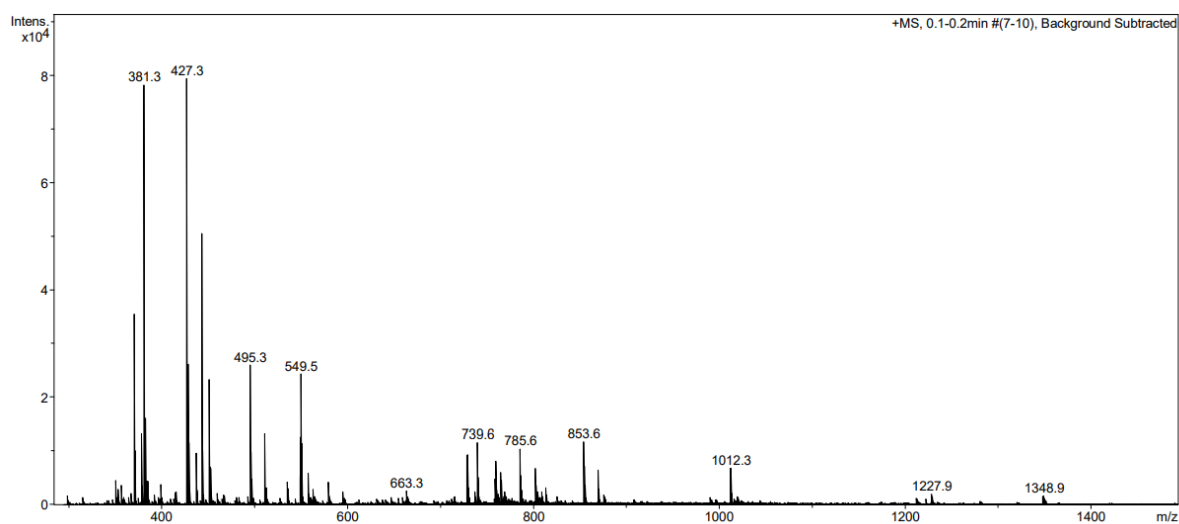
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines (**7a**) were coupled by manual SPPS. The coupling of coumarin modified Lysines (**7a**) was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalysed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 4% yield.

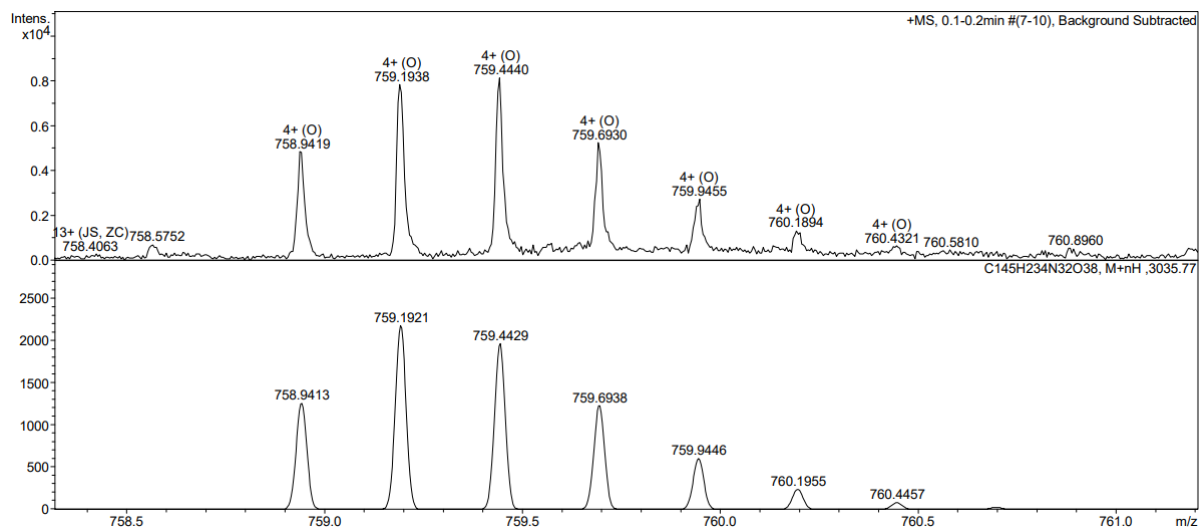
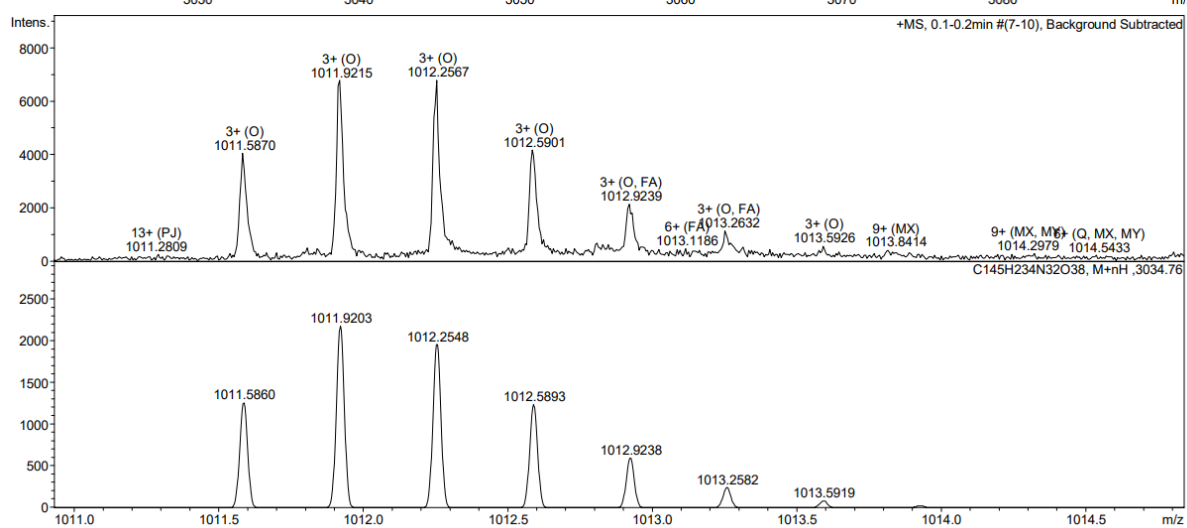
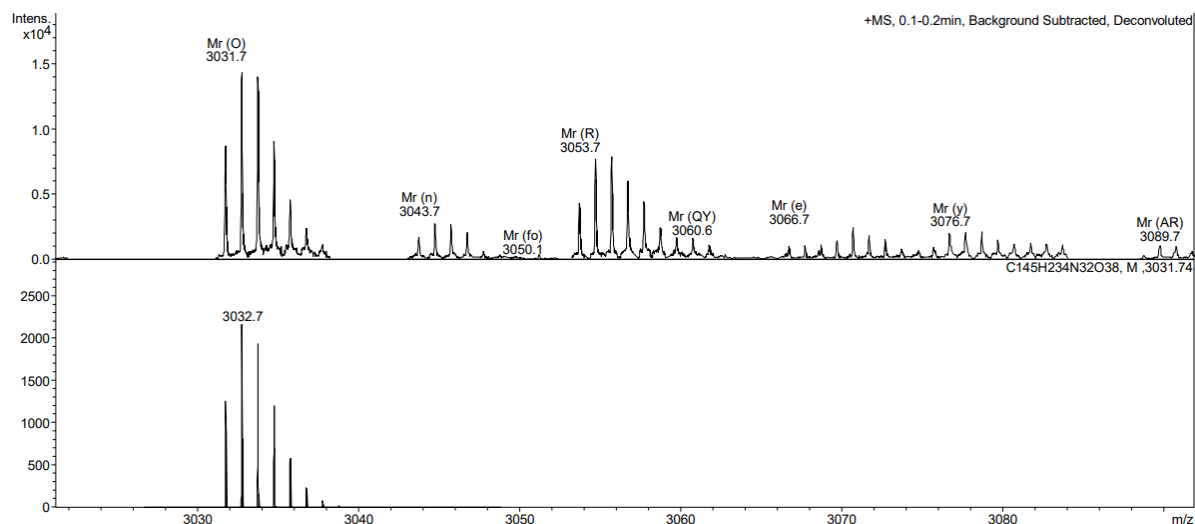
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 10.6, 10.7, 11.0 min.

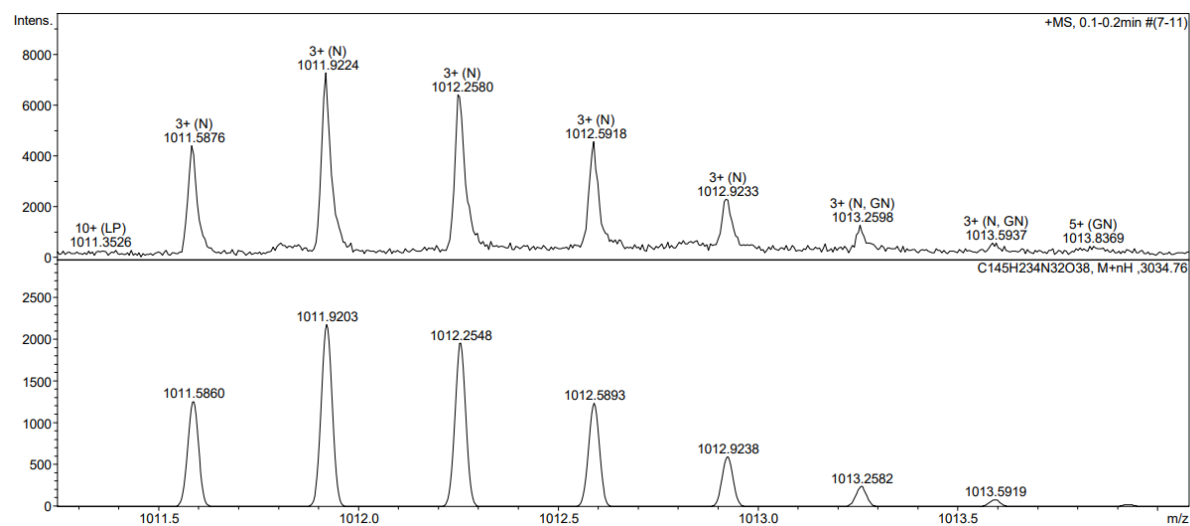
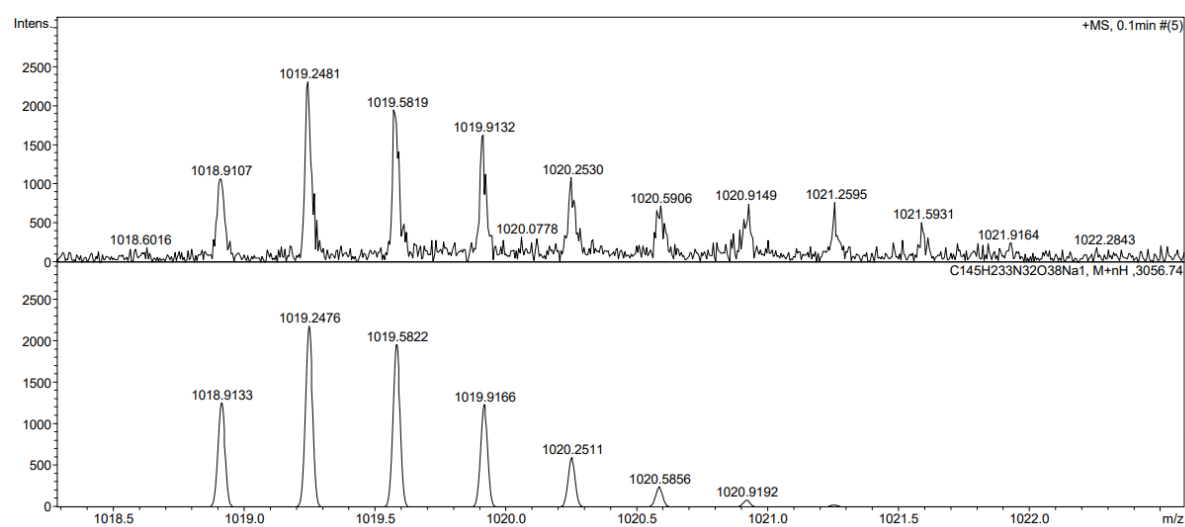
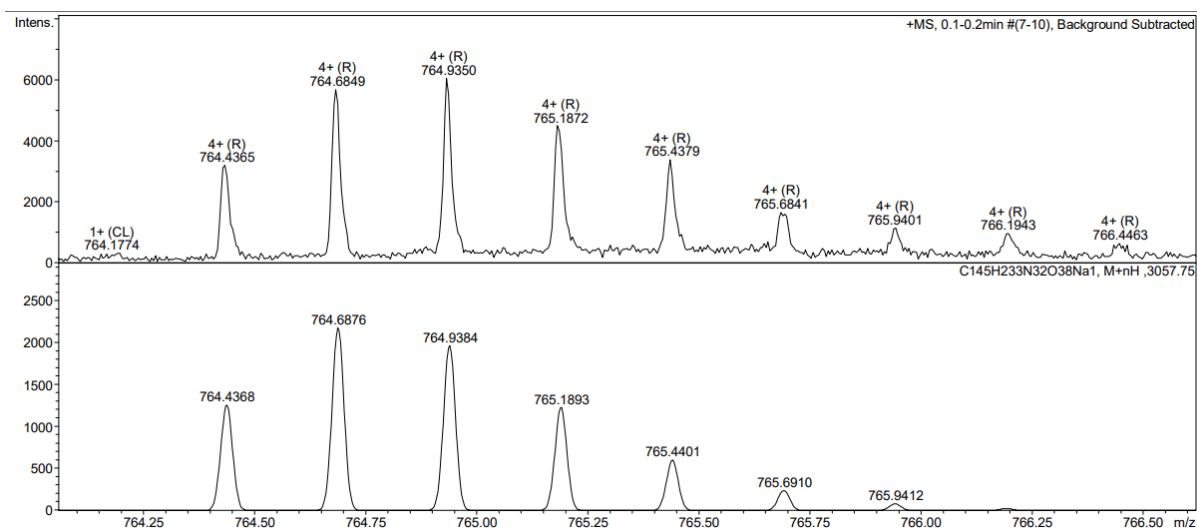


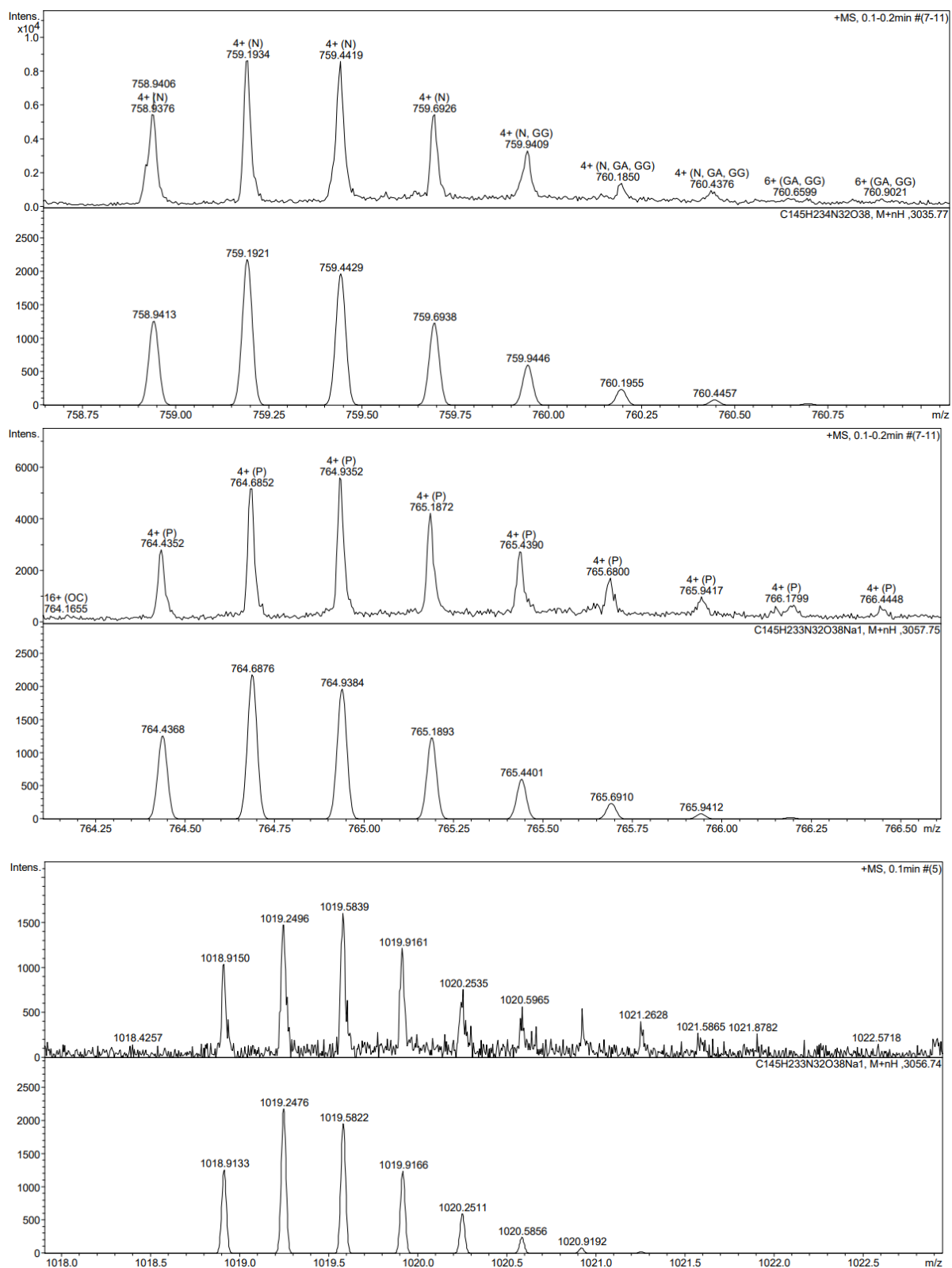
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.

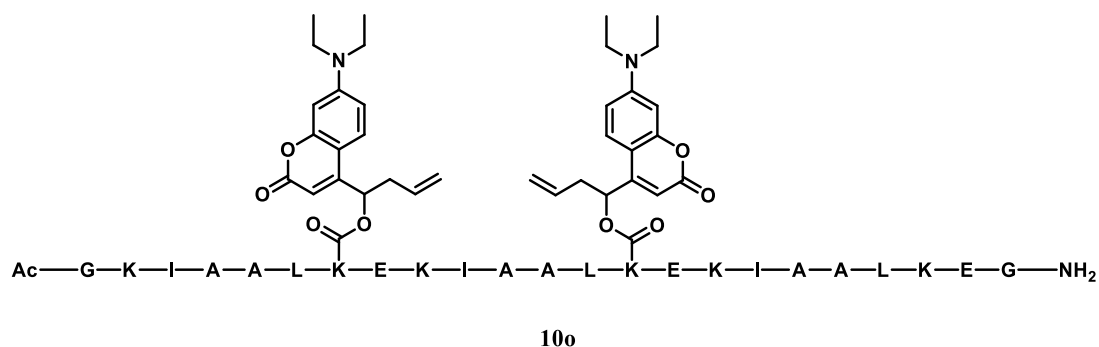






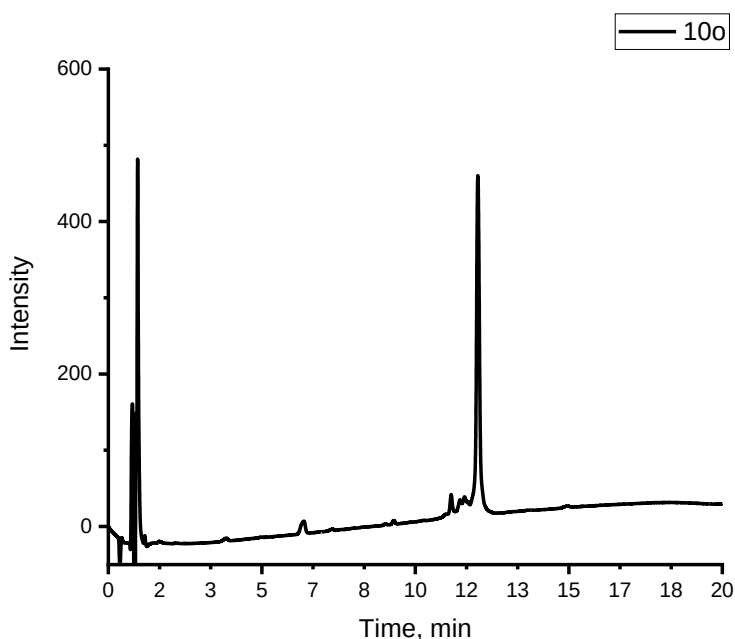


10o



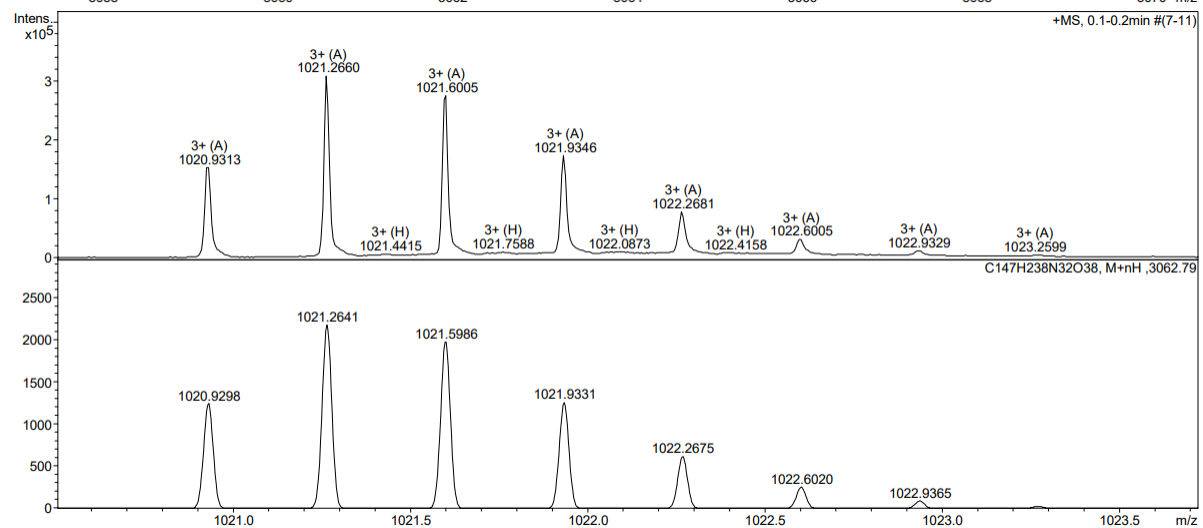
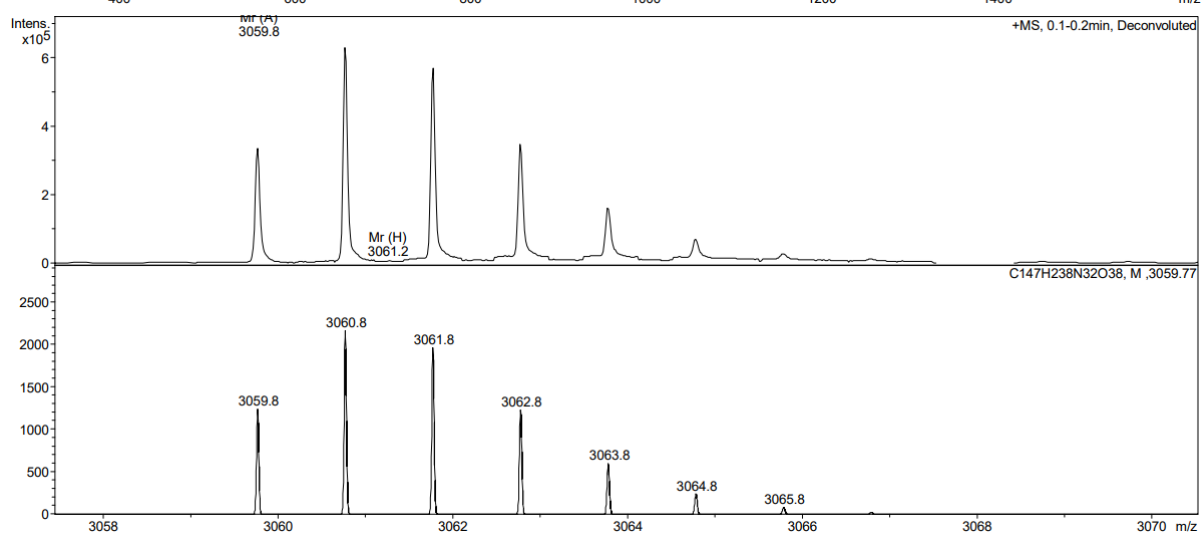
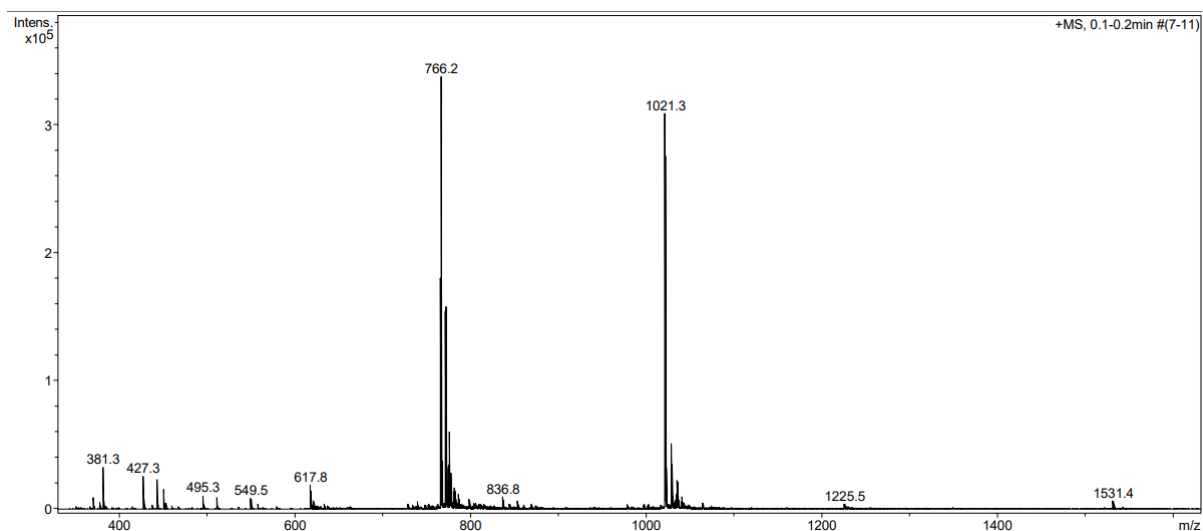
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines (**7a**) were coupled by manual SPPS. The coupling of coumarin modified Lysines (**7a**) was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalysed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 25% yield.

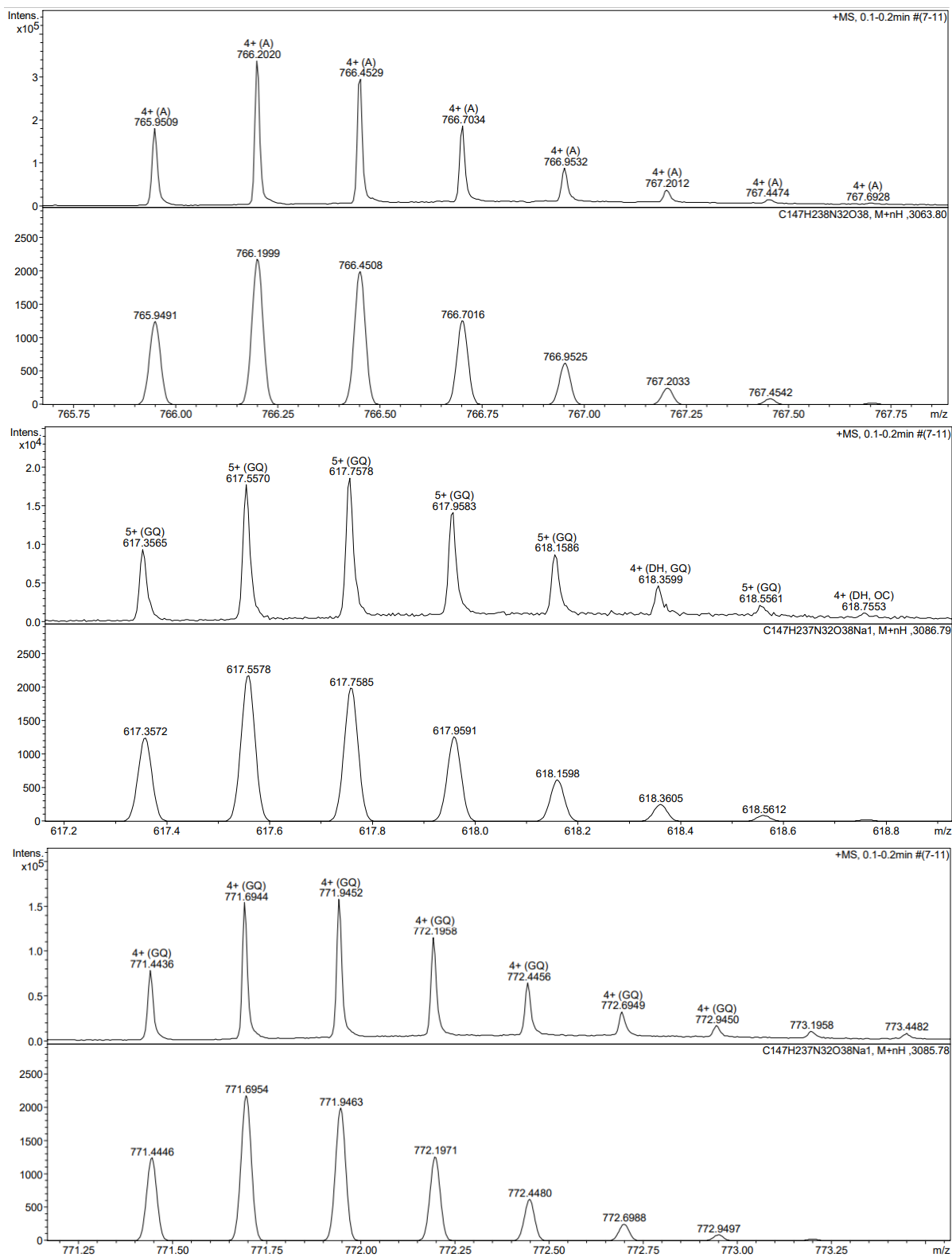
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): $R_t = 12.25$ min.

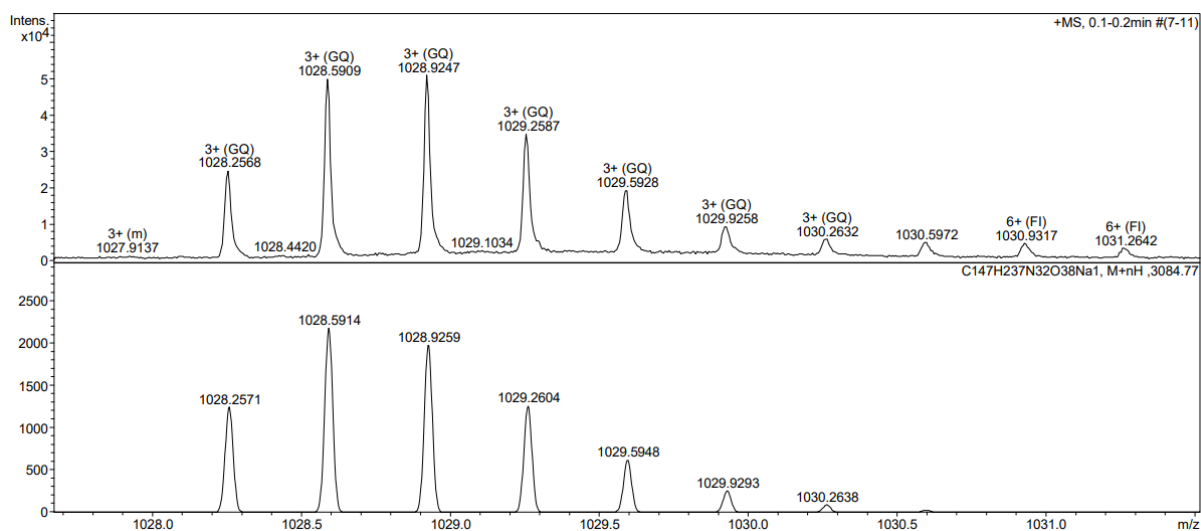


ESI-MS m/z : 3060.8 $[M+H]^+$.

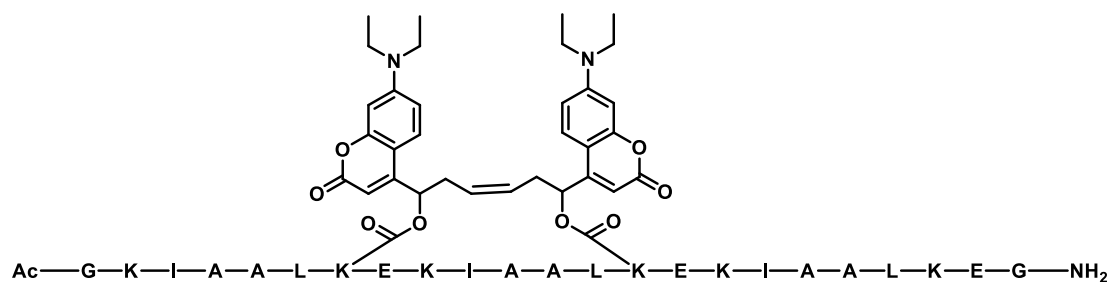
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491.







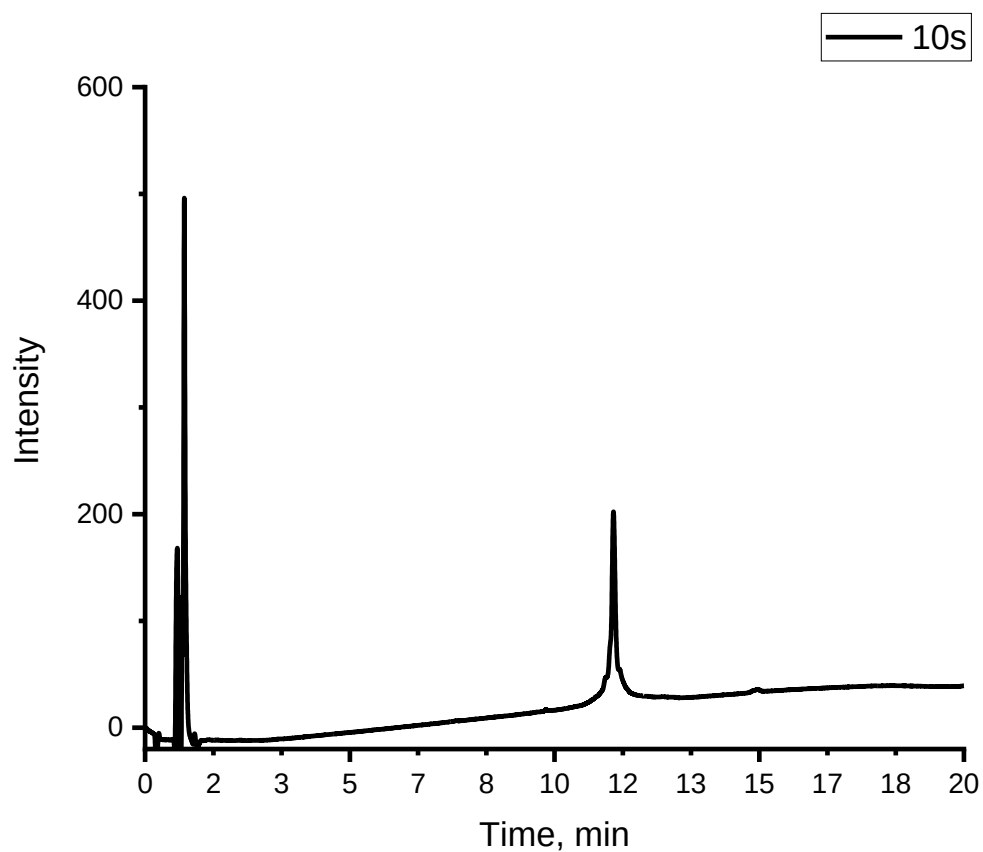
10s



10s

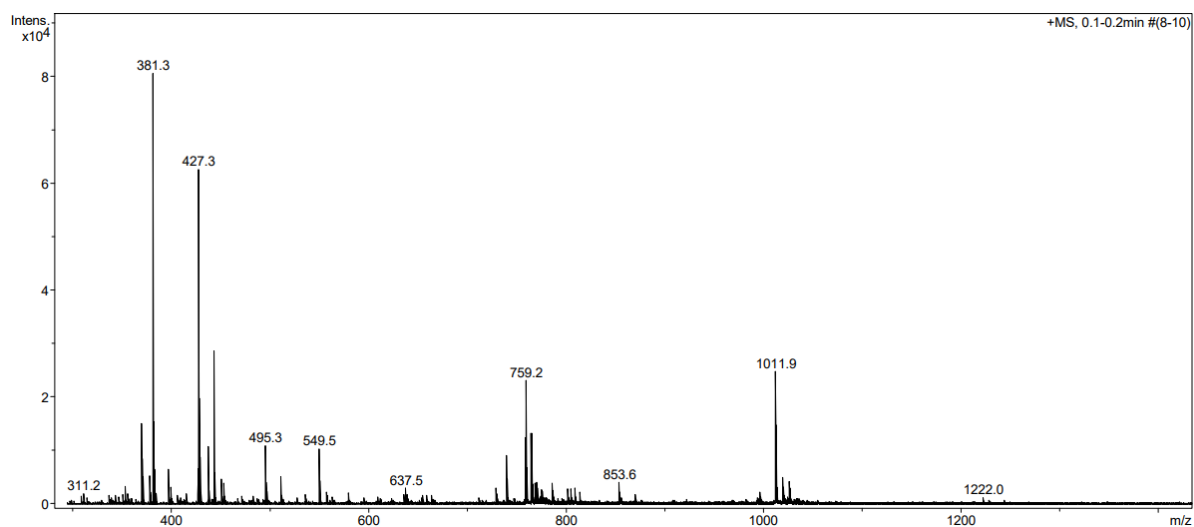
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines (**7a**) were coupled by manual SPPS. The coupling of coumarin modified Lysines (**7a**) was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalysed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 12% yield.

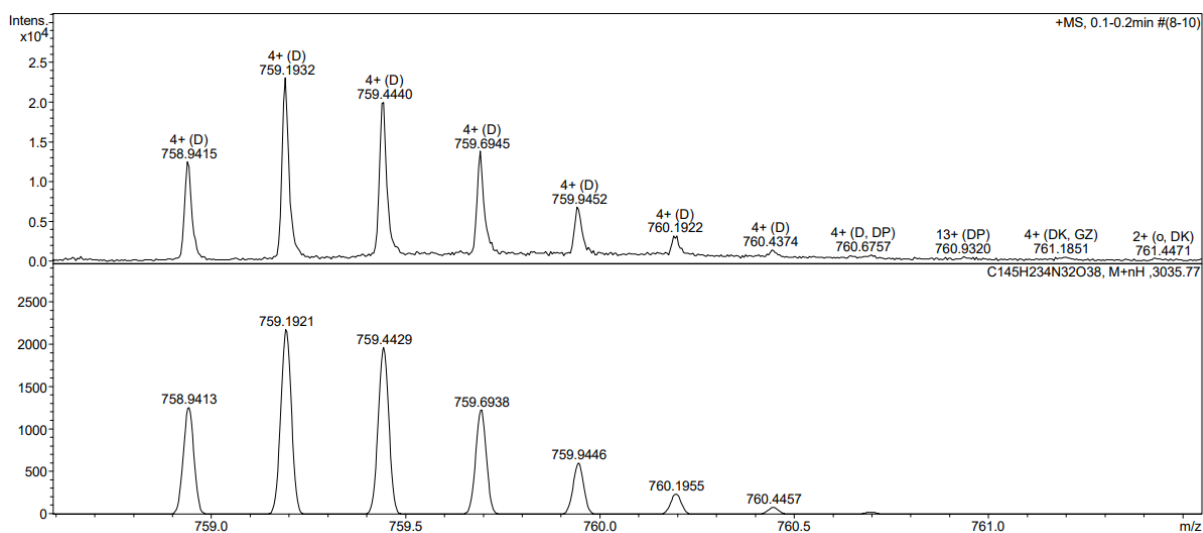
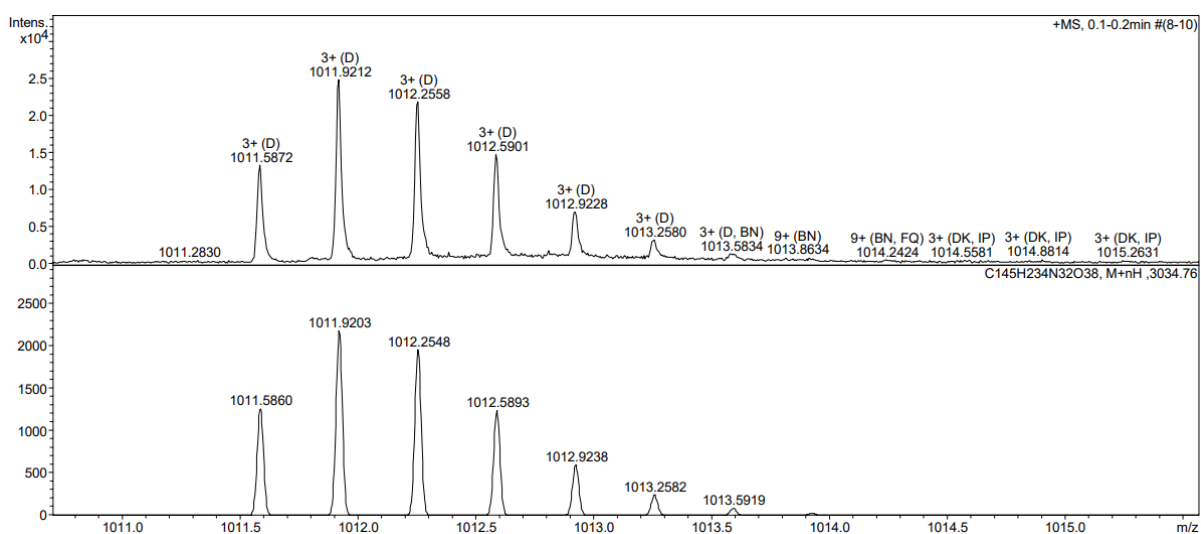
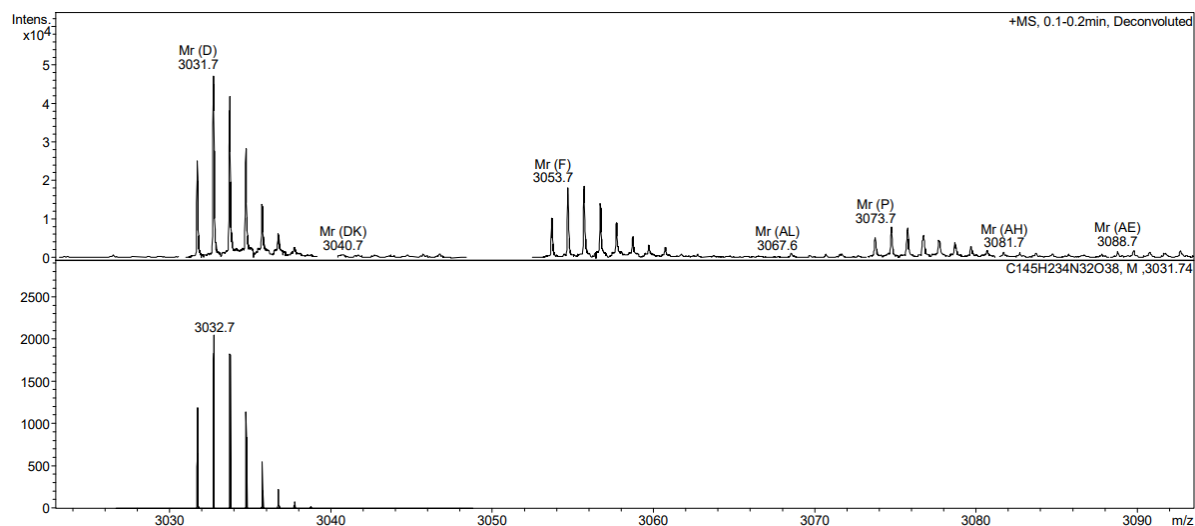
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 11.9 min.

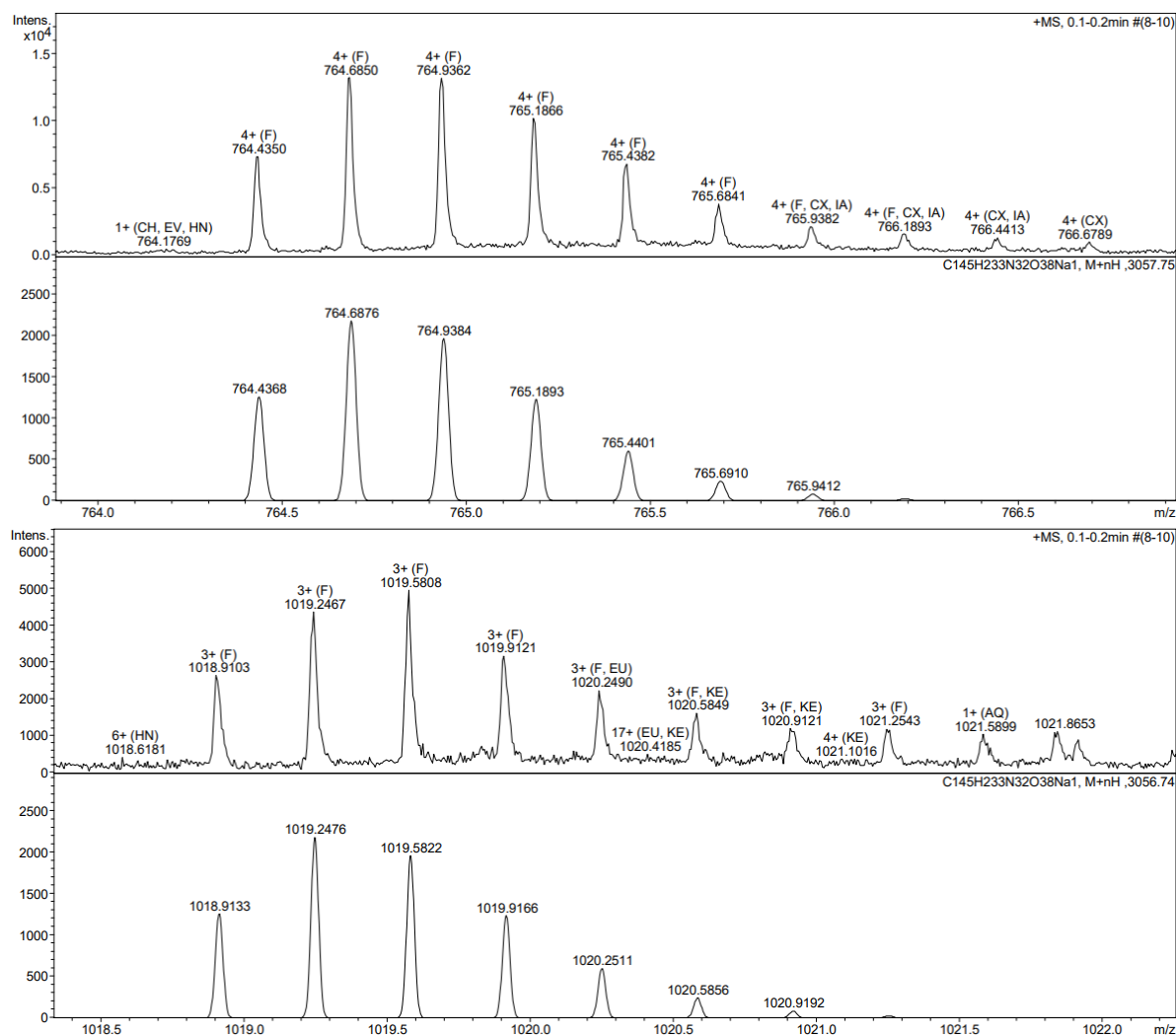


ESI-MS m/z : 3031.7 $[M+H]^+$.

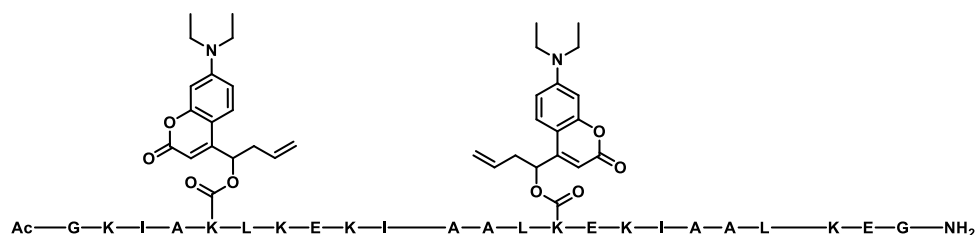
HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.







11o

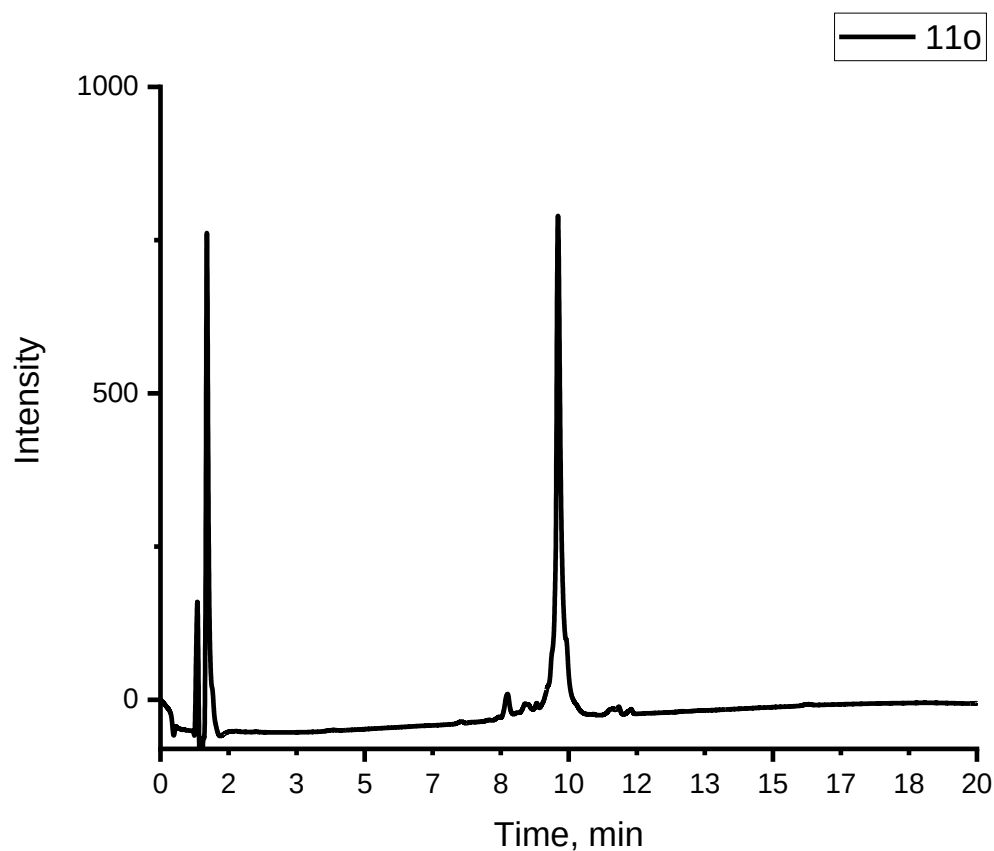


11o

The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines (**7a**) were coupled by manual SPPS. The coupling of coumarin modified Lysines (**7a**) was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalysed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing

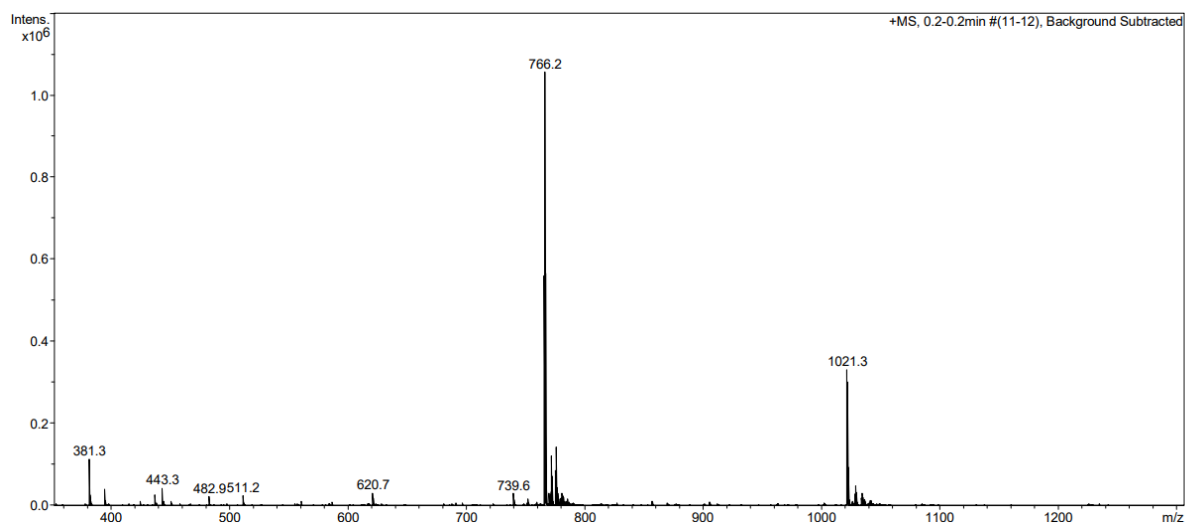
column 3 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 3% yield.

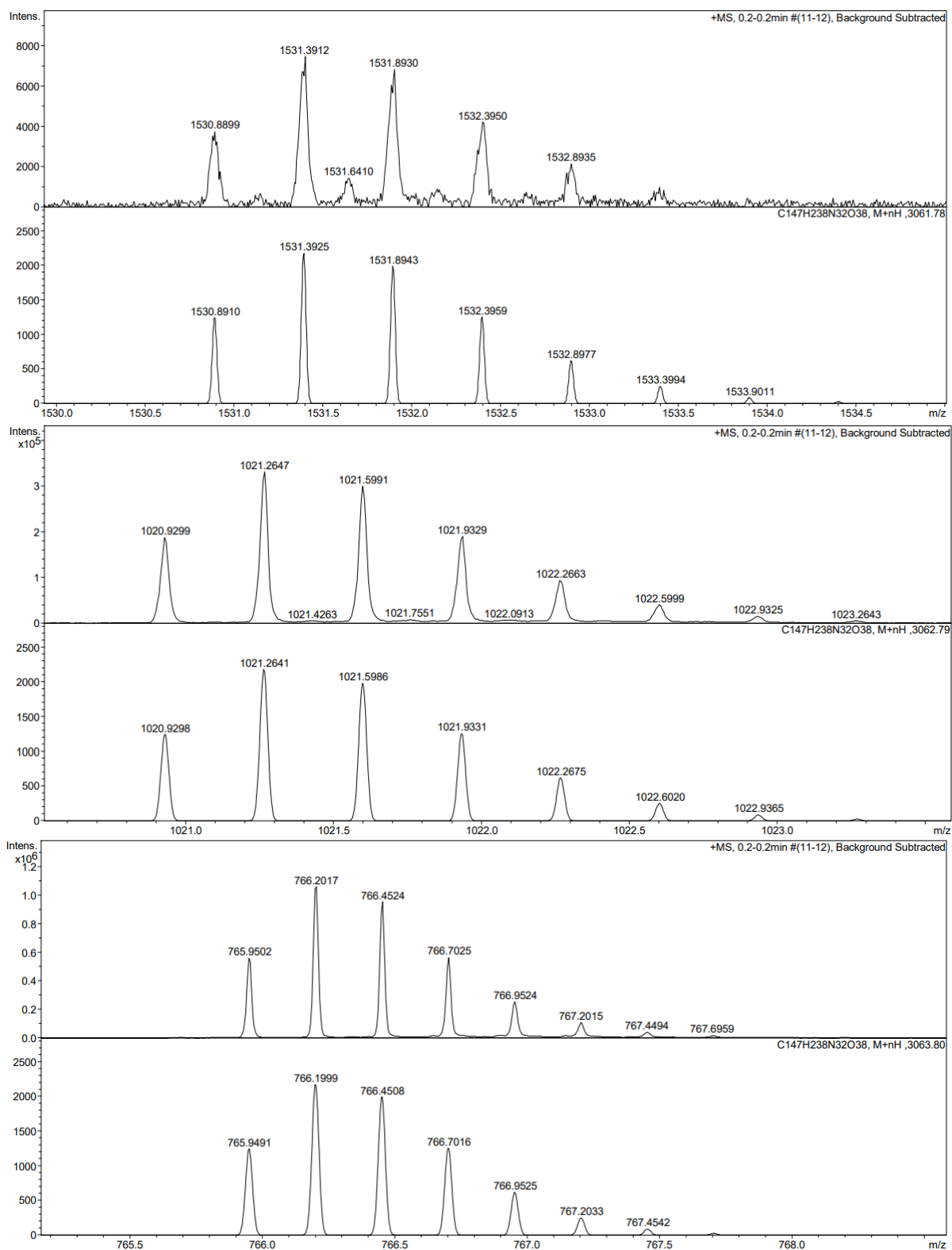
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): $R_t = 9.75$ min.

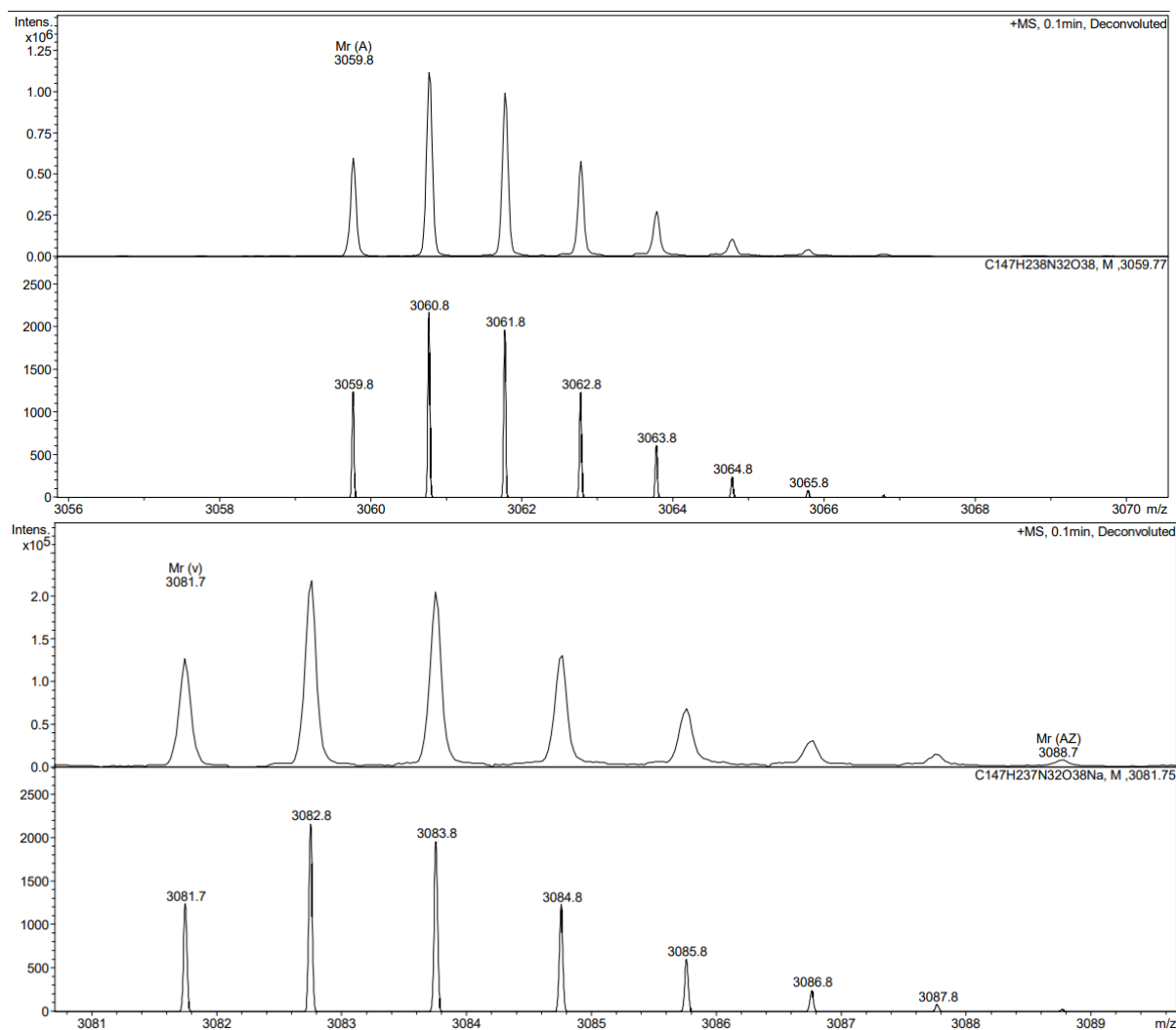


ESI-MS m/z : 3060.8 $[M+H]^+$.

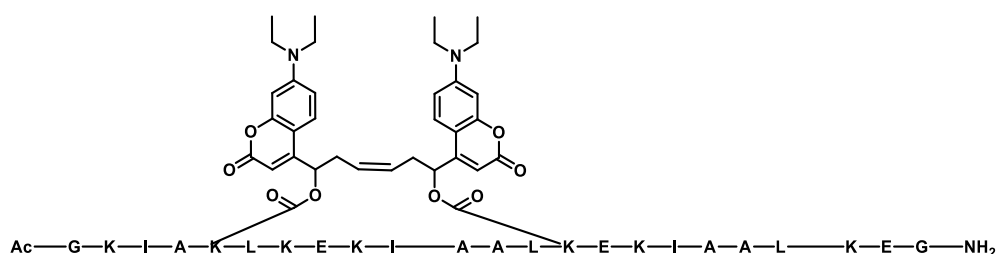
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491.







11s

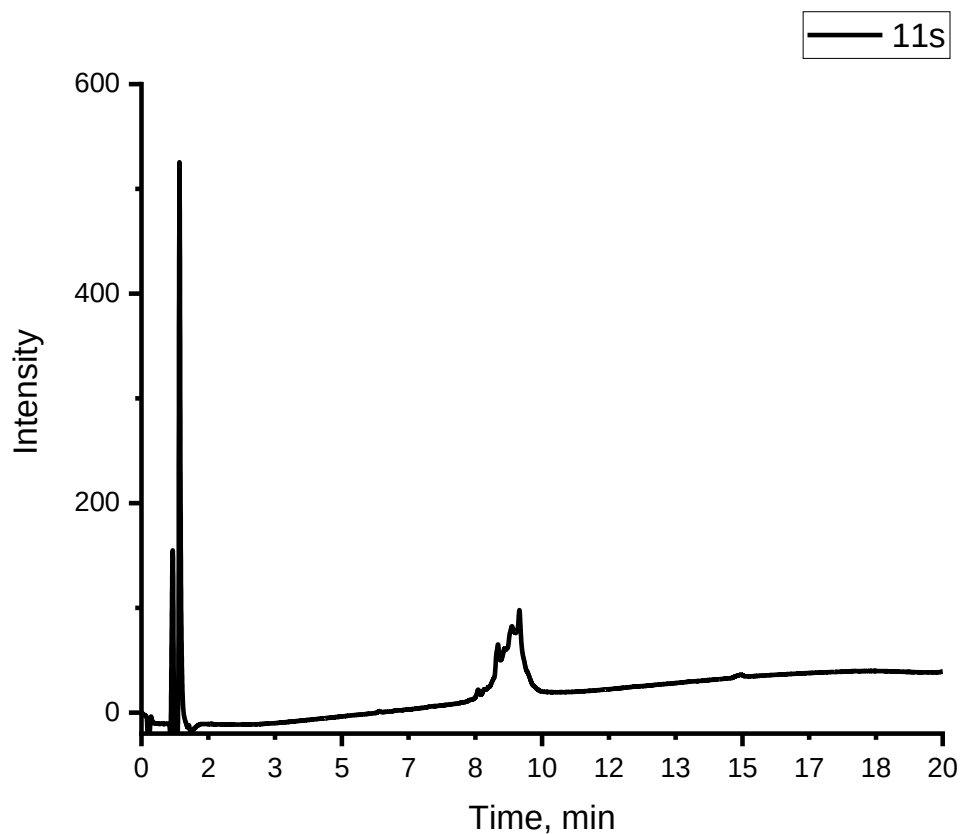


11s

The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines were coupled by manual SPPS. The coupling of coumarin modified Lysines was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the N-terminus was acylated (**SOP1**). After thorough washing

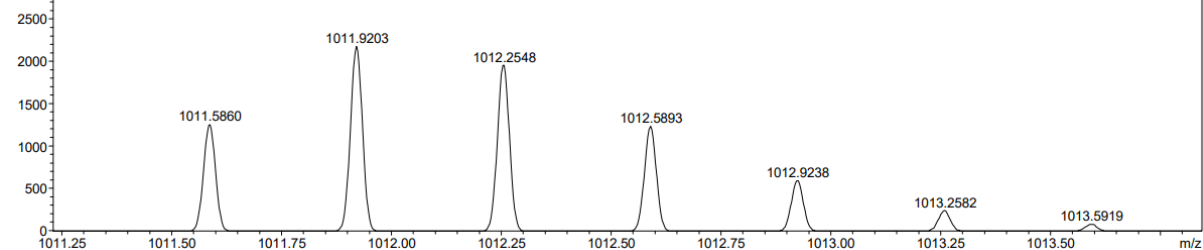
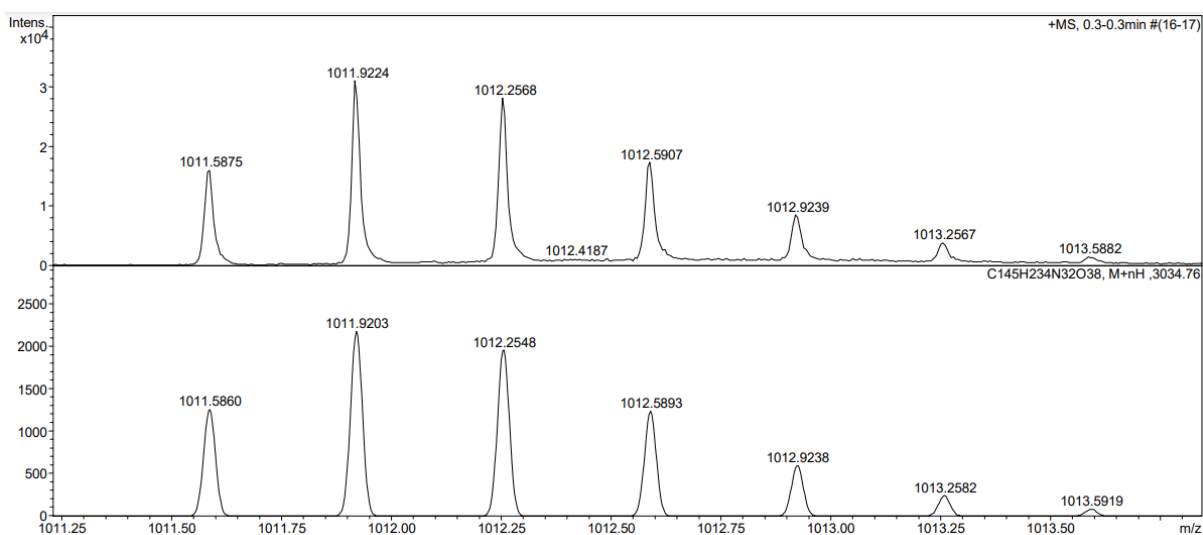
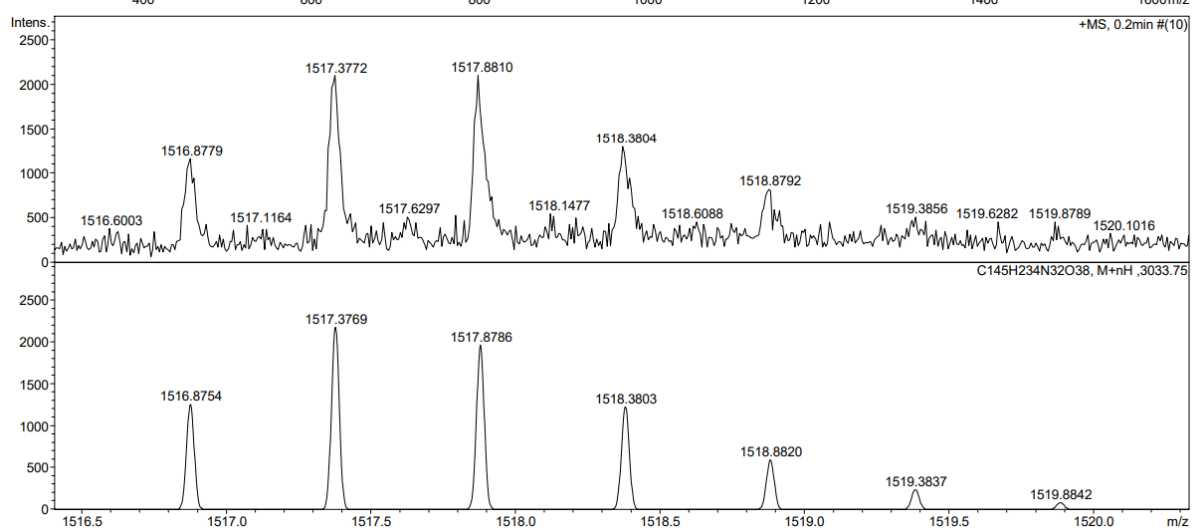
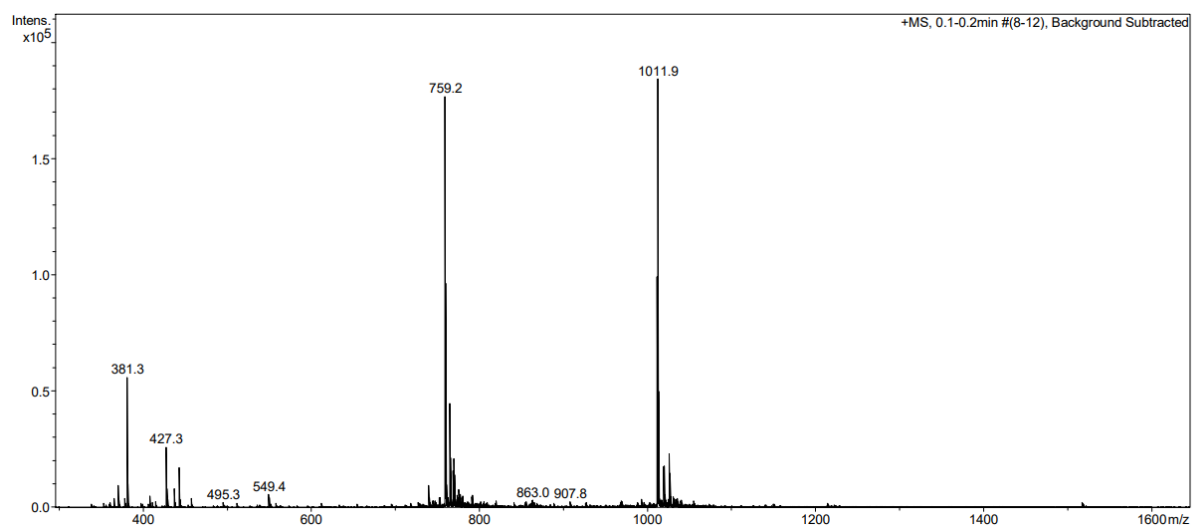
of the resin Grubbs-catalyzed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 3% yield.

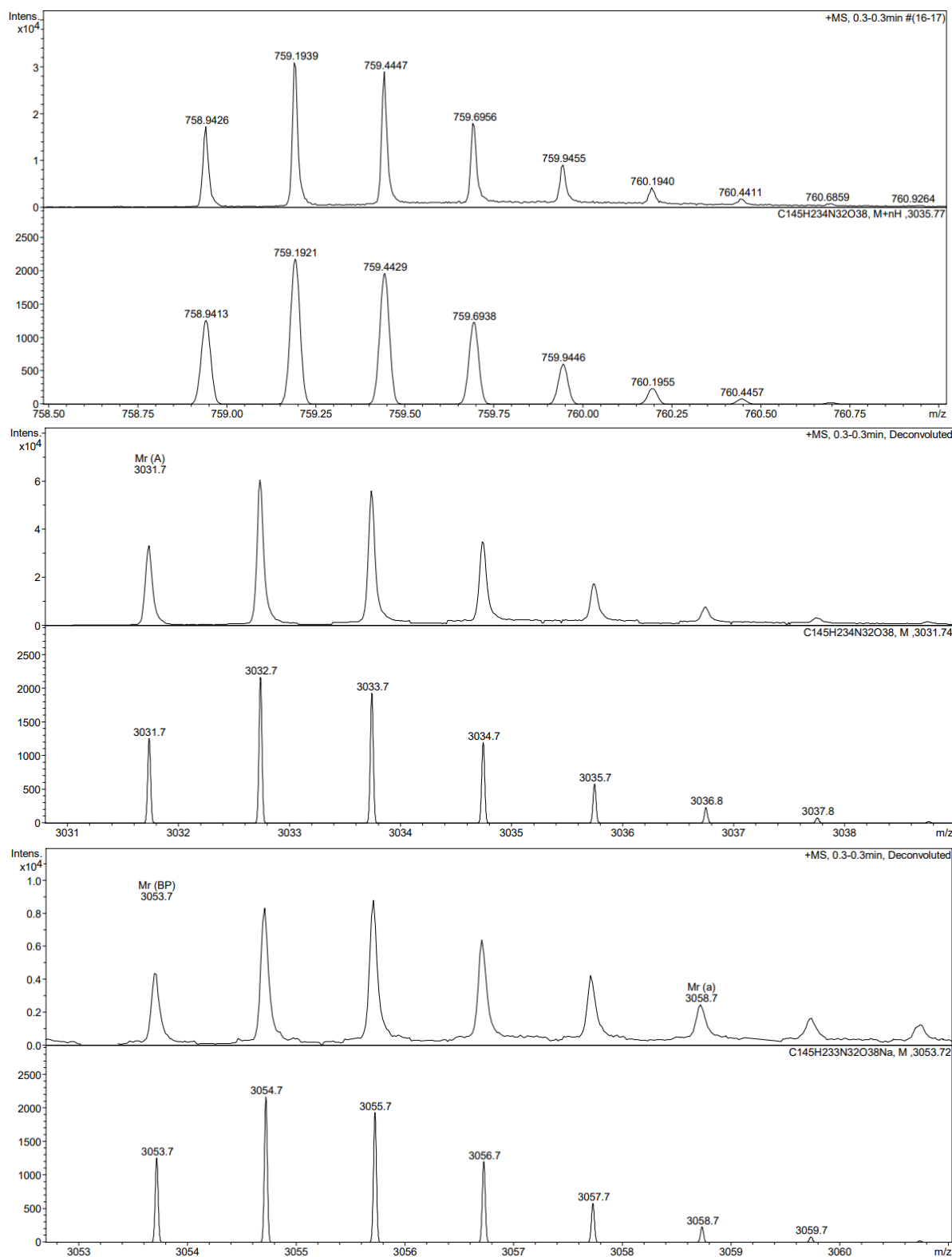
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): $R_t = 8.3, 9.5, 9.6$ min.



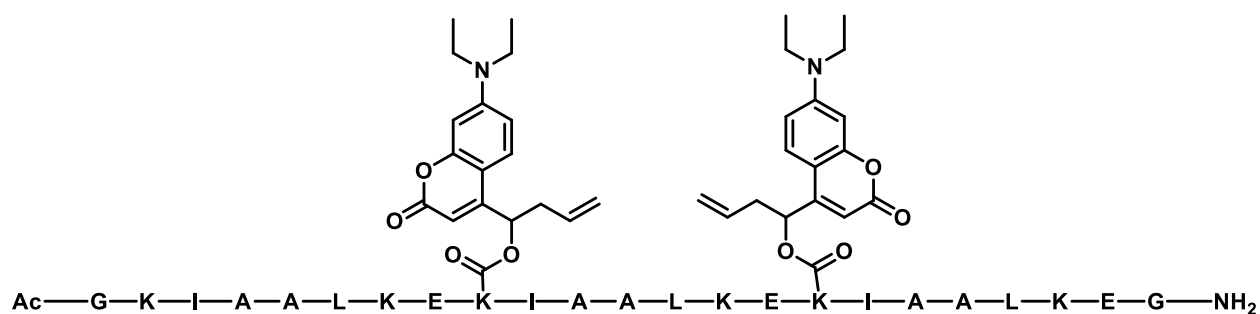
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.



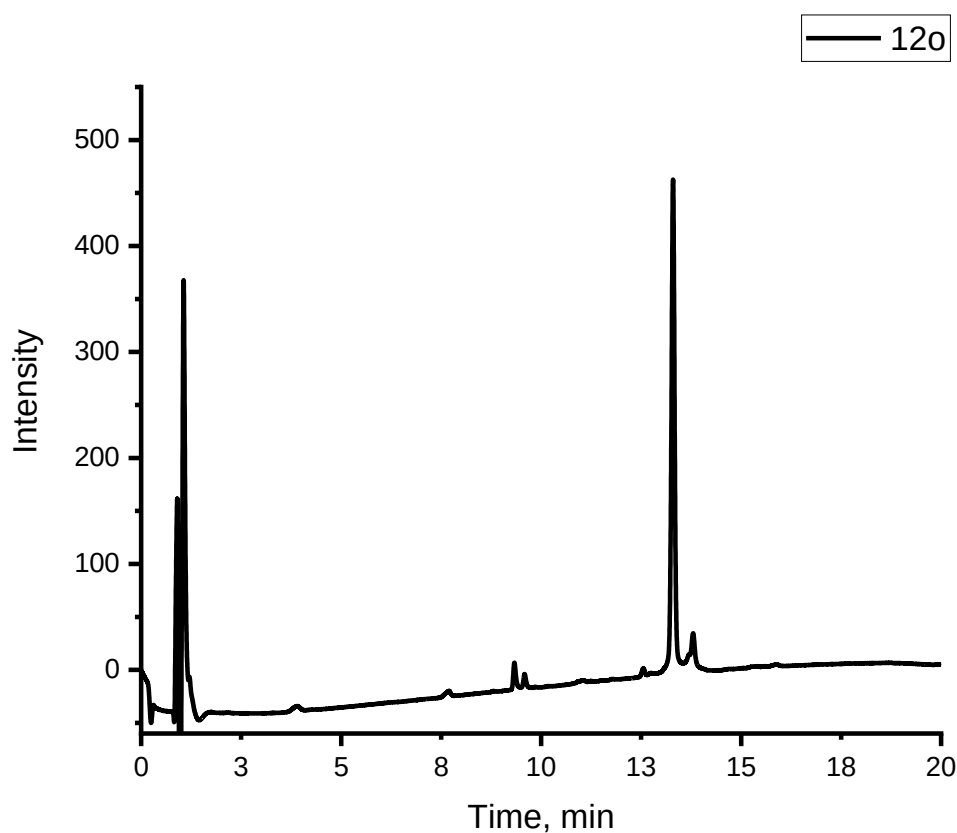


12o



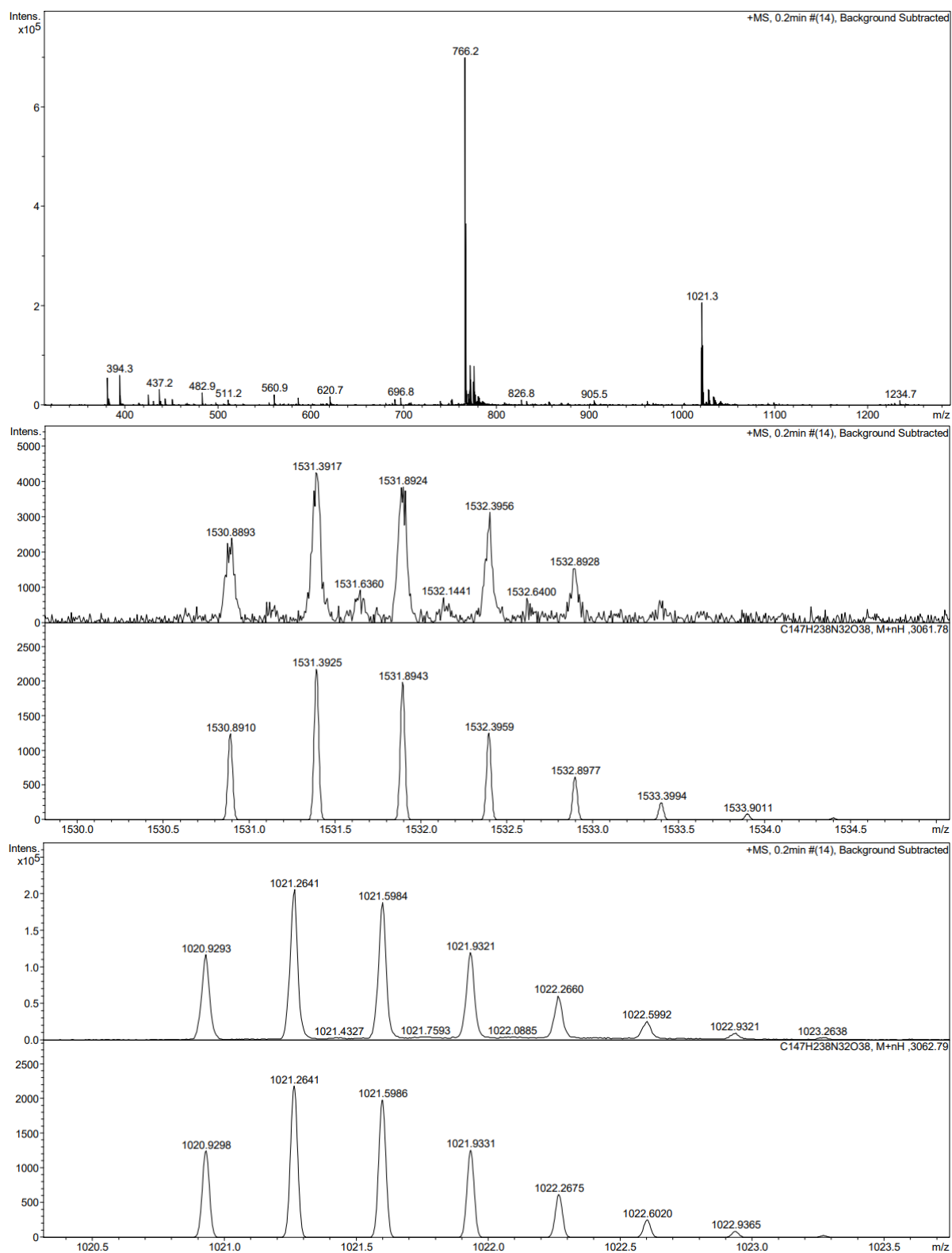
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines were coupled by manual SPPS. The coupling of coumarin modified Lysines was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalyzed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 12% yield.

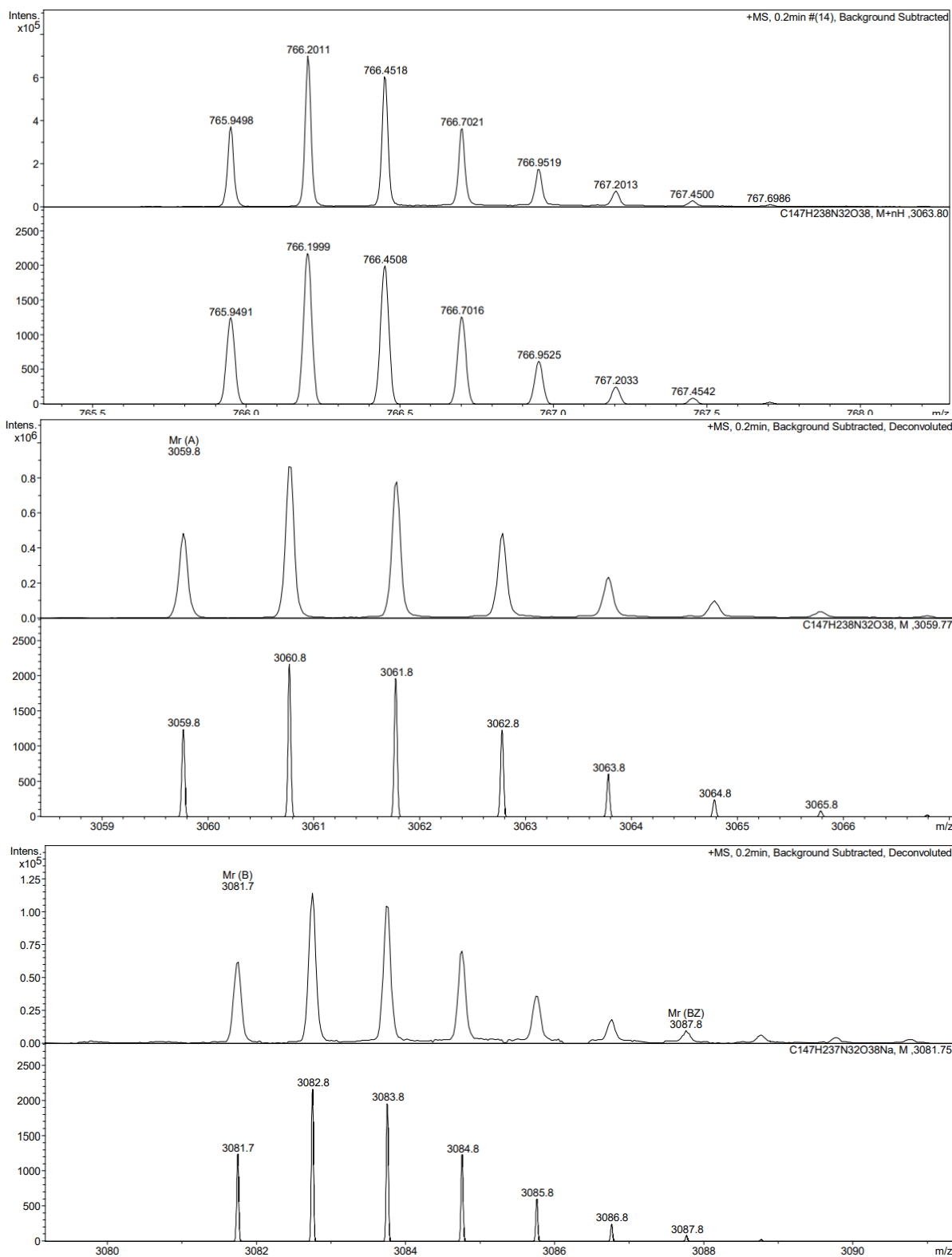
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 13.7 min.

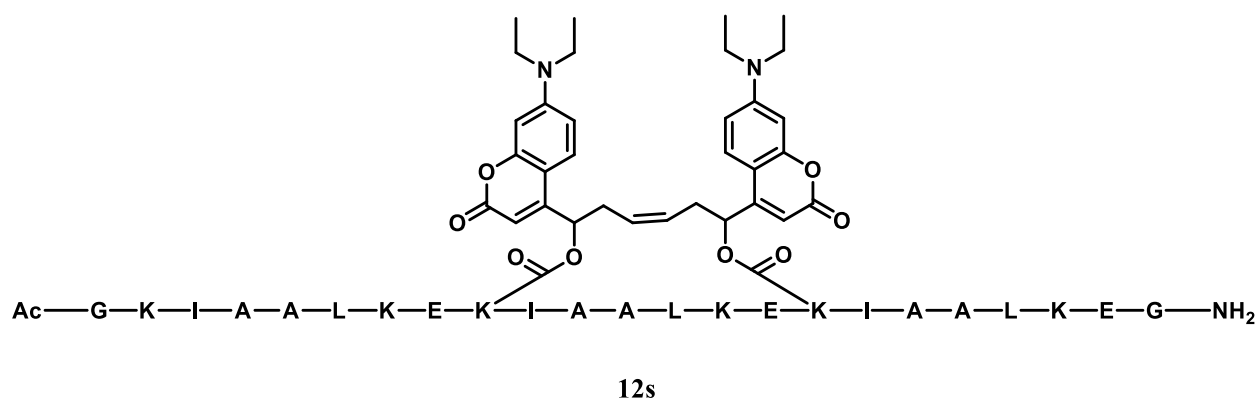


ESI-MS m/z : 3060.8 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calcd. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.949.

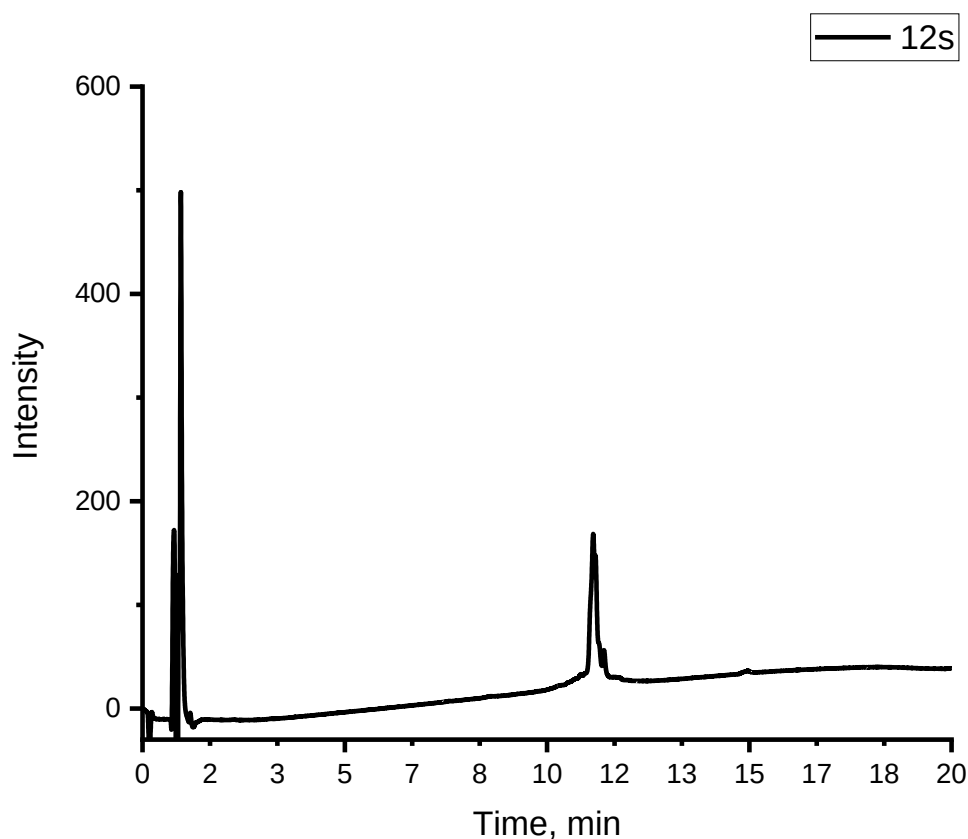




12s

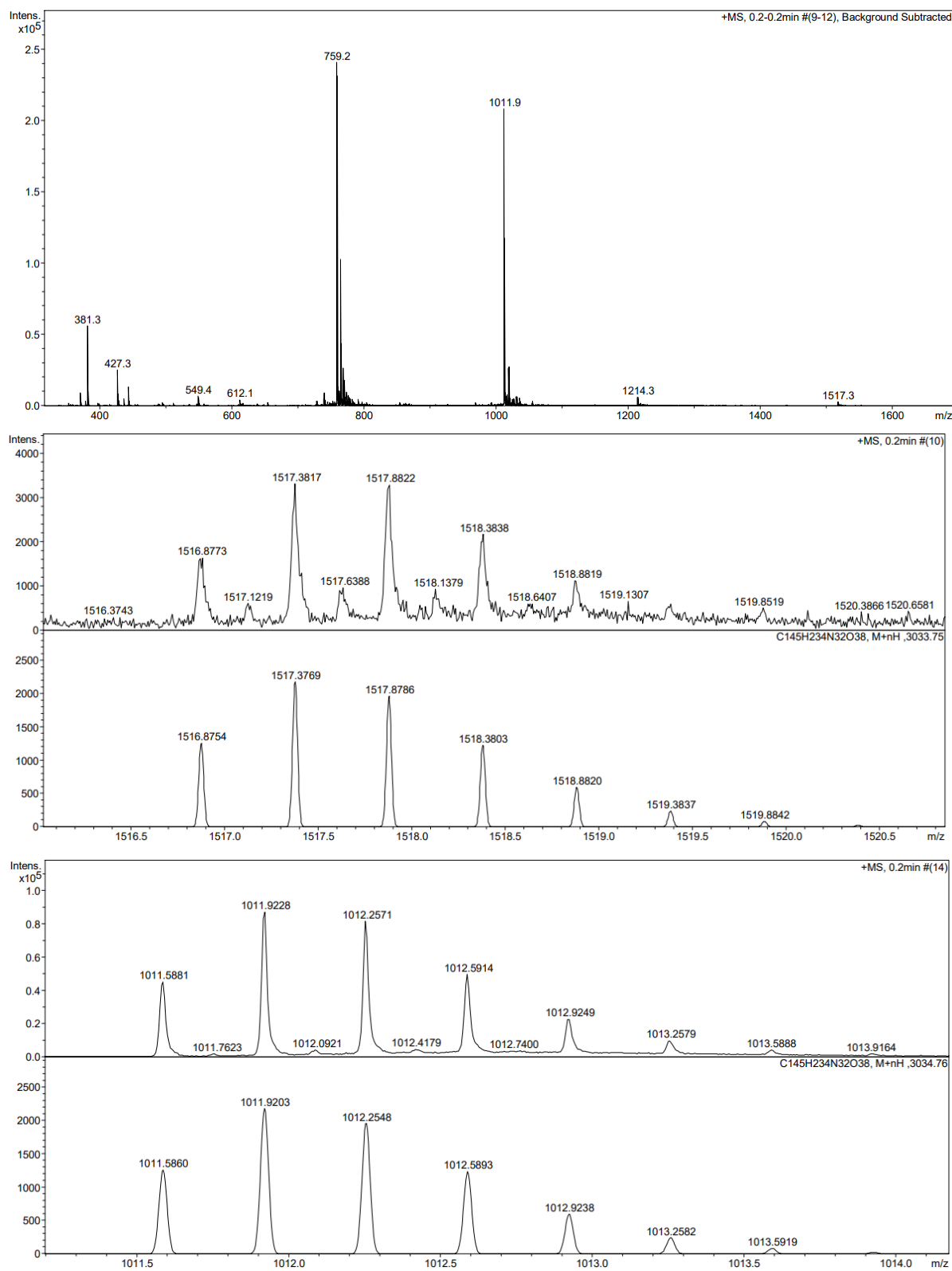
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines were coupled by manual SPPS. The coupling of coumarin modified Lysines was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalyzed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 10% yield.

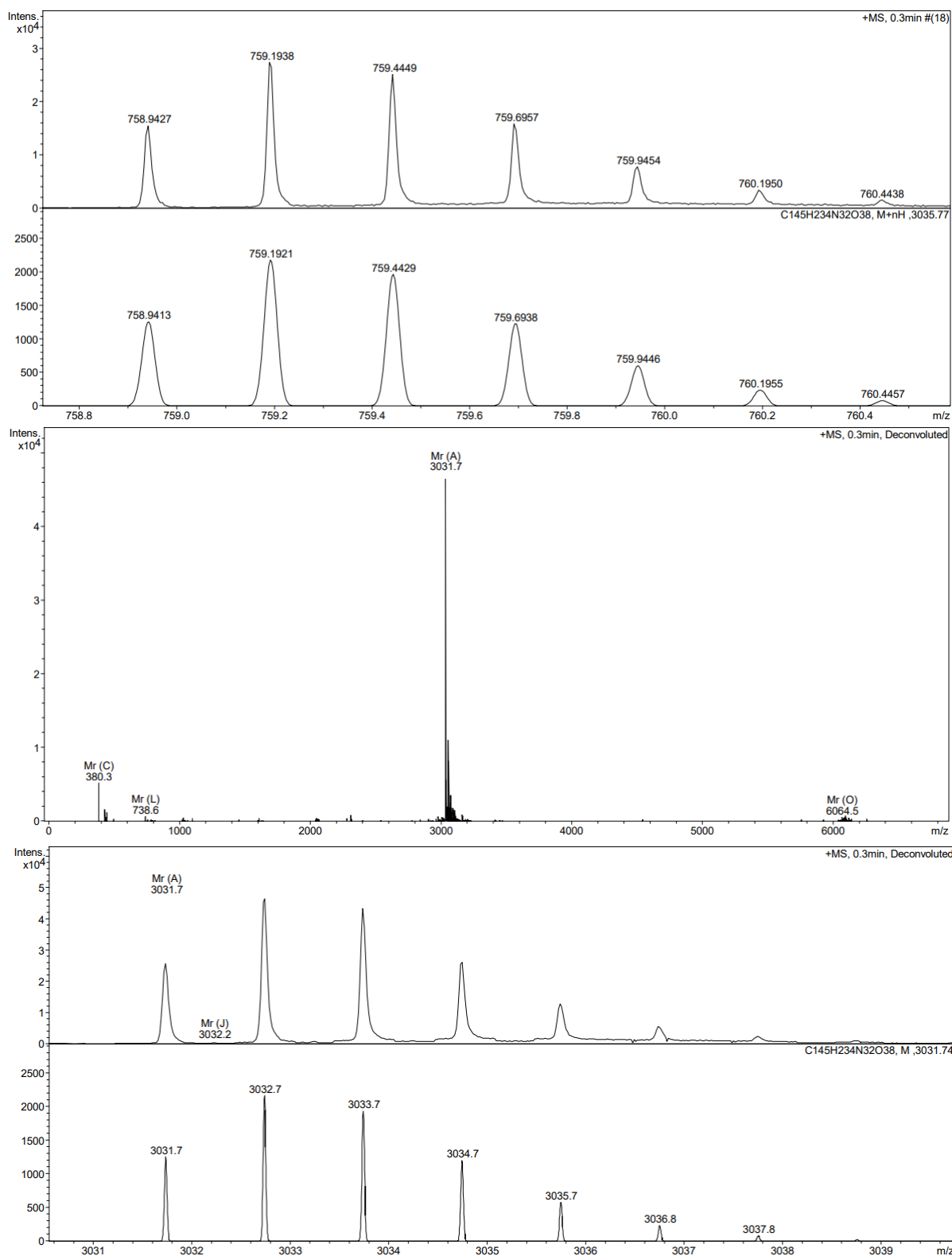
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 11.55 min.



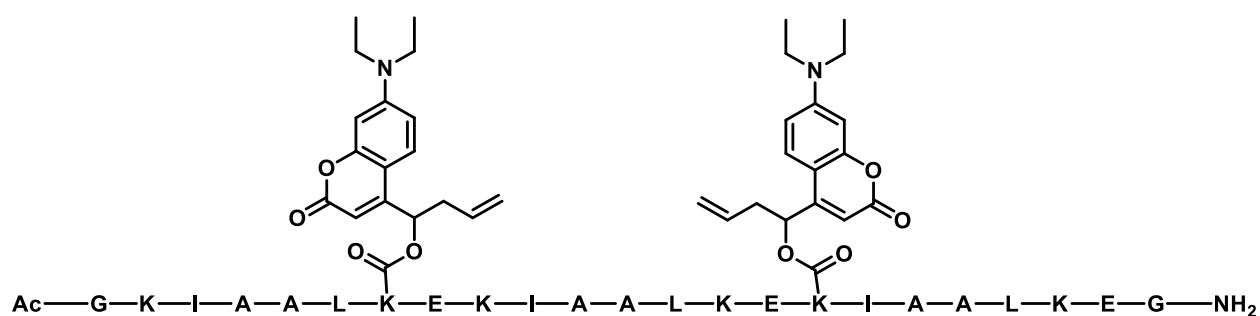
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H+Na]^{4+})$: 764.4315, found: 764.4368.



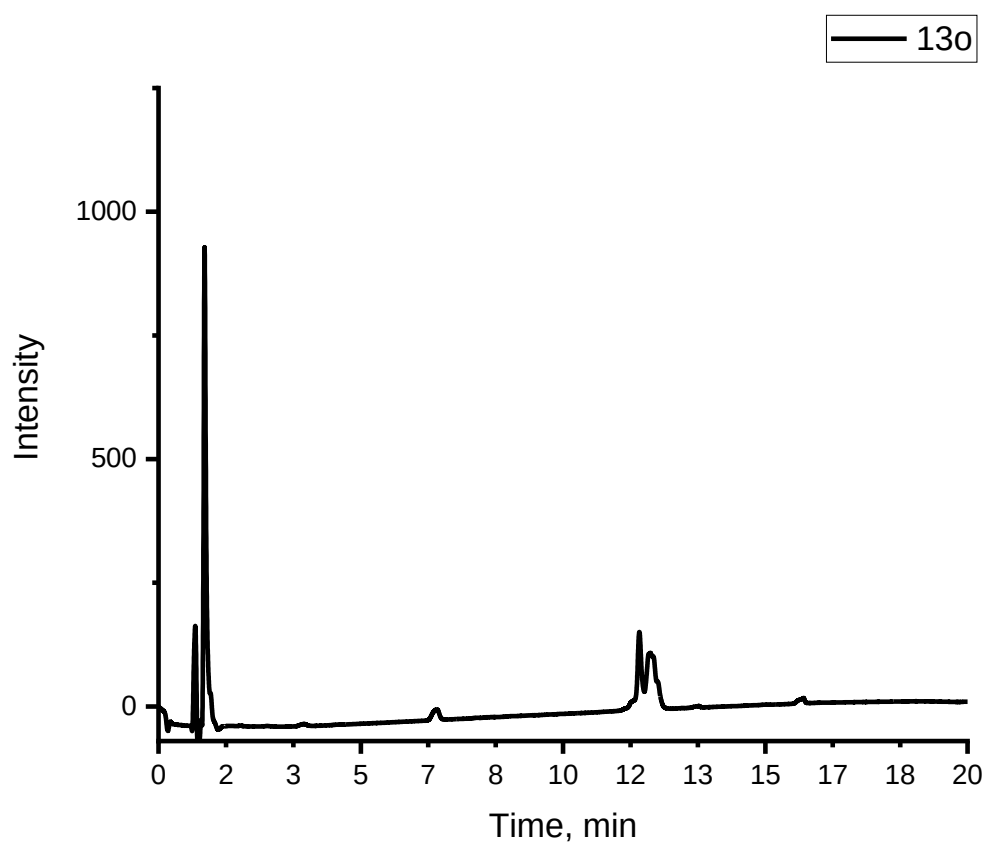


13o



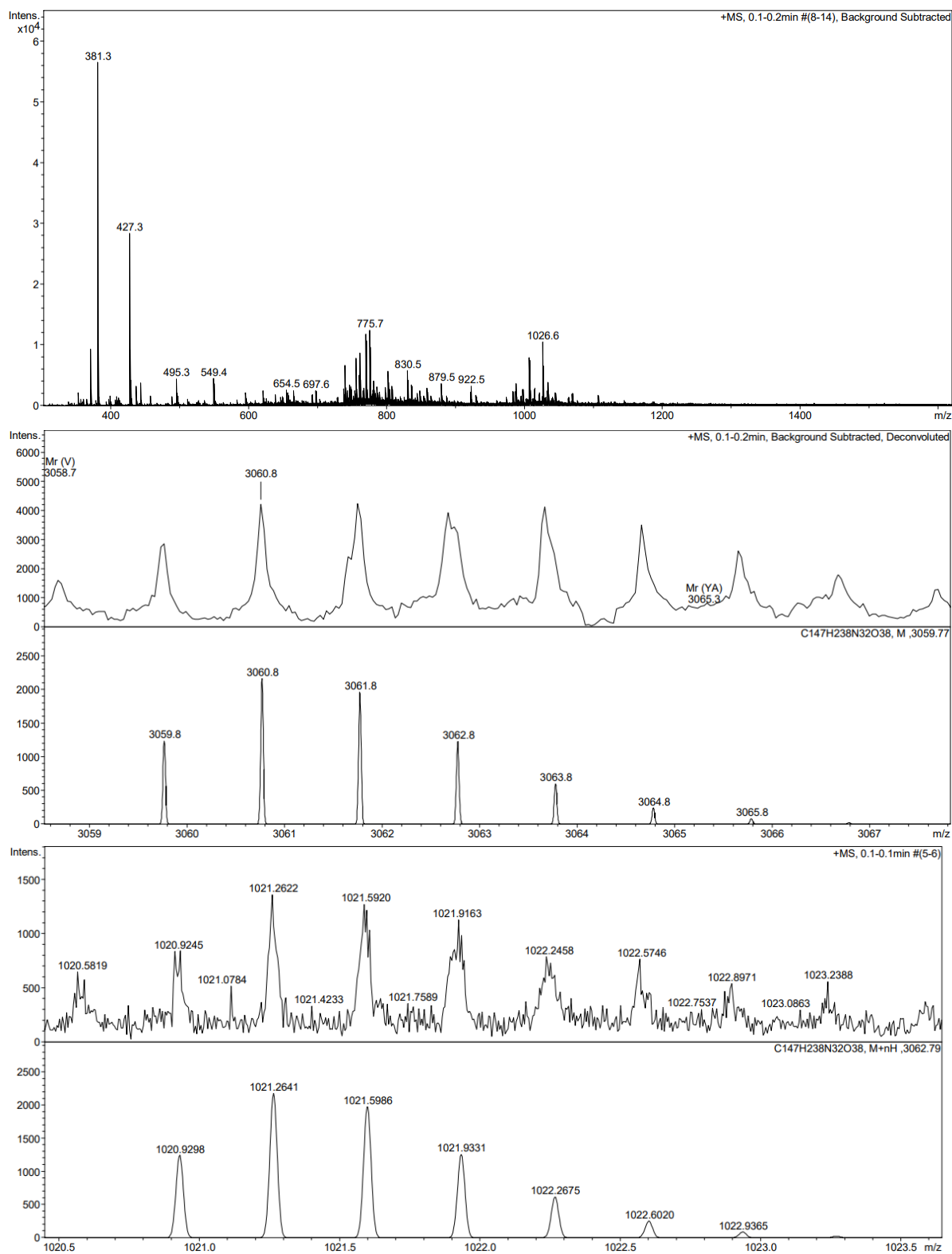
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines were coupled by manual SPPS. The coupling of coumarin modified Lysines was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalyzed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 15% yield.

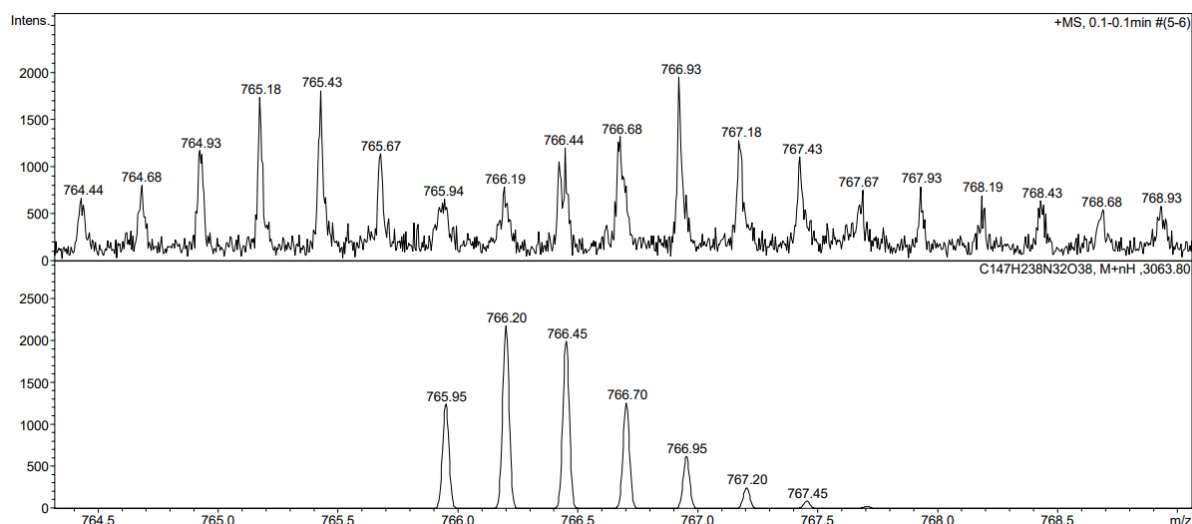
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 12.1 min.



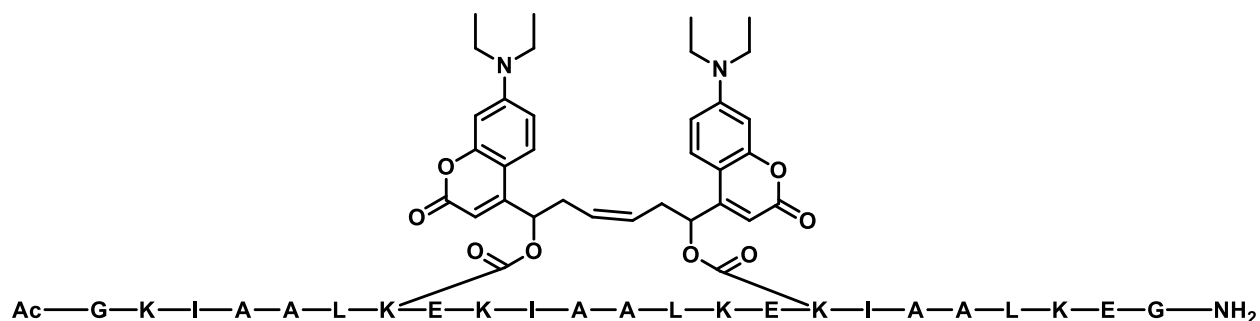
ESI-MS m/z : 3060.8 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calcd. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491





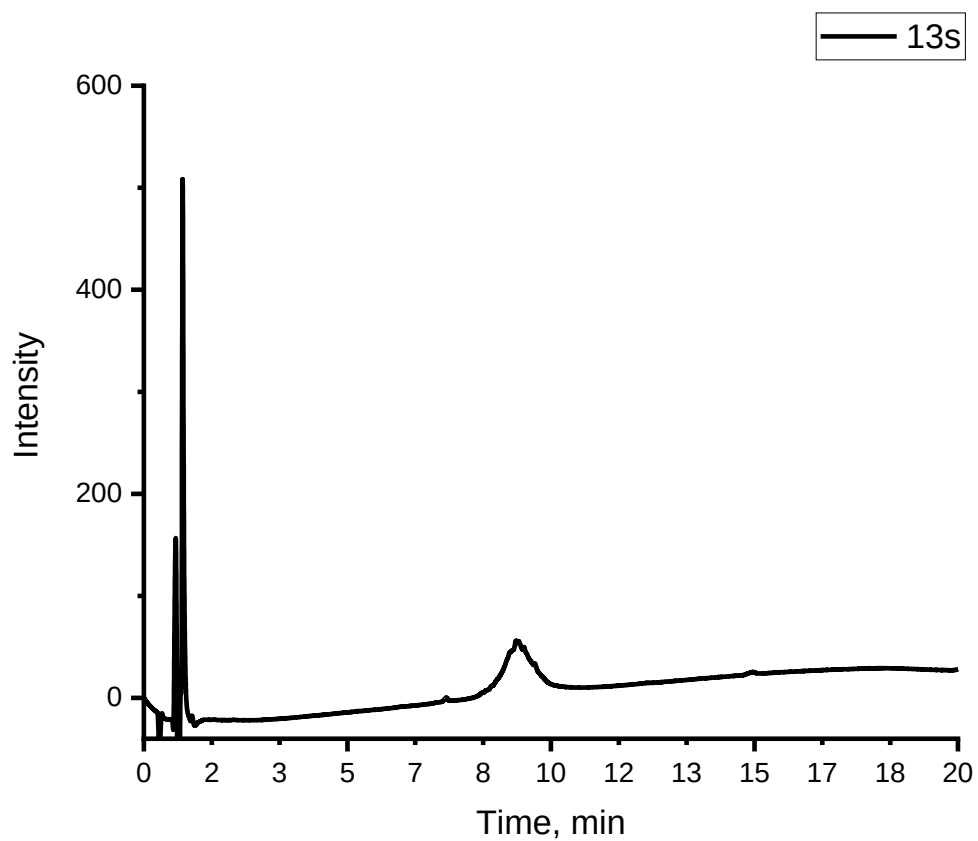
13s



13s

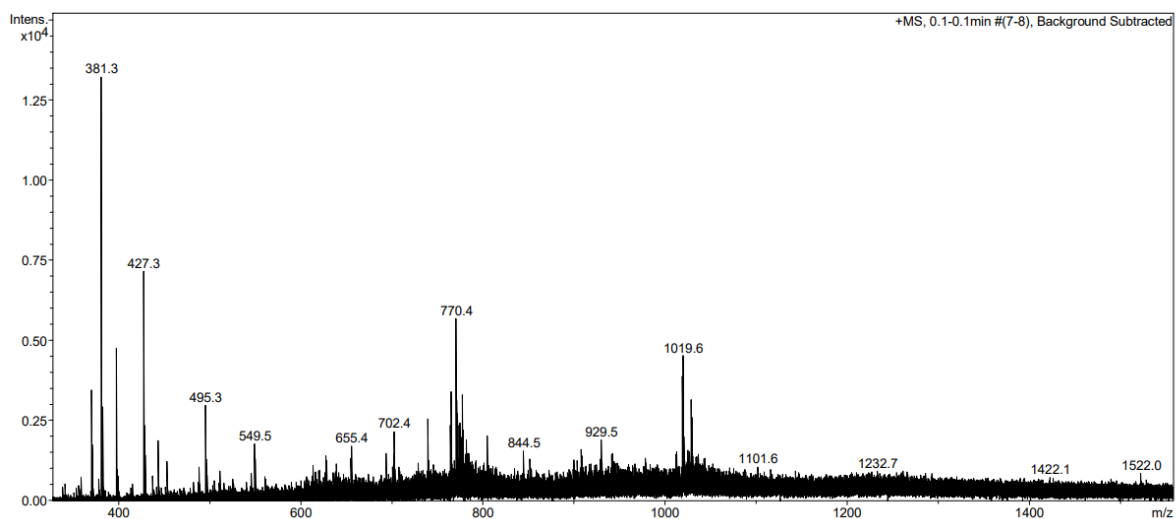
The peptide was synthesized at a 50 μ molar scale on Rink amide resin. The non-modified amino acids were coupled by automated SPPS, while the coumarin modified Lysines were coupled by manual SPPS. The coupling of coumarin modified Lysines was followed by KAISER tests and test cleavages. The final Fmoc deprotection was carried out and the *N*-terminus was acylated (**SOP1**). After thorough washing of the resin Grubbs-catalyzed macrocyclization was performed (**SOP2**). The peptide was cleaved (**SOP3**) and after lyophilization the crude product was purified by preparative HPLC utilizing column 2 with a linear gradient (20-90% MeCN + 0.1% TFA in 30 min). The product peptide was obtained in 5% yield.

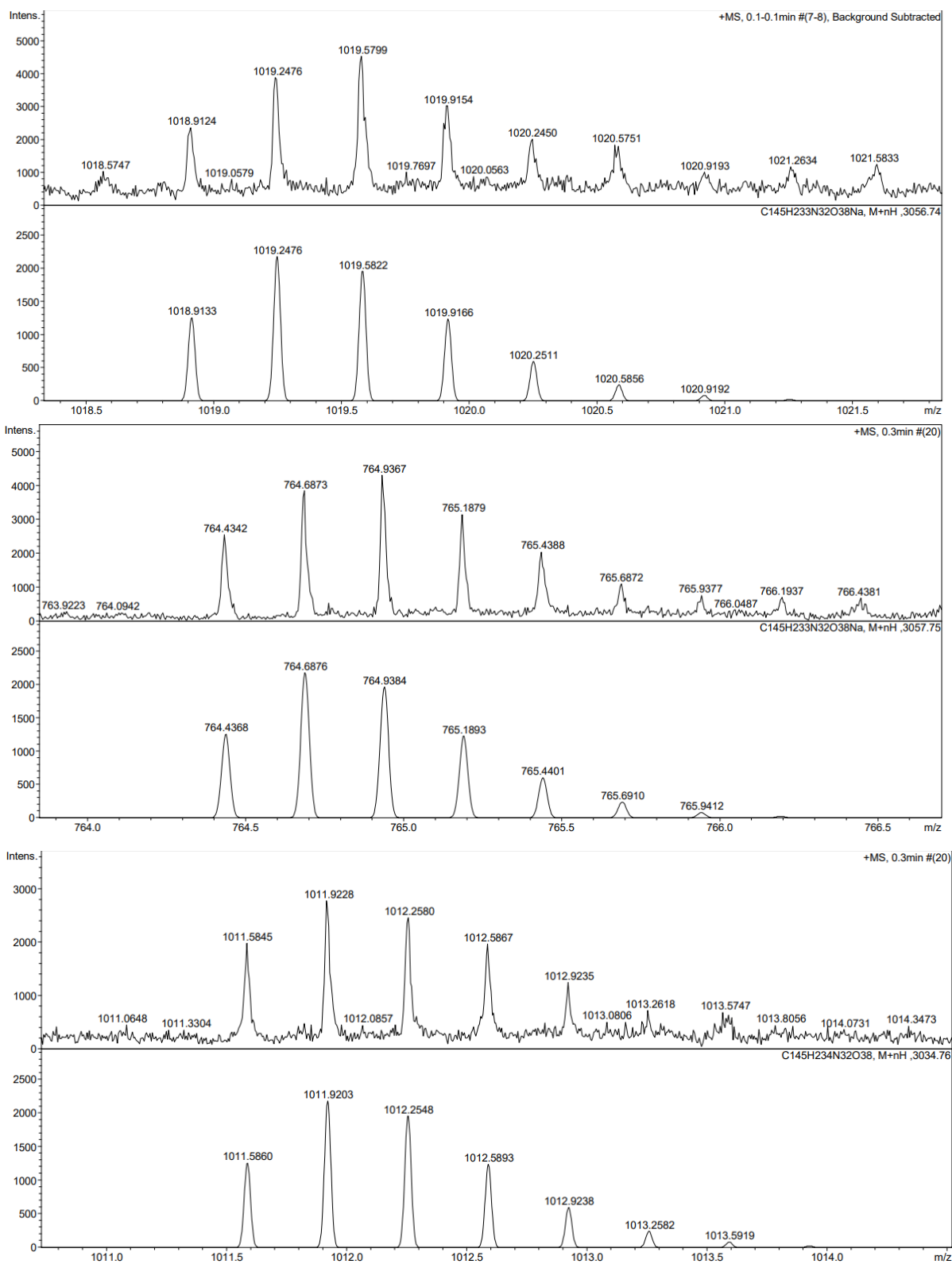
UPLC (column 1, 20-80% in 15 min, detection at 215 nm): R_t = 9.2 min.

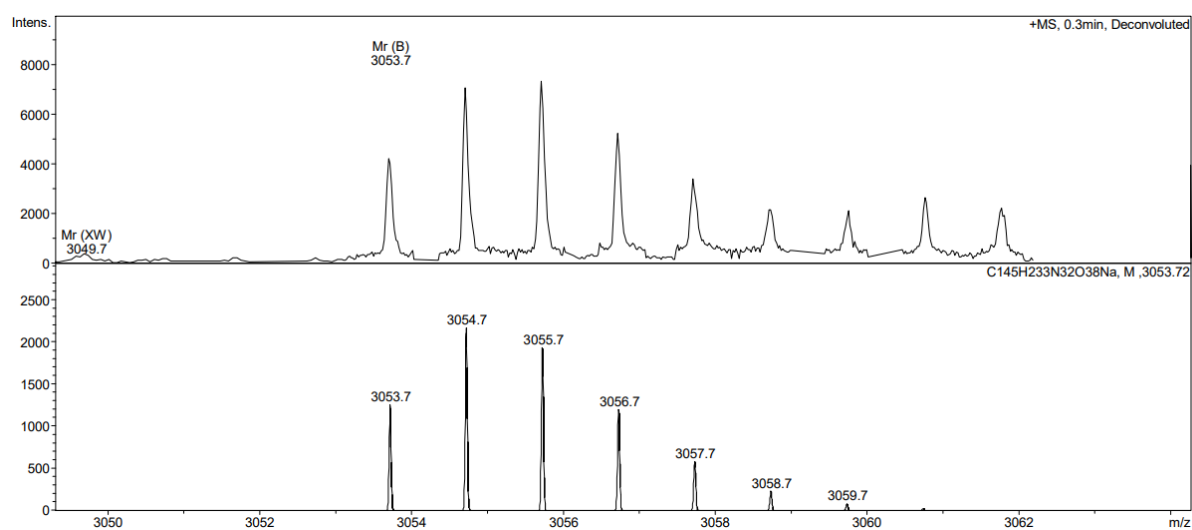
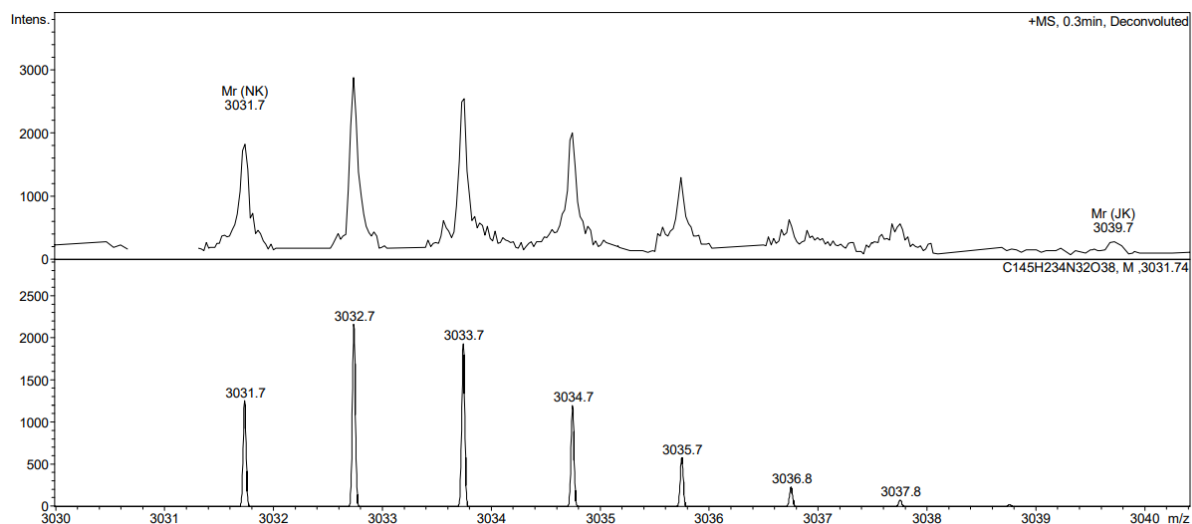


ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H+Na]^{4+})$: 764.4315, found: 764.4368.

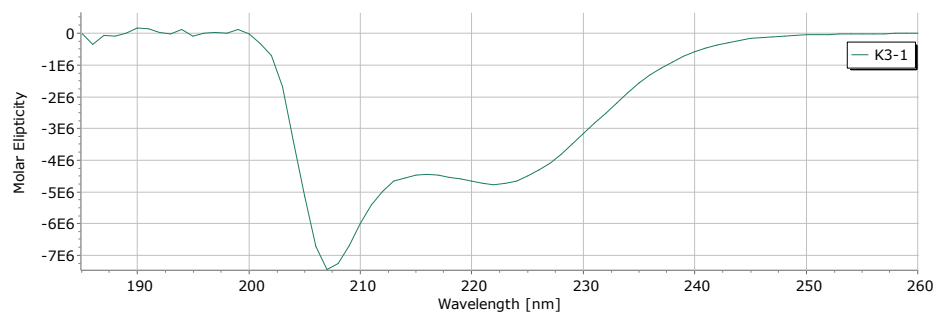




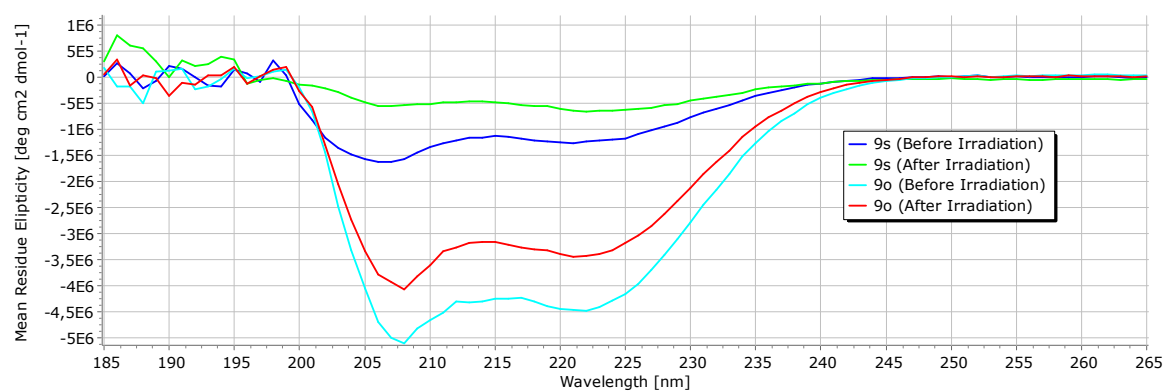


Section 4. CD Spectra

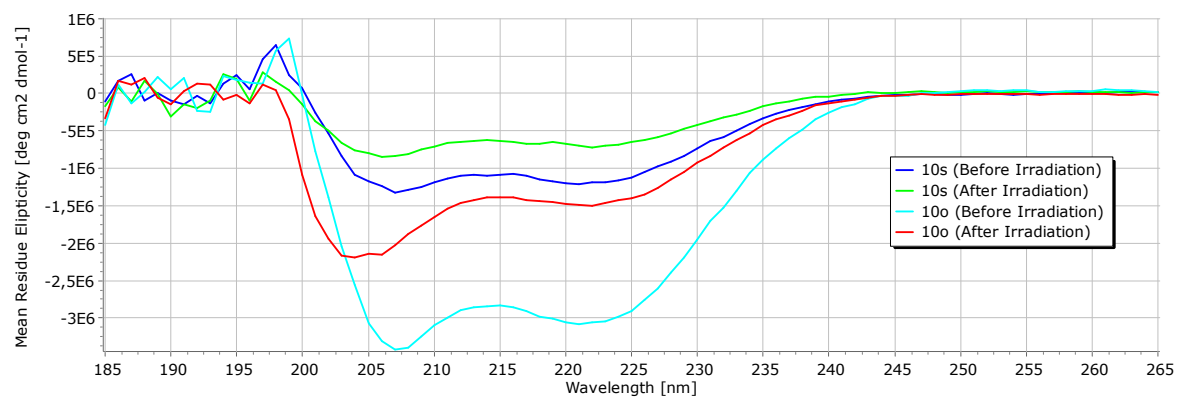
Peptide K3 (8):



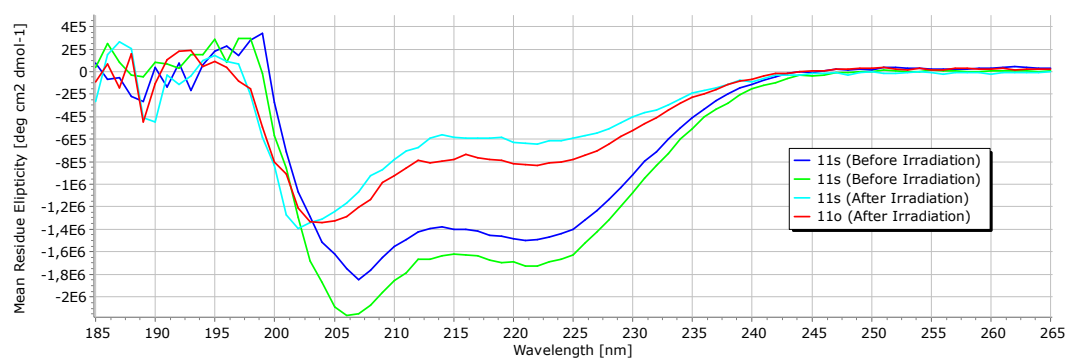
Peptides 9o and 9s before and after irradiation at 405 nm:



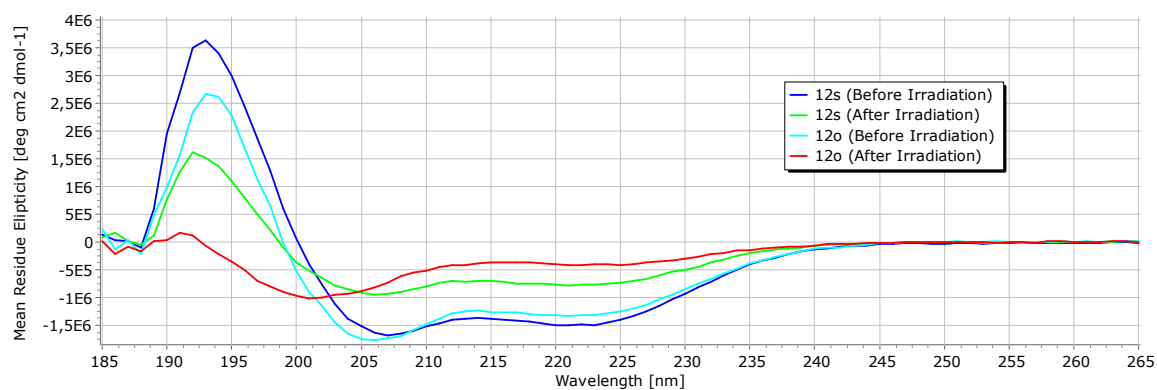
Peptides 10o and 10s before and after irradiation at 405 nm:



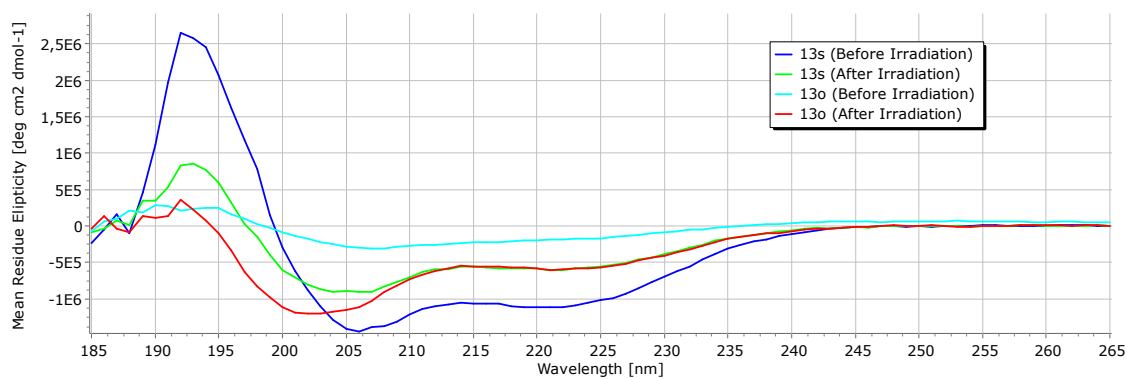
Peptides 11o and 11s before and after irradiation at 405 nm:



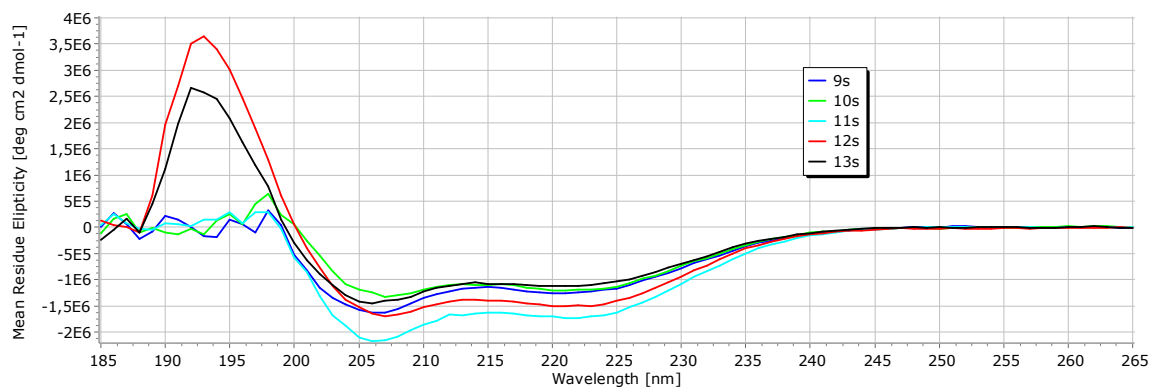
Peptides 12o and 12s before and after irradiation at 405 nm:



Peptides 13o and 13s before and after irradiation at 405 nm:

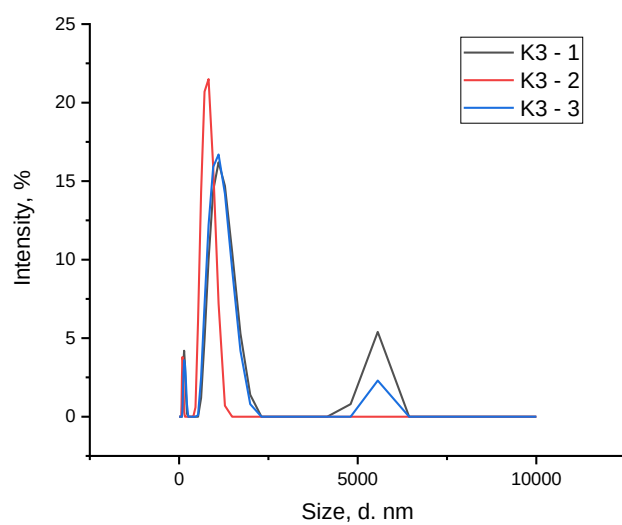


Peptides 9s, 10s, 11s, 12s and 13s:



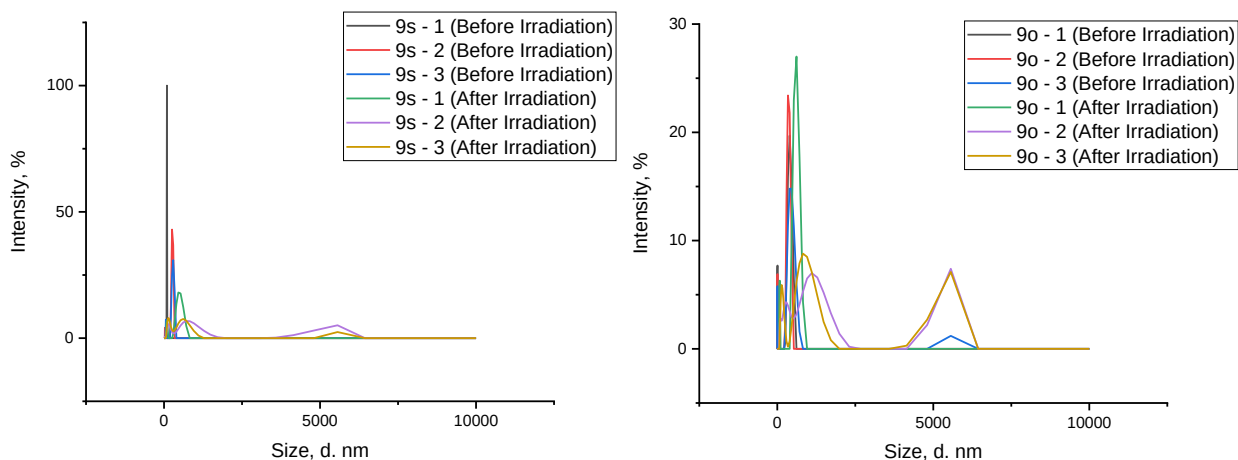
Section 5. Dynamic Light Scattering (DLS)

K3 particle size distribution:



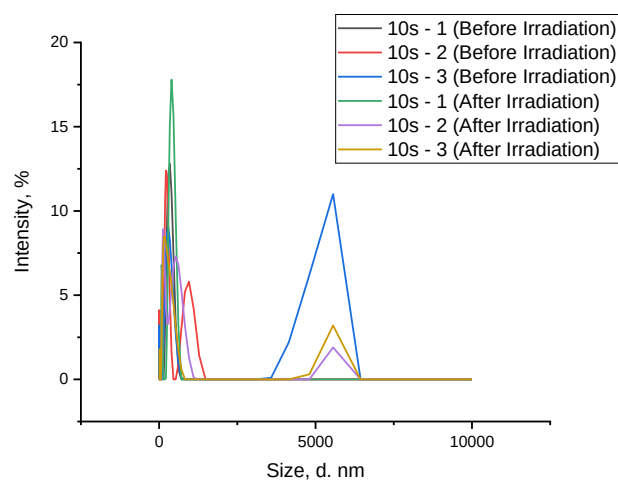
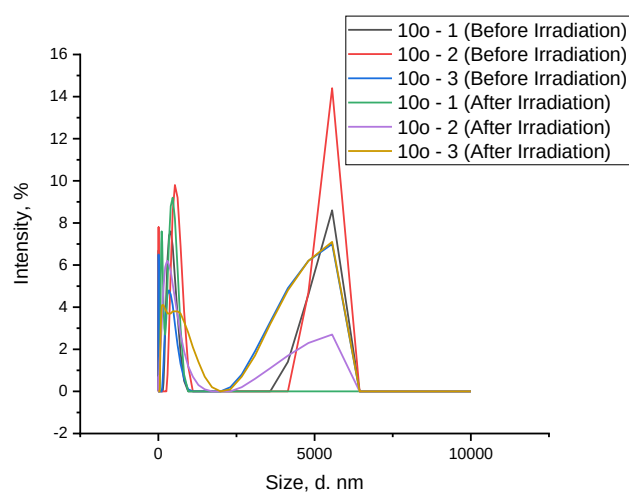
Sample Name	Z-Average (d.nm)	Pdl	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volume Mean (d.nm)	Number Mean (d.nm)
K3 - 1	1104	0,866	26,1	19,1	1269	1362	165,4
K3 - 2	1299	0,752	20,9	17,6	700,9	788,6	95,66
K3 - 3	1095	0,719	26,4	21,6	1061	1225	174

9o and 9s particle size distribution:



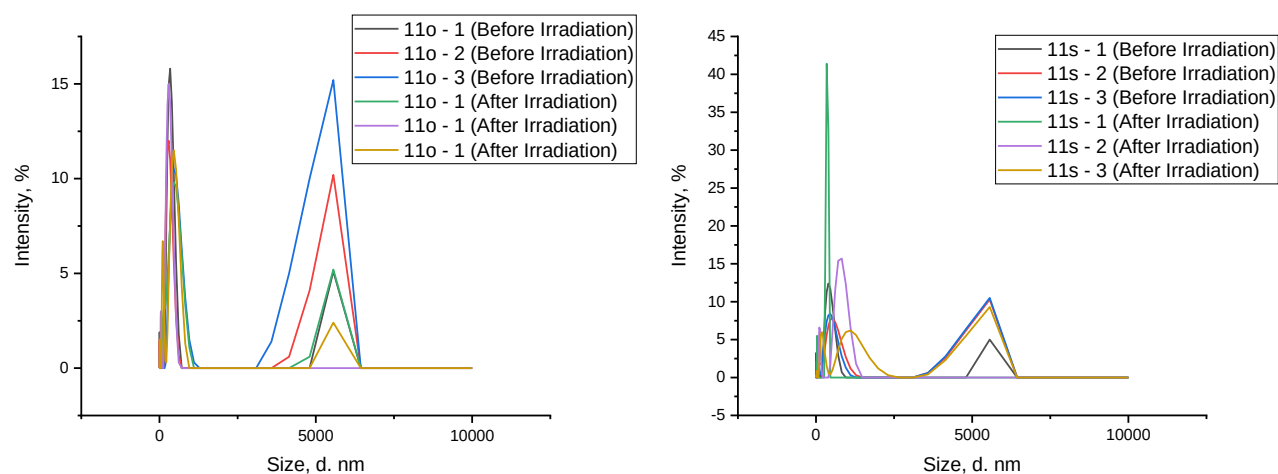
Sample Name	Z-Average (d.nm)	Pdl	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volum e Mean (d.nm)	Number Mean (d.nm)
9o – 1 (Before Irradiation)	615,3	0,735	17,3	18,1	266,7	5,125	4,532
9o – 2 (Before Irradiation)	683,7	0,642	15,3	21,4	261,5	4,947	4,319
9o – 3 (Before Irradiation)	383,4	0,518	23,8	22,9	368,6	5,1	4,215
9s – 1 (Before Irradiation)	3054	1	0	0	91,28	91,77	91,77
9s – 2 (Before Irradiation)	1285	0,899	9,8	10	247,8	72,53	27,31
9s – 3 (Before Irradiation)	1126	0,861	13,3	12,7	245,4	161,9	58,37
9o – 1 (After Irradiation)	935,4	0,745	15,9	13,7	510,1	502,9	70,54
9o – 2 (After Irradiation)	694,3	0,739	34,4	38,4	1116	1370	79,24
9o – 3 (After Irradiation)	564	0,631	32,6	34	1062	1267	106,5
9s – 1 (After Irradiation)	482,4	0,541	21,1	19,2	392,2	395	78,99
9s – 2 (After Irradiation)	315	0,771	39,7	38,6	919,9	406,5	11,77
9s – 3 (After Irradiation)	350,9	0,508	36,9	32,6	483,1	660	96,07

10o and 10s particle size distribution:



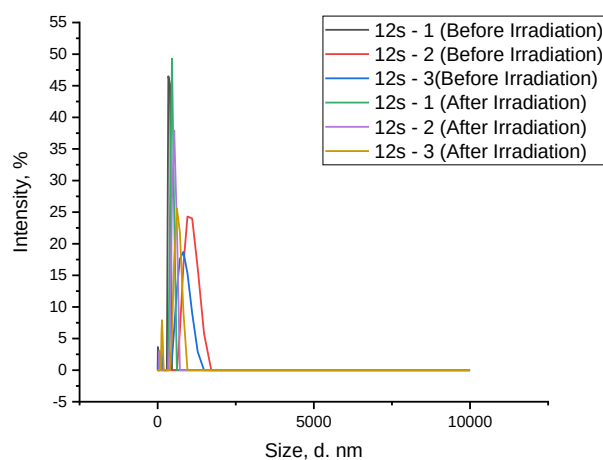
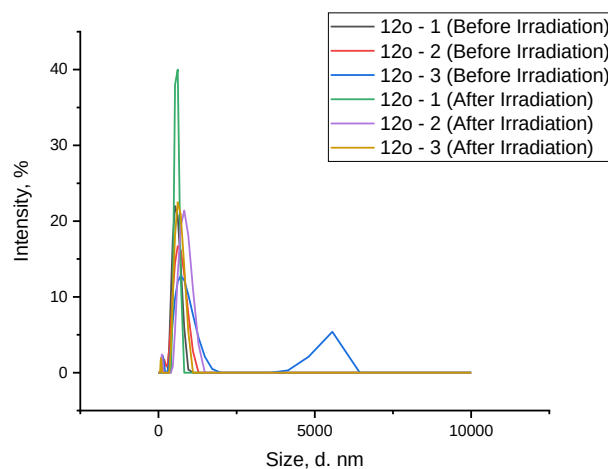
Sample Name	Z-Average (d.nm)	Pdl	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volume Mean (d.nm)	Number Mean (d.nm)
10o – 1 (Before Irradiation)	332,9	0,568	34,4	28,8	952,8	7,54	4,979
10o – 2 (Before Irradiation)	732,3	1	26,6	21,8	1302	7,929	5,184
10o – 2 (Before Irradiation)	281,1	0,42	31,6	41,9	1232	8,083	4,767
10s – 1 (Before Irradiation)	870,9	0,699	28	30,1	234,7	5,199	4,257
10s – 2 (Before Irradiation)	2638	1	26,1	18,4	335,3	5,878	4,501
10s – 3 (Before Irradiation)	469	0,576	36,9	10	1189	7,633	4,251
10o – 1 (After Irradiation)	330,2	0,363	31,4	35,4	291,1	304,1	74,71
10o – 2 (After Irradiation)	162,6	0,74	77,8	18,9	658,5	20,19	5,311
10o – 3 (After Irradiation)	291,4	0,715	50,2	43,5	1373	1749	63,66
10s – 1 (After Irradiation)	470,6	0,465	22	19,6	323,4	292,9	74,05
10s – 2 (After Irradiation)	280,1	0,486	37,1	31,2	422	575,8	102,8
10s – 3 (After Irradiation)	255,1	0,528	55,9	17,7	411,7	73,98	15,81

11o and 11s particle size distribution:



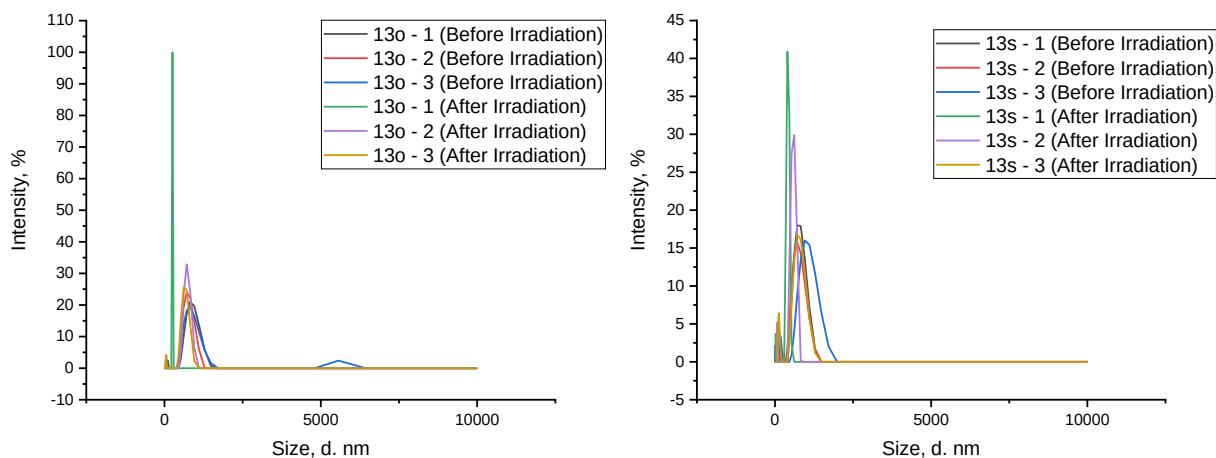
Sample Name	Z-Average (d.nm)	PdI	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volume Mean (d.nm)	Number Mean (d.nm)
11o – 1 (Before Irradiation)	740,2	0,653	29,2	23,4	579	5,086	3,594
11o – 2 (Before Irradiation)	561,9	0,531	33,8	7,7	1017	6,375	3,408
11o – 3 (Before Irradiation)	893,5	0,683	34,8	12,1	1923	2311	404,8
11s – 1 (Before Irradiation)	855,8	0,757	31,4	18,8	590,5	4,78	3,714
11s – 2 (Before Irradiation)	605,2	0,65	43,8	11,2	1333	13,18	5,046
11s – 3 (Before Irradiation)	622,1	0,564	40,6	11,3	1326	7,913	3,889
11o – 1 (After Irradiation)	504	0,665	52,3	26,4	670,5	256,4	19,79
11o – 2 (After Irradiation)	534,4	0,527	30,5	24,1	268,8	129,5	35,19
11o – 3 (After Irradiation)	480,2	0,572	28	27,1	455,2	26,18	8,987
11s – 1 (After Irradiation)	1483	0,907	10,1	10,3	317,9	109,9	31,62
11s – 2 (After Irradiation)	889	0,744	23,7	22,8	598,2	752,2	97,96
11s – 3 (After Irradiation)	597,2	0,67	33,5	36,3	1389	211	6,999

12o and 12s particle size distribution:



Sample Name	Z-Average (d.nm)	Pdl	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volume Mean (d.nm)	Number Mean (d.nm)
12o – 1 (Before Irradiation)	733,6	0,583	22,4	14,3	536,8	588,2	145,1
12o – 2 (Before Irradiation)	728,8	0,561	29,6	21,7	610,1	713,5	325,7
12o – 3 (Before Irradiation)	766,4	0,638	35,5	19,5	1074	1158	196,3
12s – 1 (Before Irradiation)	2058	1	7,3	11,2	338,8	7,051	5,759
12s – 2 (Before Irradiation)	1264	0,707	19,3	13	977,7	1069	115,1
12s – 3 (Before Irradiation)	960,6	0,672	23,9	19	696	14,34	5,049
12o – 1 (After Irradiation)	1145	0,53	12,2	0	581	598	571,5
12o – 2 (After Irradiation)	971,7	0,497	23	14,6	778,1	869,9	156,4
12o – 3 (After Irradiation)	874,5	0,562	21,8	15,6	614,6	658,8	81,39
12s – 1 (After Irradiation)	1516	0,838	10,5	0	459,8	467,9	453
12s – 2 (After Irradiation)	1421	0,853	12,5	11	491,2	439,7	56,01
12s – 3 (After Irradiation)	1383	1	16,1	12,9	536,4	624,4	174,7

13o and 13s particle size distribution:



Sample Name	Z-Average (d.nm)	PdI	%Pd Peak 1 (Percent)	%Pd Peak 2 (Percent)	Intensity Mean (d.nm)	Volume Mean (d.nm)	Number Mean (d.nm)
13o – 1 (Before Irradiation)	1029	0,653	23	16,8	809,5	903,2	103,7
13o – 2 (Before Irradiation)	974,1	0,662	21,8	0	752,9	806,8	705,2
13o – 3 (Before Irradiation)	804,6	0,633	27,1	1,2	946,8	988,3	747,2
13s – 1 (Before Irradiation)	928,4	0,839	24	22,3	662,2	10,78	4,738
13s – 2 (Before Irradiation)	771,8	0,631	26,9	25,8	595,5	10,9	5,17
13s – 3 (Before Irradiation)	934,9	0,711	25,9	22,2	831,1	13,24	4,403
13o – 1 (After Irradiation)	2194	1	0	0	255	256,4	256,4
13o – 2 (After Irradiation)	1634	0,722	15,1	12,9	676,7	484,8	32,47
13o – 3 (After Irradiation)	1192	0,709	17,4	15,3	588,4	542,9	53,37
13s – 1 (After Irradiation)	1050	0,75	11,2	10,4	383,3	96,44	22,88
13s – 2 (After Irradiation)	1150	0,749	14	13,5	512,3	502,6	71,84
13s – 3 (After Irradiation)	706,6	0,711	23,5	20,9	609,5	758,7	116,1

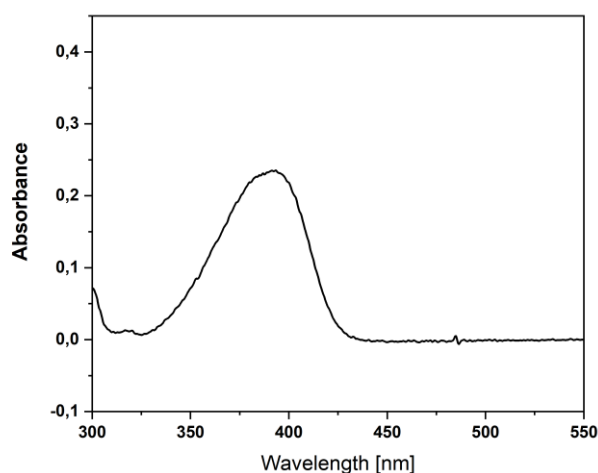
Section 6. Photophysical and Photochemical Properties

UV-Vis Spectra

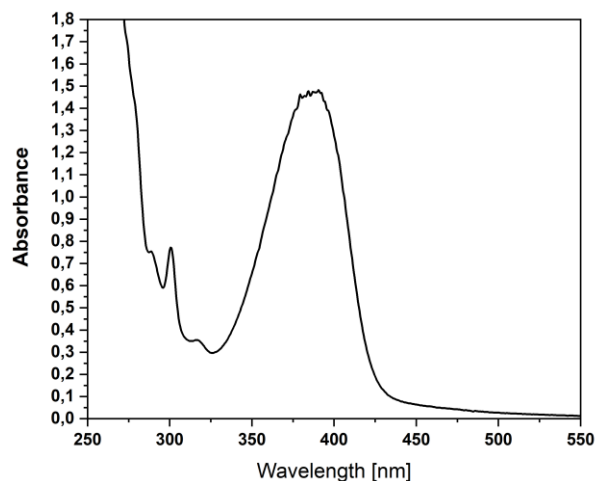
The UV-Vis spectra of the coumarin cores **7a** and **7b** and the obtained peptide conjugates were recorded in chloroform (**7a** and **7b**) or phosphate buffer (pH=7.0, for all peptides) and are displayed below. The spectra of the coumarin cores were recorded in 10x10 mm quartz cuvettes and the peptides in 10x4 mm quartz cuvettes (4 mm pathlength for detection and 10 mm for irradiation orthogonal to the observation pathway). The data were recorded on a specord S600 UV-Vis spectrometer and processed and the SpectraGryph 1.2 and OriginPro 2020 software including baseline correction and line smoothening. An overview of the lowest electronic transition maximum that can be attributed to the coumarin photolabile staple is given in the following table.

	7a	7b	9o	9s	10o	10s	11o	11s	12o	12s	13o	13s
λ_{max}	394	387	382	387	381	385	387	386	385	385	384	387

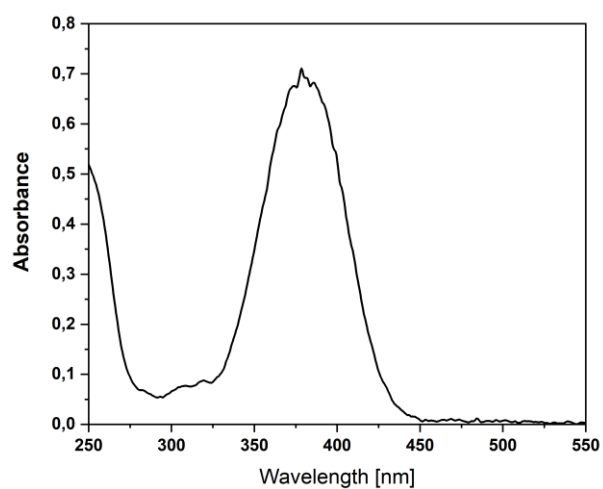
UV-Vis spectrum of **7a** (100 μM , chloroform):



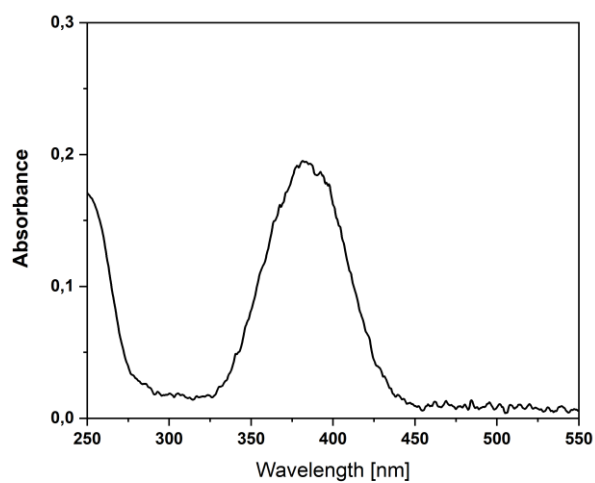
UV-Vis spectrum of **7b** (200 μM , chloroform):



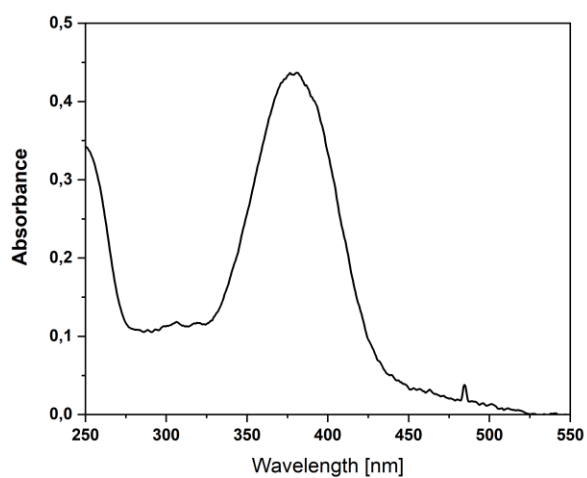
UV-Vis spectrum of **9o** (100 μ M, phosphate buffer, pH=7.0):



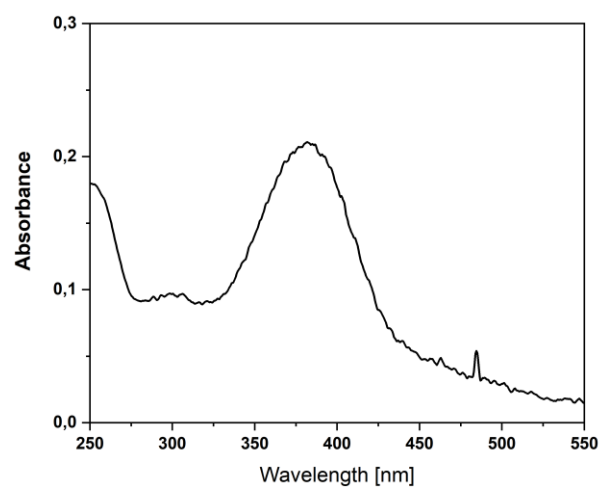
UV-Vis spectrum of **9s** (100 μ M, phosphate buffer, pH=7.0):



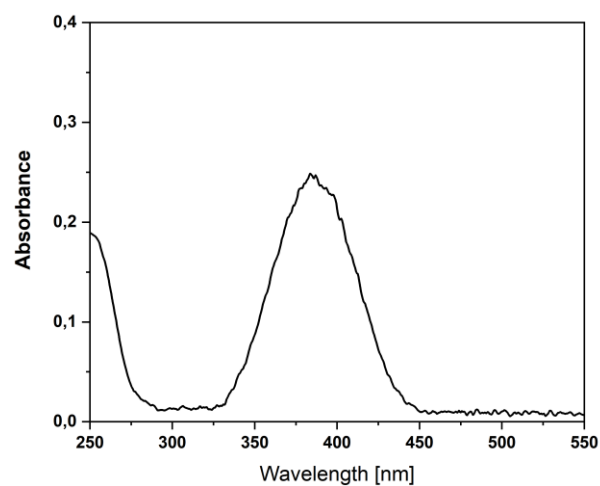
UV-Vis spectrum of **10o** (100 μ M, phosphate buffer, pH=7.0):



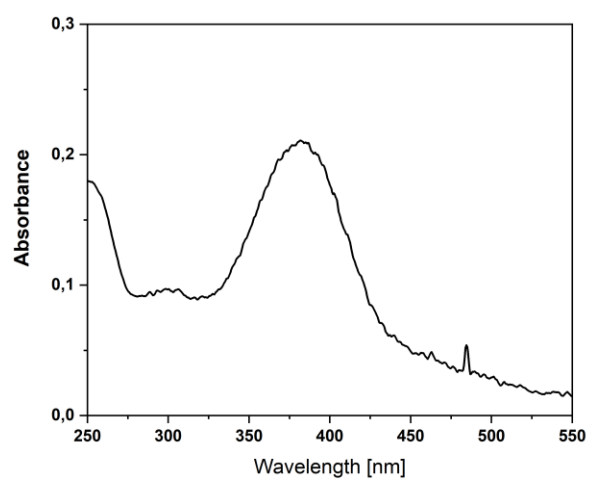
UV-Vis spectrum of **10s** (100 μ M, phosphate buffer, pH=7.0):



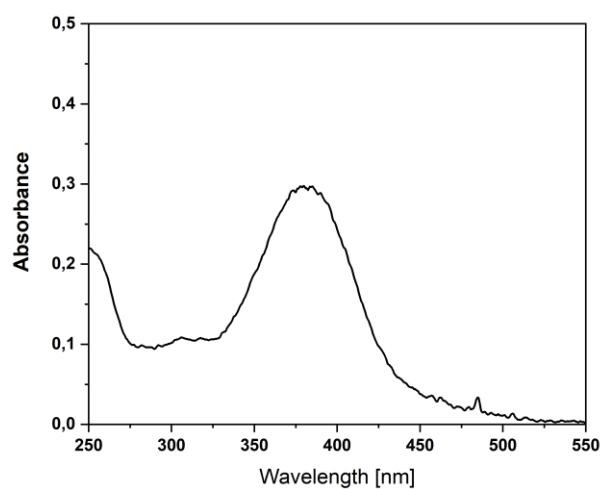
UV-Vis spectrum of **11o** (100 μ M, phosphate buffer, pH=7.0):



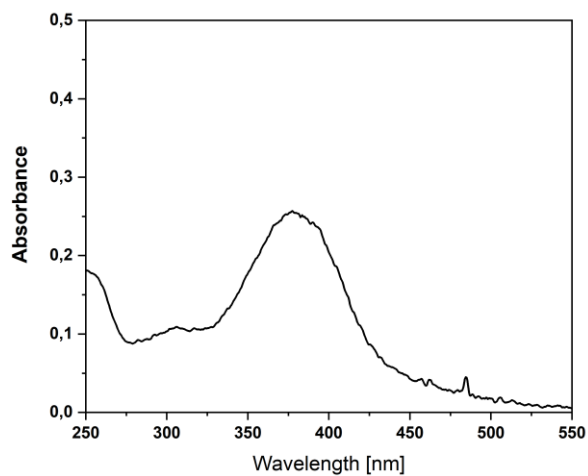
UV-Vis spectrum of **11s** (100 μ M, phosphate buffer, pH=7.0):



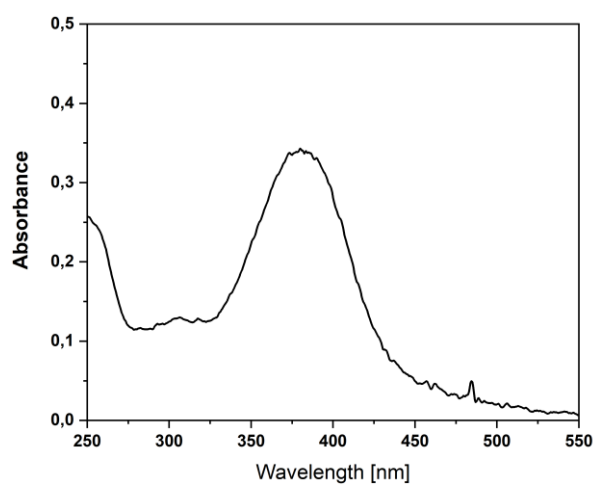
UV-Vis spectrum of **12o** (100 μ M, phosphate buffer, pH=7.0):



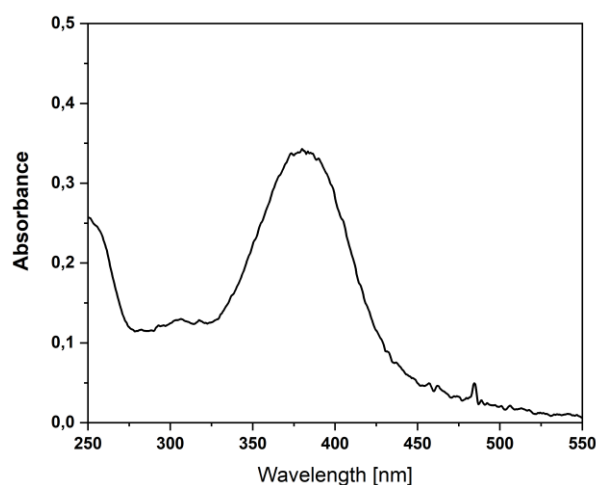
UV-Vis spectrum of **12s** (100 μ M, phosphate buffer, pH=7.0):



UV-Vis spectrum of **13o** (100 μ M, phosphate buffer, pH=7.0):



UV-Vis spectrum of **13o** (100 μ M, phosphate buffer, pH=7.0):



Quantum Yields

Chemical actinometry

A modification of a standard protocol was applied for the determination of the photon flux.^[1] An aqueous H_2SO_4 solution (0.05 M) containing freshly recrystallized $\text{K}_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ (41 mM, 2 mL) was irradiated at 20 °C for a given period of time in the dark with a 365 nm and 405 nm LED at a controlled, fixed, low intensity. The solution was then diluted with 1.0 mL of an aqueous H_2SO_4 solution (0.5 M) containing phenanthroline (1 g/L) and NaOAc (122.5 g/L) and left to react for 10 min. The absorption at 510 nm was measured and compared to an identically prepared non-irradiated sample. The concentration of $[\text{Fe}(\text{phenanthroline})_3]^{2+}$ complex was calculated using its molar absorptivity ($\epsilon = 11100 \text{ M}^{-1} \text{ cm}^{-1}$) and considering the dilution. The quantity of Fe^{2+} ions expressed in mol was plotted against time and the slope, obtained by linear fitting the data points to the equation $y = ax + b$, equals the rate of formation of the Fe^{2+} ion at the given wavelengths. The rate can be converted into the photon flux (I) by dividing it by the quantum yield of $[\text{Fe}(\text{phenanthroline})_3]^{2+}$ complex ($\Phi_{365\text{nm}} = 1.29$, $\Phi_{405\text{nm}} = 1.13$). The obtained photon flux was to $I_{405\text{nm}} = 9.9543508 \times 10^{-5} \text{ mE s}^{-1}$.

Quantum yield

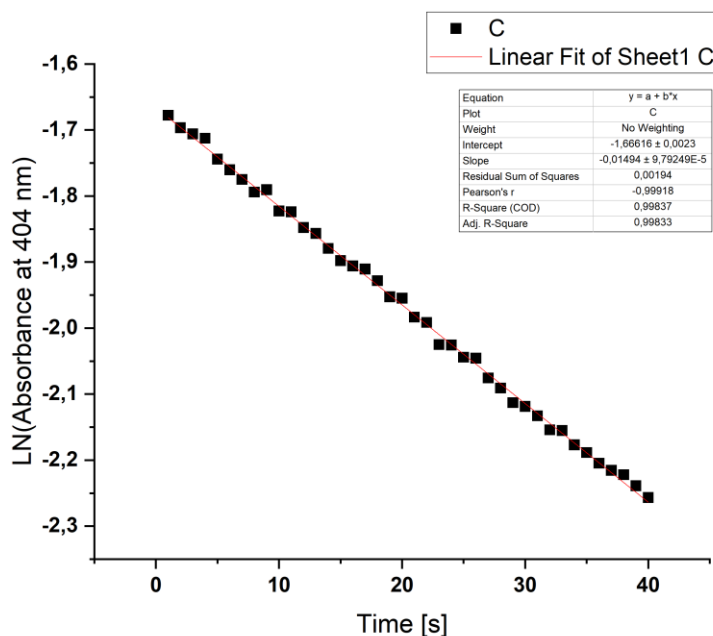
The quantum yield of the photochemical ring opening is determined using an initial slope method. The sample is irradiated with an LED and the natural logarithm of the absorbance data is plotted against time. From equation^[1] 1, we can determine the quantum yield of the photochemical bond cleavage.

$$\text{Eq. 1} \quad \phi = \frac{-k [X]_0 V}{I (1 - 10^{-\text{Abs}(0)})}$$

Where ϕ is the quantum yield; $-k$ is the reaction rate; $[X]$ is the concentration of the coumarin-lysine conjugate in M^{-1} ; V is the volume in mL and I is the photon flux in mE s^{-1} . Applying first order kinetics, the rate $-k$ can be taken from the initial slope of the natural logarithm of absorbance vs. time.

Quantum yield Determination for UAA 7a

The quantum yield (QY) was determined for the photochemically active coumarin core **7a** (100 μ M in chloroform) to be to be *ca.* 4%, a value that is in agreement with literature values.^[2]



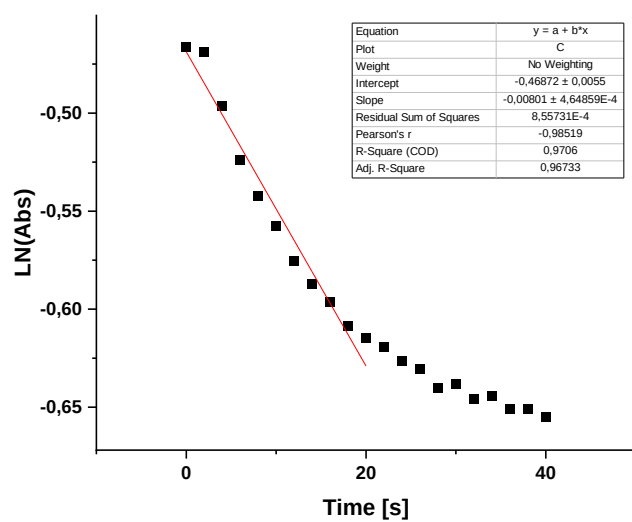
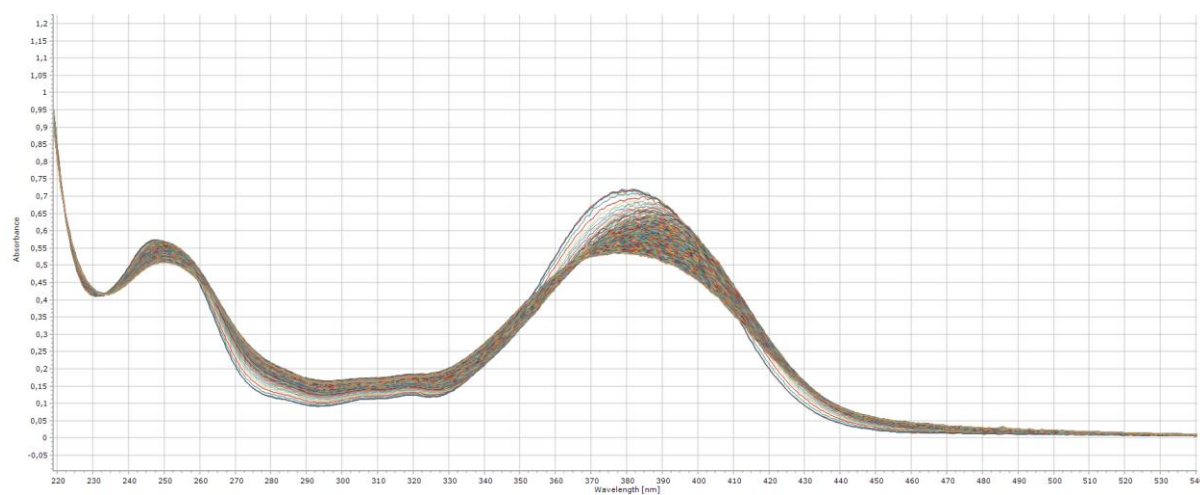
Quantum yield Determination for the Isolated Peptides

The QYs of peptides **9–12** in both the open and the unstapled form were approximated under irradiation with 405 nm light in PBS buffer (8 mM Na₂HPO₄, and 2 mM KH₂PO₄, pH 7.0, 200 μ M, 1 mL). Time-resolved UV-Vis spectra (*vide infra*) and UPLC measurements (*vide infra*) show that all photocaged peptides uncage efficiently.

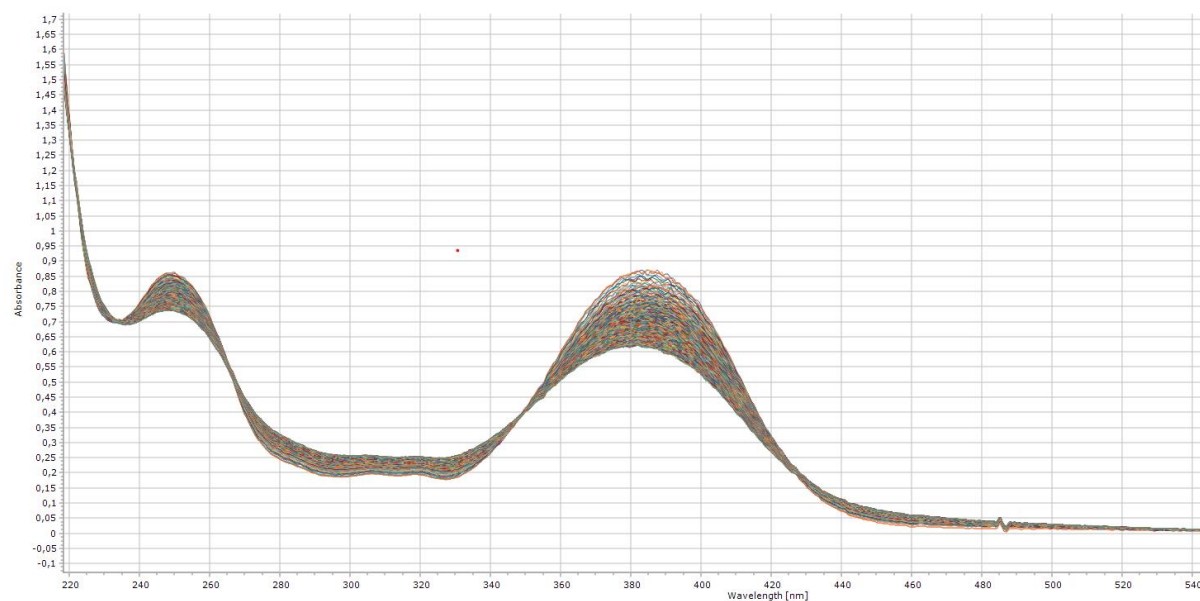
All QYs are in the range of 1 or 2% (see table below) and thus, in the same range as the Fmoc-protected UAA (*vide supra*) and comparable molecules the literature considering the inaccuracy of QY measurements especially with small amount of samples in the low absorption regime. The same sample amount in compound **12s** did not allow an analysis of it.

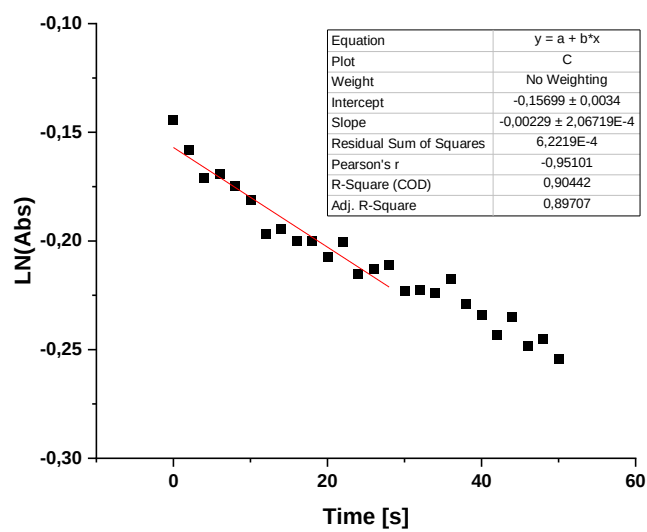
Peptide	Concentration [μ mol]	Solvent	Wavelength of Irradiation	Quantum Yield [%]
9o	200	PBS	405 nm	2
9s	200	PBS	405 nm	1
10o	200	PBS	405 nm	2
10s	200	PBS	405 nm	1
11o	200	PBS	405 nm	1
11s	200	PBS	405 nm	1
12o	200	PBS	405 nm	1
13o	200	PBS	405 nm	1
13s	200	PBS	405 nm	1

90

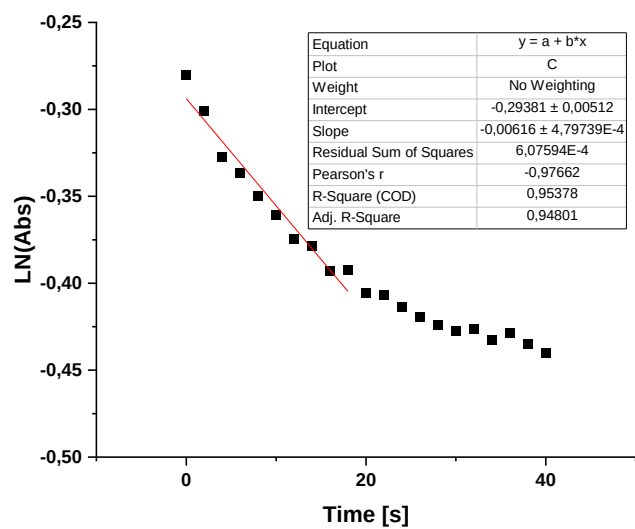
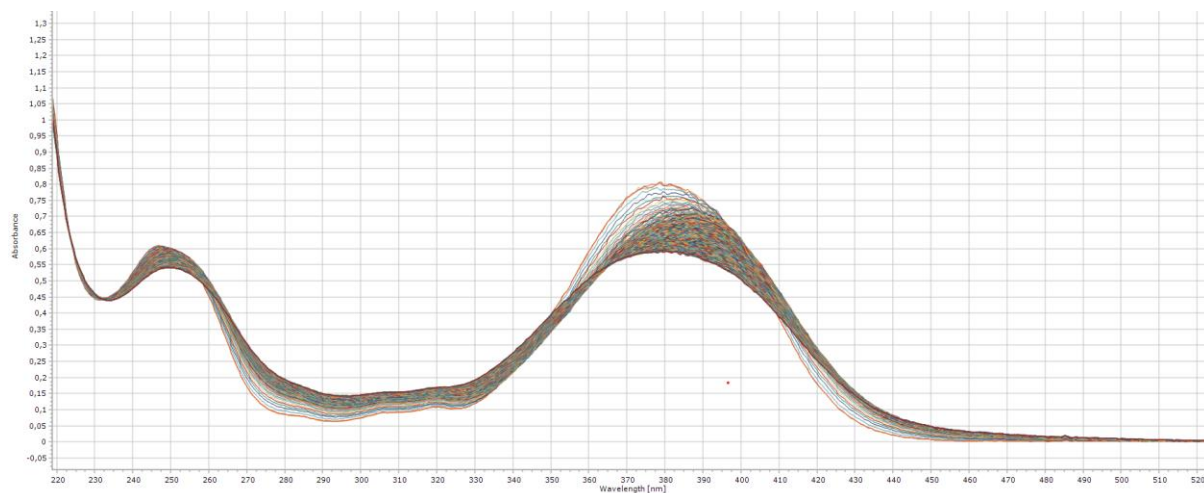


9s

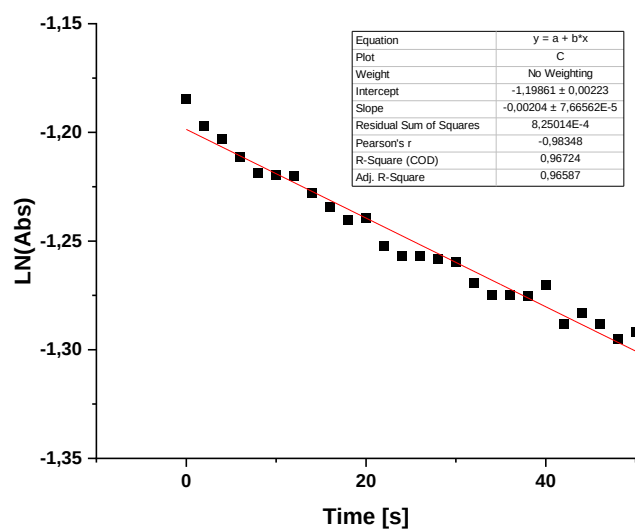
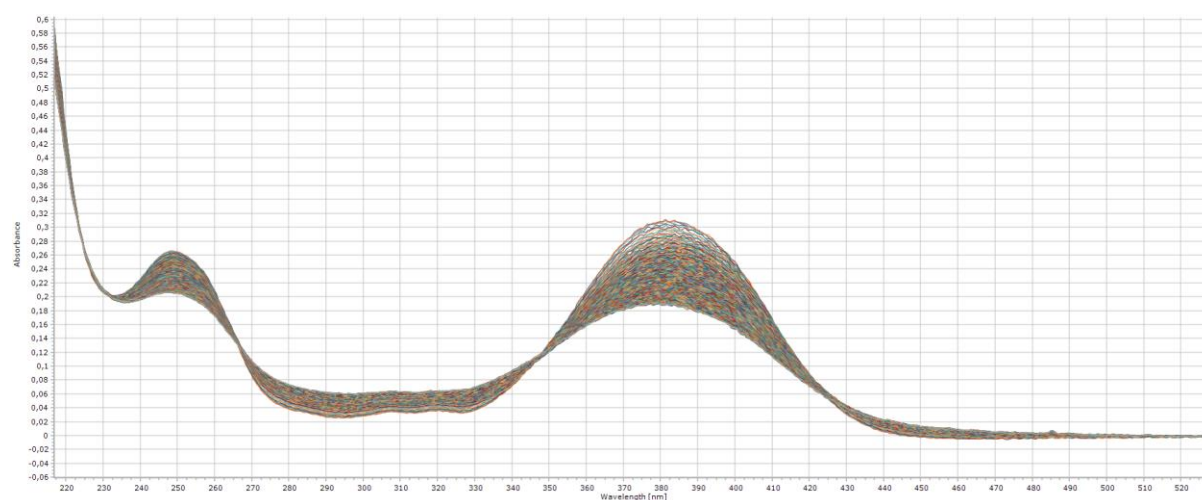




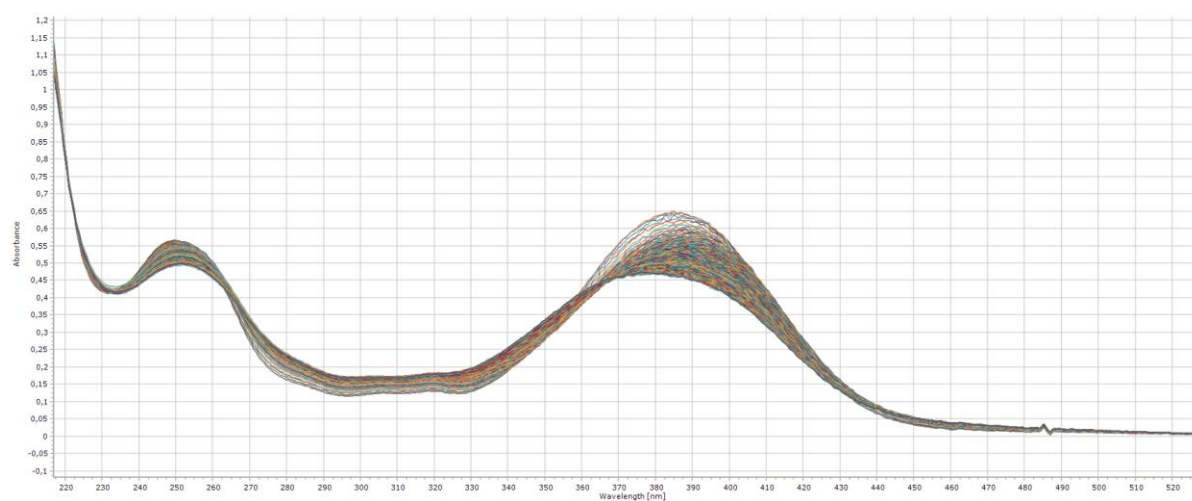
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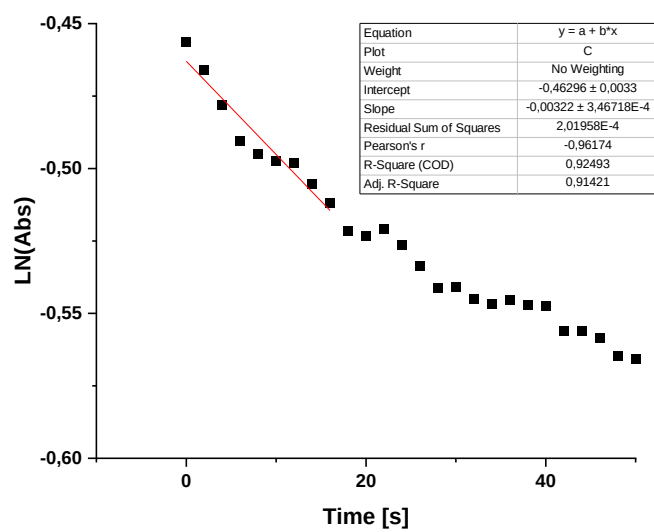


10s

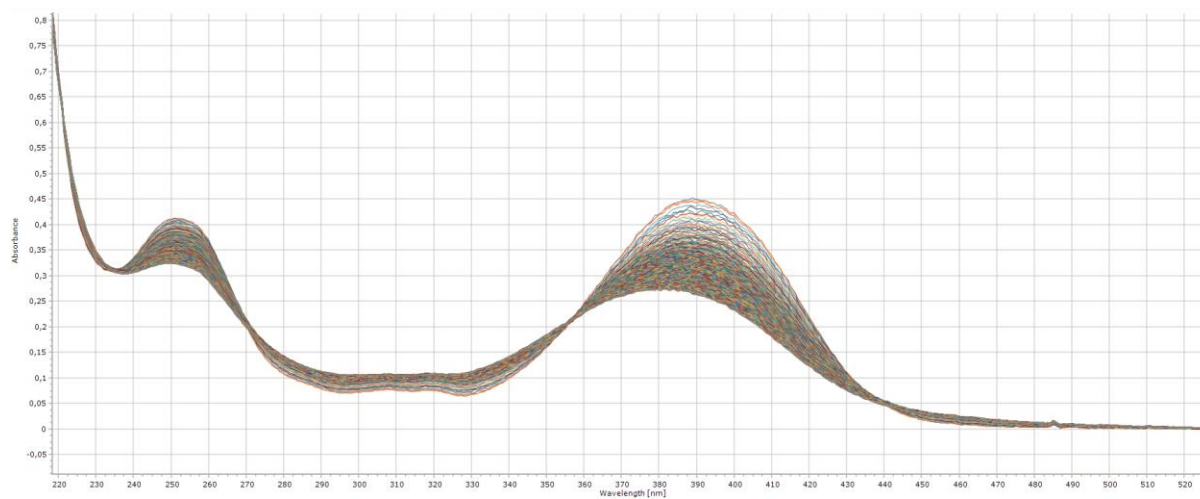


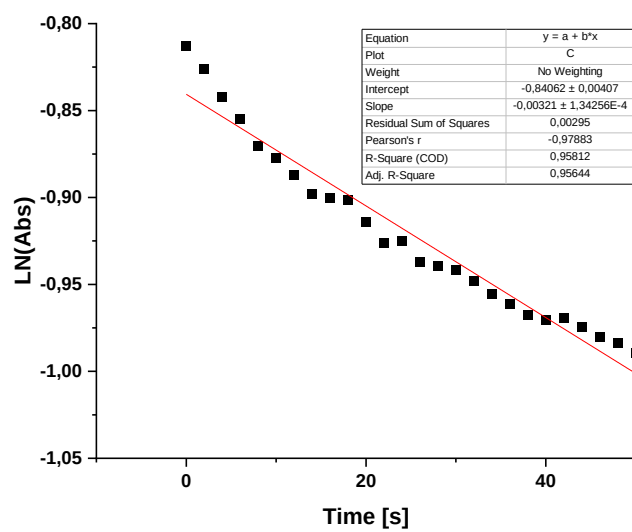
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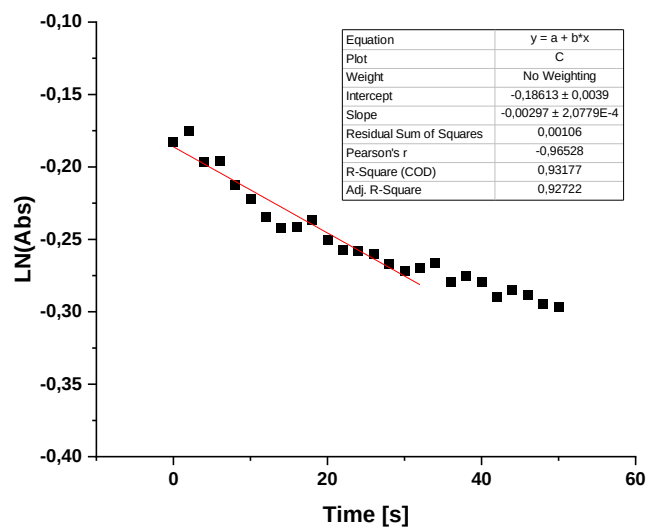
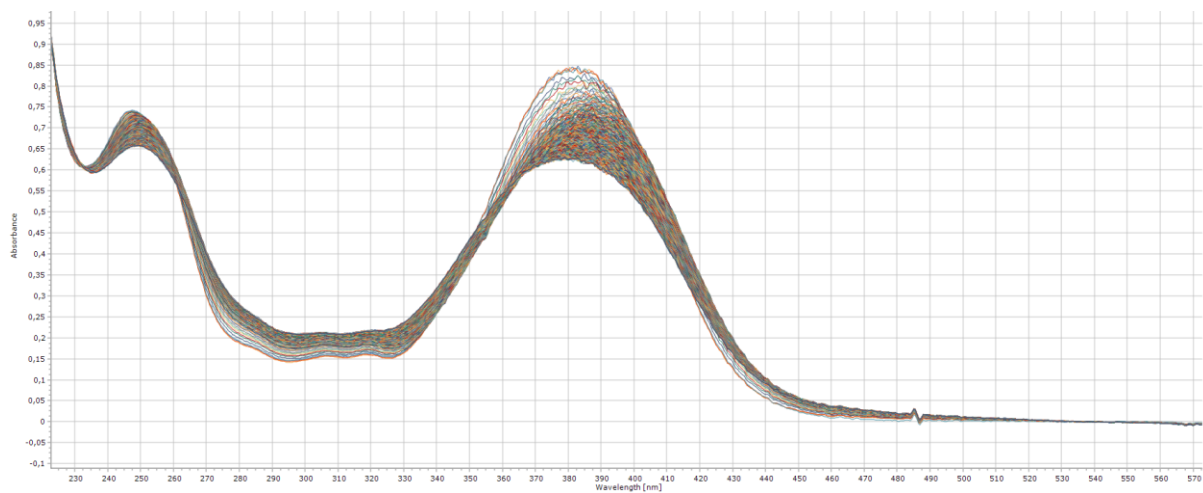


11s

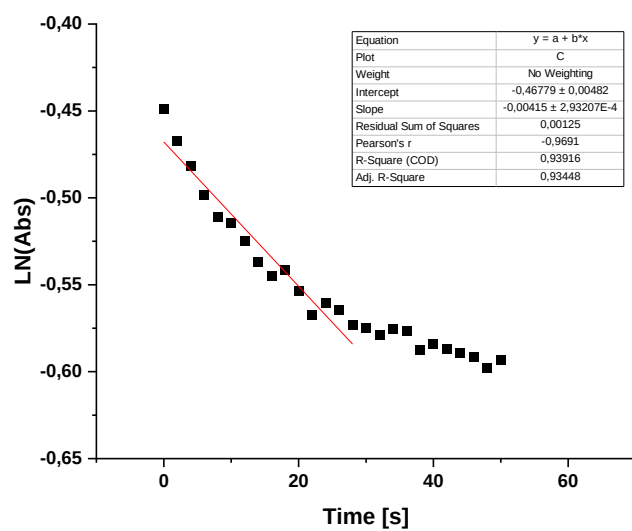
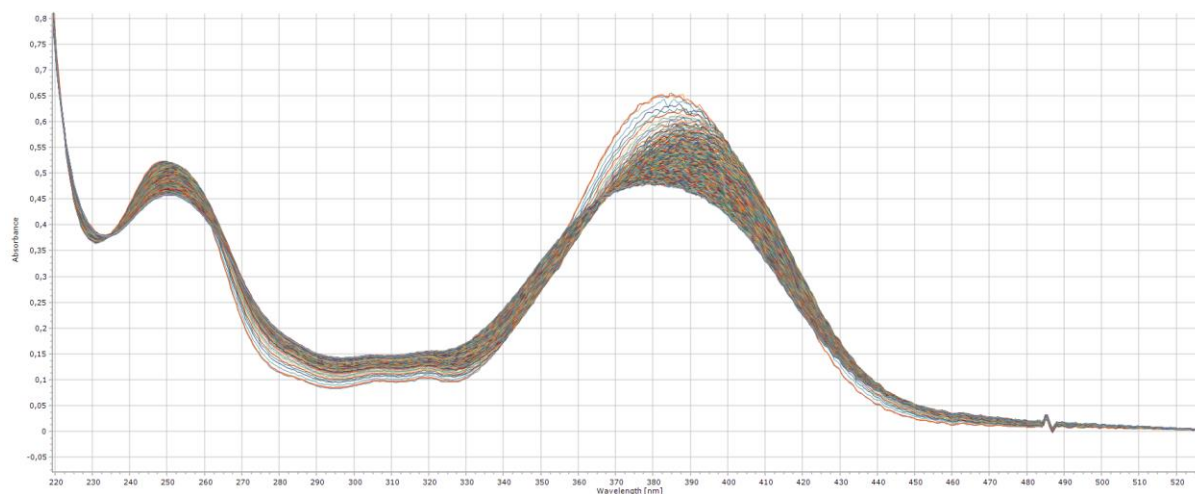




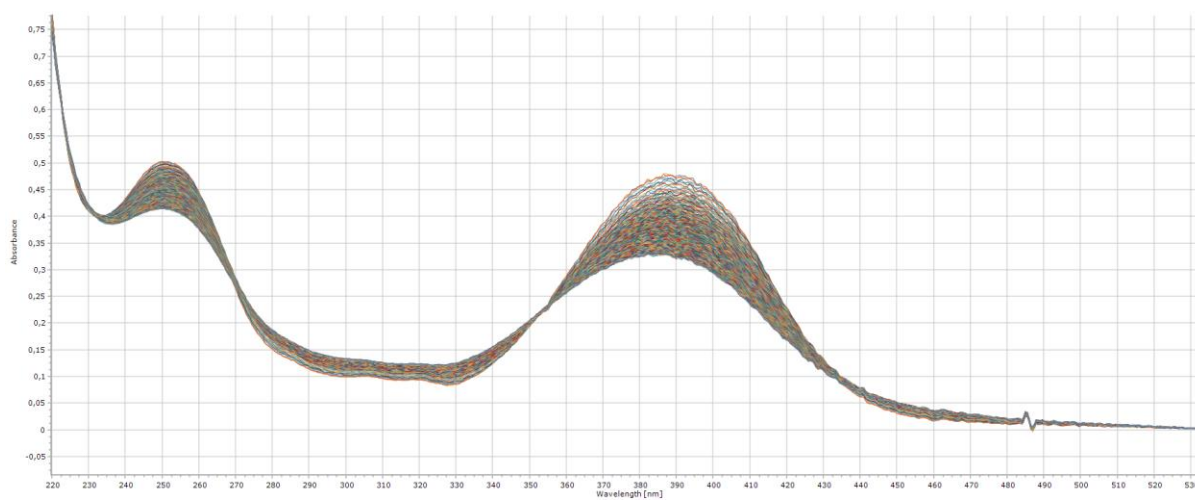
12o

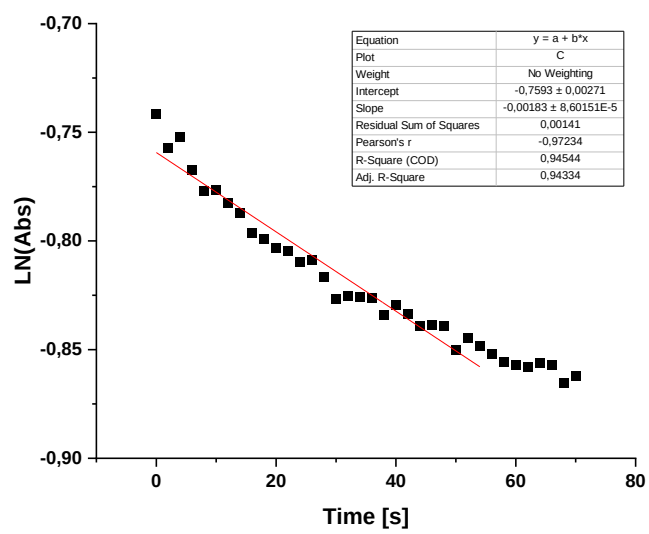


13o



13s





Section 7. Computational Methods

QM calculations:

Conformer-rotamer ensemble sampling tool (CREST)^[3], was used to generate the initial conformations of the coumarin-photocage. Out of 273 conformations, the two most stable ones were optimized using Gaussian16 package^[4] at the B3LYP/def2-TZVP level and the D3 dispersion correction from Grimme and co-workers.^[5–8] The most stable conformation is shown below. The distance between the oxygen atoms that are the connection sites to peptides is 11.25 Å.

Parametrization:

The coumarin-photocage, and the HPO_4^{2-} and H_2PO_4^- molecules were parameterised on the basis of Gaussian16 software package calculations.^[4] Geometry and frequency calculations were performed at the B3LYP/def2-TZVP level using the D3 dispersion correction from Grimme and co-workers.^[5–8] The point charges were computed with the RESP procedure at the HF/6-31G* level, as established in the standard protocol, employing the AMBER atom types.^[9] The Seminario approach (implemented in CartHess2FC.py) is then employed to obtain the force constant and the equilibrium angle for the OH-P-OH and O2-P-OH angles.^[10]

System set up:

All molecular dynamics simulations were carried out with the AMBER 20 software package,^[11] using sander for minimizations and pmemd for the simulation of the trajectories, employing the FF19SB force field.^[12] for the protein and the Generalized AMBER force field (GAFF) for the staple^[13]. All structures were set in a periodic cuboid box of 12 Å, between the protein and the periodic box wall, of OPCBOX water molecules^[14] with 15 molecules of HPO_4^{2-} and H_2PO_4^- . The resultant system is then neutralized with Na^+ counter ions.

System preparation:

The initial structure of the K3 reference was modeled using *AlphaFold*^[15] and was used to build the initial structure of the stapled peptides. The following procedure is then employed for all the peptides.

The hydrogen atoms were first minimized for 2000 cycles (1000 with steepest descent and 1000 with conjugate gradient), by restraining the rest of the atoms with a $10.0 \text{ kcal/mol} \cdot \text{\AA}^2$ force constant. Following another 8000 cycle minimization (4000 cycles with steepest descent and 4000 with conjugate gradient), constraining everything except the solvent and backbone atoms with a $5.0 \text{ kcal/mol} \cdot \text{\AA}^2$ force constant. Finally, the system was minimized for 10000 cycles (2000 with steepest descent and 8000 with conjugate gradient) without restraints.

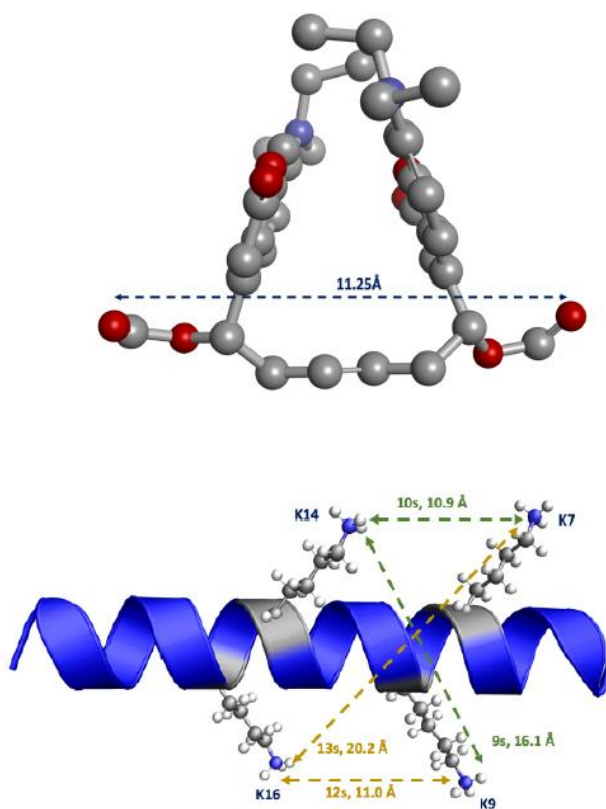
Simulation of the trajectories:

All the molecular dynamic simulations were performed with a 2 fs time step, using the SHAKE algorithm to constrain the bond lengths involving hydrogen atoms^[16]. Periodic boundary

conditions were employed together with the particle mesh Ewald (PME) method for treating the long-range electrostatic interactions with a cutoff distance of 8 Å for the non-bonded interactions.

An initial heating step was carried out over a period of 1000 ps. The simulation was performed in the NVT ensemble using the Langevin dynamics, with a collision frequency of 5.0 ps⁻¹. The temperature was gradually increased from 0K to 300K over the first 400,000 time steps, and then maintained at 300K for the remaining 100,000 time steps. Then, the equilibration was performed in the NPT ensemble with isotropic position scaling with a pressure relaxation time of 5.0 ps. The temperature was maintained at 300K using the Langevin thermostat with a collision frequency of 5.0 ps⁻¹. The simulation was performed for a total time of 1000 ps.

We performed a 400 ns production simulation of all peptides using the NPT ensemble. The production simulation was initiated from the final frame of the equilibration simulation with the same values as set for the equilibration phase. The heating and production phase were performed using GPUs^[17].

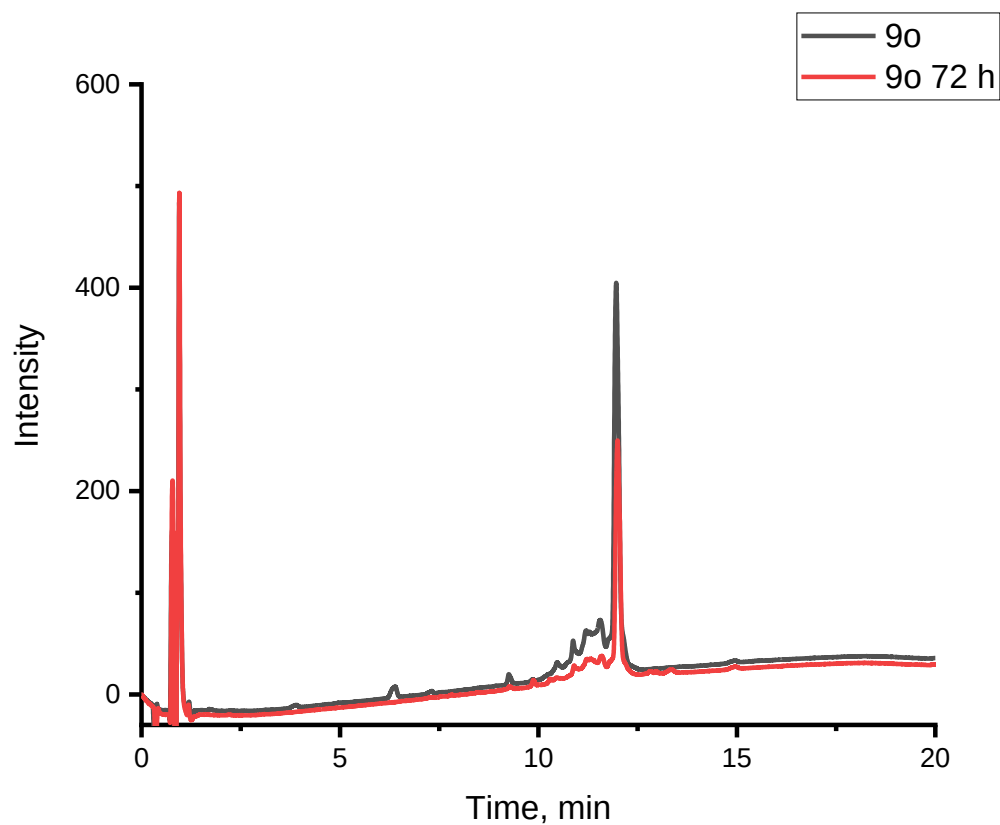


Section 8. Peptide Stability

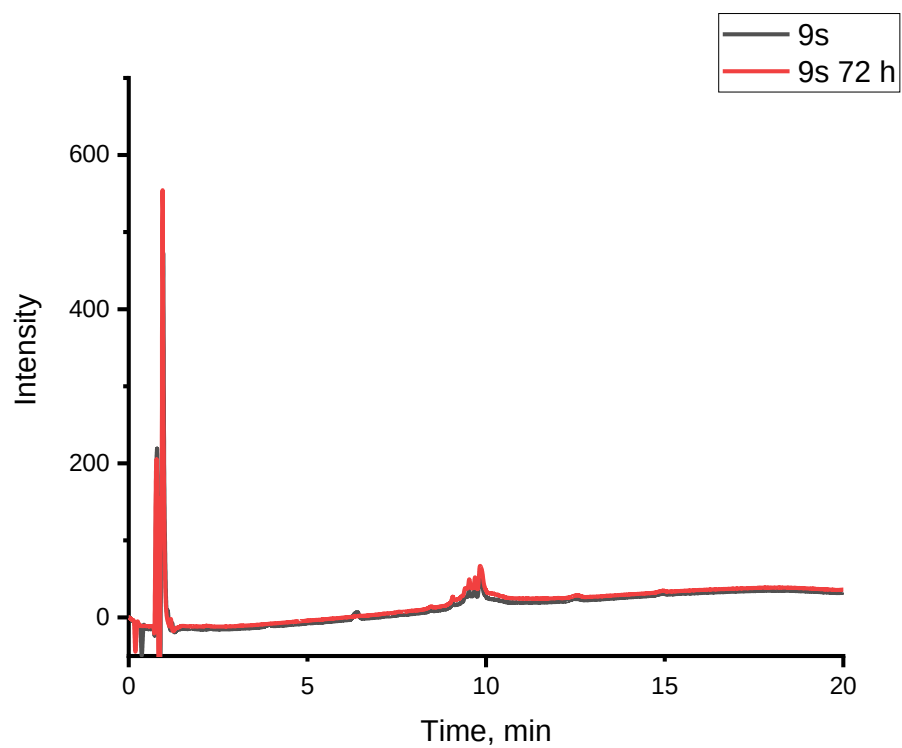
The stability of the synthesized peptides in physiological conditions was followed by UPLC. The peptides were dissolved in 10 mM PBS buffer, pH 7.4 to obtain a 200 μ M solution. The sample was shaken for 72 h at 37 °C. UPLC (column 1, 20-80% in 15 min, detection at 215 nm).

UPLC Traces at the start and after 72 h

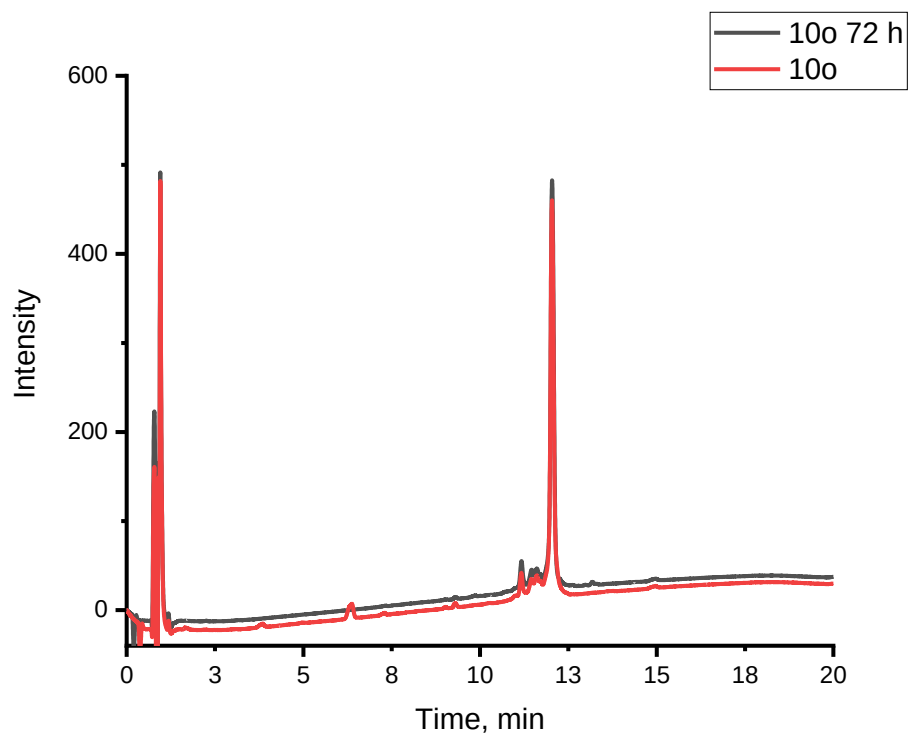
9o



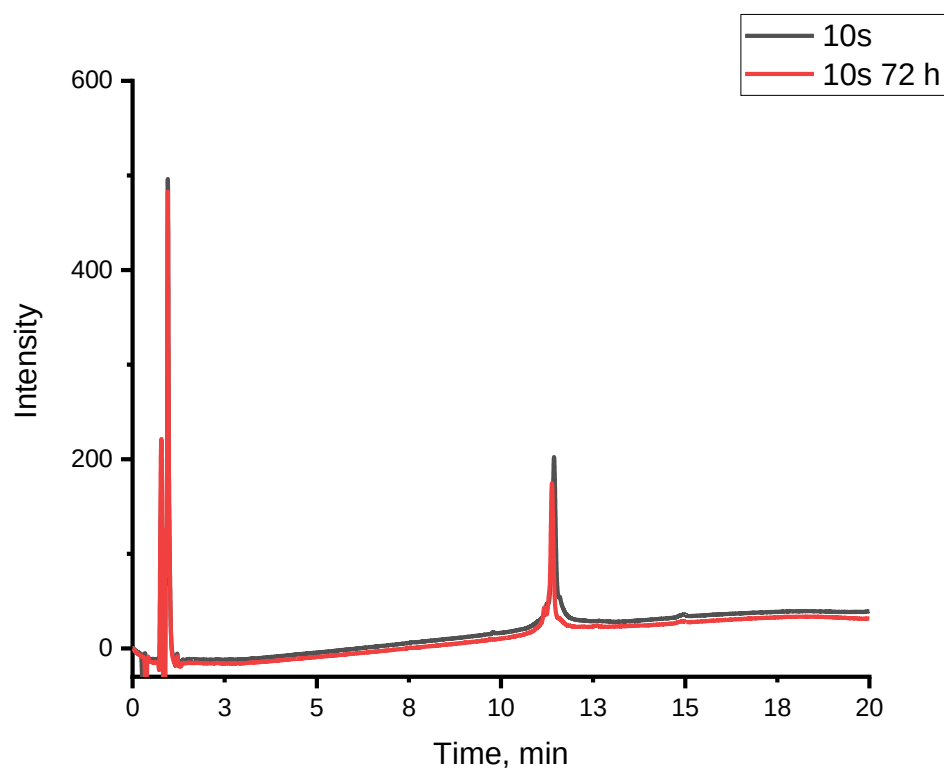
9s



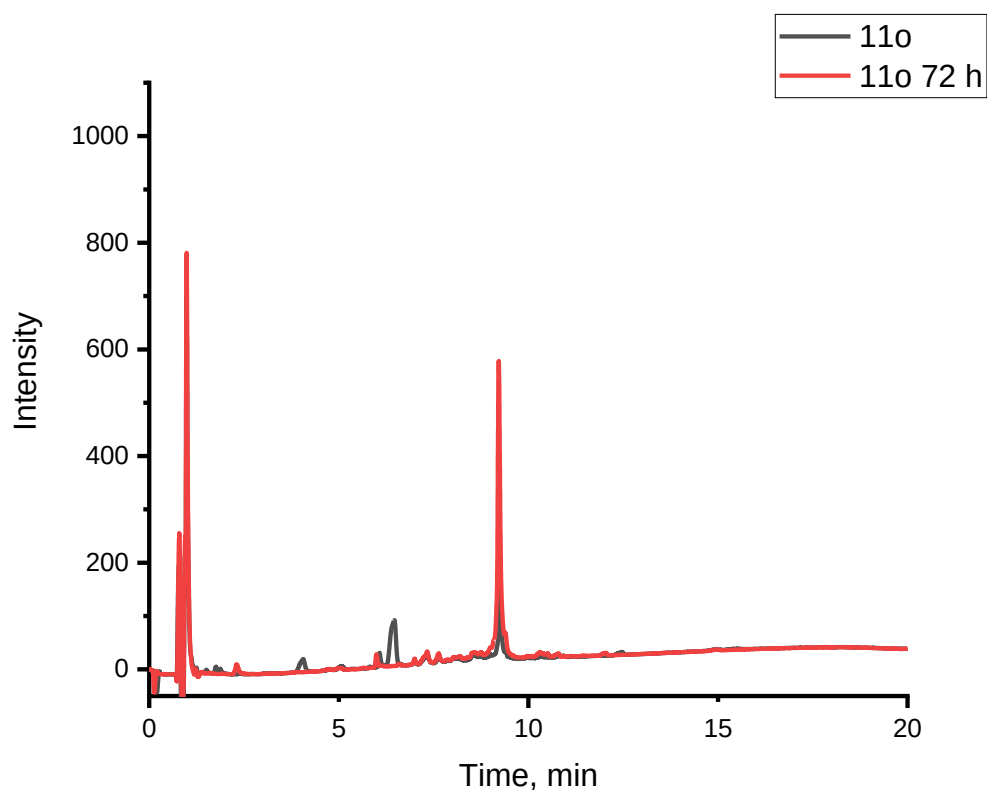
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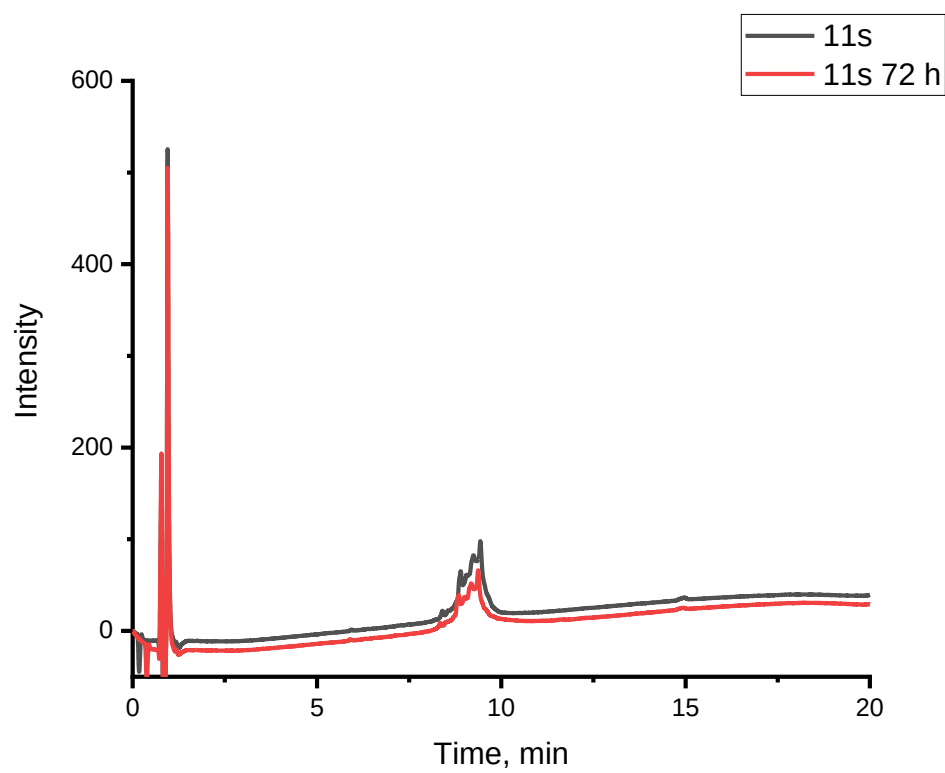
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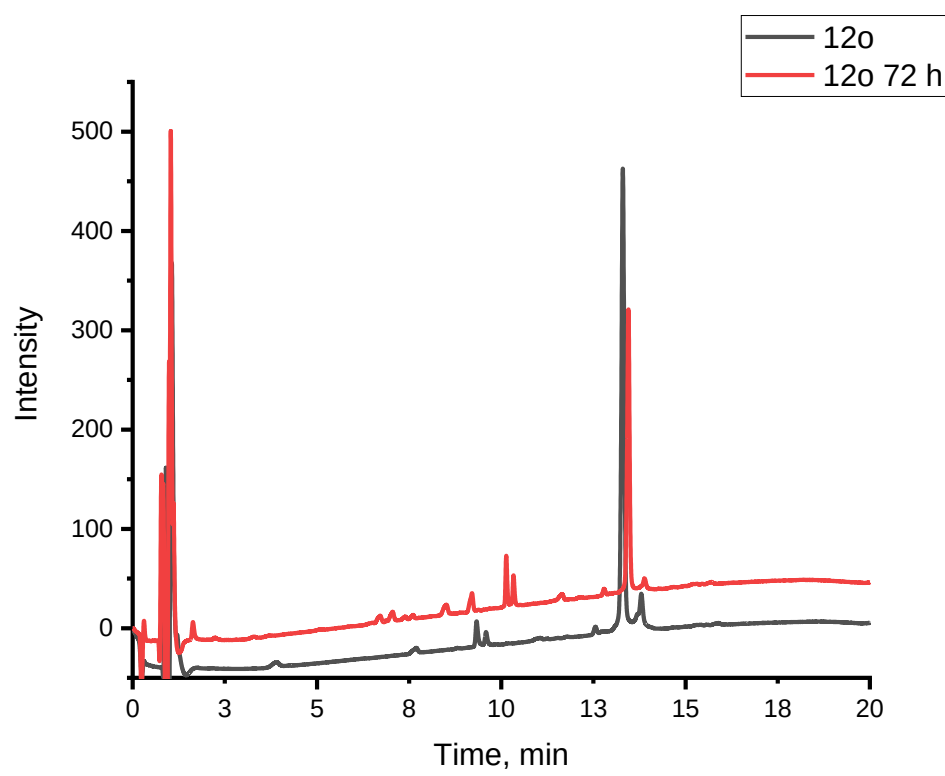
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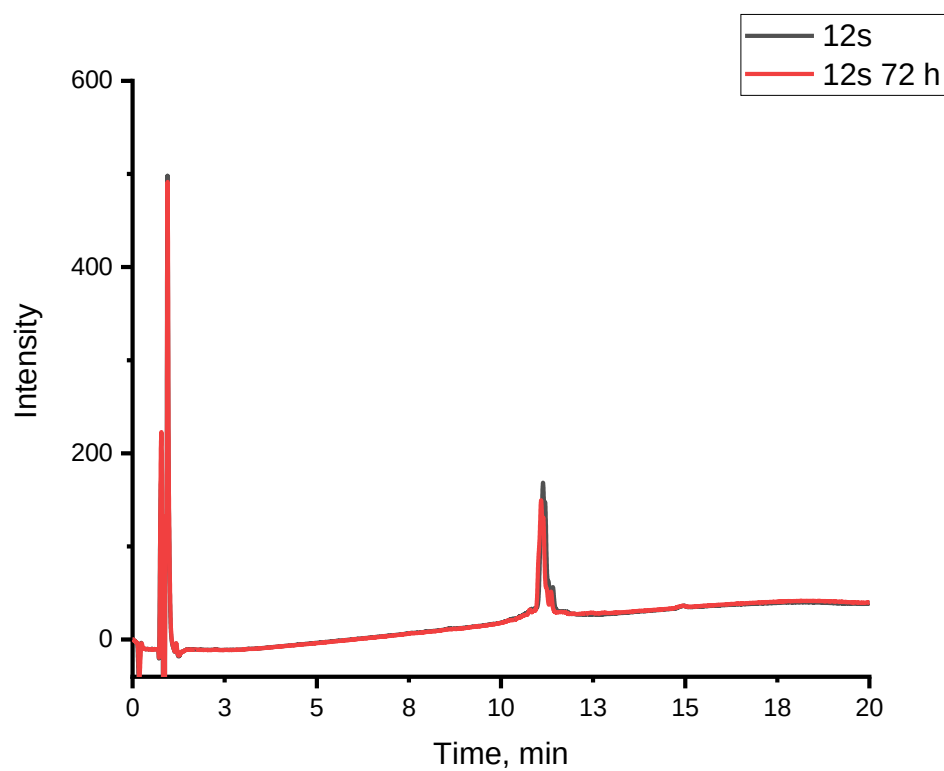
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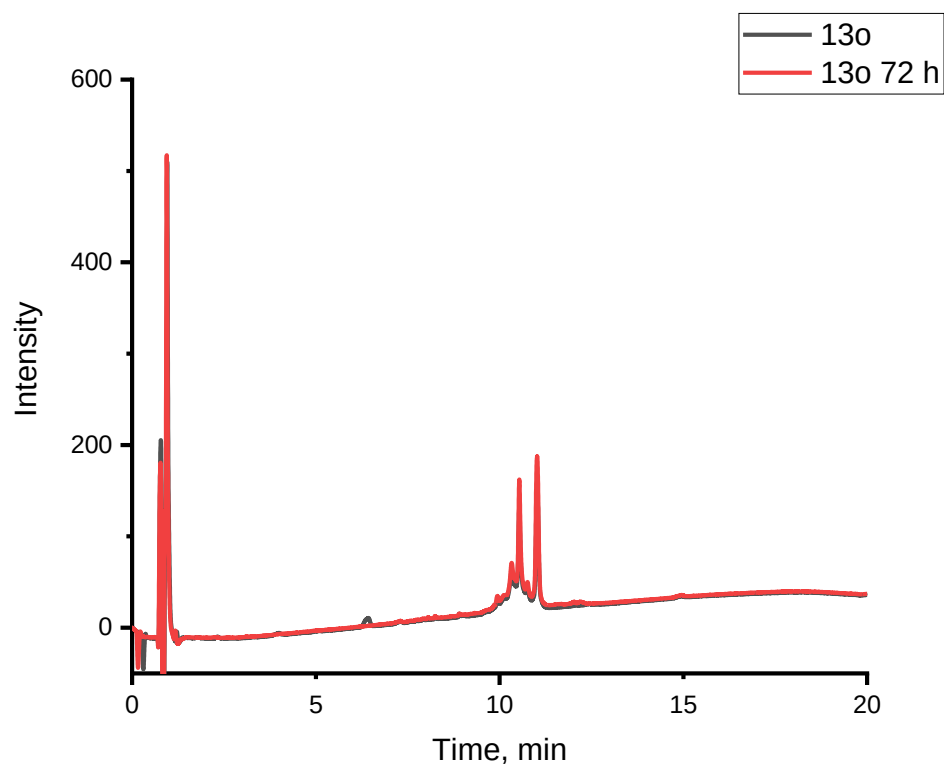
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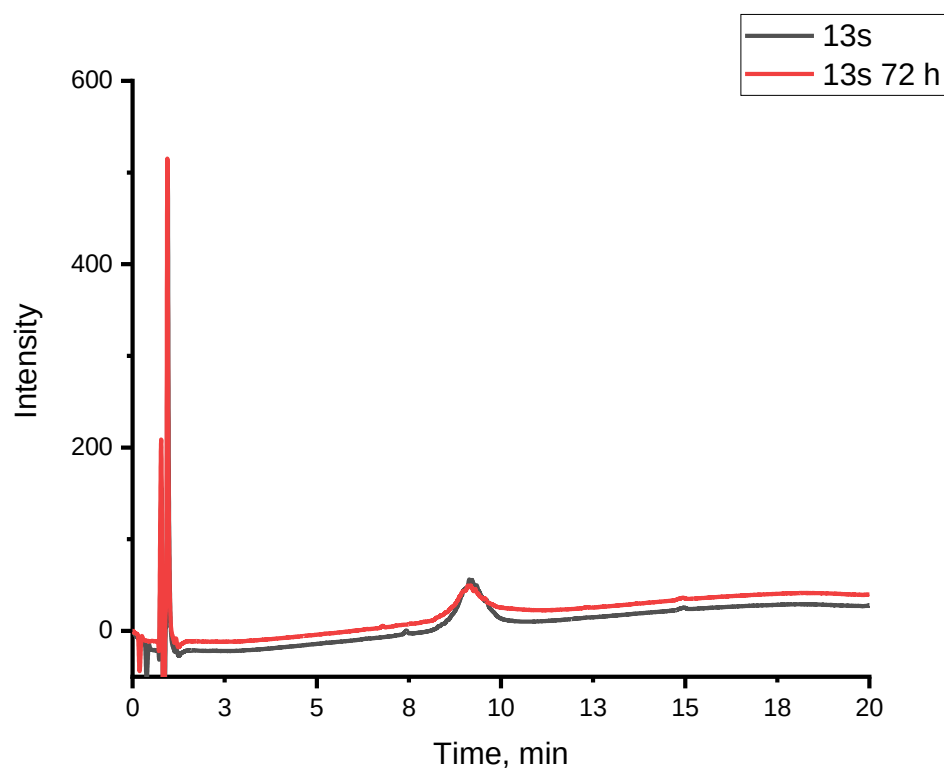
12s



13o



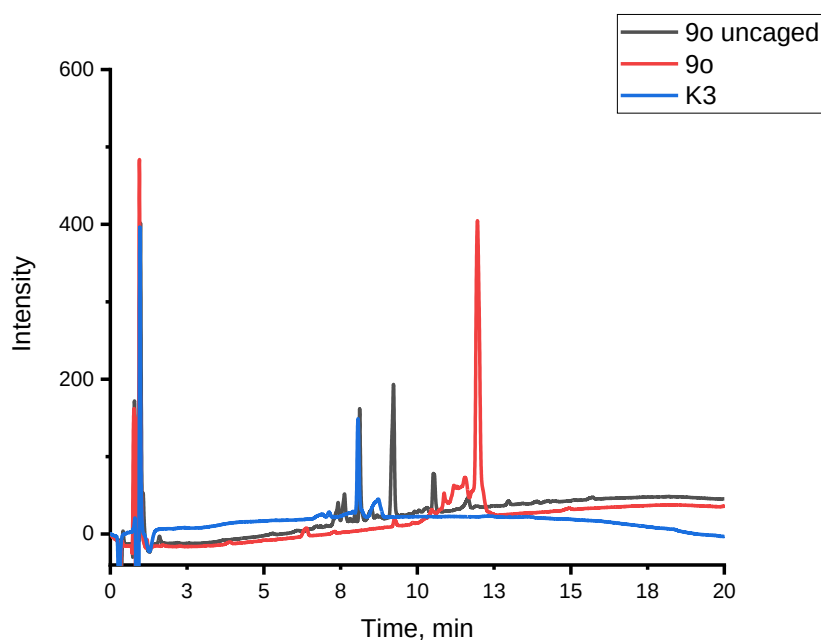
13s



Section 9. UPLC Analysis of Peptide Uncaging

Peptides **9–13** after uncaging were confirmed via UPLC and ESI-MS. The samples were dissolved in 10mM PBS buffer with pH 7.0 at the concentration 200 μ M. The uncaging was done at 405 nm wavelength as described previously. UPLC (column 1, 20-80% in 15 min, detection at 215 nm)

UPLC Traces and Mass Spectra of **9o** after uncaging



K3

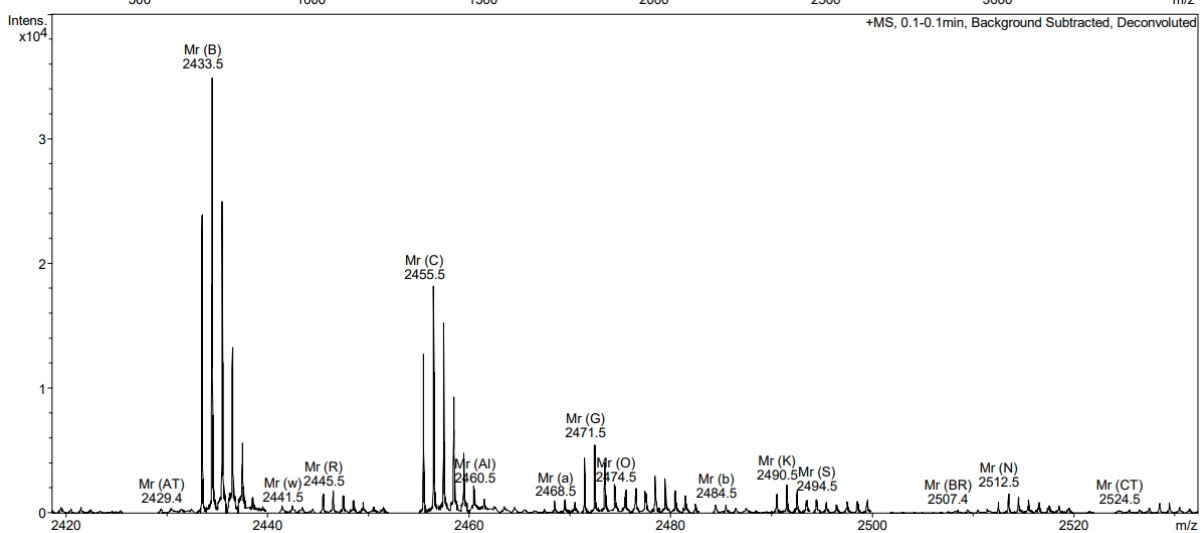
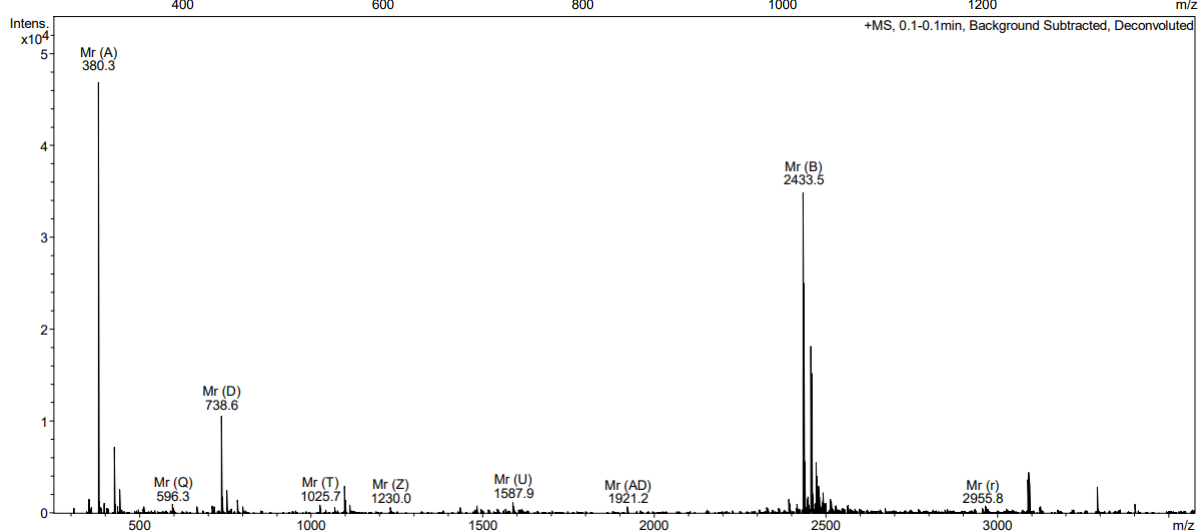
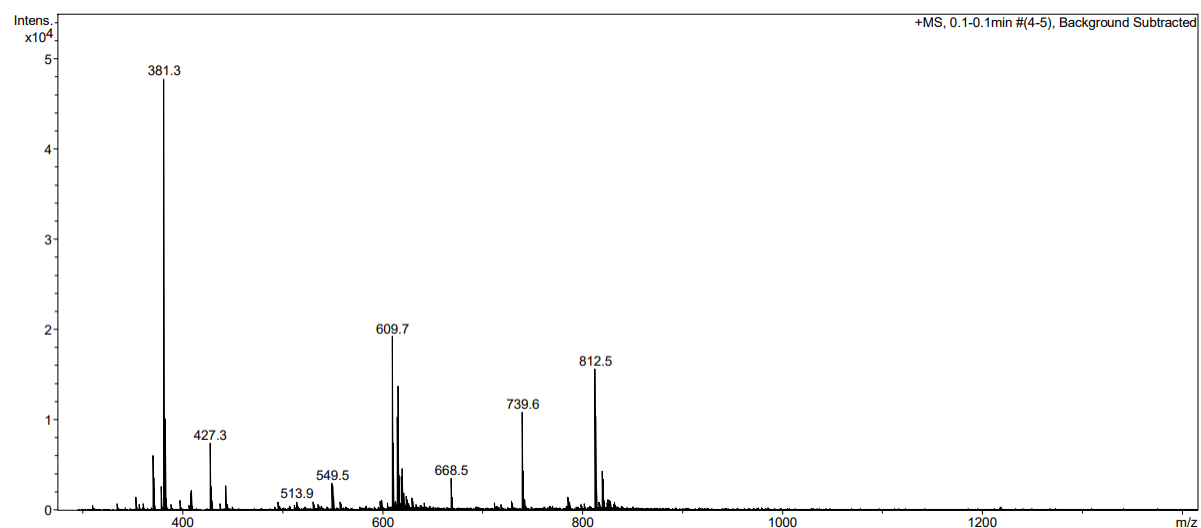
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

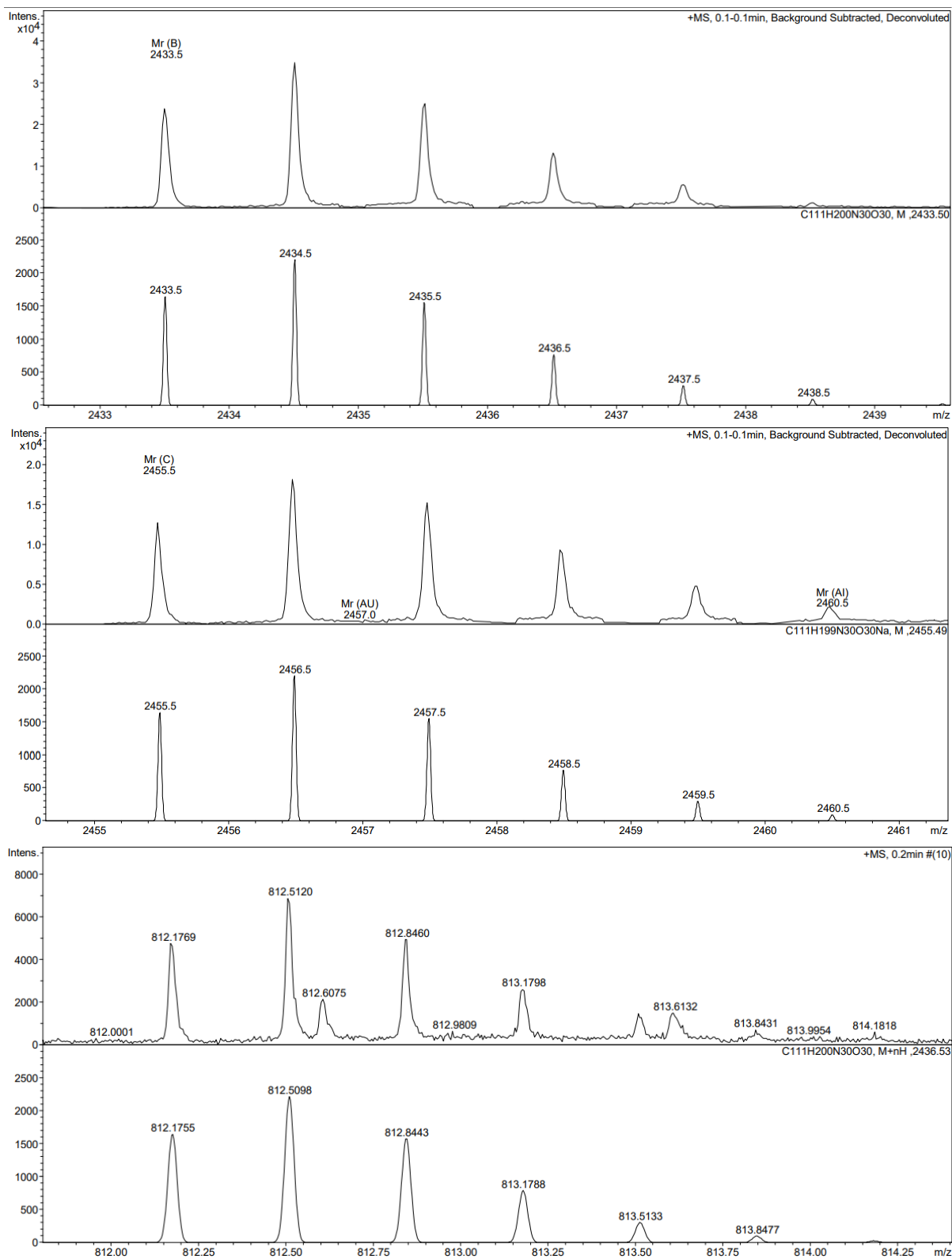
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

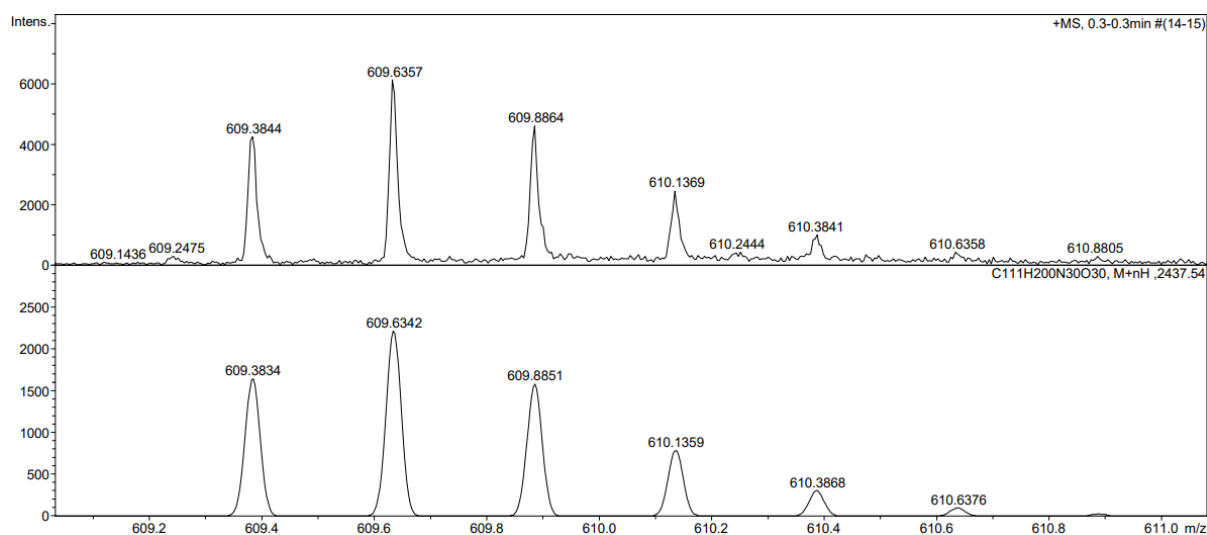
9o

ESI-MS m/z : 3060.8 $[M+H]^+$.

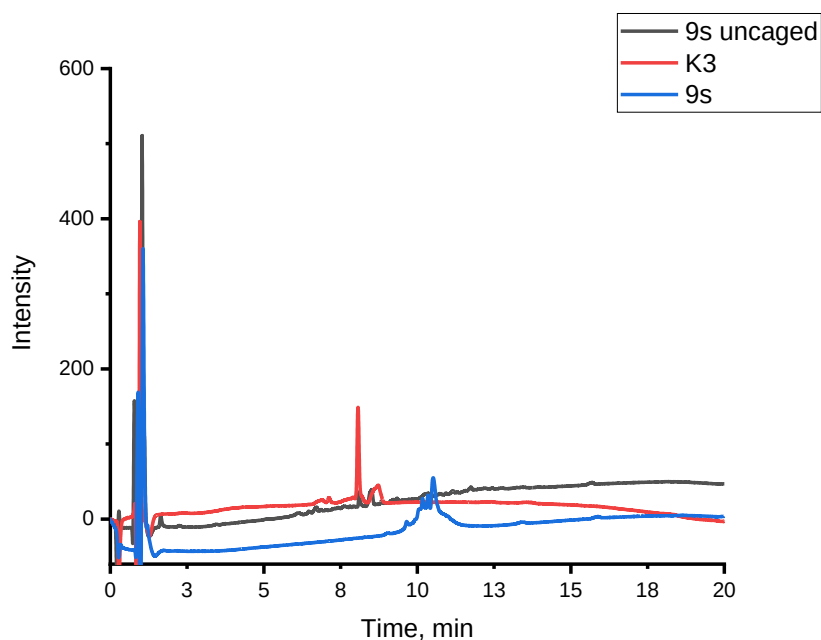
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9478.







UPLC traces and Mass Spectra of 9s after uncaging



K3

ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

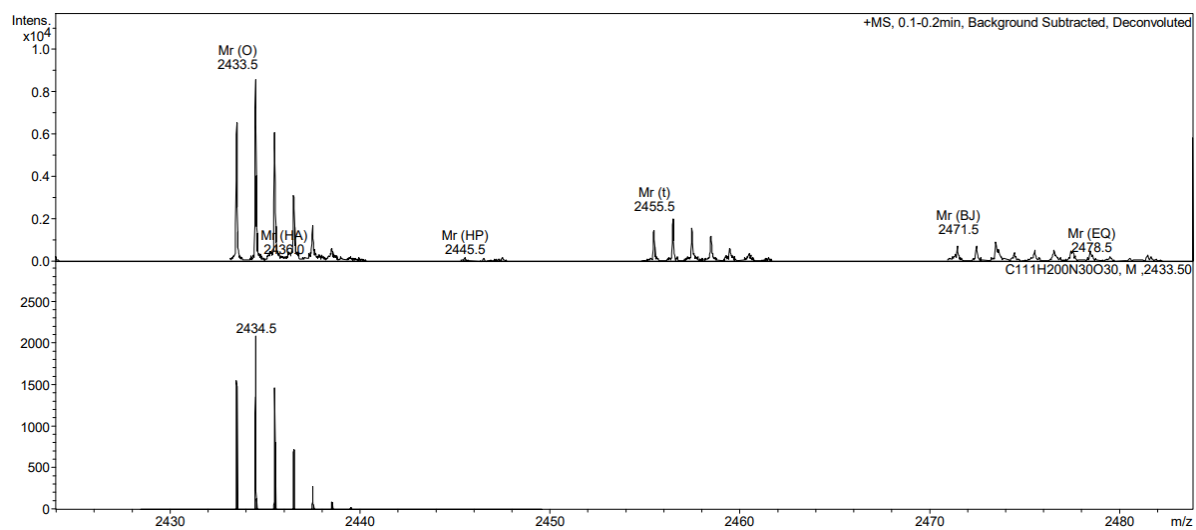
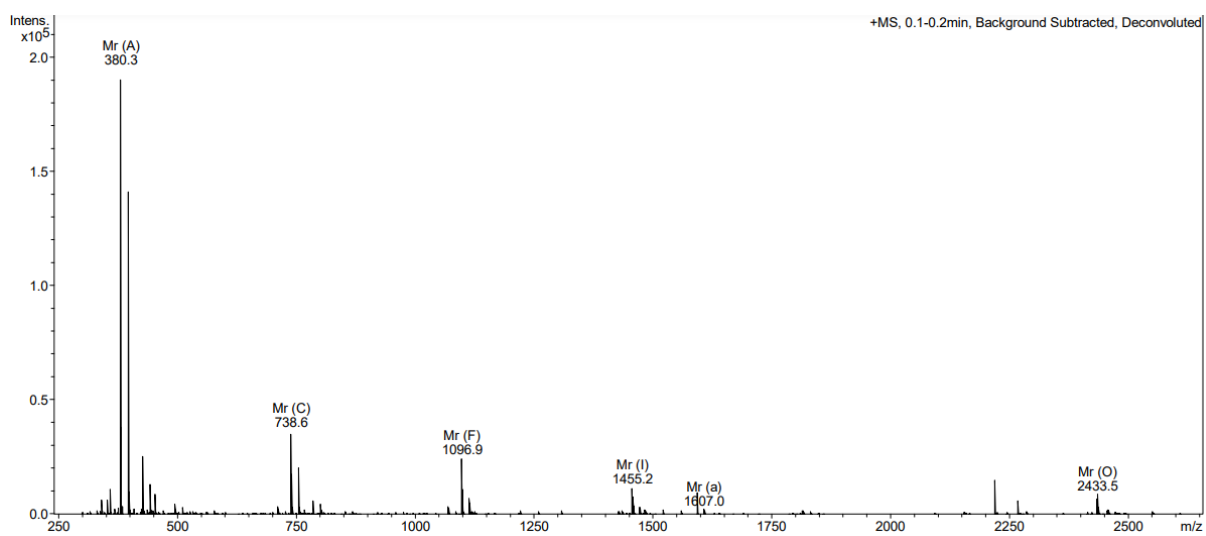
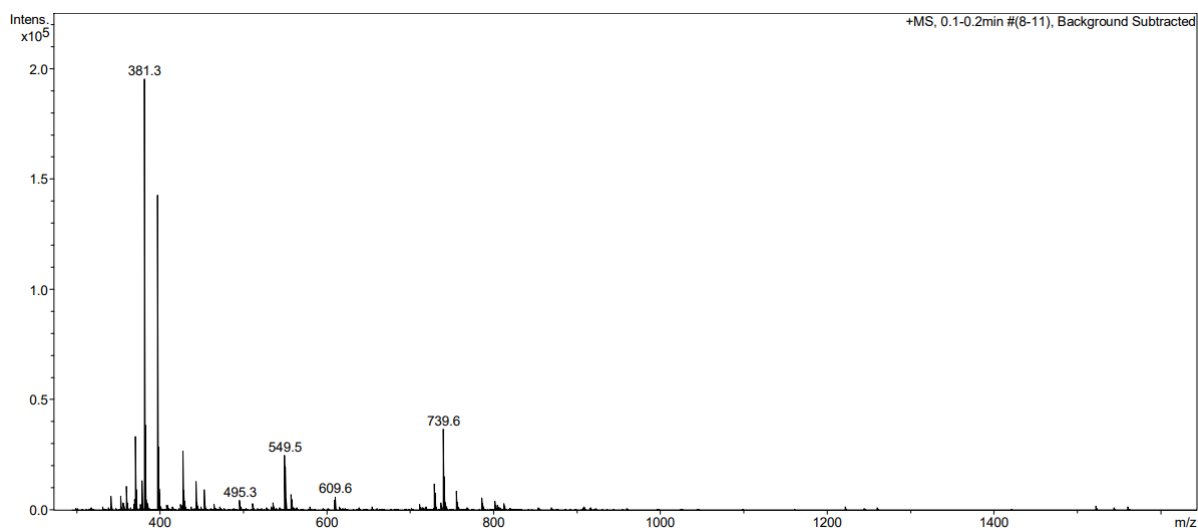
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

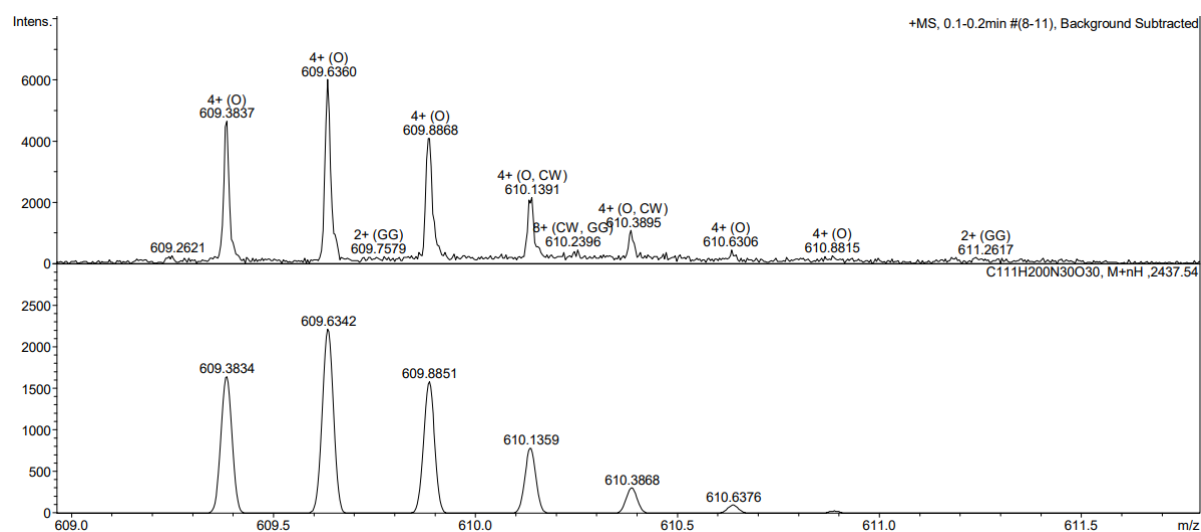
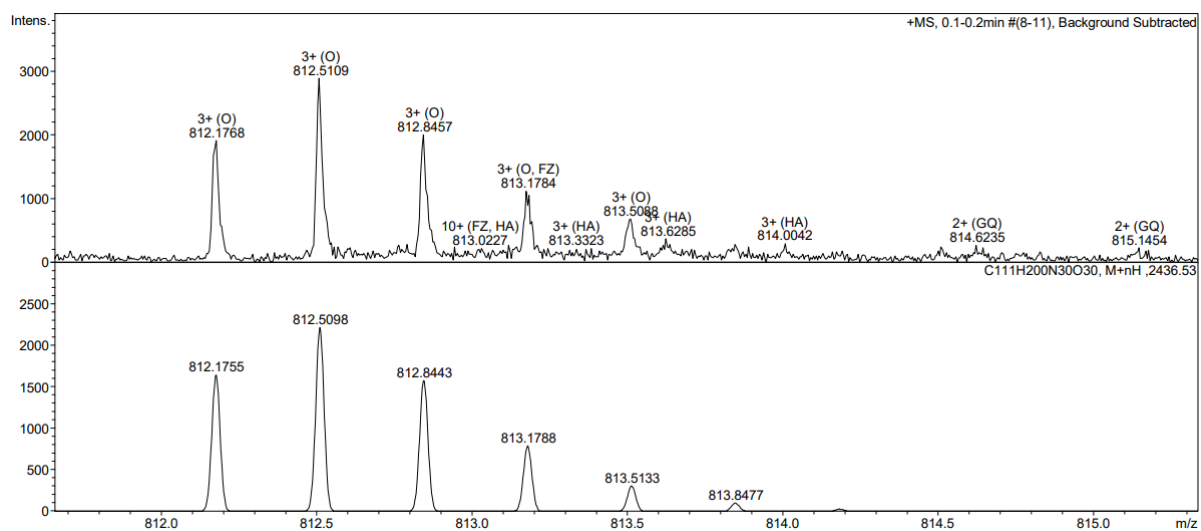
9s

ESI-MS m/z : 3031.7 $[M+H]^+$.

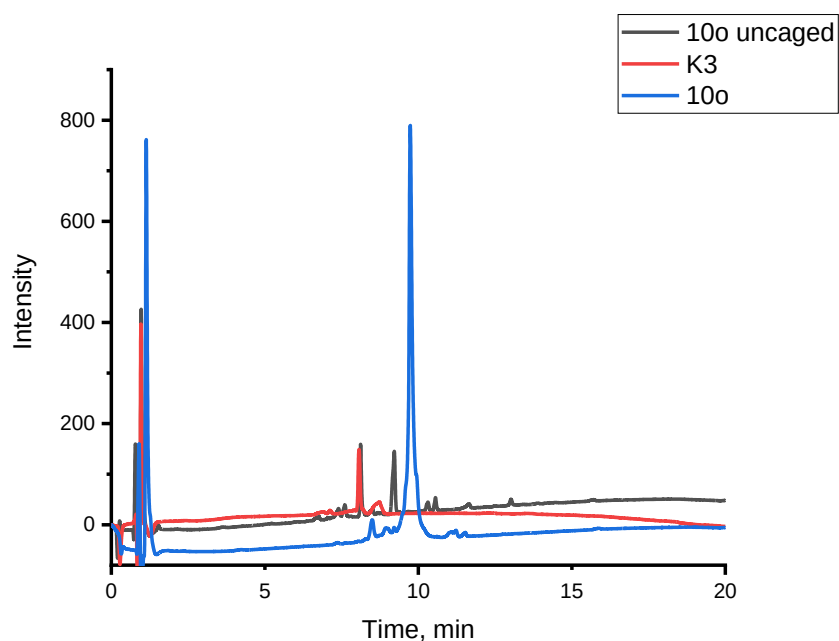
HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.

[NS1][IL2]





UPLC traces and Mass Spectra of 10o after uncaging



K3

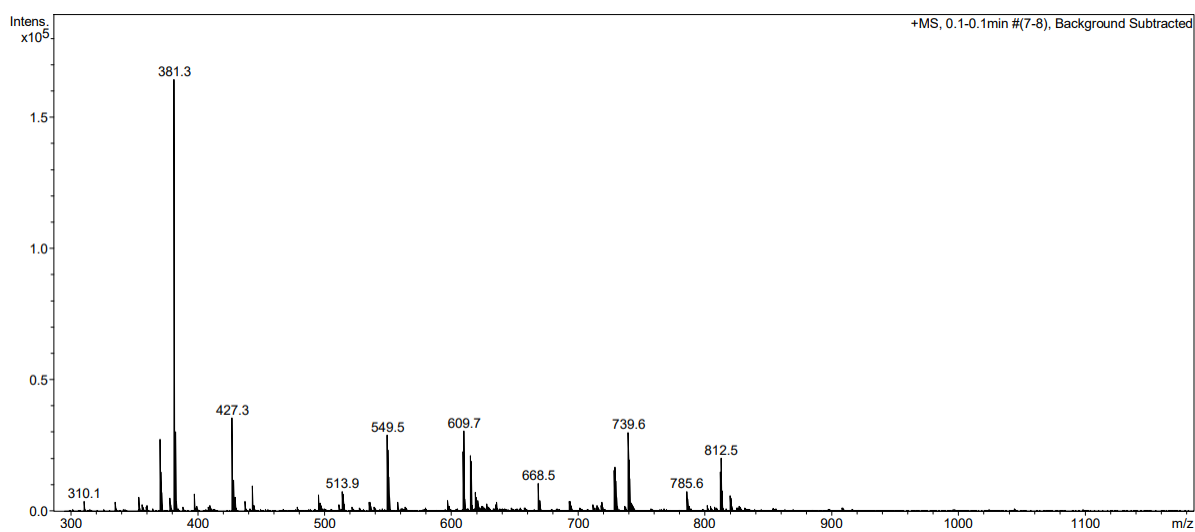
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

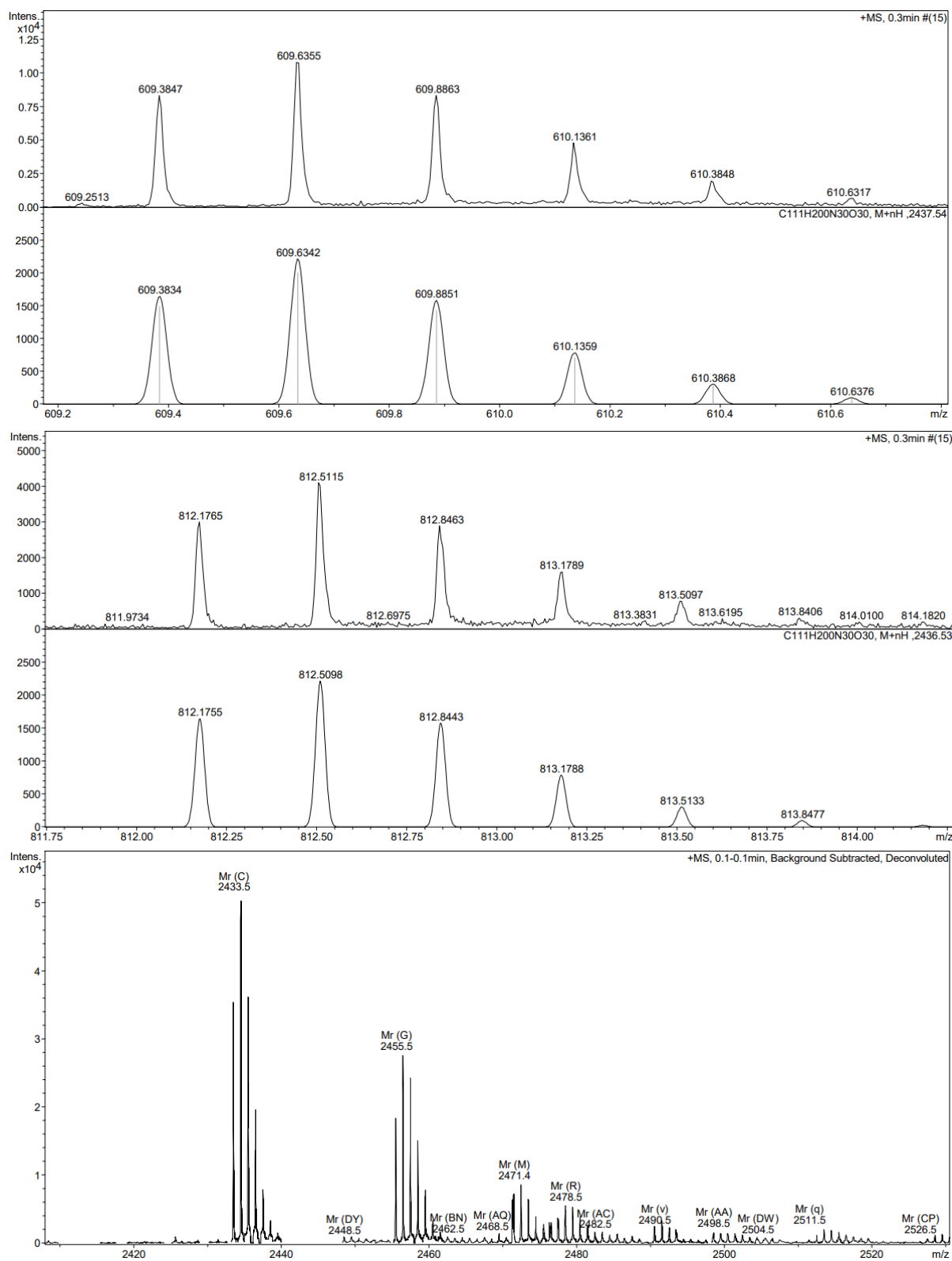
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

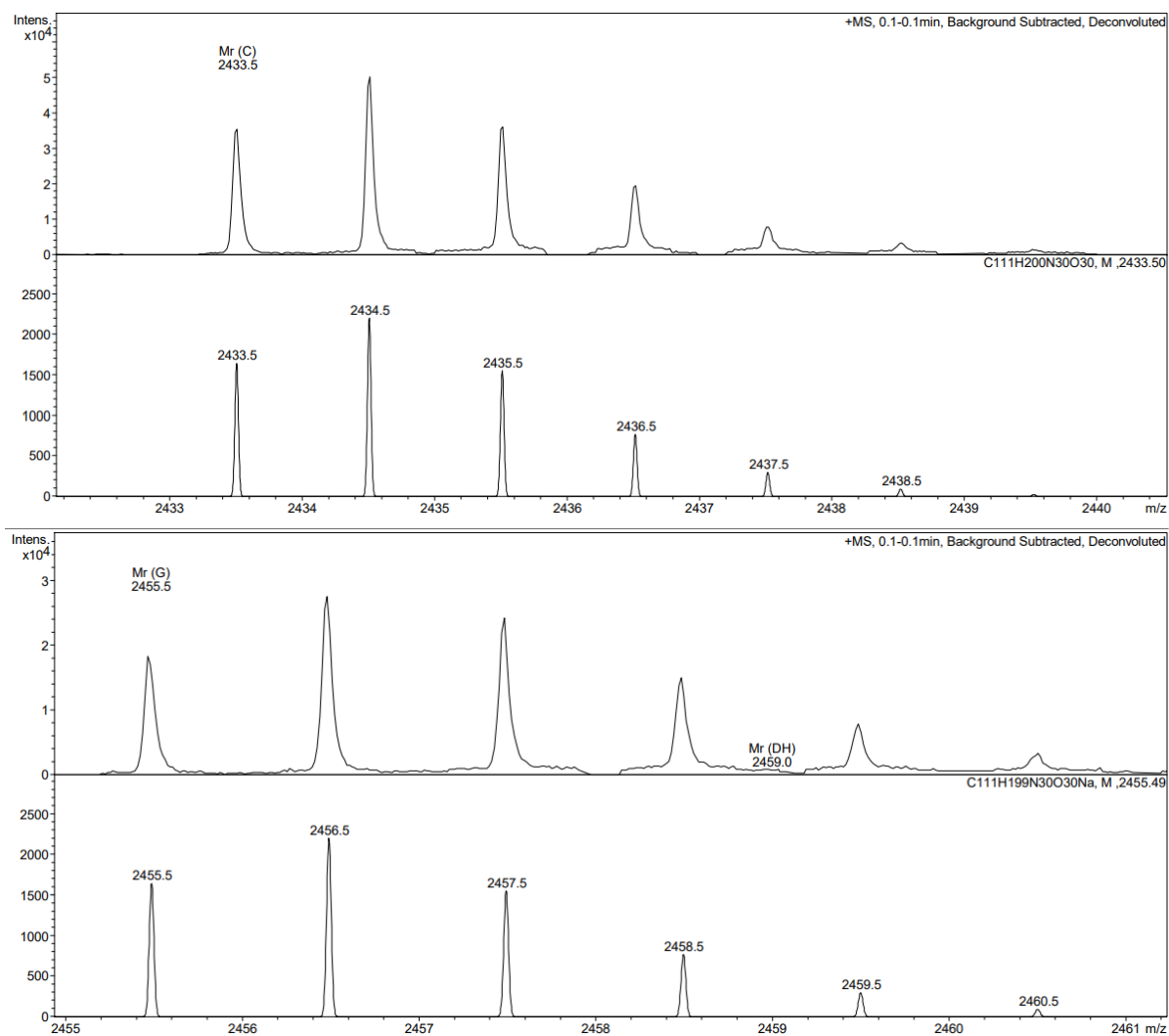
10o

ESI-MS m/z : 3060.8 $[M+H]^+$.

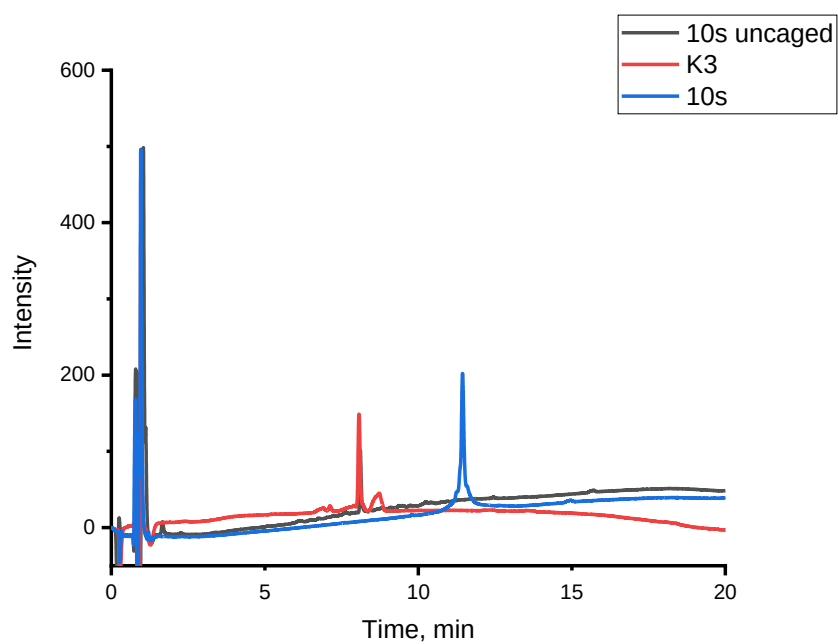
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491.







UPLC traces and Mass Spectra of 10s after uncaging



K3

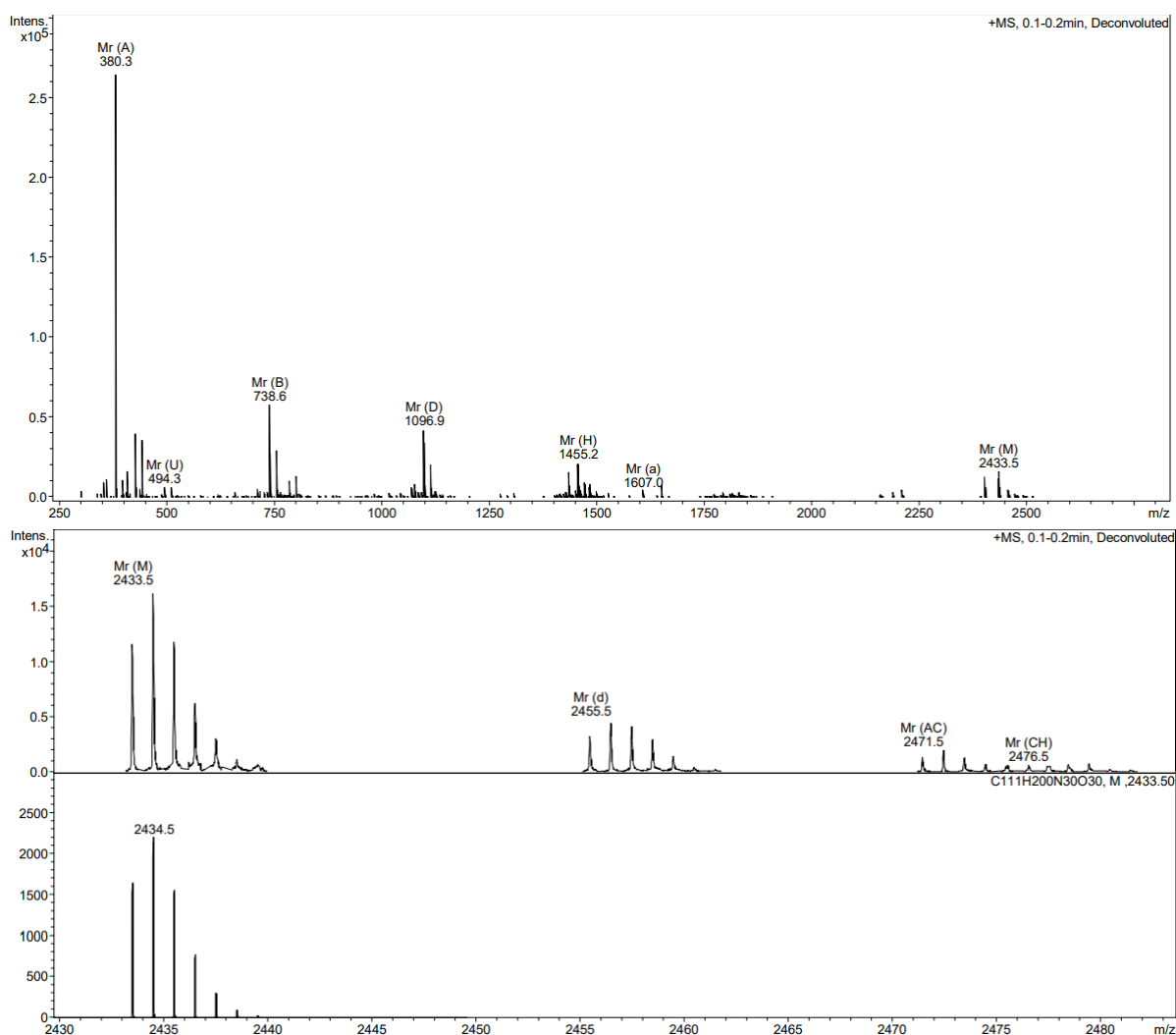
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

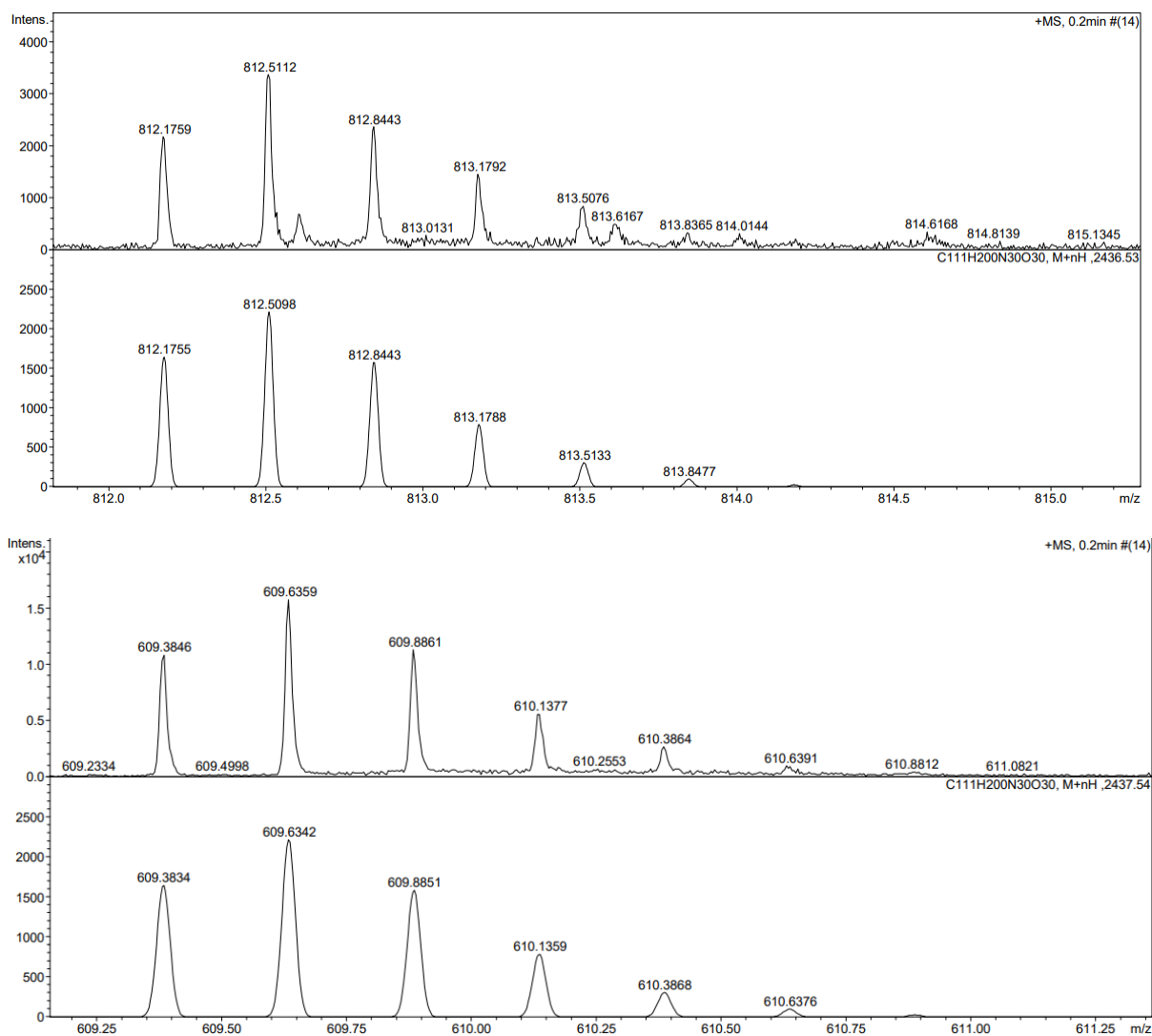
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

10s

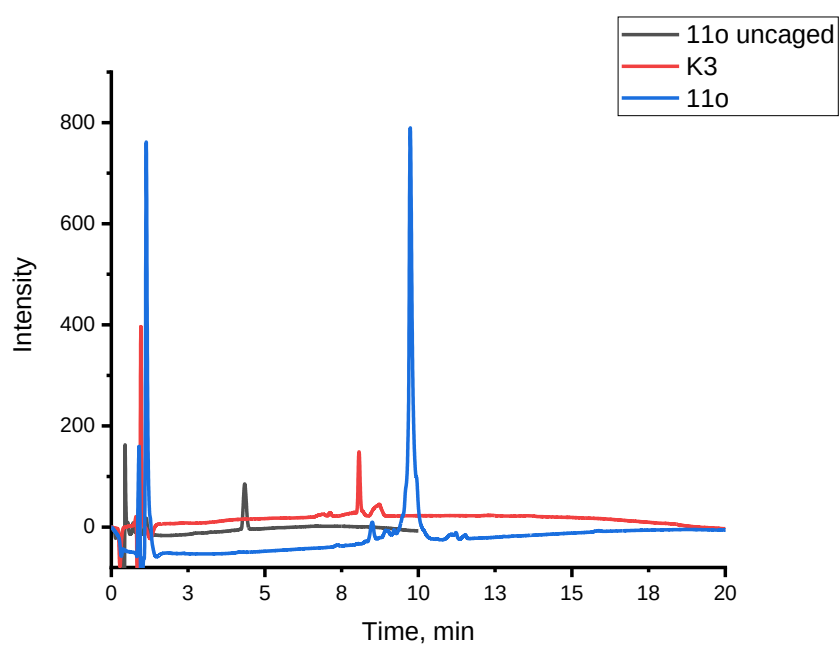
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.





UPLC traces and Mass Spectra of 11o after uncaging



K3

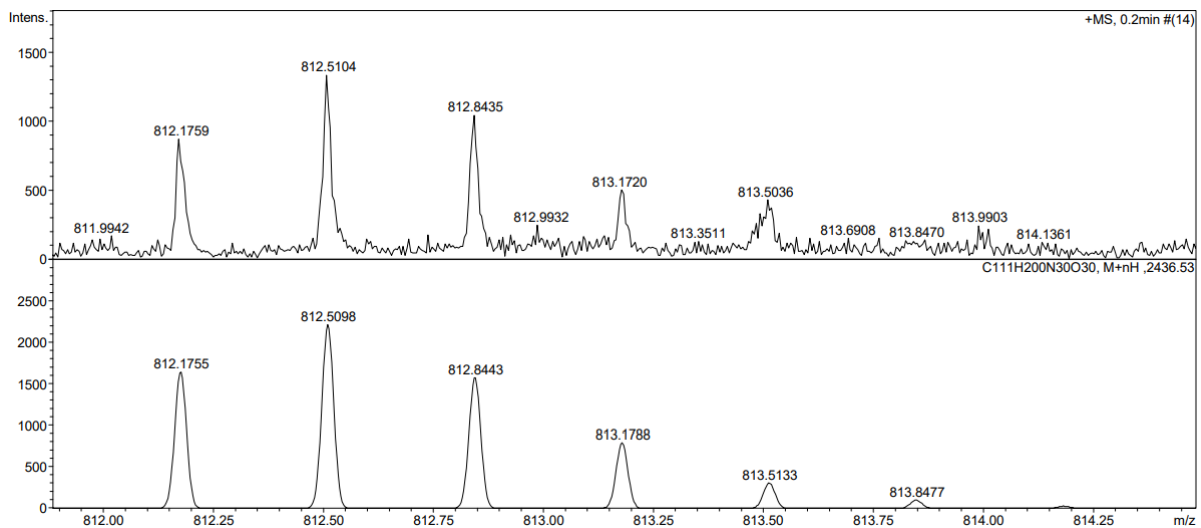
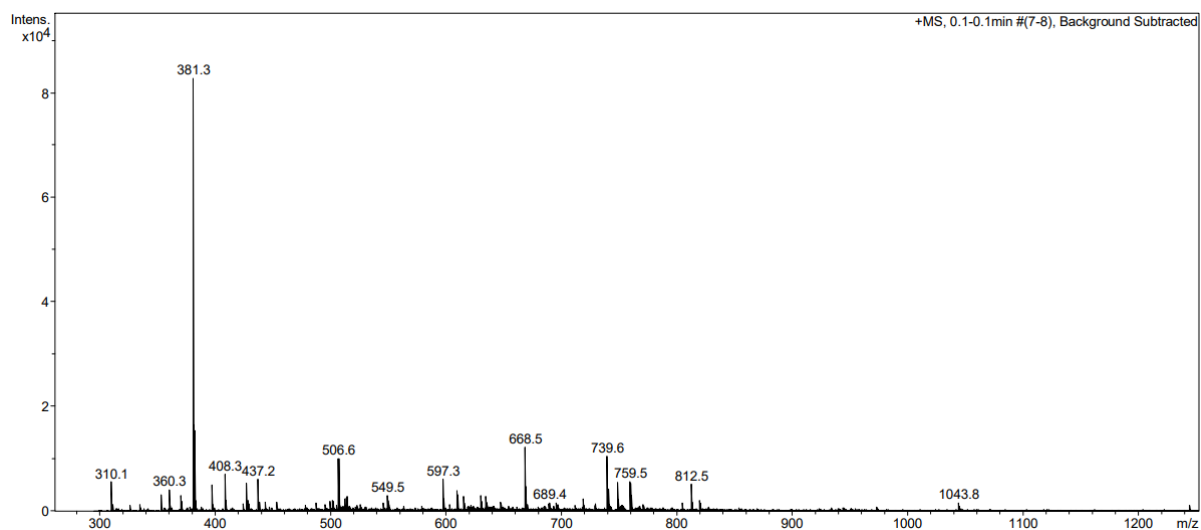
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

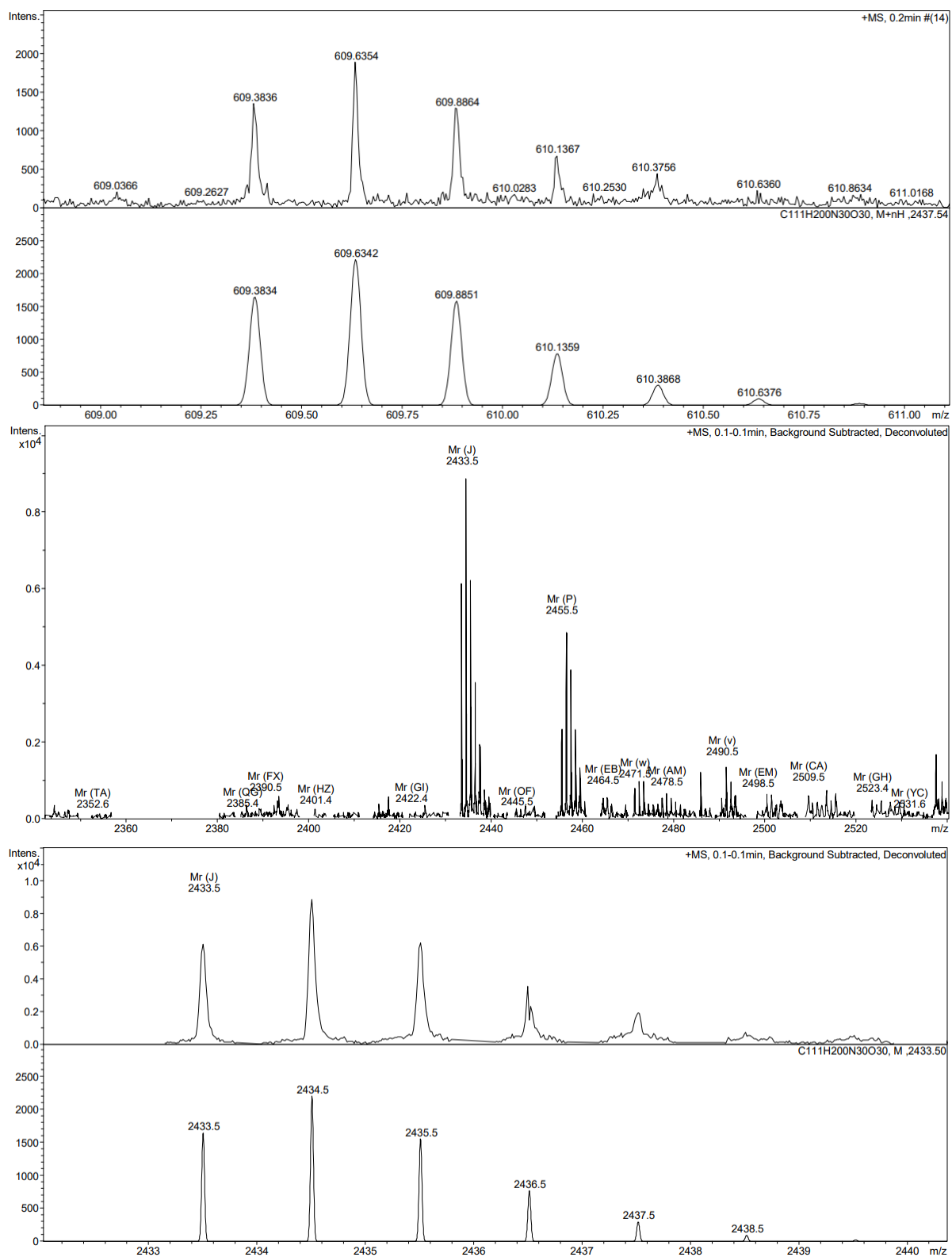
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

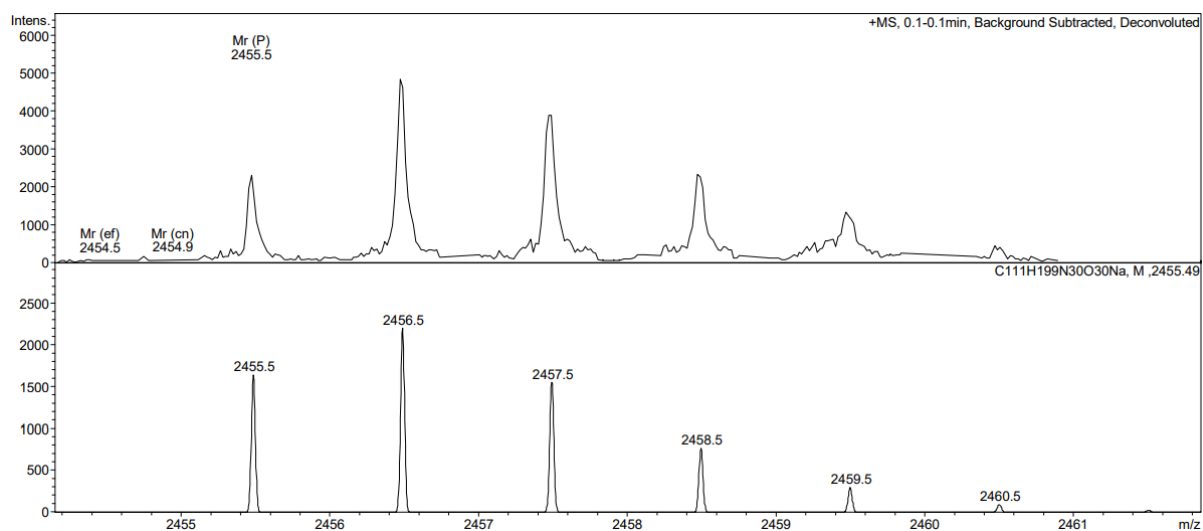
11o

ESI-MS m/z : 3060.8 $[M+H]^+$.

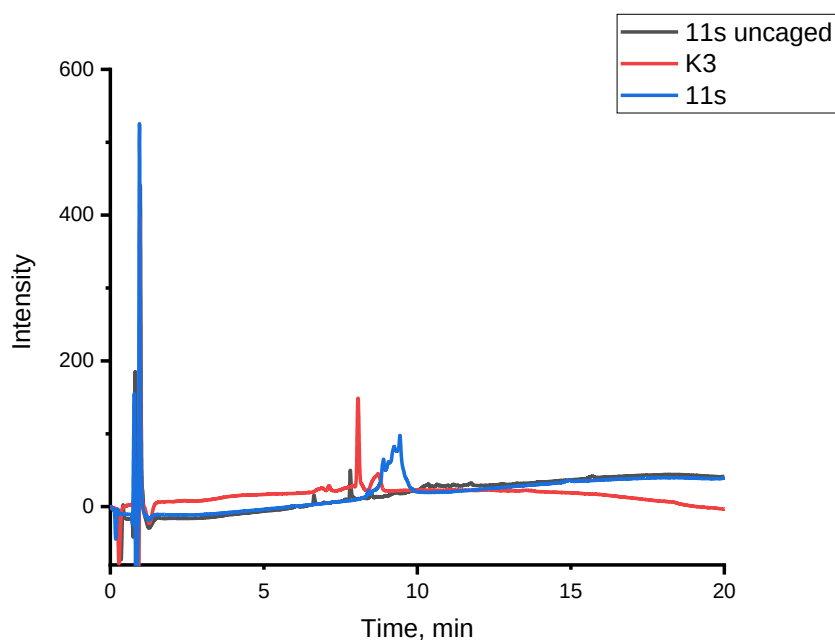
HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calc. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491.







UPLC traces and Mass Spectra of 11s after uncaging



K3

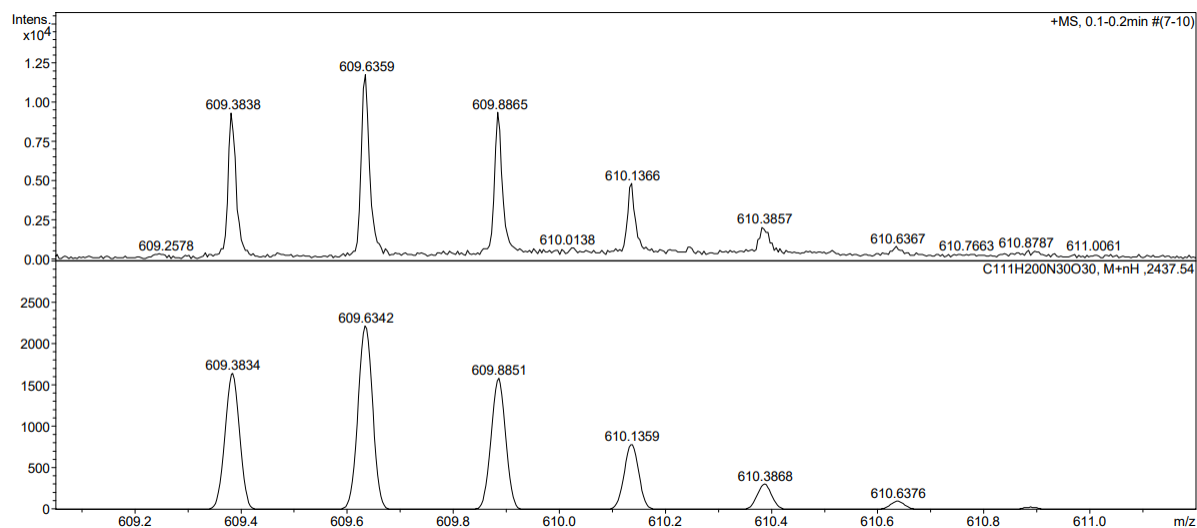
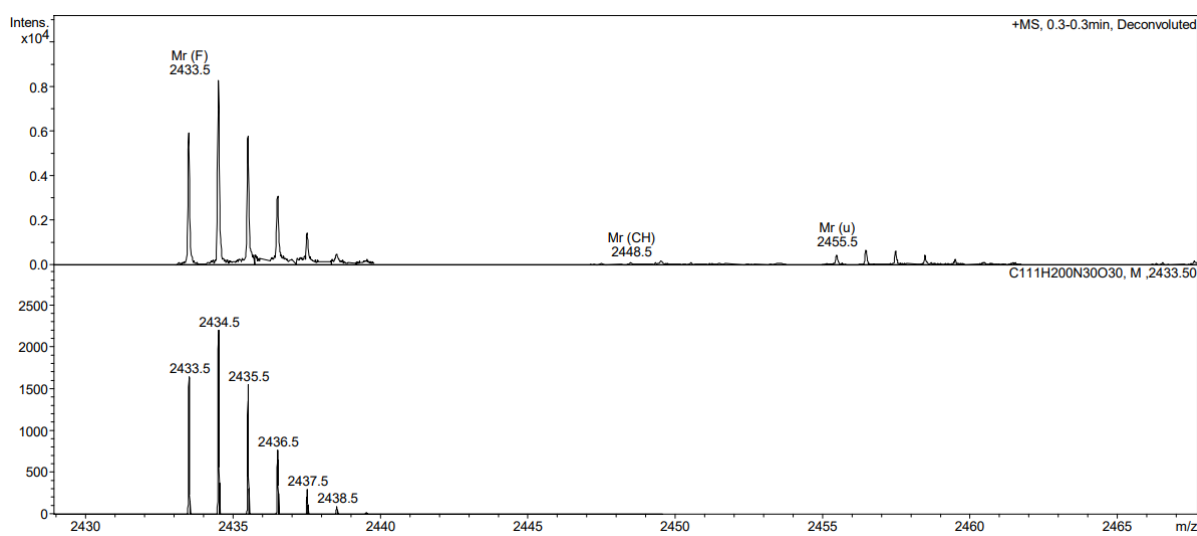
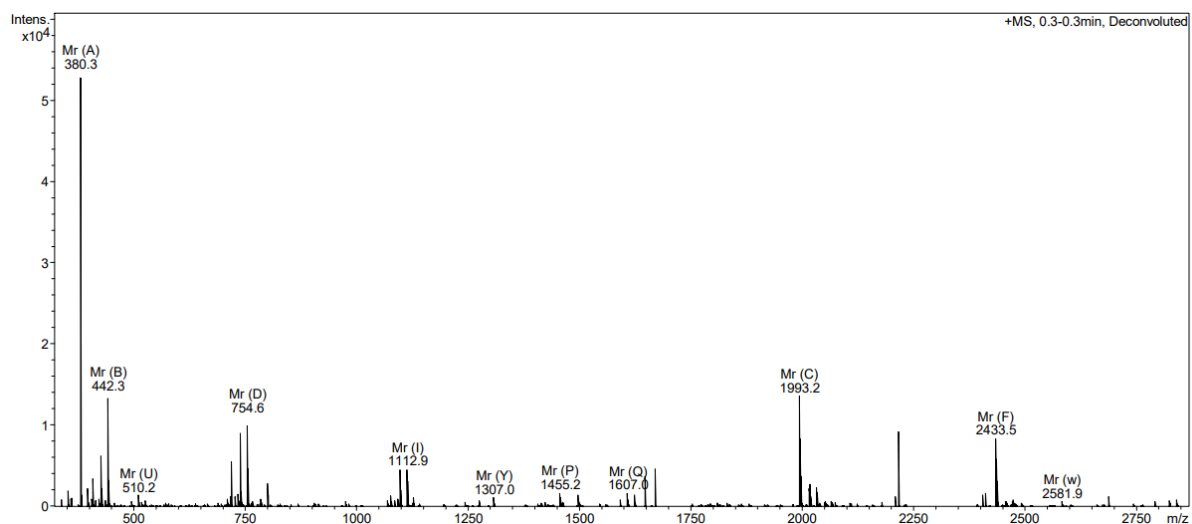
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

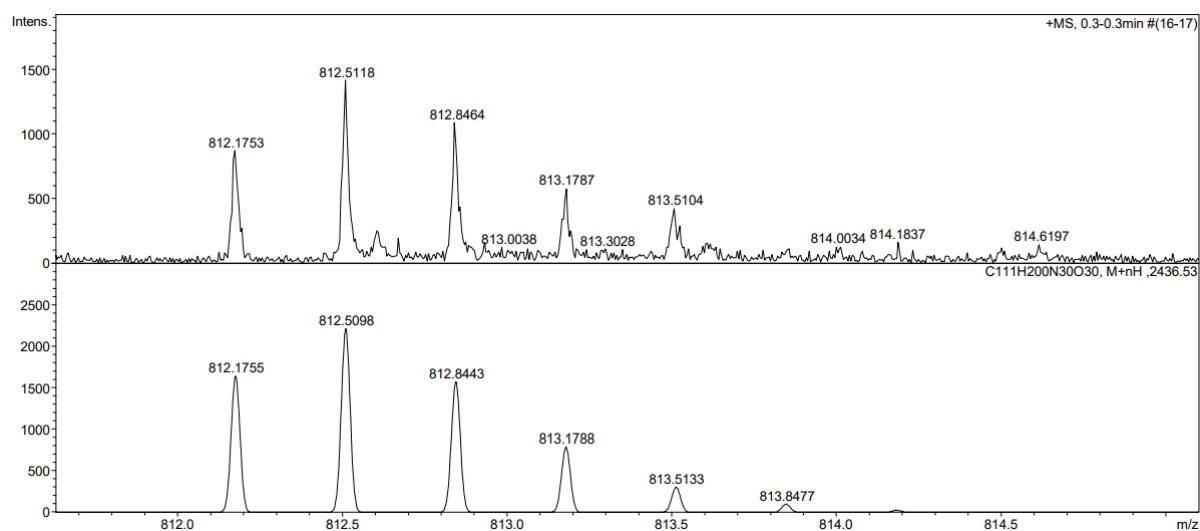
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

11s

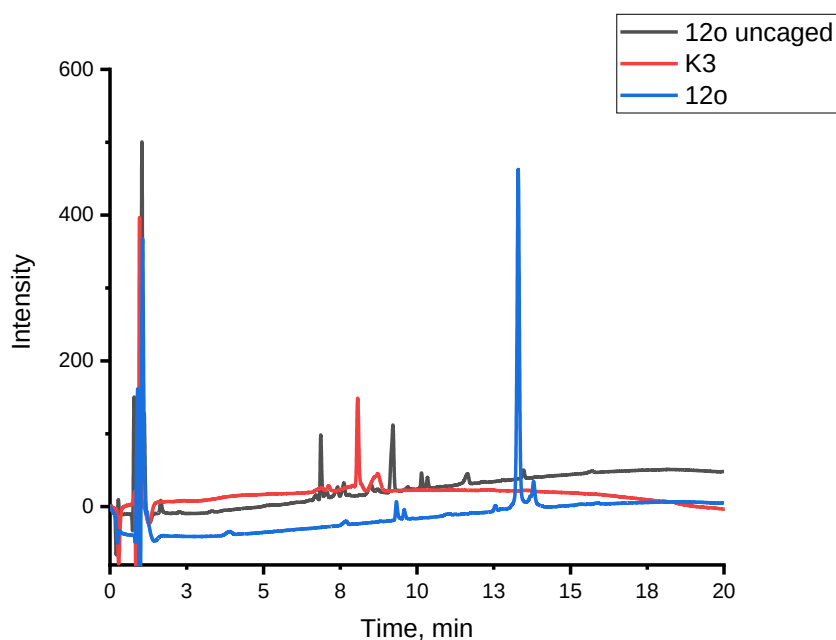
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+4H]^{4+})$: 758.9341, found: 758.9413.





UPLC traces and Mass Spectra of 12o after uncaging



K3

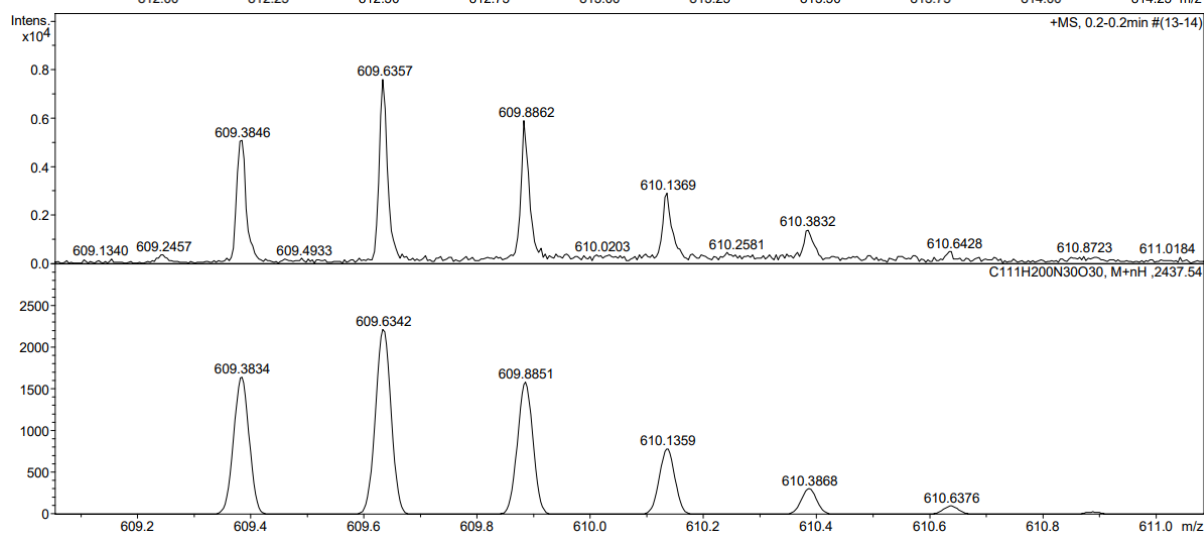
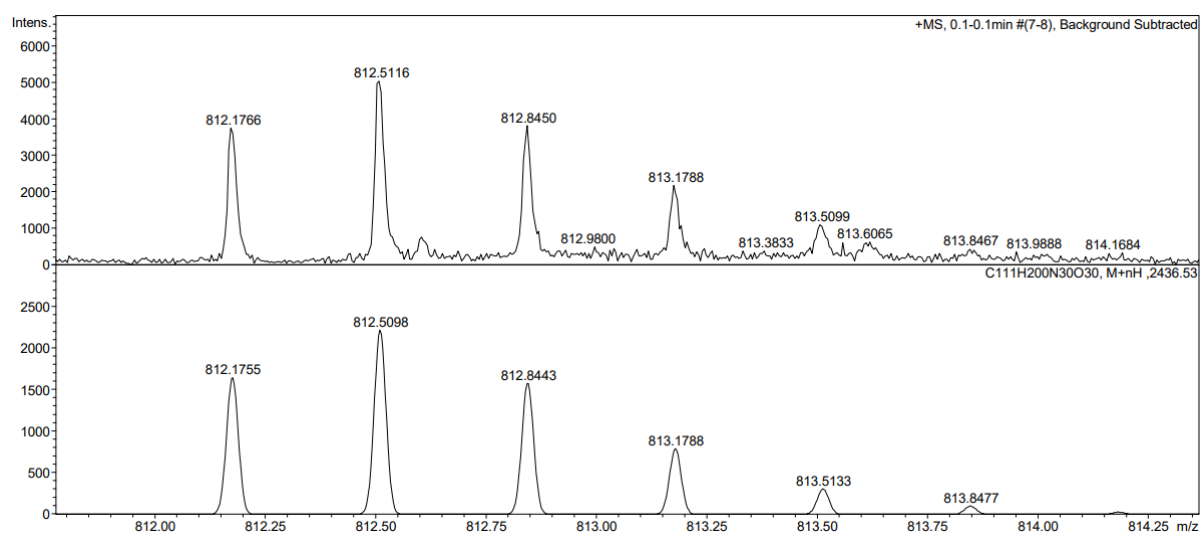
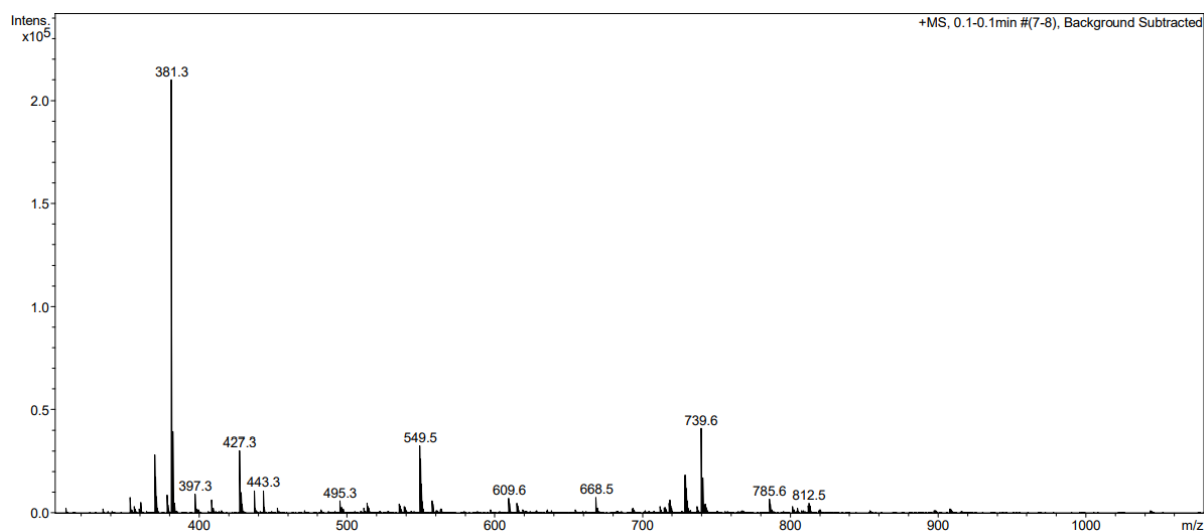
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

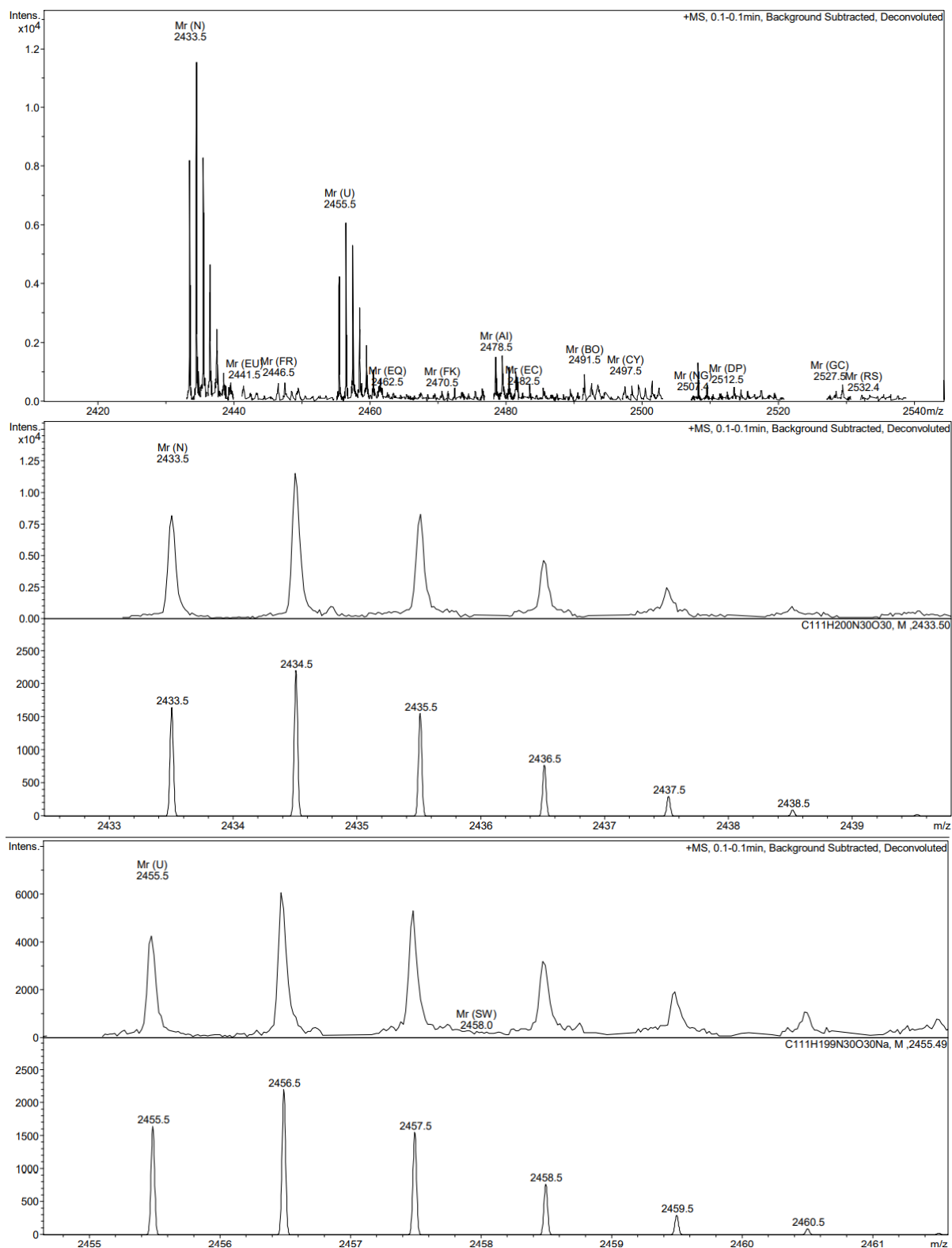
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

12o

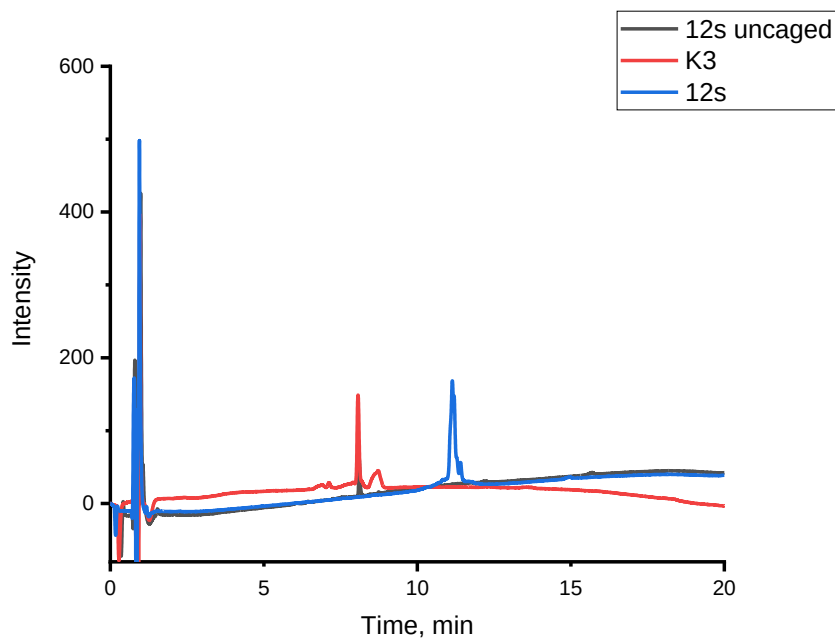
ESI-MS m/z : 3060.8 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calcd. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.949.





UPLC traces and Mass Spectra of 12s after uncaging



K3

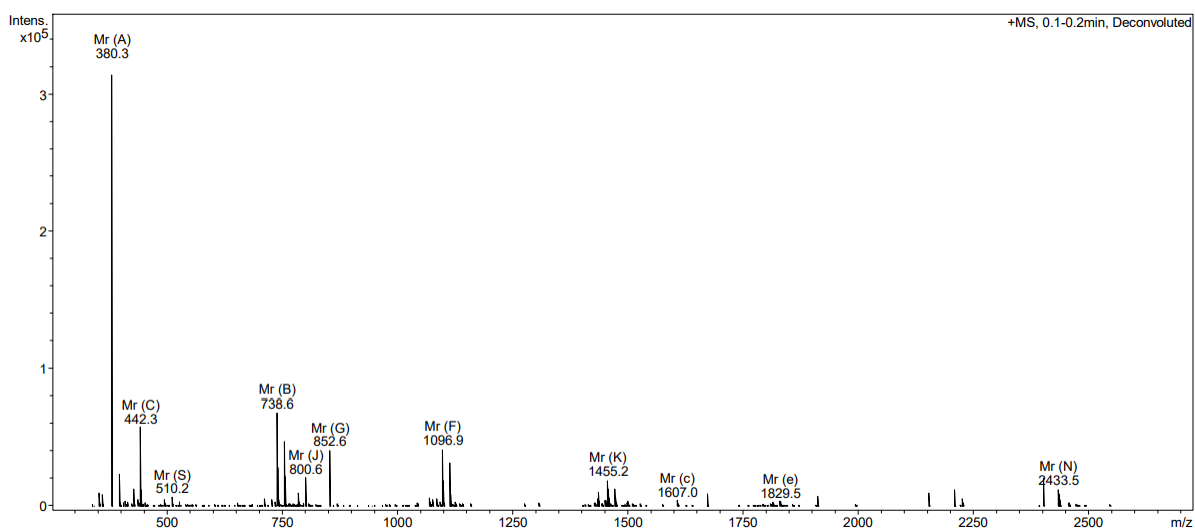
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

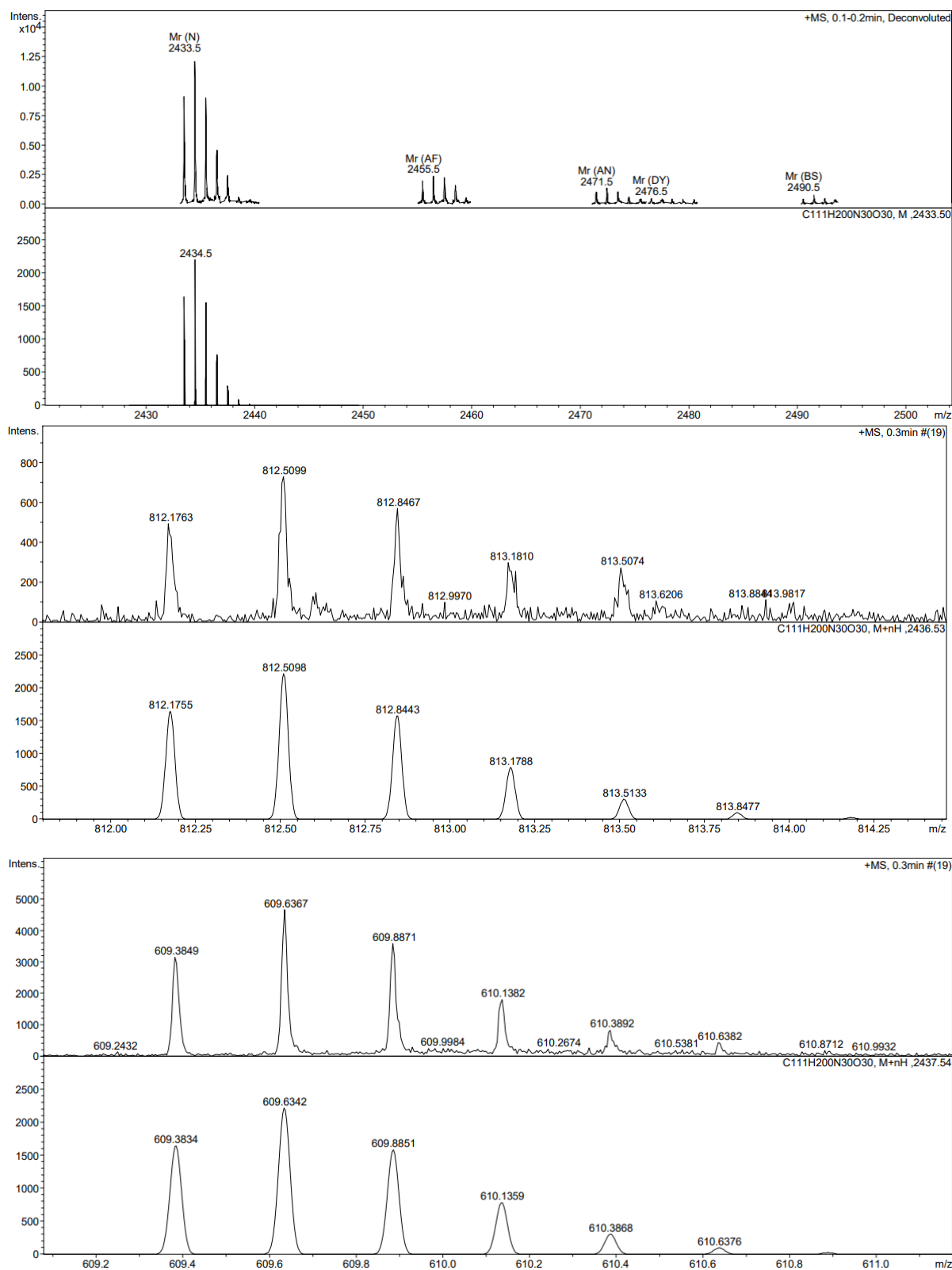
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

12s

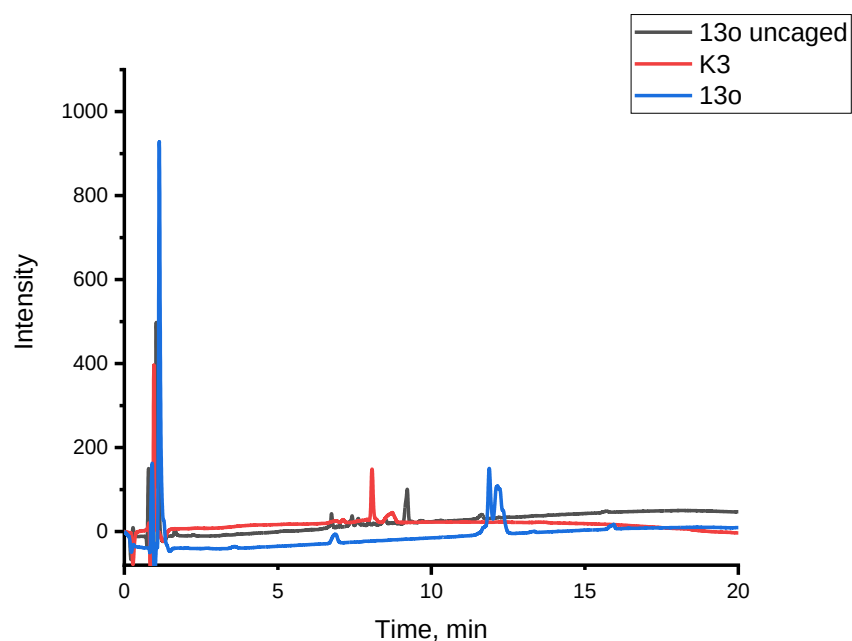
ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H+Na]^{4+})$: 764.4315, found: 764.4368.





UPLC traces and Mass Spectra of 13o after uncaging



K3

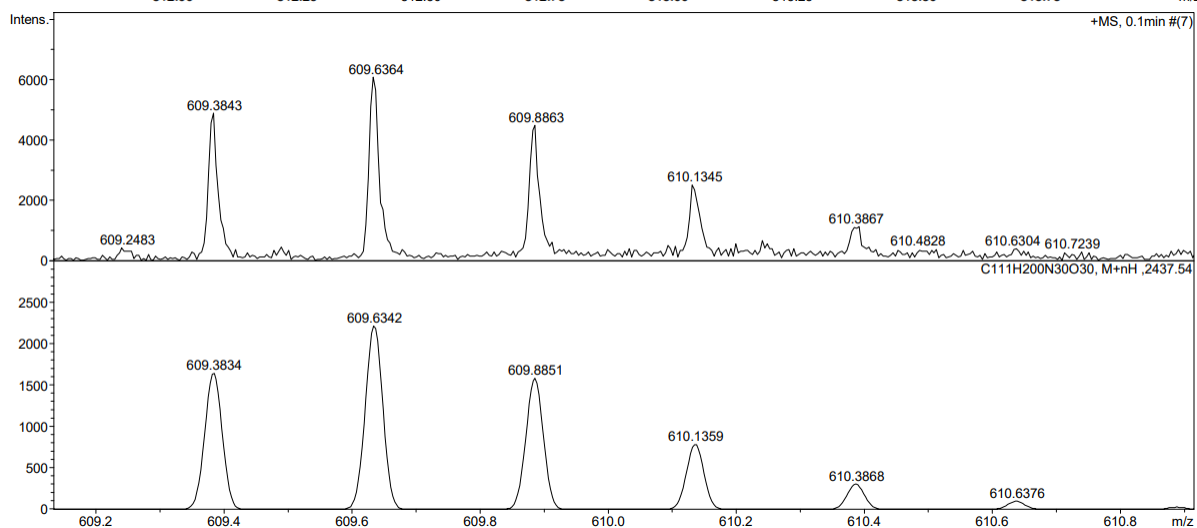
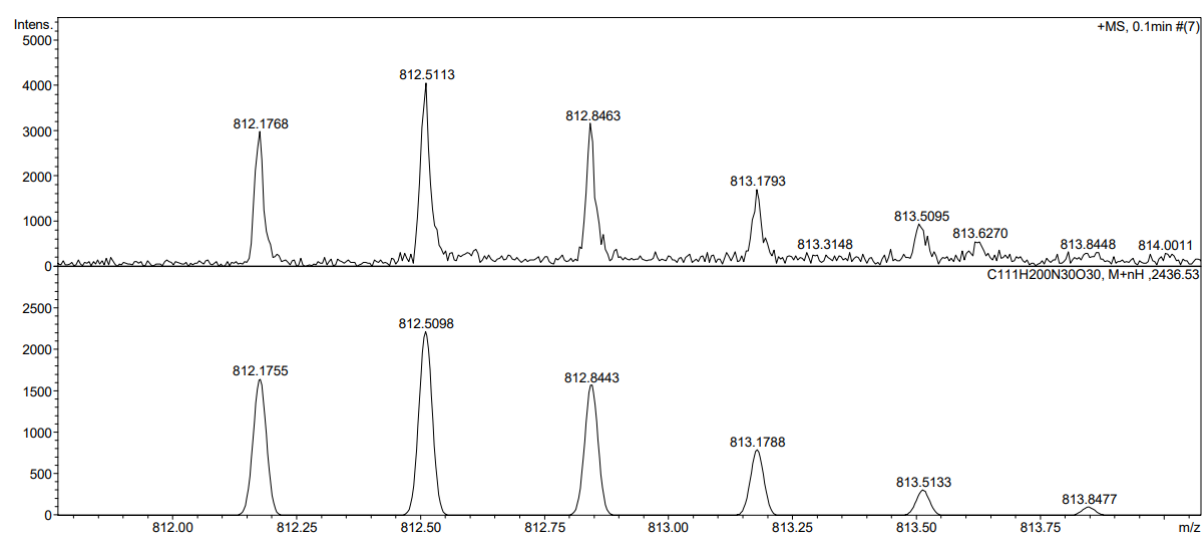
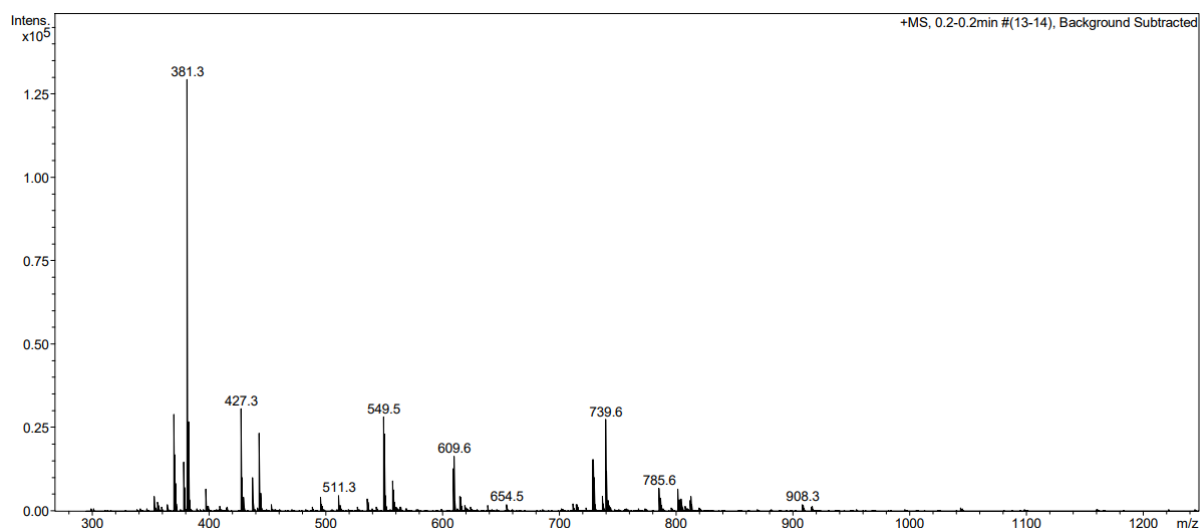
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

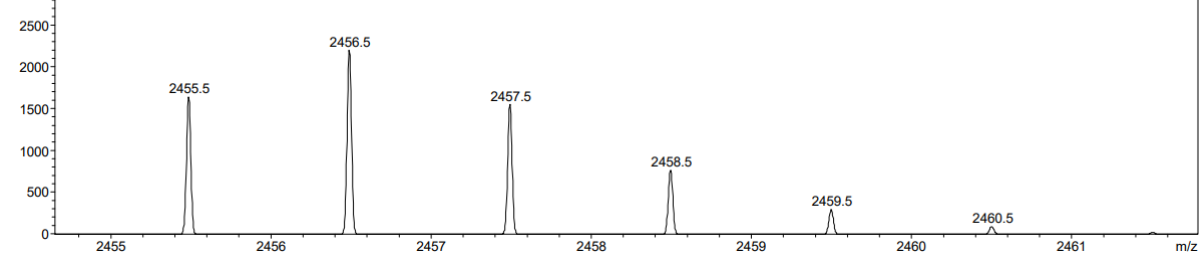
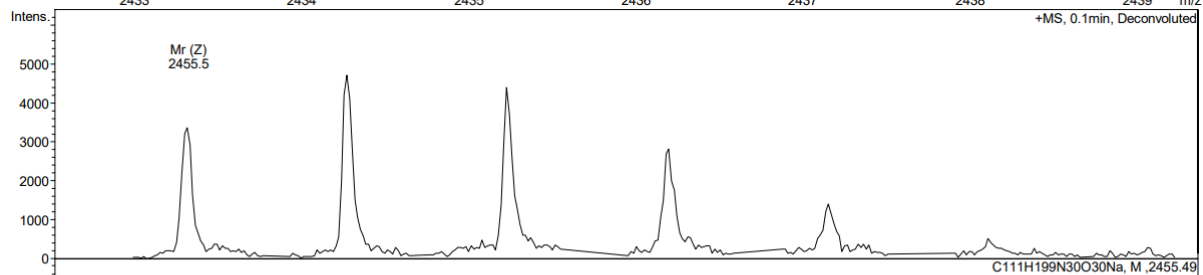
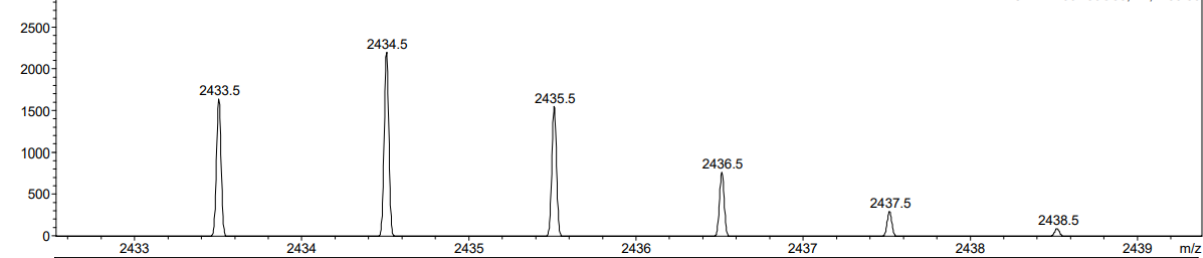
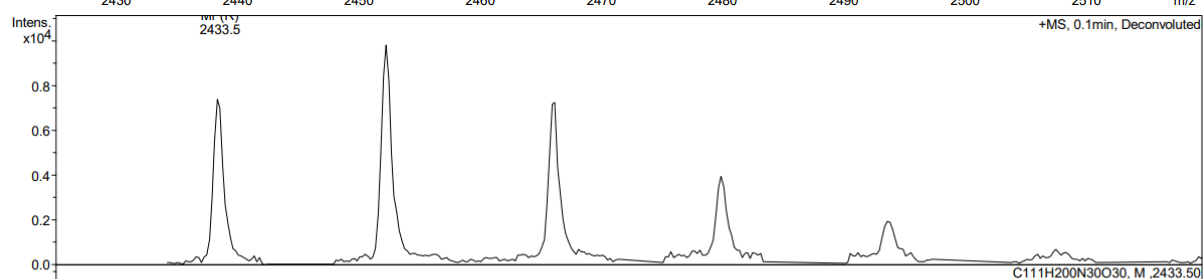
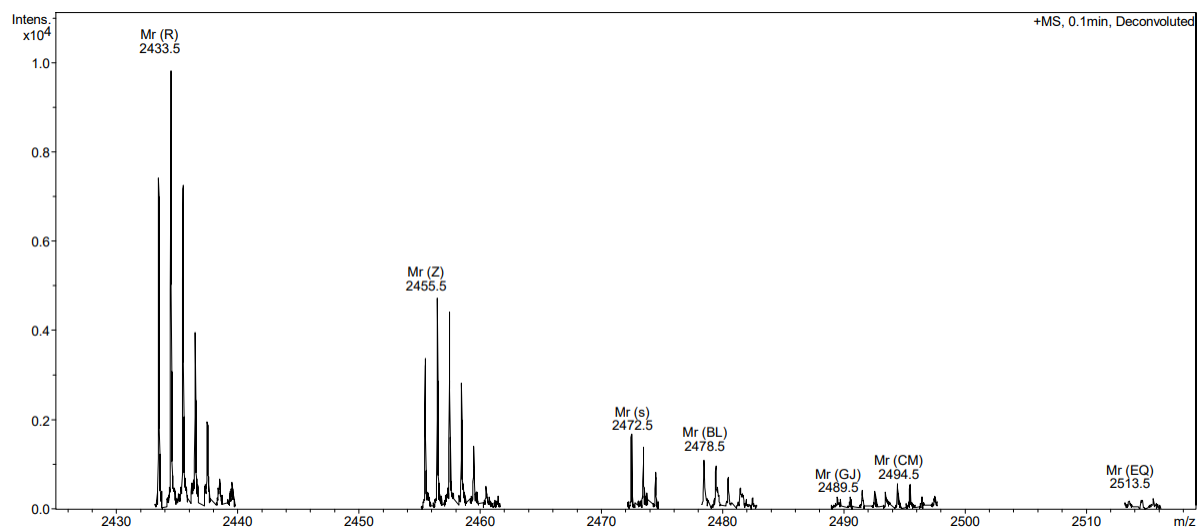
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.1275.

13o

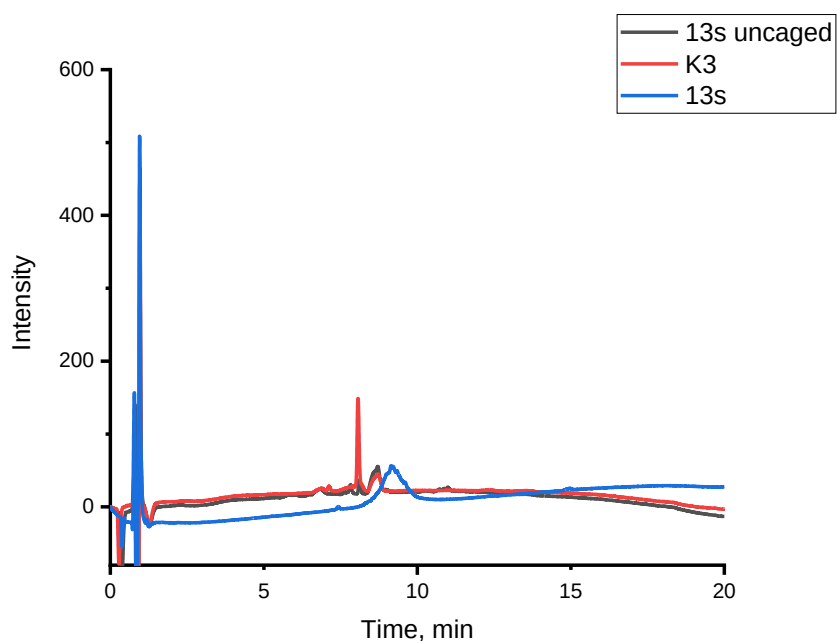
ESI-MS m/z : 3060.8 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{147}H_{238}N_{32}O_{38}$, $([M+3H]^{3+})$: 1020.9225 found: 1020.9298; calcd. for $C_{279}H_{485}N_{73}O_{66}S_3$, $([M+4H]^{4+})$: 765.9419, found: 765.9491





UPLC traces and Mass Spectra of 13s after uncaging



K3

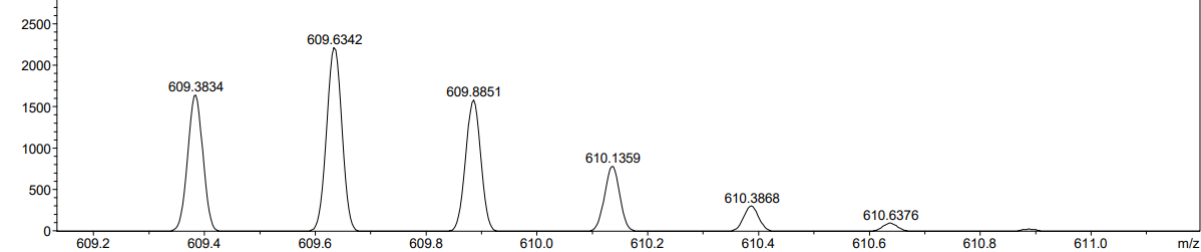
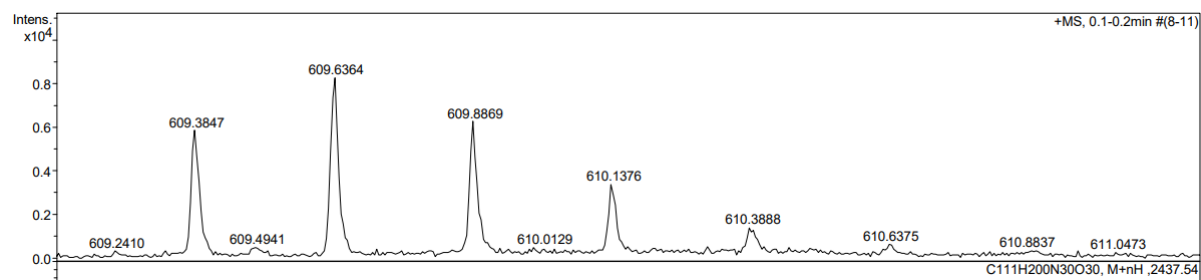
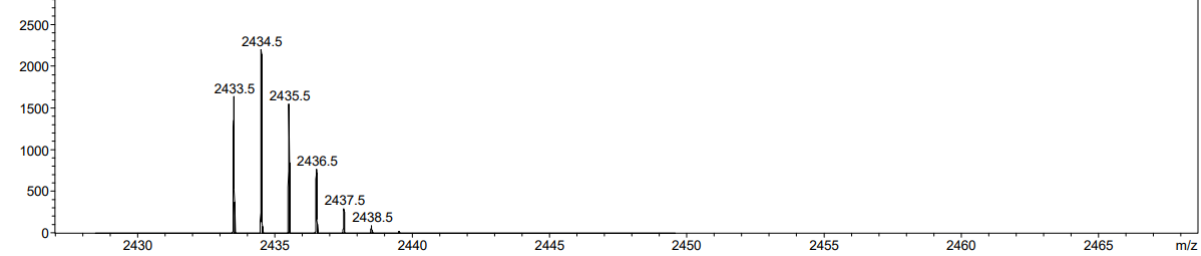
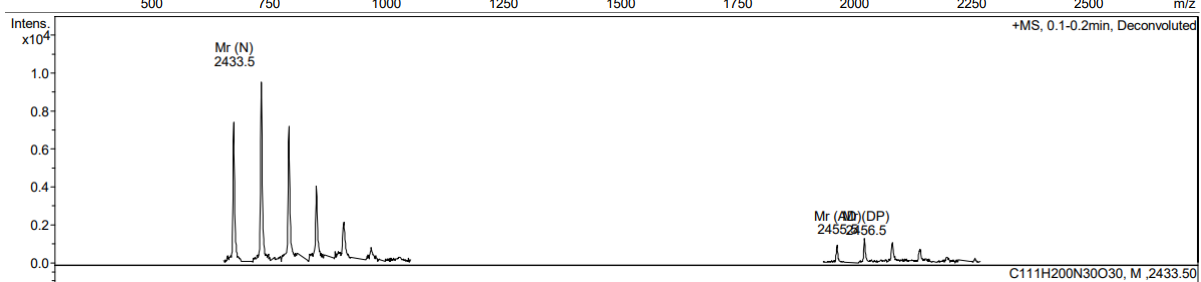
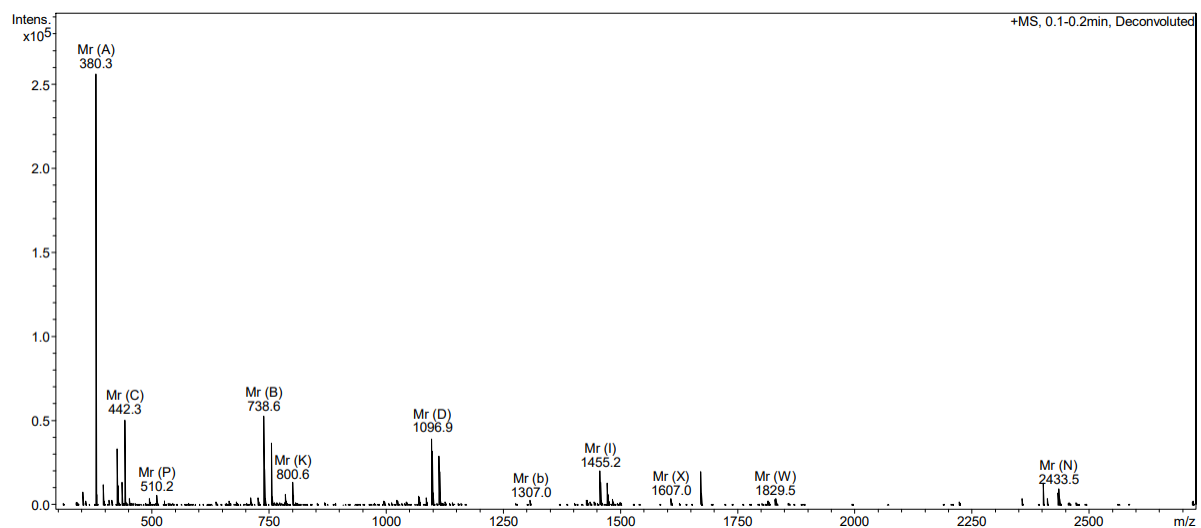
ESI-MS m/z : 2433.5 $[M+H]^+$, 2455.5 $[M+Na]^+$.

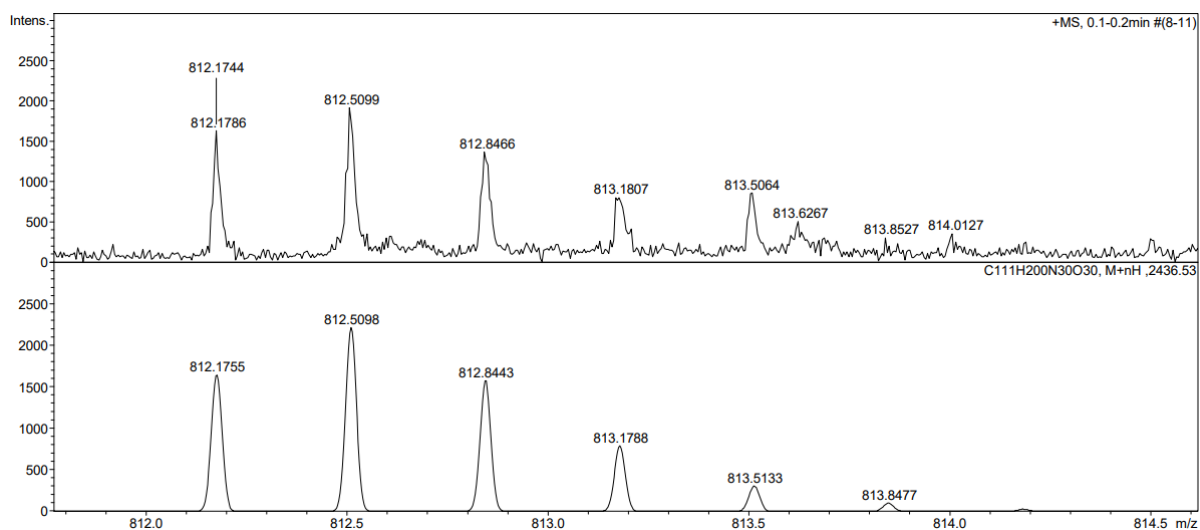
HR-MS (ESI): calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+2H]^{2+})$: 1217.7524 found: 1217.7596; calc. for $C_{117}H_{203}N_{31}O_{28}$, $([M+3H]^{3+})$: 812.1682, found: 812.12765.

13s

ESI-MS m/z : 3031.7 $[M+H]^+$.

HR-MS (ESI): calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H]^{3+})$: 1011.5787 found: 1011.5860; calc. for $C_{145}H_{234}N_{32}O_{38}$, $([M+3H+Na]^{4+})$: 764.4315, found: 764.4368.





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