

Supplementary Information

A pH-Tuned Reactivity Strategy Enables a 2-Acylbenzaldehyde-Acylhydrazine-Thiophenol (AAT) Three-Component Click Reaction for Unprotected Peptide Macrocyclization and Conjugation

Jinyu Liu,¹⁺ Die Xia,³⁺ Ting Ye,²⁺ Qun Zhang,² Luyao Zhang,⁴ Hanmei Zhou,⁵ Wai Yin Yau,^{5*} Xiao Jia,^{2,3,4,5*} Hongxiang Wu^{1,2,3,4,5*}

1 School of Pharmaceutical Sciences, Guizhou Medical University, No.9 Beijing Road, Guiyang City, 550000, PR China

2 School of Traditional Chinese Medicine, Nanjing University of Chinese Medicine, Xianlin Rd, Nanjing, 210023, P. R. China

3 School of Pharmacy, Zunyi Medical University, Jinwan District, Zhuhai, 519000, P. R. China

4 School of Pharmaceutical Sciences, Southern Medical University, No.1023-1063 South Shatai Road, Baiyun District, Guangzhou City, 510515, PR China.

5 Zhongshan Institute for Drug Discovery, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Zhongshan, 528400, P. R. China

General Information

All commercial materials (purchased from bidepharm, Accela ChemBio, CSBio, Energy Chemical, Aikonchem, Adamas-beta, Leyan, Shanghai Titan Scientific,) were used without further purification. All solvents were reagent grade or HPLC grade. HPLC was performed on a ELAY TECH IdealChrom 920 Prep HPLC system. LPLC was performed on a Santai SepaBean machine. High-resolution mass spectral (HR-MS) analyses were performed on a Thermo Scientific Exactive Plus MS coupled with a Thermo Scientific Vanquish LC system and an Accucore RP-MS column (2.6 μ m, 100 x 2.1 mm). Mobile phases were 0.1% trifluoroacetic acid (TFA) in acetonitrile (MeCN) and 0.1% trifluoroacetic acid (TFA) in water (H₂O) (v/v). Flow rate was set at 0.3 mL/min and gradient was 20% to 95% MeCN in 7 min. Analytical TLC was performed on Merck silica gel 60 F254 plates and visualized under UV light (254 nm). Silica flash column chromatography was performed using 200-300 mesh silica (Huanghai). ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded at 298 K on a BRUKER AVANCE NEO Ascend 600 NMR spectrometer at 600 MHz for ¹H NMR and 151 MHz for ¹³C NMR. Chemical shifts were reported in parts per million (ppm) and referenced to solvent residual signals ¹H: CDCl₃: δ = 7.26 ppm, *d*₆-DMSO: δ = 2.50 ppm, ¹³C: CDCl₃: δ = 77.16 ppm, *d*₆-DMSO: δ = 39.52 ppm. ¹H NMR data was reported as chemical shift (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet) and coupling constant (*J* Hz).

Abbreviations

HR-ESI-MS = high resolution-electrospray ionization-mass, HPLC = high pressure liquid chromatography, LPLC = low pressure liquid chromatography, TFA = trifluoroacetic acid, DMF = dimethylformamide, DMSO = dimethyl sulfoxide, DIEA = diisopropylethylamine, HATU = 2-(7-Azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate, MPAA = 4-mercaptophenylacetic acid, RB = round bottom flask, CDMT = 2-chloro-4,6-dimethoxy-1,3,5-triazine, NMM = 4-methylmorpholine, r.t. = room temperature, EA = ethyl acetate, PE = petroleum ether.

1 Synthesis of peptides.

Standard Protocol of Solid-phase Peptide Synthesis (SPPS)

The commercially available 2-chlorotrityl resin (CS Biochem, loading: ~ 0.55 mmol/g) was employed in the solid phase peptide synthesis of peptides. FmocHN-Xaa-COOH (4.0 equiv.) and diisopropylethylamine (8.0 equiv) were dissolved in dichloromethane, then this solution was poured into a reaction vial containing 2-chlorotrityl resin, and the mixture was shaken at room temperature (r.t.) for 2 hr. After that, this amino acid bound resin was washed with dimethylformamide and dichloromethane. The resin was subjected to Fmoc-SPPS to give the corresponding peptide products.

The obtained resin bound peptides were treated with TFA cocktail for 1.5 hr. After that, the peptide TFA solution was poured into 50 ml diethyl ether, and the resulting suspension was centrifuged to give a white pellet. After decanting diethyl ether, the remaining solid was ready for HPLC purification.

Preparation of hydrazine 2-chlorotrityl chloride resin

2-chlorotrityl chloride resin (1 g, loading = ~ 0.5 mmol/g) was swelled in 10 mL $\text{CH}_2\text{Cl}_2/\text{DMF}$ (1/1, v/v). Then 10 mL $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}/\text{DMF}$ (1/20, v/v) were added. The reaction was conducted for 30 min. 10 mL of methanol/DMF (1/20, v/v) was added to quench the remaining 2-chlorotrityl chloride resin. After 30 min, resin was washed with DMF and CH_2Cl_2 and ready for iterative peptide assembly (Fmoc-SPPS).

Synthesis of Peptide **1a**



Peptide **1a** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected

and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (2-40% ACN/H₂O over 35 min) and lyophilization to give **1a** (40.7 mg, 14%) as a white powder.

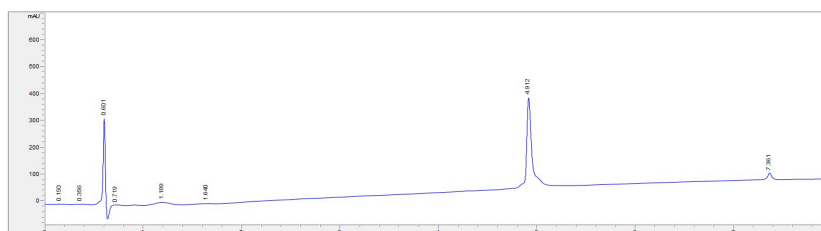


Figure 1.1 UV (205nm) trace from LC-MS analysis of purified **1a**. Gradient: 2%-40% ACN/H₂O with 0.1% TFA over 8 min.

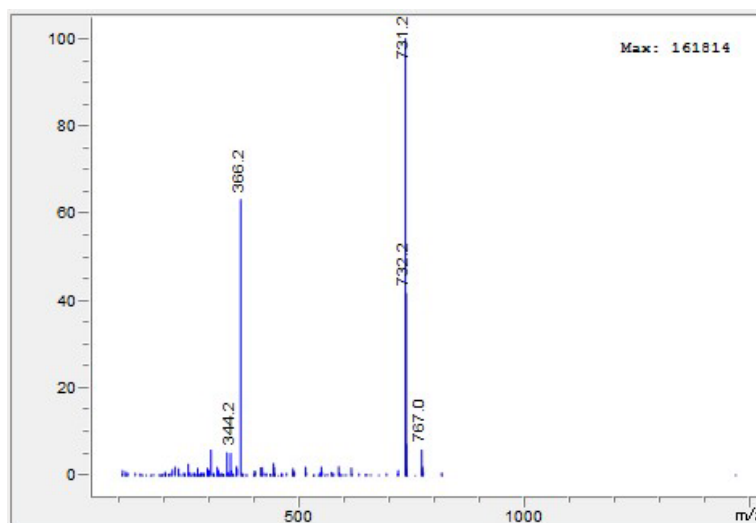


Figure 1.2 ESI-MS calcd. for **1a** (C₂₈H₅₄N₁₄O₉) [M+1H]¹⁺ m/z = 731.8, found 731.2; [M+2H]²⁺ m/z = 366.4, found 366.2.

Synthesis of Peptide **1b**



Peptide **1b** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected

and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification 10-50% ACN/H₂O over 35 min) and lyophilization to give **1b** (114.1 mg, 23%) as a white powder.



Figure 2.1 UV (205nm) trace from LC-MS analysis of purified **1b**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

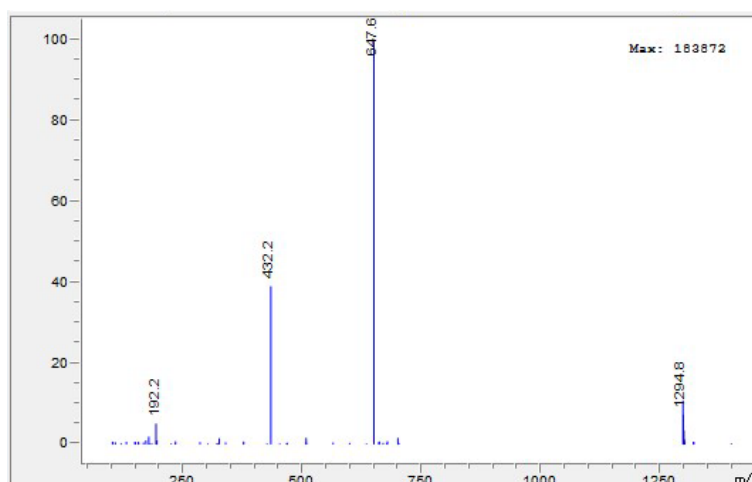


Figure 2.2 ESI-MS calcd. for **1b** (C₅₈H₁₀₃N₁₇O₁₄S) [M+1H]¹⁺ m/z = 1294.8, found 1294.9; [M+2H]²⁺ m/z = 647.9, found 647.6; [M+3H]³⁺ m/z = 432.3, found 432.2.

Synthesis of Peptide **1c**



Peptide **1c** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1c** (76.7 mg, 13%) as a white powder.

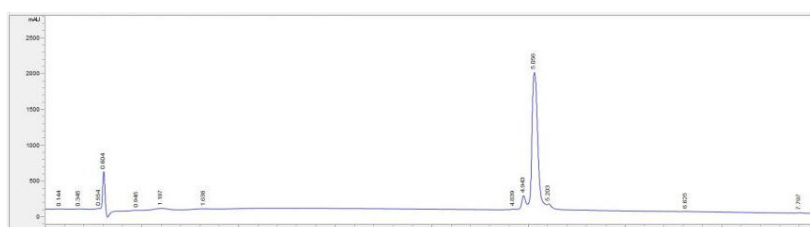


Figure 3.1 UV (205nm) trace from LC-MS analysis of purified **1c**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

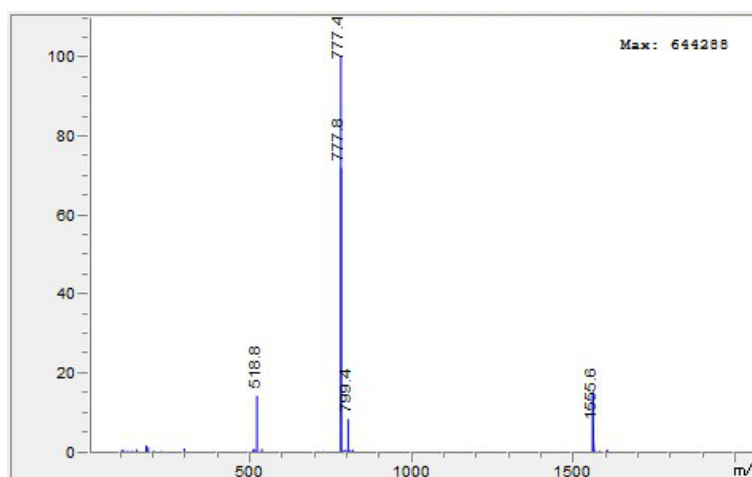


Figure 3.2 ESI-MS calcd. for **1c** (C₇₅H₉₉N₁₉O₁₈) [M+1H]¹⁺ m/z = 1555.4, found 1555.6; [M+2H]²⁺ m/z = 778.2, found 777.8; [M+3H]³⁺ m/z = 519.1, found 518.8.

Synthesis of Peptide **1d**



Peptide **1d** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1d** (62.8 mg, 10%) as a white powder.

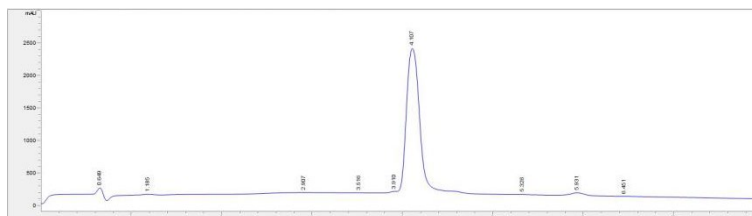


Figure 4.1 UV (205nm) trace from LC-MS analysis of purified **1d**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

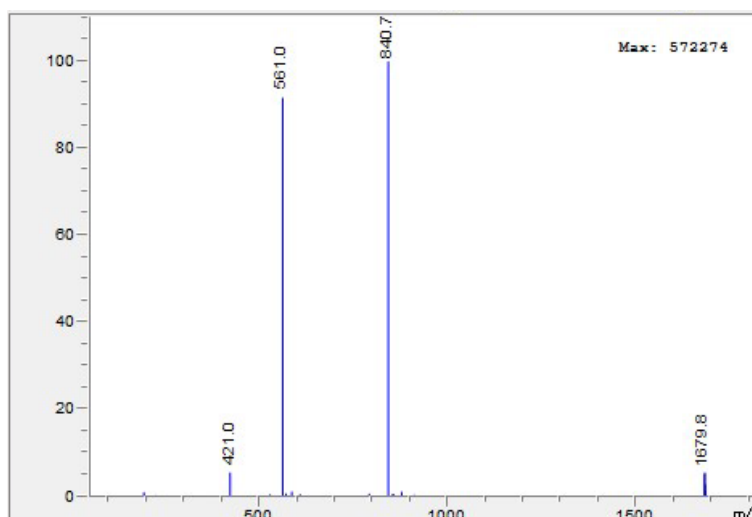


Figure 4.2 ESI-MS calcd. for **1d** (C₇₇H₁₀₁N₂₅O₁₇S) [M+1H]¹⁺ m/z = 1680.7, found 1679.8; [M+2H]²⁺ m/z = 840.9, found 840.7; [M+3H]³⁺ m/z = 560.9, found 561.0; [M+4H]⁴⁺ m/z = 421.0, found 421.0.

Synthesis of Peptide **1e**



Peptide **1e** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1e** (91.6 mg, 15%) as a white powder.

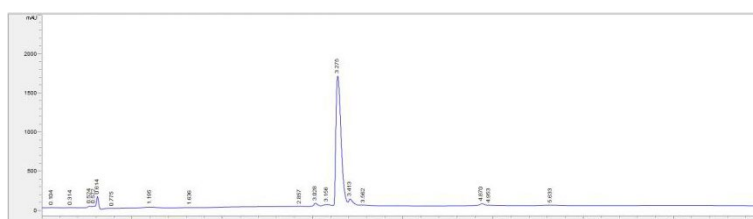


Figure 5.1 UV (205nm) trace from LC-MS analysis of purified **1e**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

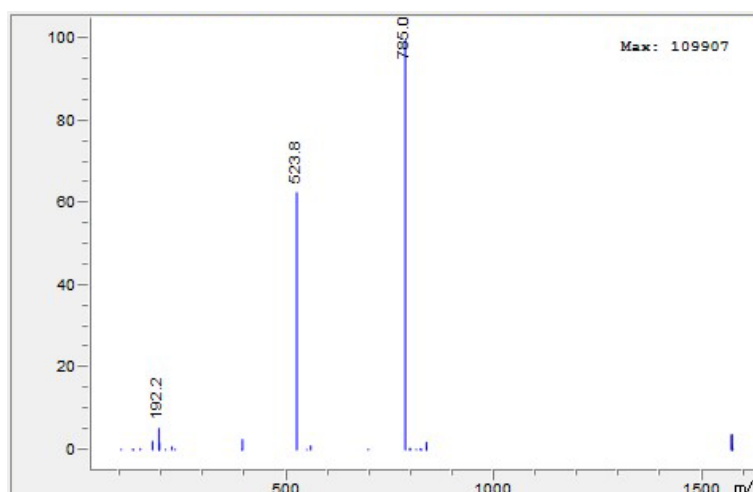


Figure 5.2 ESI-MS calcd. for **1e** (C₇₀H₁₀₀N₂₂O₂₀) [M+2H]²⁺ m/z = 785.1, found 785.0; [M+3H]³⁺ m/z = 523.7, found 523.8.

Synthesis of Peptide **1f**



Peptide **1f** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1f** (79.4 mg, 15%) as a white powder.

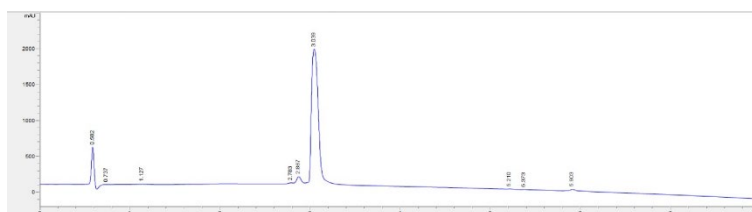


Figure 6.1 UV (205nm) trace from LC-MS analysis of purified **1f**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

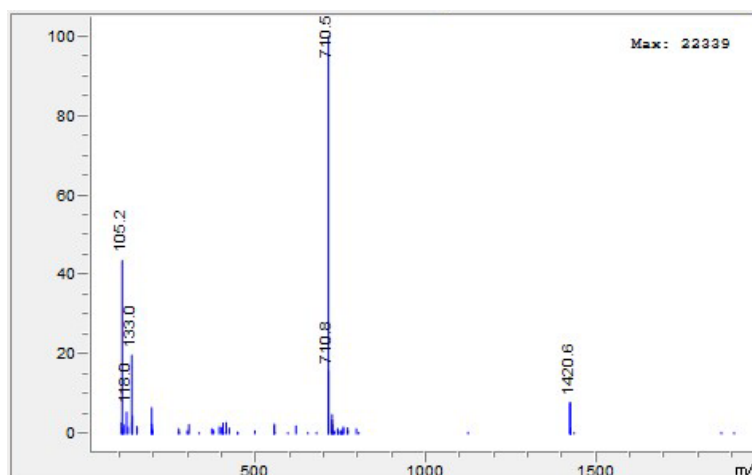


Figure 6.2 ESI-MS calcd. for **1f** (C₆₀H₈₉N₂₃O₁₈) [M+1H]¹⁺ m/z = 1420.8, found 1420.6; [M+2H]²⁺ m/z = 710.9, found 710.9.

Synthesis of Peptide **1g**



Peptide **1g** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether,

the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1g** (56.7 mg, 9%) as a white powder.

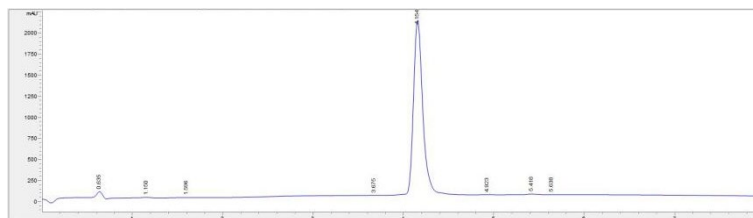


Figure 7.1 UV (205nm) trace from LC-MS analysis of purified **1g**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

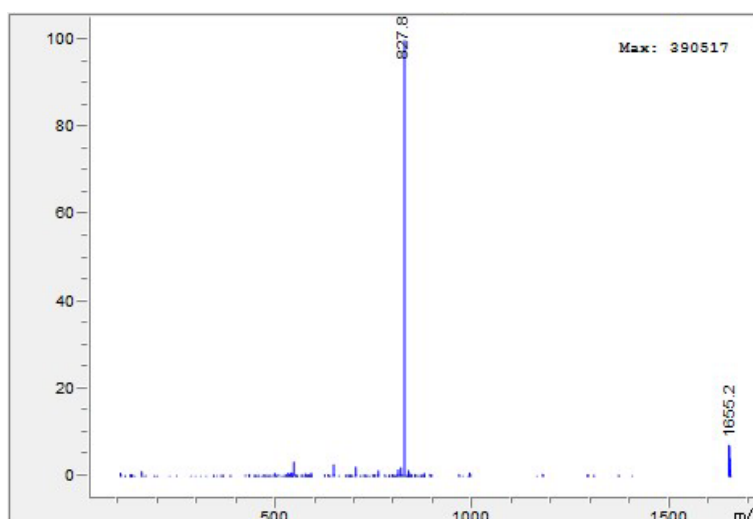


Figure 7.2 ESI-MS calcd. for **1g** (C₇₇H₁₀₃N₂₃O₁₉) [M+1H]¹⁺ m/z = 1655.8, found 1655.2; [M+2H]²⁺ m/z = 828.1, found 827.8.

Synthesis of Peptide **1h**



Peptide **1h** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1h** (65.8 mg, 14%) as a white powder.

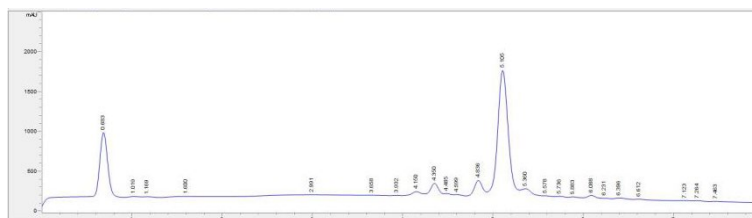


Figure 8.1 UV (205nm) trace from LC-MS analysis of purified **1h**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

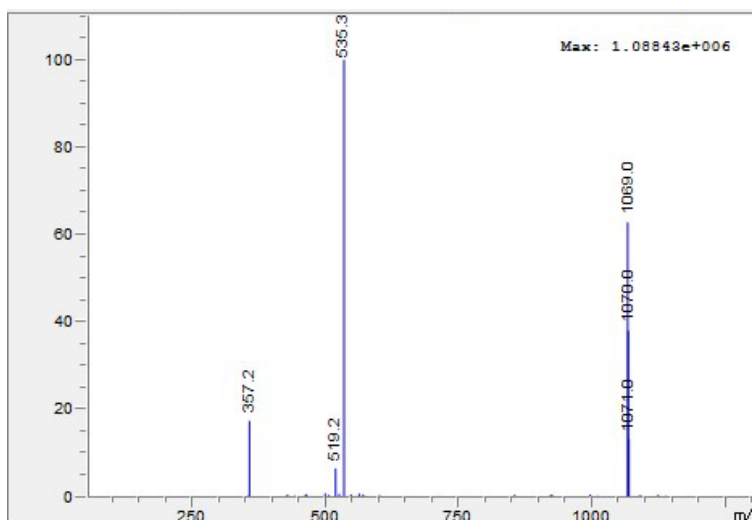


Figure 8.2 ESI-MS calcd. for **1h** (C₄₈H₈₈N₁₄O₁₃) [M+1H]¹⁺ m/z = 1069.7, found 1069.0; [M+2H]²⁺ m/z = 535.4, found 535.3; [M+3H]³⁺ m/z = 357.2, found 357.2.

Synthesis of Peptide **1i**



Peptide **1i** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (5-50% ACN/H₂O over 30min) and lyophilization to give **1i** (80.0 mg, 18%) as a white powder.

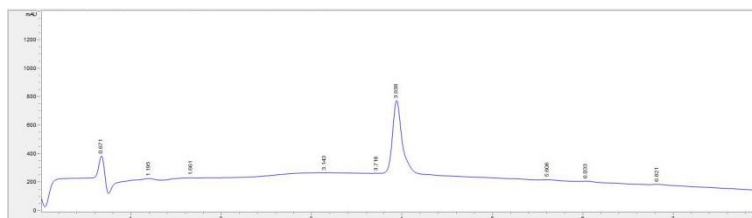


Figure 9.1 UV (205nm) trace from LC-MS analysis of purified **1i**. Gradient: 5%-50% ACN/H₂O with 0.1% TFA over 8 min.

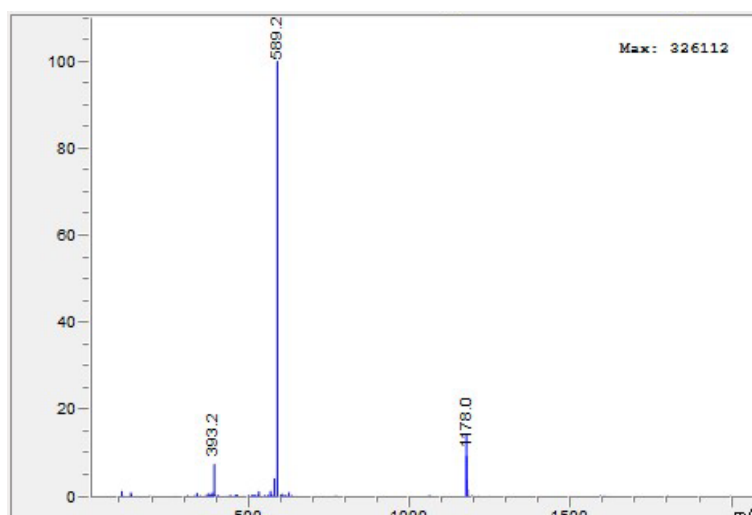


Figure 9.2 ESI-MS calcd. for **1i** (C₄₄H₈₄N₂₂O₁₂S₂) [M+1H]¹⁺ m/z = 1178.4, found 1178.0; [M+2H]²⁺ m/z = 589.7, found 589.2; [M+3H]³⁺ m/z = 393.5, found 393.2.

Synthesis of Peptide **1j**



Peptide **1j** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 2% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (2-30% ACN/H₂O over 30min) and lyophilization to give **1j** (30.7 mg, 14%) as a white powder.

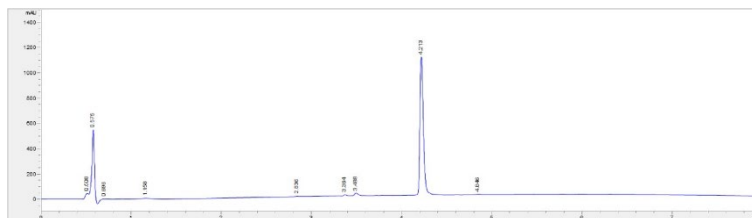


Figure 10.1 UV (205nm) trace from LC-MS analysis of purified **1j**. Gradient: 2%-30% ACN/H₂O with 0.1% TFA over 8 min.

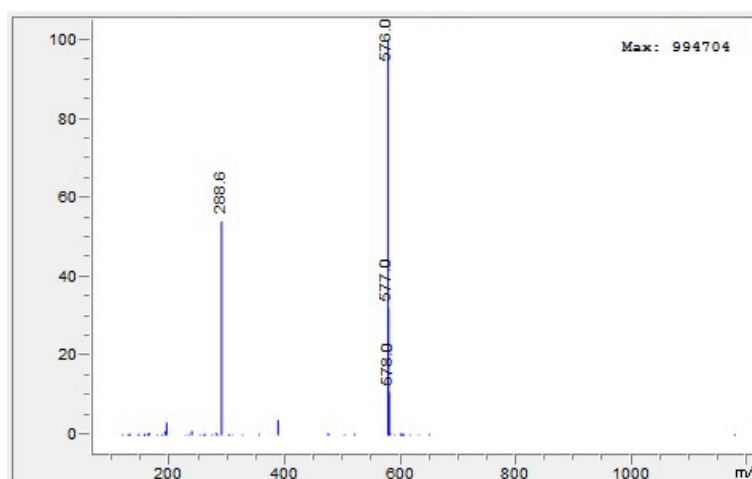


Figure 10.2 ESI-MS calcd. for **1j** (C₂₂H₄₁N₉O₇S) [M+1H]¹⁺ m/z =576.7, found 577.0; [M+2H]²⁺ m/z = 5288.9, found 288.6.

Synthesis of Peptide **1k**



Peptide **1k** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1k** (136.7 mg, 15%) as a white powder.

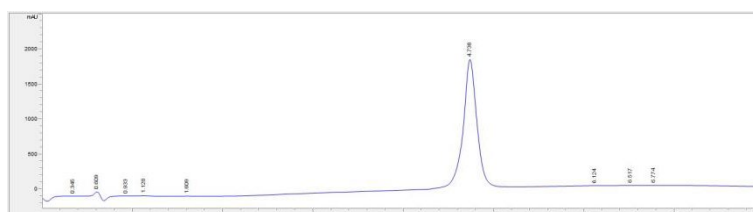


Figure 11.1 UV (205nm) trace from LC-MS analysis of purified **1k**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

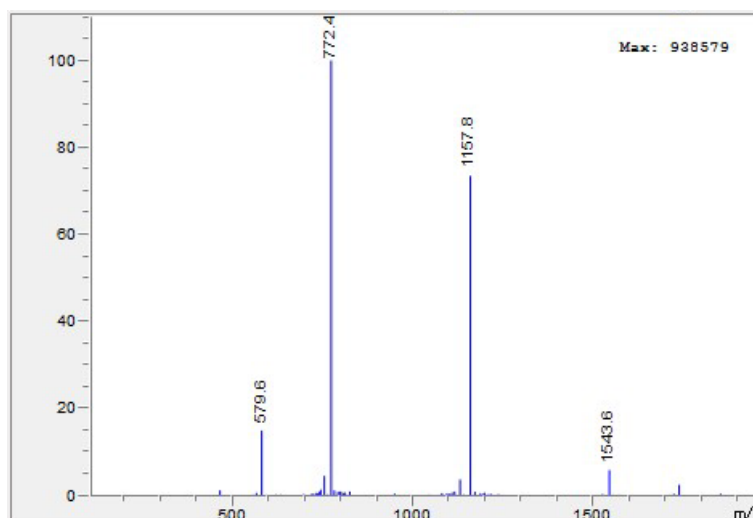


Figure 11.2 ESI-MS calcd. for **1k** (C₁₀₄H₁₅₁N₃₁O₃₀) [M+2H]²⁺ m/z = 1158.7, found 1157.8; [M+3H]³⁺ m/z = 772.8, found 772.4; [M+4H]⁴⁺ m/z = 579.9, found 579.6.

Synthesis of Peptide **1l**



Peptide **1l** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **11** (98.4 mg, 13%) as a white powder.

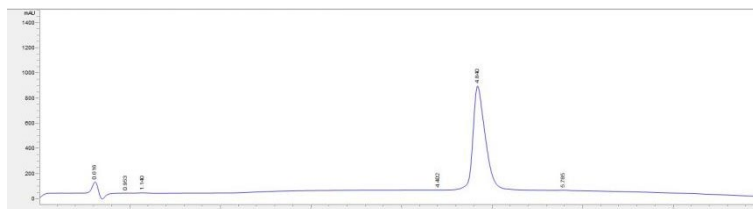


Figure 12.1 UV (205nm) trace from LC-MS analysis of purified **11**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

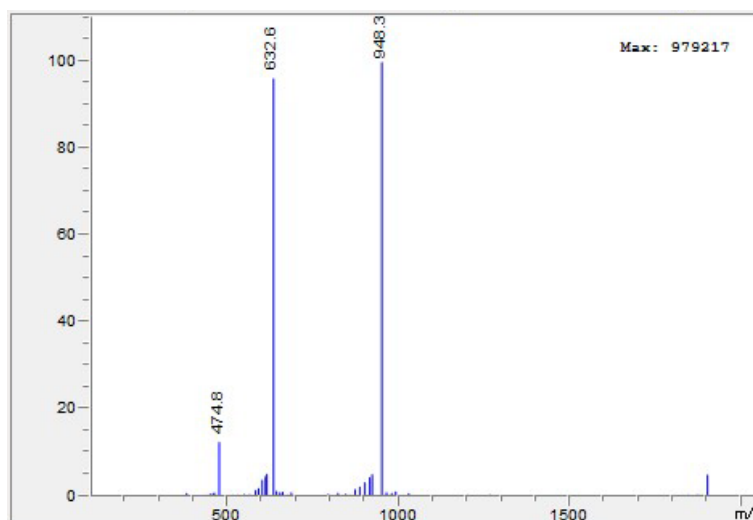


Figure 12.2 ESI-MS calcd. for **11** (C₈₃H₁₃₈N₂₈O₂₃) [M+2H]²⁺ m/z = 949.1, found 948.3; [M+3H]³⁺ m/z = 633.1, found 632.6; [M+4H]⁴⁺ m/z = 475.0, found 474.8.

Synthesis of Peptide **1m**



Peptide **1m** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 5% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **1m** (124.7 mg, 10%) as a white powder.

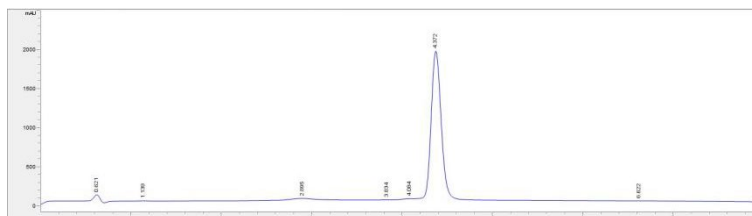


Figure 13.1 UV (205nm) trace from LC-MS analysis of purified **1m**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

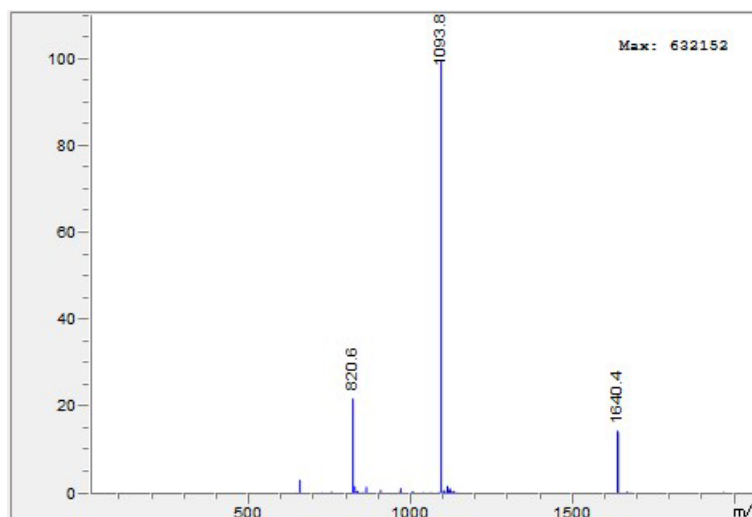


Figure 13.2 ESI-MS calcd. for **1m** (C₁₄₁H₂₁₉N₄₅O₄₂S₂) [M+2H]²⁺ m/z = 1640.3, found 1640.4; [M+3H]³⁺ m/z = 1093.9, found 1093.8; [M+4H]⁴⁺ m/z = 820.6, found 820.6.

Synthesis of Peptide **1n**



Peptide **1n** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the

resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1n** (94.6 mg, 9%) as a white powder.

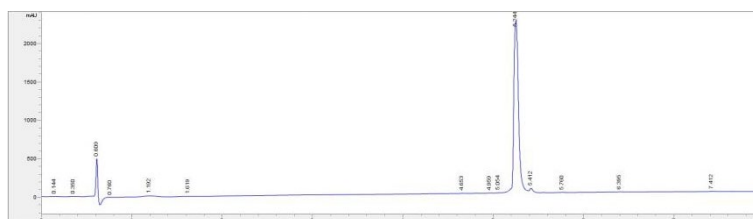


Figure 14.1 UV (205nm) trace from LC-MS analysis of purified **1n**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

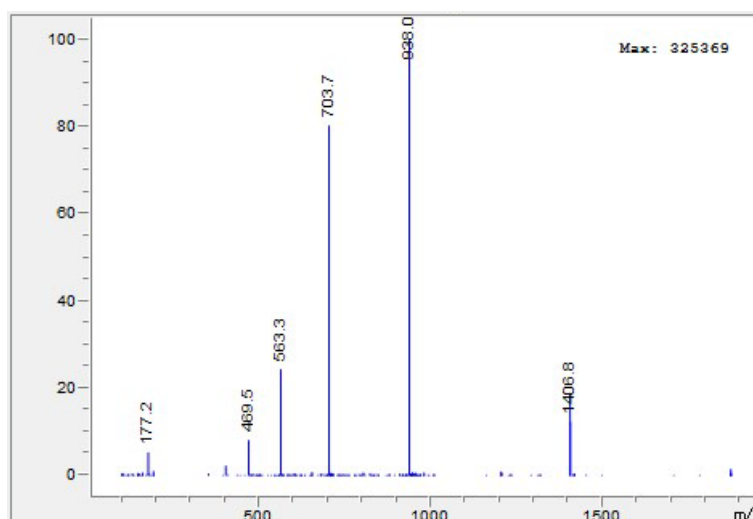


Figure 14.2 ESI-MS calcd. for **1n** (C₁₂₈H₂₁₉N₄₁O₂₈S) [M+2H]²⁺ m/z = 1407.2, found 1406.8; [M+3H]³⁺ m/z = 938.5, found 938.0; [M+4H]⁴⁺ m/z = 704.1, found 703.7; [M+5H]⁵⁺ m/z = 563.5, found 563.3; [M+6H]⁶⁺ m/z = 469.7, found 469.5.

Synthesis of Peptide **1o**



Peptide **1o** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1o** (104.8 mg, 11%) as a white powder.

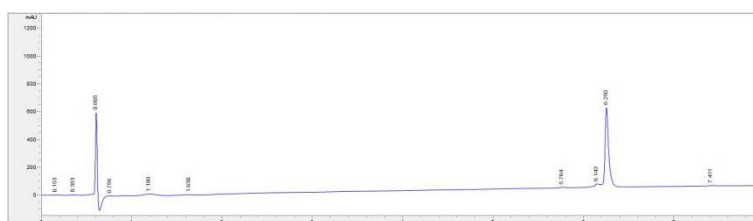


Figure 15.1 UV (205nm) trace from LC-MS analysis of purified **1o**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

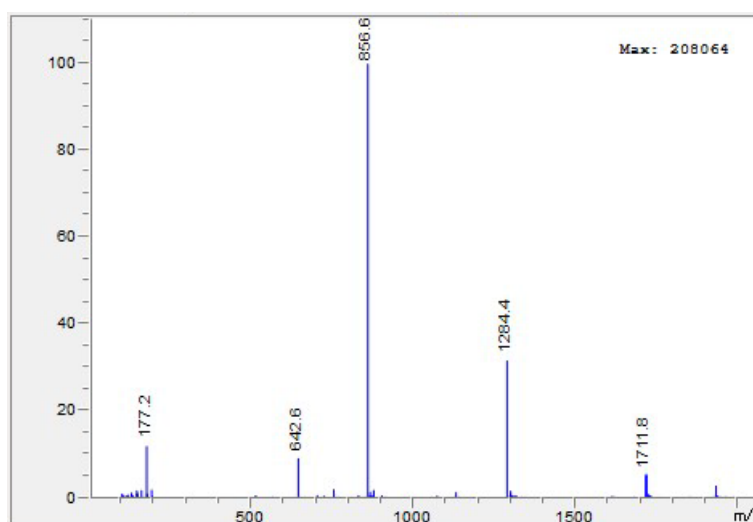


Figure 15.2 ESI-MS calcd. for **1o** (C₁₁₇H₁₈₇N₃₃O₃₀S) [M+2H]²⁺ m/z = 1284.2, found 1284.4; [M+3H]³⁺ m/z = 856.5, found 856.6; [M+4H]⁴⁺ m/z = 642.6, found 642.6.

Synthesis of Peptide **1p**



Peptide **1p** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1p** (89.5 mg, 9%) as a white powder.

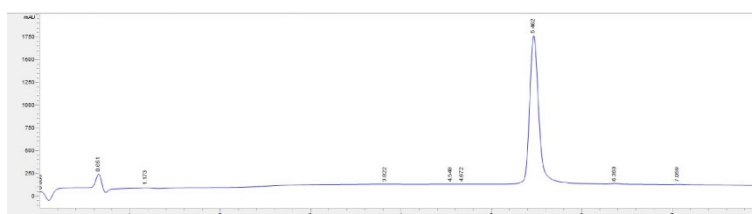


Figure 16.1 UV (205nm) trace from LC-MS analysis of purified **1p**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

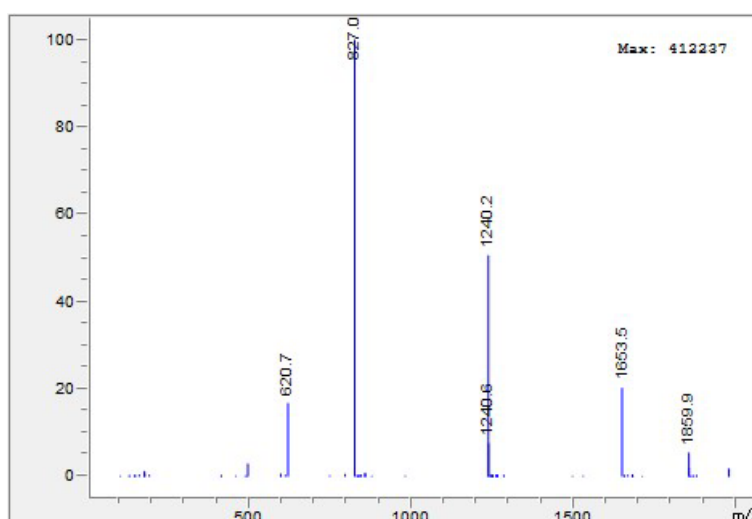


Figure 16.2 ESI-MS calcd. for **1p** (C₁₁₁H₁₈₇N₃₃O₃₁) [M+2H]²⁺ *m/z* = 1240.2, found 1240.2; [M+3H]³⁺ *m/z* = 827.1, found 827.0; [M+4H]⁴⁺ *m/z* = 620.6, found 620.7.

Synthesis of Peptide **1q**



Peptide **1q** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1q** (137.2 mg, 16%) as a white powder.

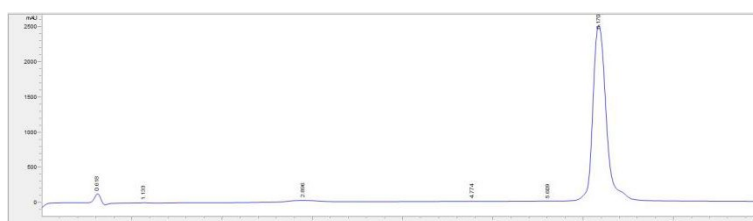


Figure 17.1 UV (205nm) trace from LC-MS analysis of purified **1q**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

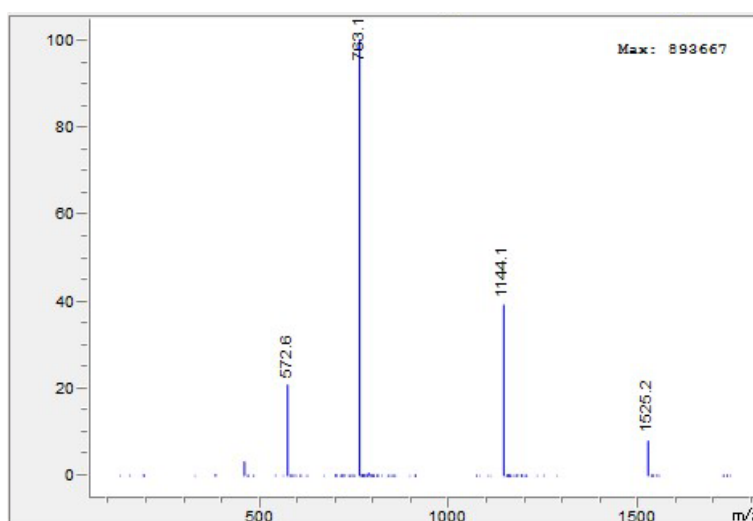


Figure 17.2 ESI-MS calcd. for **1q** (C₁₀₉H₁₆₇N₃₅O₁₈S) [M+2H]²⁺ m/z = 1144.2, found 1144.1; [M+3H]³⁺ m/z = 763.1, found 763.1; [M+4H]⁴⁺ m/z = 572.6, found 572.6.

Synthesis of Peptide **1r**



Peptide **1r** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1r** (115.3 mg, 14%) as a white powder.

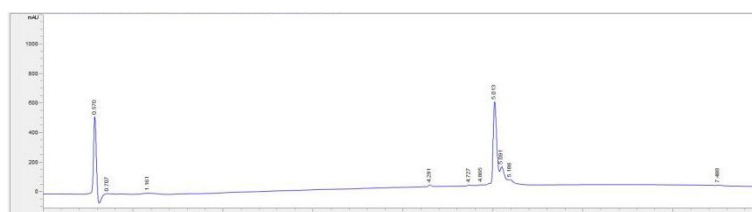


Figure 18.1 UV (205nm) trace from LC-MS analysis of purified **1r**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

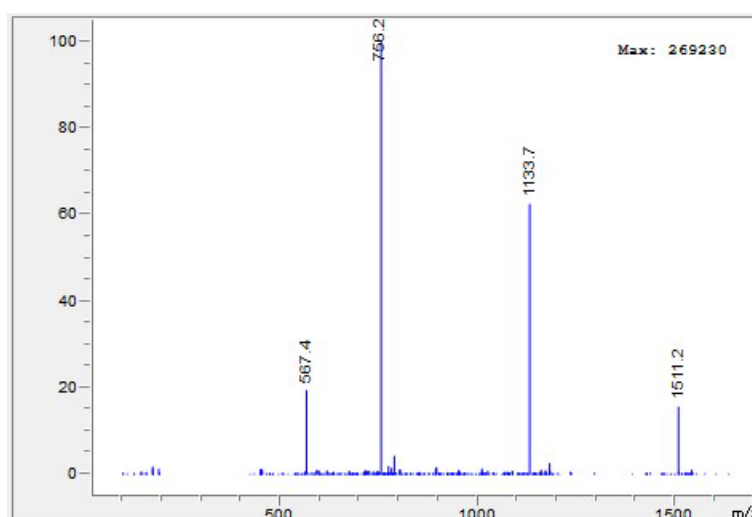


Figure 18.2 ESI-MS calcd. for **1r** (C₁₀₃H₁₇₆N₃₀O₂₅S) [M+2H]²⁺ m/z = 1133.7, found 1133.7; [M+3H]³⁺ m/z = 756.1, found 756.2; [M+4H]⁴⁺ m/z = 567.3, found 567.4.

Synthesis of Peptide **1s**



Peptide **1s** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for

1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1s** (98.7 mg, 10%) as a white powder.

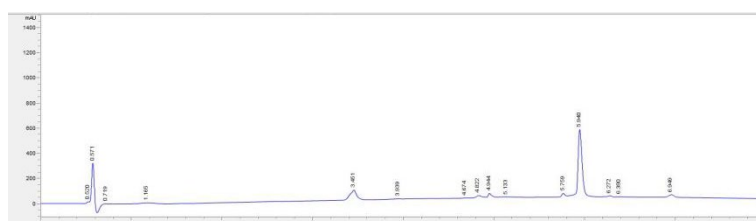


Figure 19.1 UV (205nm) trace from LC-MS analysis of purified **1s**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

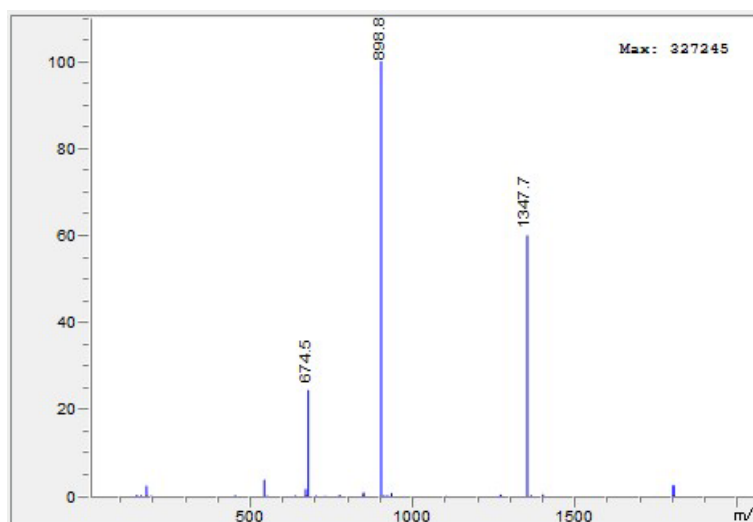
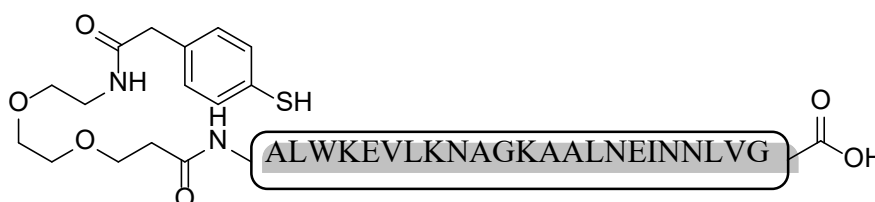


Figure 19.2 ESI-MS calcd. for **1s** (C₁₂₃H₂₀₄N₃₀O₃₅S) [M+2H]²⁺ m/z = 1348.3, found 1347.7; [M+3H]³⁺ m/z = 899.2, found 898.8; [M+4H]⁴⁺ m/z = 674.6, found 674.5.

Synthesis of Peptide **3g**



Peptide **3g** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3g** (103.8 mg, 10%) as a white powder.

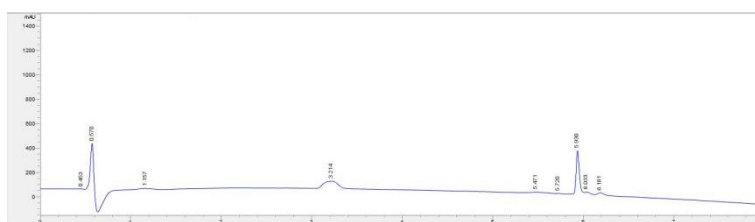


Figure 20.1 UV (205nm) trace from LC-MS analysis of purified **3g**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

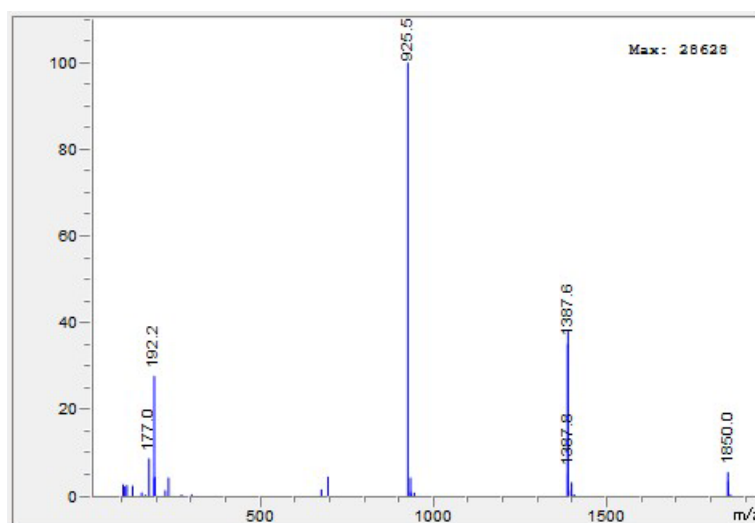
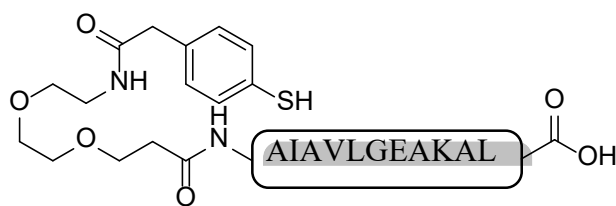


Figure 20.2 ESI-MS calcd. for **3g** (C₁₂₆H₂₀₅N₃₃O₃₅S) [M+2H]²⁺ m/z = 1388.2, found 1387.6; [M+3H]³⁺ m/z = 925.8, found 925.5.

Synthesis of Peptide **3h**



Peptide **3h** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (10-50% ACN/H₂O over 30min) and lyophilization to give **3h** (78.5 mg, 15%) as a white powder.

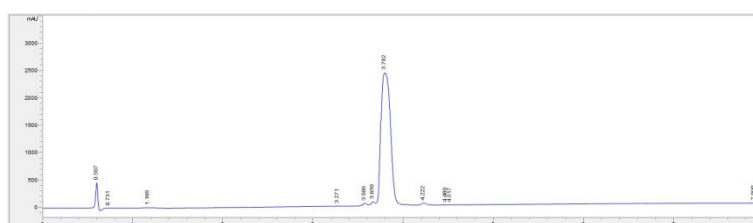


Figure 21.1 UV (205nm) trace from LC-MS analysis of purified **3h**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

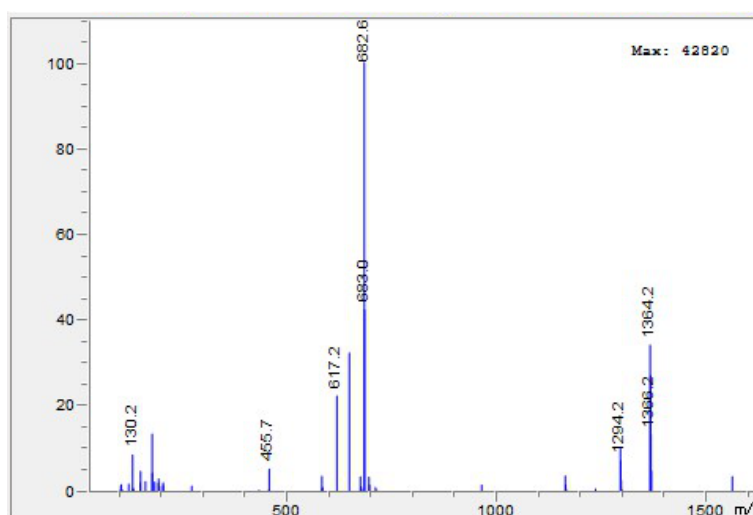


Figure 21.2 ESI-MS calcd. for **3h** (C₆₃H₁₀₆N₁₄O₁₇S) [M+1H]¹⁺ m/z = 1363.7, found 1364.2; [M+2H]²⁺ m/z = 682.4, found 682.6.

Synthesis of Peptide **3i**



Peptide **3i** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg). Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3i** (87.4 mg, 8%) as a white powder.

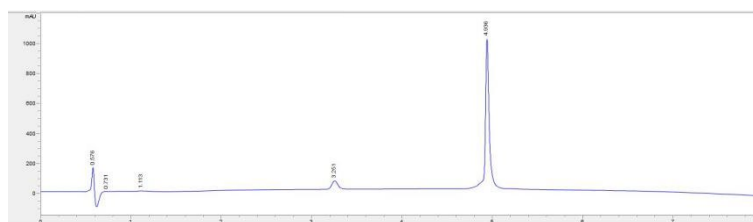


Figure 22.1 UV (205nm) trace from LC-MS analysis of purified **3i**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

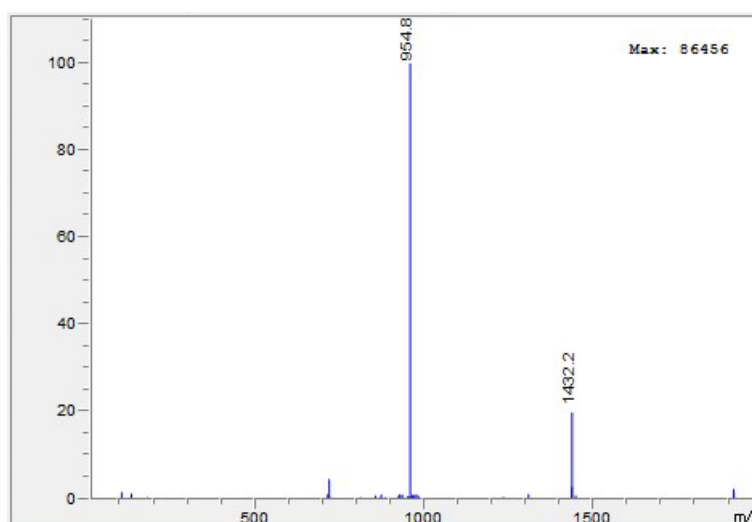


Figure 22.2 ESI-MS calcd. for **3i** (C₁₃₂H₂₀₄N₃₂O₃₅S₂) [M+2H]²⁺ m/z = 1432.4, found 1432.2; [M+2H]²⁺ m/z = 955.2, found 954.8.

Synthesis of Peptide **3j**



Peptide **3j** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg). Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3j** (78.3 mg, 8%) as a white powder.

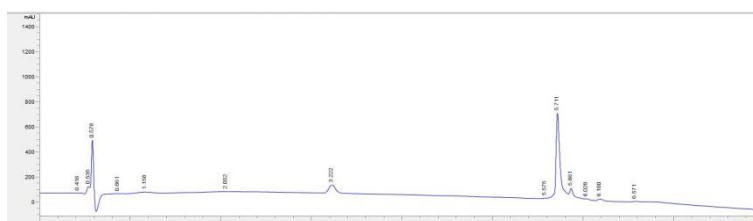
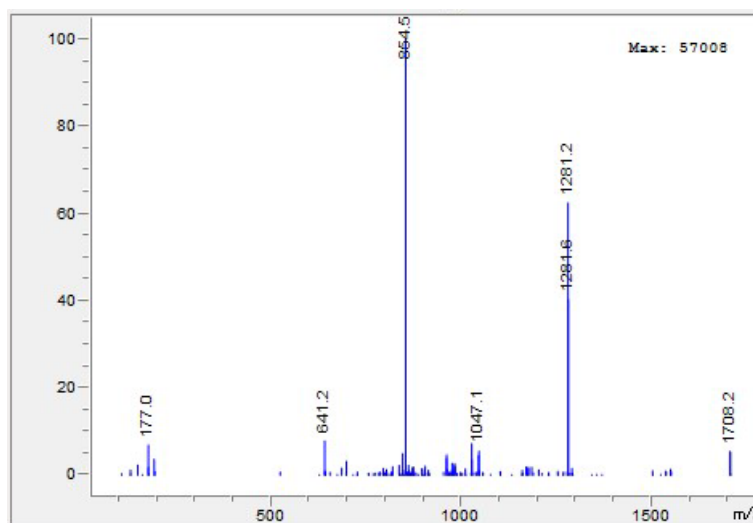


Figure 23.1 UV (205nm) trace from LC-MS analysis of purified **3j**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.



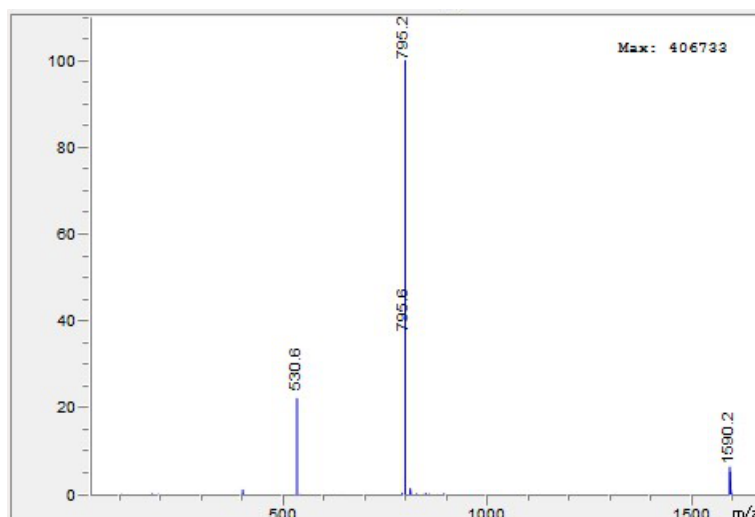
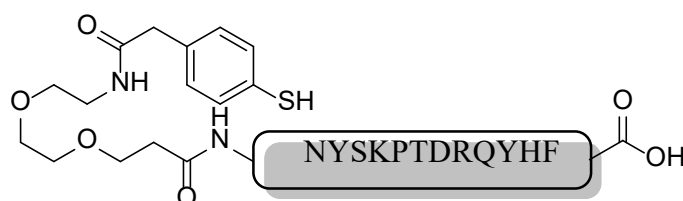


Figure 24.2 ESI-MS calcd. for **3k** ($C_{73}H_{120}N_{16}O_{19}S_2$) $[M+1H]^+$ $m/z = 1589.8$, found 1590.2; $[M+2H]^{2+}$ $m/z = 795.4$, found 795.2; $[M+3H]^{3+}$ $m/z = 530.6$, found 530.6.

Synthesis of Peptide **3l**



Peptide **3l** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/ H_2O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold ($-20\text{ }^{\circ}C$) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/ H_2O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE $0.22\mu m$), subjected to preparative HPLC purification (20-70% ACN/ H_2O over 30min) and lyophilization to give **3l** (86.2 mg, 12%) as a white powder.



Figure 25.1 UV (205nm) trace from LC-MS analysis of purified **3l**. Gradient: 20%-70% ACN/ H_2O with 0.1% TFA over 8 min.

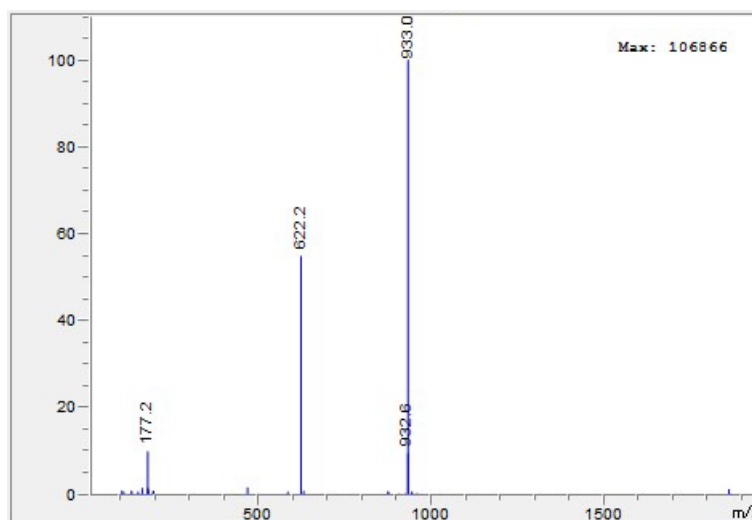
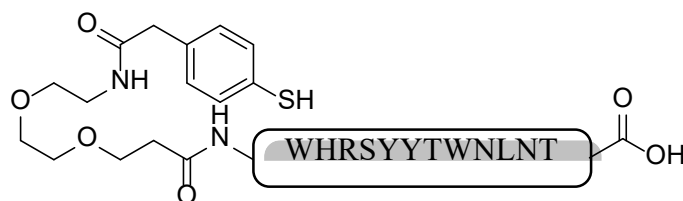


Figure 25.2 ESI-MS calcd. for **3l** (C₈₅H₁₁₇N₂₁O₂₅S) [M+2H]²⁺ m/z =932.9, found 933.0; [M+3H]³⁺ m/z =622.3, found 622.2.

Synthesis of Peptide **3m**



Peptide **3m** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3m** (115.7 mg, 15%) as a white powder.

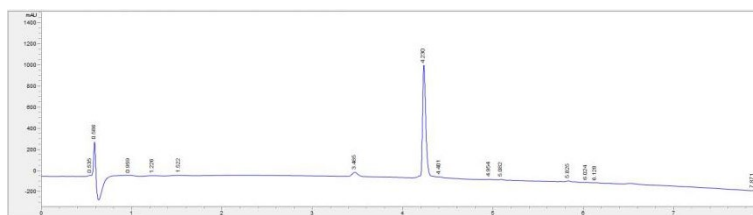


Figure 26.1 UV (205nm) trace from LC-MS analysis of purified **3m**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

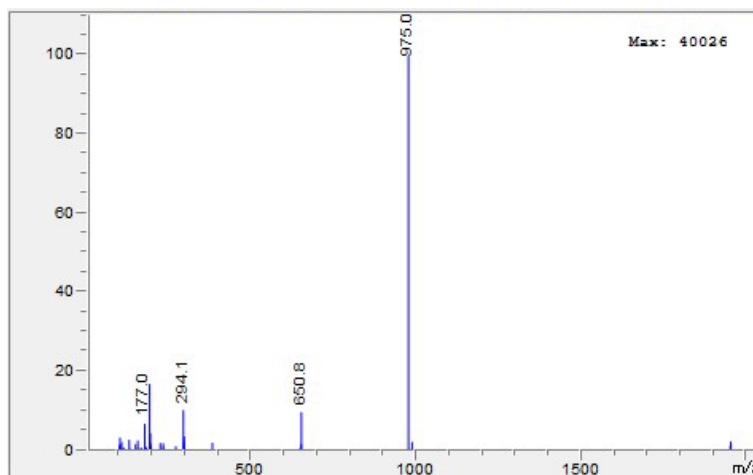
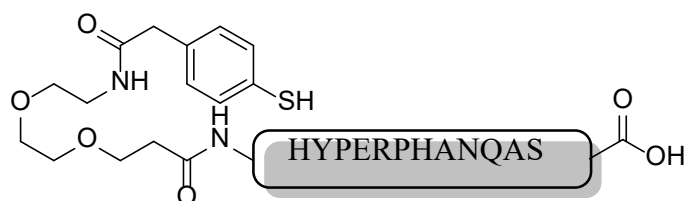


Figure 26.2 ESI-MS calcd. for **3m** (C₉₂H₁₂₀N₂₂O₂₄S) [M+2H]²⁺ m/z =975.4, found 975.0; [M+3H]³⁺ m/z =650.6, found 650.8.

Synthesis of Peptide **3n**



Peptide **3n** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (15-60% ACN/H₂O over 30min) and lyophilization to give **3n** (93.5 mg, 14%) as a white powder.

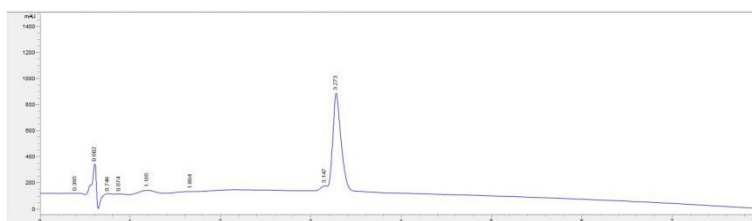


Figure 27.1 UV (205nm) trace from LC-MS analysis of purified **3n**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

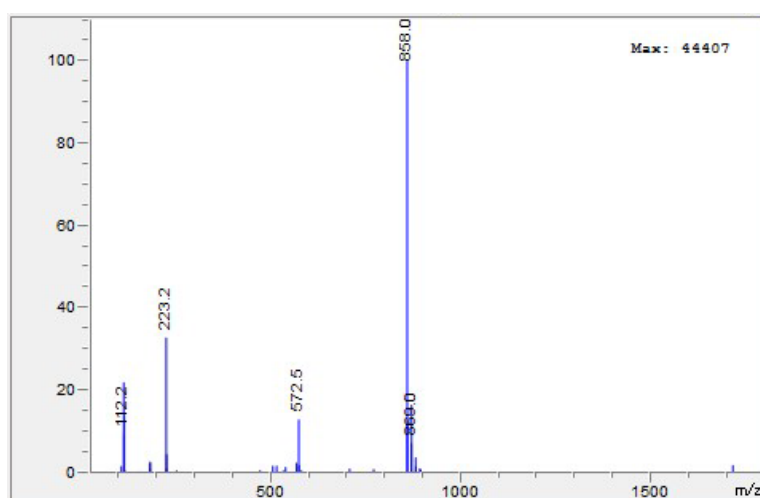
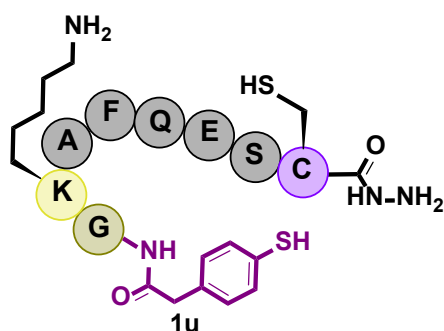


Figure 27.2 ESI-MS calcd. for **3n** (C₇₅H₁₀₆N₂₂O₂₃S) [M+2H]²⁺ m/z =858.4, found 858.0; [M+3H]³⁺ m/z =572.6, found 572.5

Synthesis of Peptide **1u**



Peptide **1u** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether,

the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (15-60% ACN/H₂O over 30min) and lyophilization to give **1u** (78.4 mg, 20%) as a white powder.

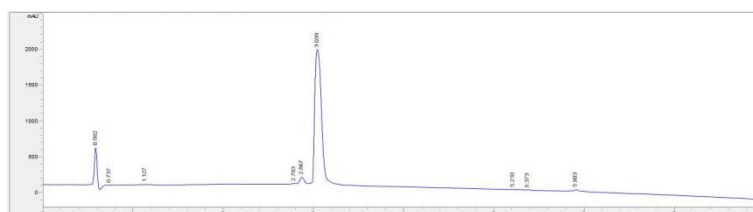


Figure 28.1 UV (205nm) trace from LC-MS analysis of purified **1u**. Gradient: 15%-60% ACN/H₂O with 0.1% TFA over 8 min.

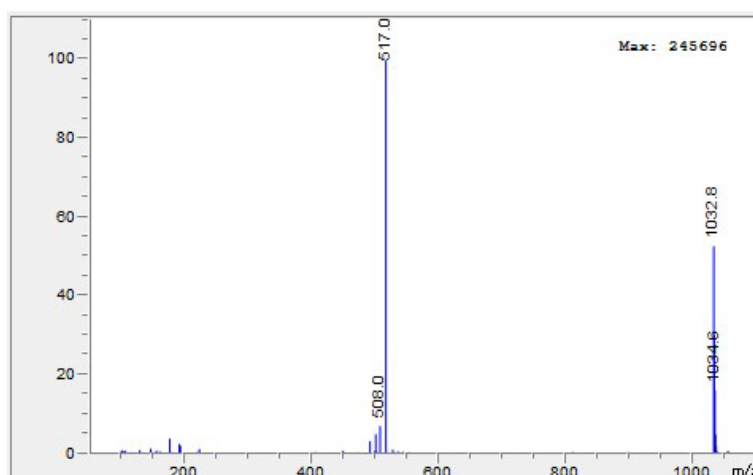
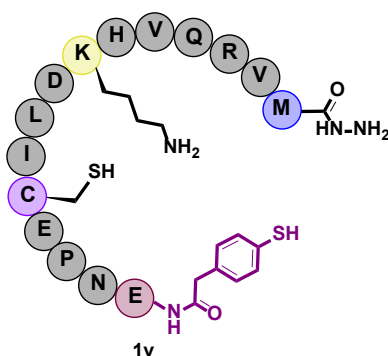


Figure 28.2 ESI-MS calcd. for **1u** (C₄₄H₆₄N₁₂O₁₃S₂) [M+1H]¹⁺ m/z =1033.4, found 1032.8; [M+2H]²⁺ m/z =517.2, found 517.0.

Synthesis of Peptide **1v**



Peptide **1v** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1v** (86.4 mg, 11%) as a white powder.

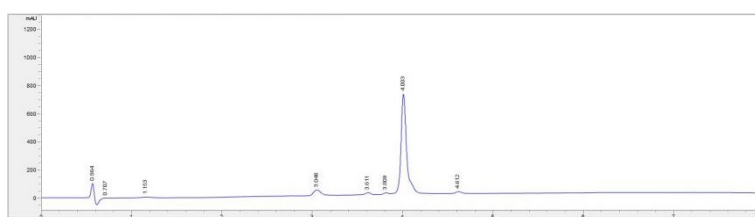


Figure 29.1 UV (205nm) trace from LC-MS analysis of purified **1v**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

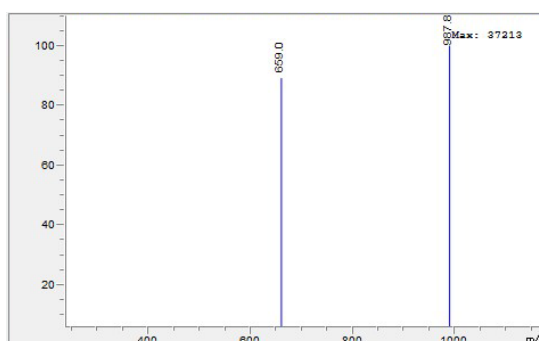
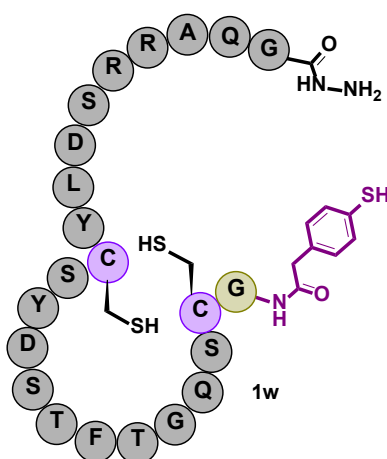


Figure 29.2 ESI-MS calcd. for **1v** (C₈₄H₁₃₅N₂₅O₂₄S₃) [M+2H]²⁺ m/z =987.9, found 987.8; [M+3H]³⁺ m/z =659.0, found 659.0.

Synthesis of Peptide **1w**



Peptide **1w** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22µm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1w** (93.4 mg, 9%) as a white powder.

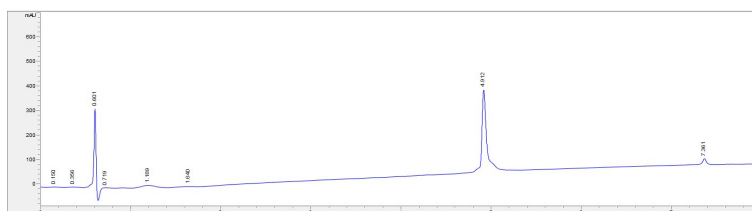


Figure 30.1 UV (205nm) trace from LC-MS analysis of purified **1w**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

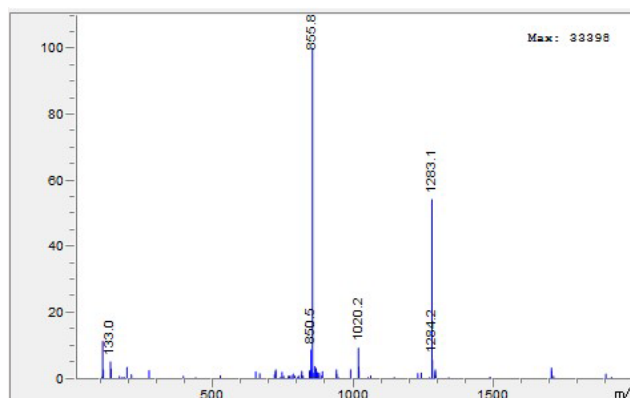
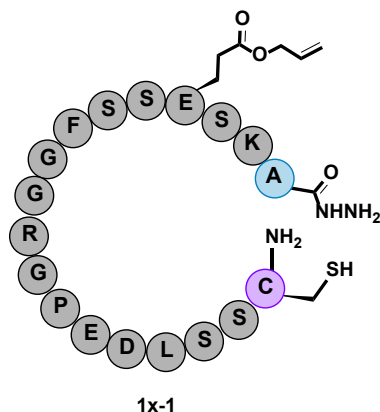


Figure 30.2 ESI-MS calcd. for **1w** (C₁₀₆H₁₅₆N₃₂O₃₇S₃) [M+2H]²⁺ *m/z* =1283.5, found 1283.1; [M+3H]³⁺ *m/z* =856.0, found 855.8.

Synthesis of Peptide **1x-1**



Peptide **1x-1** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1x-1** (135.7 mg, 19%) as a white powder.

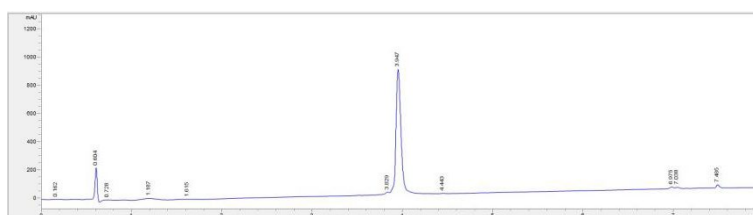


Figure 31.1 UV (205nm) trace from LC-MS analysis of purified **1x-1**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

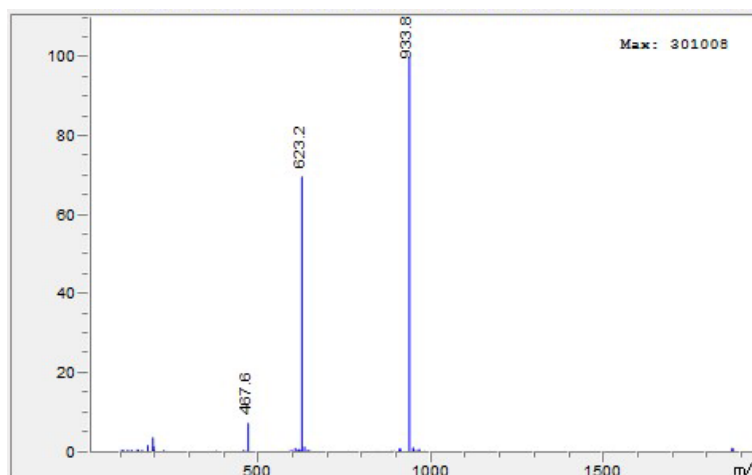
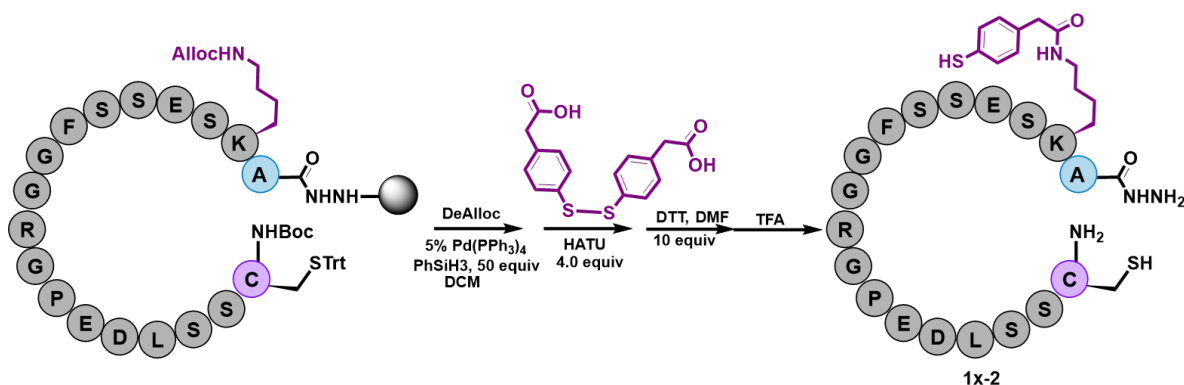


Figure 31.2 ESI-MS calcd. for **1x-1** ($C_{76}H_{122}N_{24}O_{29}S$) $[M+2H]^{2+}$ m/z =933.9, found 933.8; $[M+3H]^{3+}$ m/z =622.9, found 623.2.

Synthesis of Peptide **1x-2**



Peptide **1x-2** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **1x-2** (127.6 mg, 17%) as a white powder.

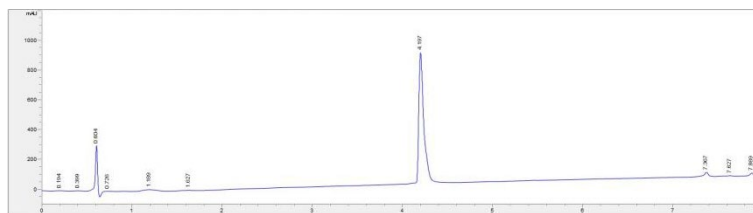


Figure 32.2 UV (205nm) trace from LC-MS analysis of purified **1x-2**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

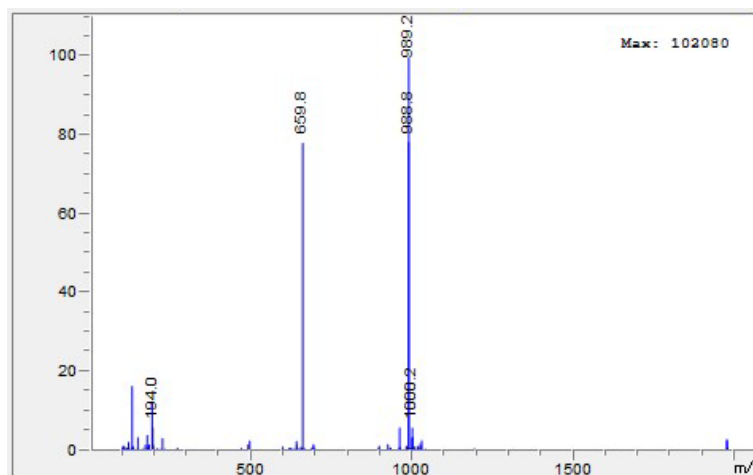
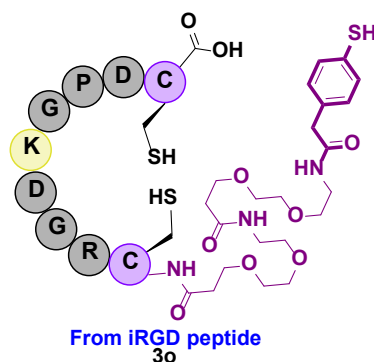


Figure 32.2 ESI-MS calcd. for **1x-2** (C₈₁H₁₂₄N₂₄O₃₀S₂) [M+2H]²⁺ m/z =989.4, found 989.2; [M+3H]³⁺ m/z =659.9, found 659.8.

Synthesis of Peptide **3o**



Peptide **3o** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg) Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was

filtrated by Syringe Filters (PTFE 0.22 μ m), subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3o** (125 mg, 23%) as a white powder.

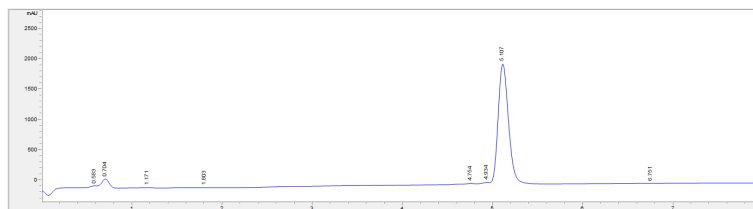


Figure 33.1 UV (205nm) trace from LC-MS analysis of purified **3o**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

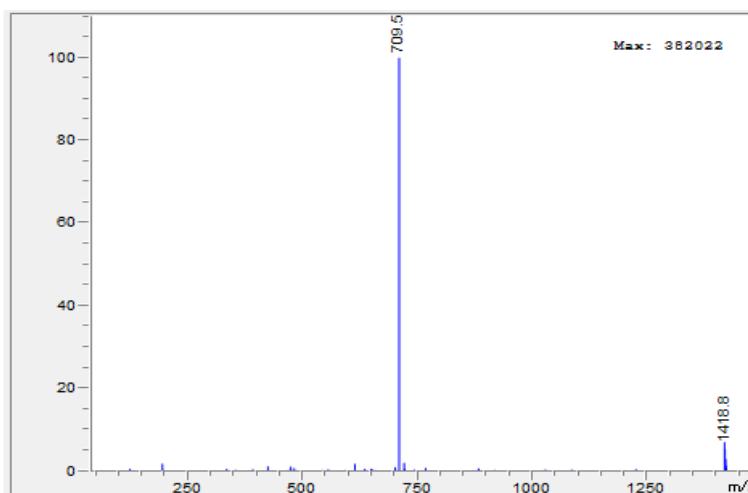
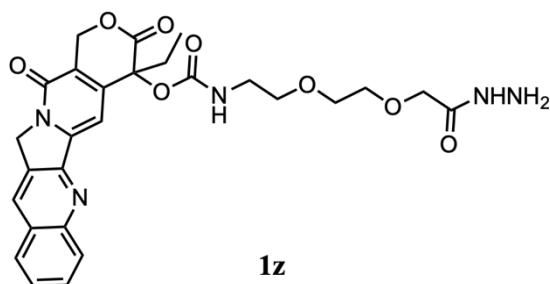


Figure 33.2 ESI-MS calcd. for **3o** (C₅₇H₉₁N₁₅O₂₁S₃) [M+1H]¹⁺ m/z =1418.5, found 1418.8; [M+2H]²⁺ m/z =709.7, found 709.5.

Synthesis of 1z



To synthesize Camptothecin-PEG-Acylhydrazine **1z**, hydrazine 2-chlorotrityl chloride resin was prepared by shaking 2-chlorotrityl chloride resin (SUNRESIN, 700 mg, 0.385 mmol, 1 equiv) with 10 mL of $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ /DMF (1/10, v/v) for 1.5 hr,

followed by washing with CH_2Cl_2 , EtOH and DMF. To couple Fmoc-AEEA-OH, Fmoc-AEEA-OH (2 mmol, 5.2 equiv), HATU (2 mmol, 5.2 equiv) and DIEA (4 mmol, 10.4 equiv) were dissolved into 8 mL DMF and shaken with resin for overnight. Resin was then washed by CH_2Cl_2 , EtOH, and DMF, followed by shaking with 25% 4-methylpiperidine in DMF for 12 min to remove Fmoc. To couple camptothecin, the cooresponding carbonate derivative was synthesized according to the liturature.¹ Reaction solution was prepared by dissolving camptothecin carbonate derivative (0.22 mmol, 2.0 equiv) and MPAA (0.165 mmol, 1.5 equiv) into 6 mL CH_2Cl_2 /DMF (2/1, v/v), followed by addition of DEIA (0.22 mmol, 2.0 equiv). Reation solution was shaken with resin (200 mg, 0.11 mmol, 1 equiv) for 2 hr, followed by washing by CH_2Cl_2 , EtOH, and DMF. Product was released by shaking resin with 2 mL 95% TFA in H_2O for 1 hr. Reaction mixture was then added, 6 mL MeCN/ H_2O (1/1, v/v) and filtered for preparative HPLC purification (10-50% MeCN/ H_2O over 30 min). Lyophilization of the product fractions gave a yellow powder (19.3 mg, 32%). ^1H NMR (600 MHz, d_6 -DMSO, 298 K) (ppm): 10.48 (s, 1H), 8.71 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.84-7.88 (m, 2H), 7.71-7.74 (m, 1H), 7.04 (s, 1H), 5.44-5.45 (m, 2H), 5.31-5.32 (m, 2H), 4.00 (s, 2H), 3.50-3.55 (m, 6H), 3.36 (t, J = 5.9 Hz, 2H), 3.03-3.11 (m, 2H), 2.06-2.13 (m, 2H), 0.88-0.90 (t, J = 7.4 Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, d_6 -DMSO, 298 K) (ppm): 168.6, 168.1, 158.1, 156.6, 154.4, 152.4, 147.9, 146.3, 145.7, 131.7, 130.4, 129.8, 128.9, 128.6, 128.0, 127.7, 118.9, 94.8, 74.7, 70.3, 69.3, 68.9, 68.7, 66.2, 50.2, 30.7, 7.5. HR-ESI-MS (+ve): calcd. for $\text{C}_{27}\text{H}_{29}\text{N}_5\text{O}_8$, $[\text{M}+\text{H}]^+$ m/z = 552.2094, found 552.2093.

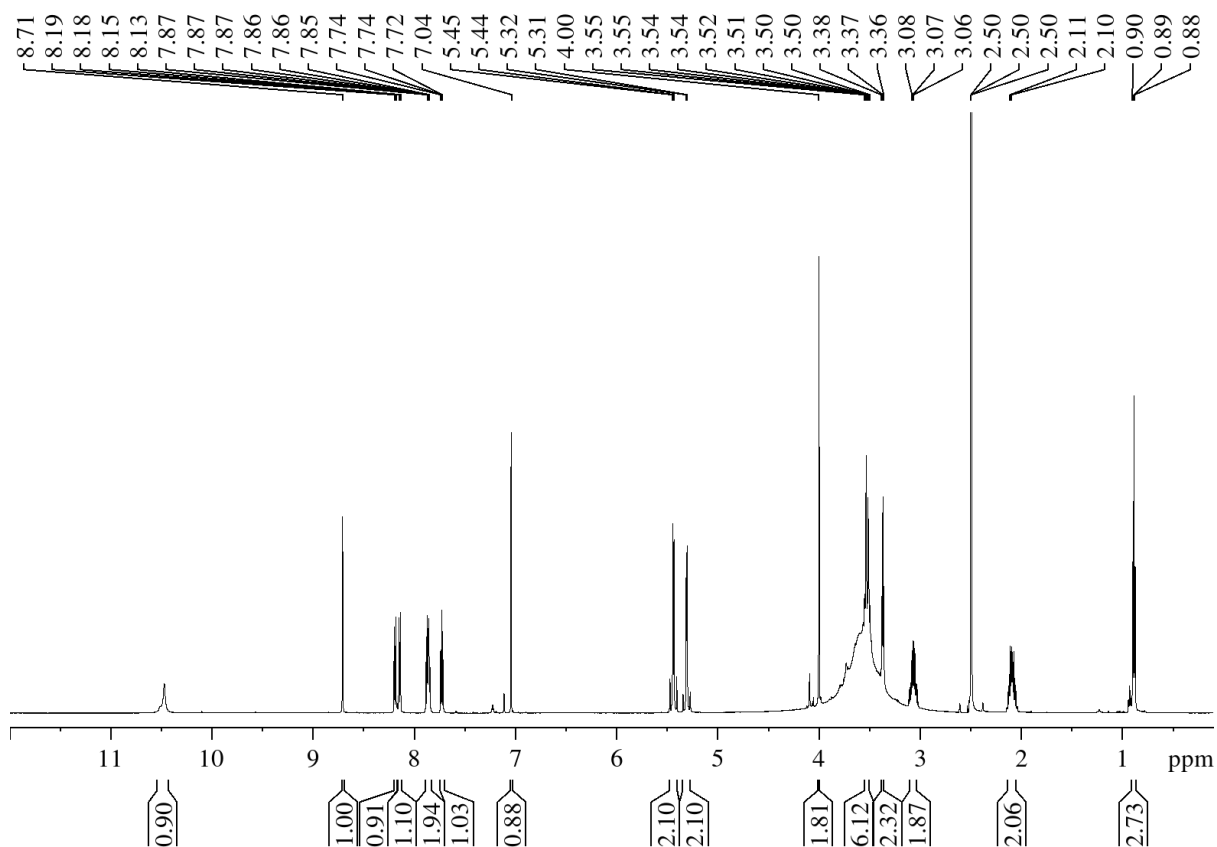


Figure 34.1 ¹H NMR (600 MHz, *d*₆-DMSO, 298 K) of **1z**.

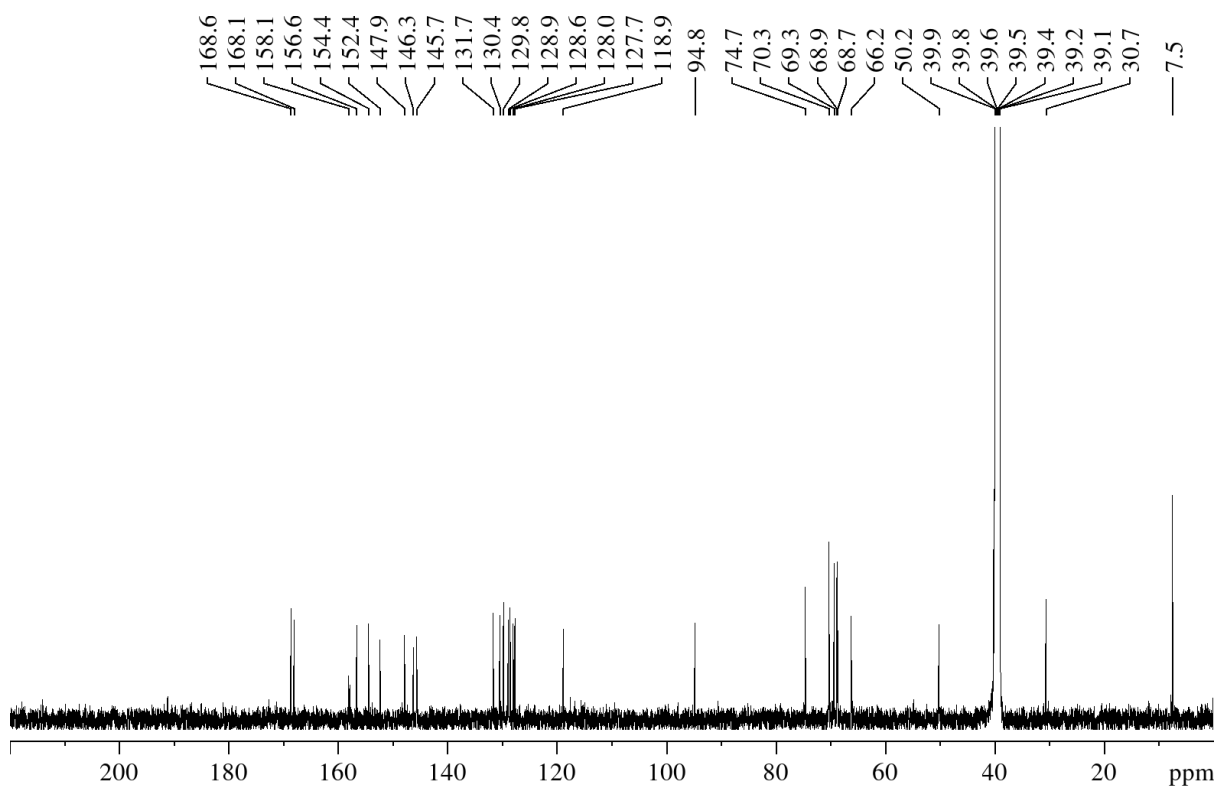


Figure 34.2 ¹³C{¹H} NMR (151 MHz, *d*₆-DMSO, 298 K) of **1z**.

Camptothecin12 #605 RT: 2.70 AV: 1 NL: 3.21E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]

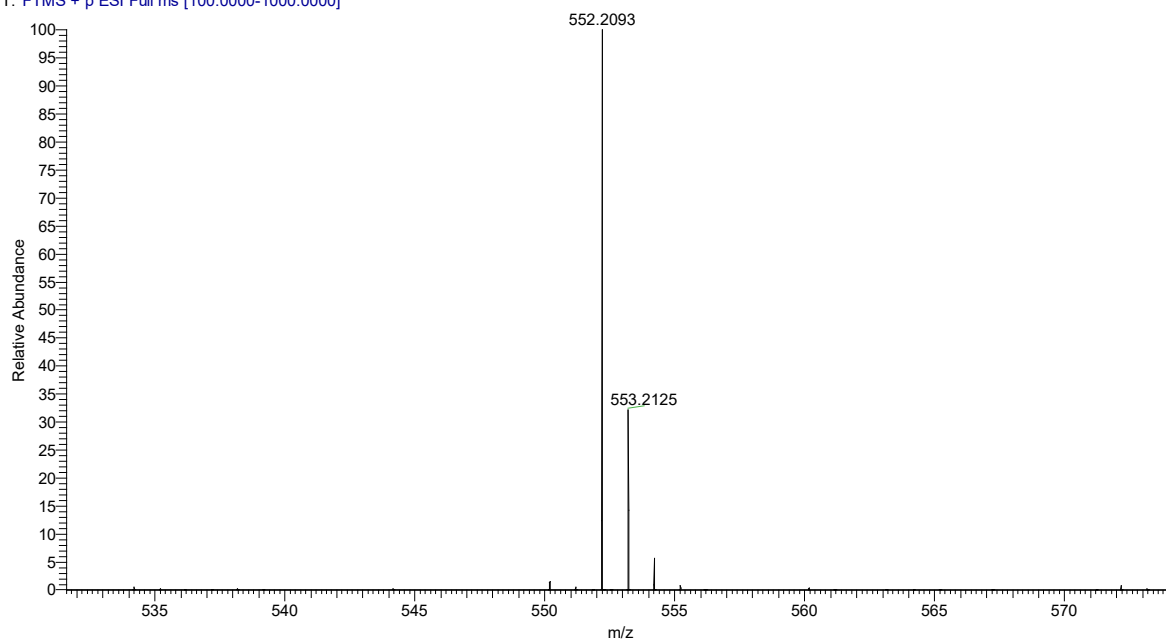


Figure 34.3 HR-ESI-MS of **1z**.

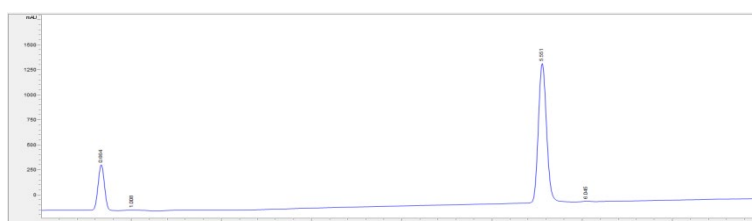


Figure 34.4 UV (205nm) trace from LC-MS analysis of purified **1z**. Gradient: 10%-50% ACN/H₂O with 0.1% TFA over 8 min.

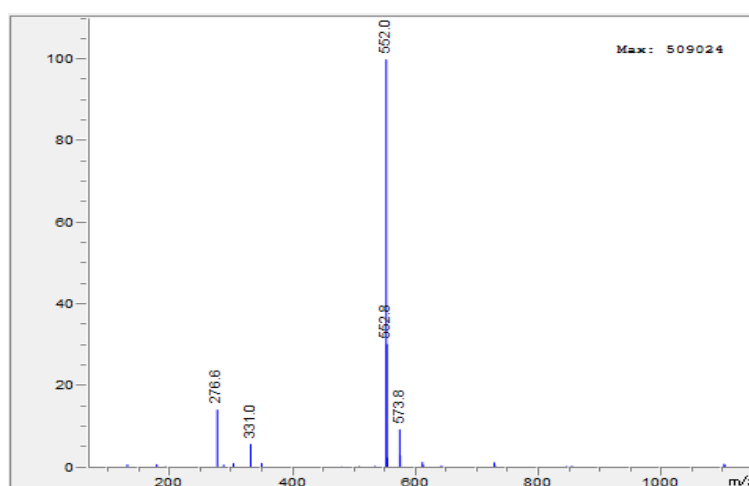
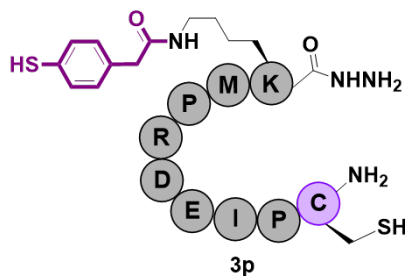


Figure 34.5 ESI-MS calcd. for **1z** (C₂₇H₂₉N₅O₈) [M+1H]¹⁺ m/z =552.2, found 552.0; [M+2H]²⁺ m/z =276.6, found 276.6.

Synthesis of Peptide **3p**



Peptide **3p** was synthesized according to the general procedure using hydrazine 2-chlorotrityl chloride resin (700 mg). Upon completion of synthesis, the crude peptide was then deprotected and cleaved from the resin using 30 mL TFA/DODT/TIPS/H₂O (95.5/1.5/1.5/1.5, v/v/v/v) for 1 h. The reaction solution was poured into cold (-20 °C) diethyl ether (120 mL), and the resulting suspension was centrifuged to give a white pellet. After decantation of diethyl ether, the residual solid was dissolved in 10% ACN/H₂O aqueous solution, then this solution was filtrated by Syringe Filters (PTFE 0.22μm) and subjected to preparative HPLC purification (20-70% ACN/H₂O over 30min) and lyophilization to give **3p** (143 mg, 30%) as a white powder.

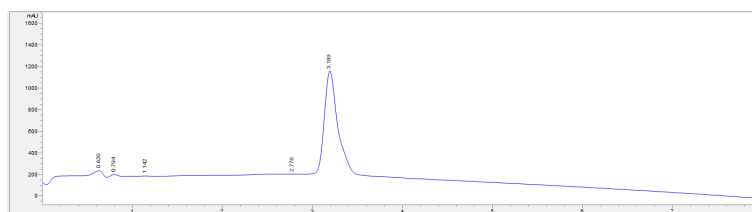


Figure 35.1 UV (205nm) trace from LC-MS analysis of purified **3p**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

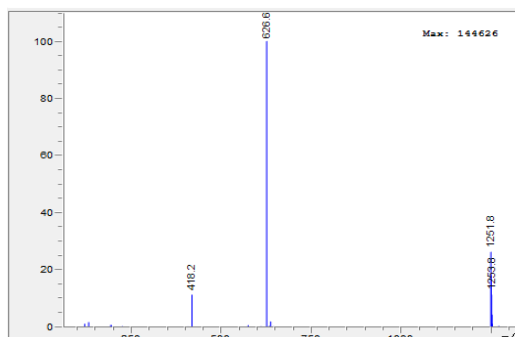
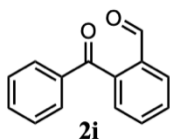


Figure 35.2 ESI-MS calcd. for **3p** (C₅₃H₈₅N₁₅O₁₄S₃) [M+1H]¹⁺ m/z =1251.6, found 1251.8; [M+2H]²⁺ m/z =626.3, found 626.6; [M+3H]³⁺ m/z =417.9, found 418.2.

2 Synthesis of 2i-2s



Synthesis of **2i**. To a 250 mL RB was added benzoic acid (2 mmol, 300 mg), CDMT (2 mmol, 350 mg), toluene (10 mL), and NMM (2 mmol, 0.22 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (4 mmol, 848 mg), (2-formylphenyl)boronic acid (2 mmol, 244 mg) and $Pd(P(Ph_3)_2)Cl_2$ (0.02 mmol, 14 mg). Reaction mixture was stirred at 110°C for 6 hr, cooled down and concentrated under a reduced pressure at 70 °C. Residue was redissolved into minimal amount of MeCN, filtered and product was isolated using a C18 column (SEPAFLASHTM, 20-45 μm , 100 Å), 10-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 36 mg pale yellow solid, 8.6%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.03 (s, 1H), 8.03-8.04 (m, 1H), 7.80-7.81 (m, 2H), 7.67-7.71 (m, 2H), 7.59-7.62 (m, 1H), 7.51-7.52 (m, 1H), 7.46-7.49 (m, 2H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 196.6, 190.7, 141.5, 137.2, 135.5, 133.8, 133.5, 130.7, 130.2, 130.1, 129.0, 128.8. HR-ESI-MS (+ve): calcd. for $C_{14}H_{10}O_2$, $[M+H]^+$ m/z = 211.0759, found 211.0757.

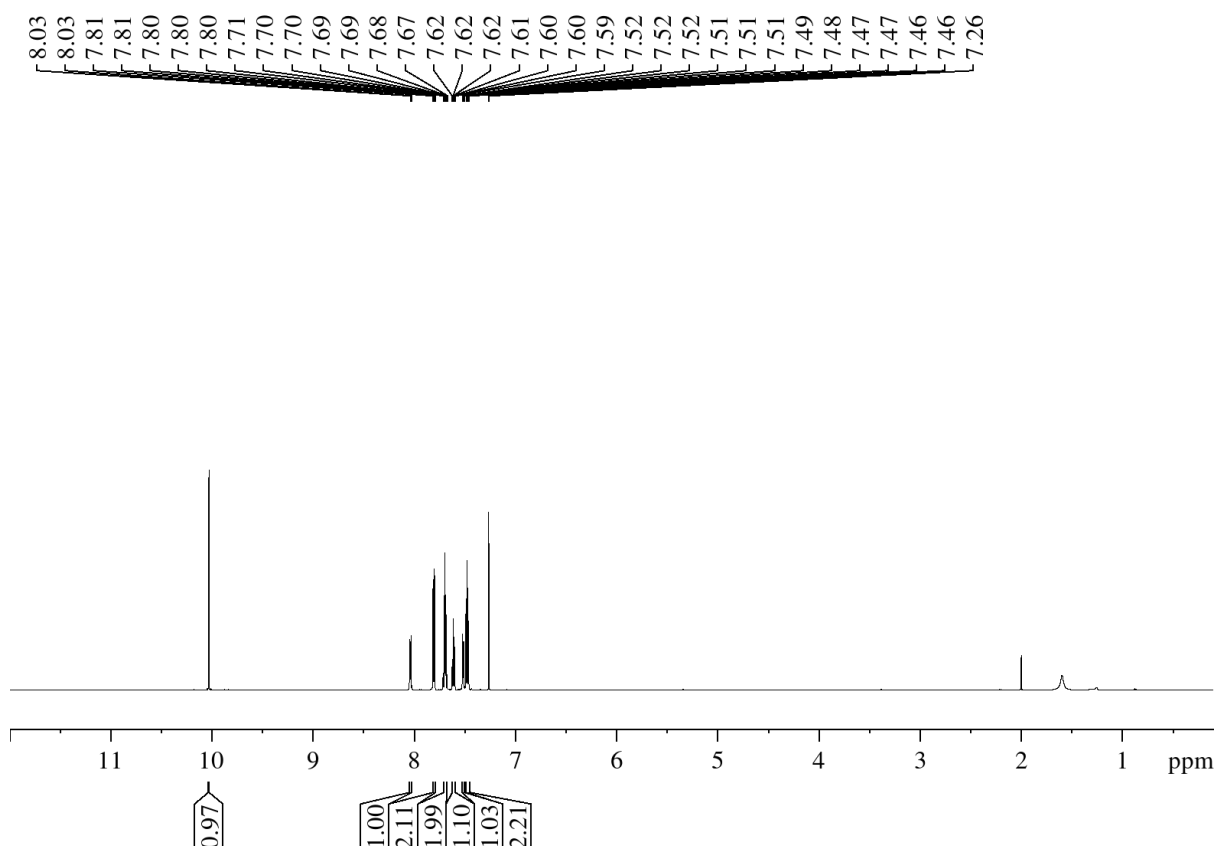


Figure 36.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2i**.

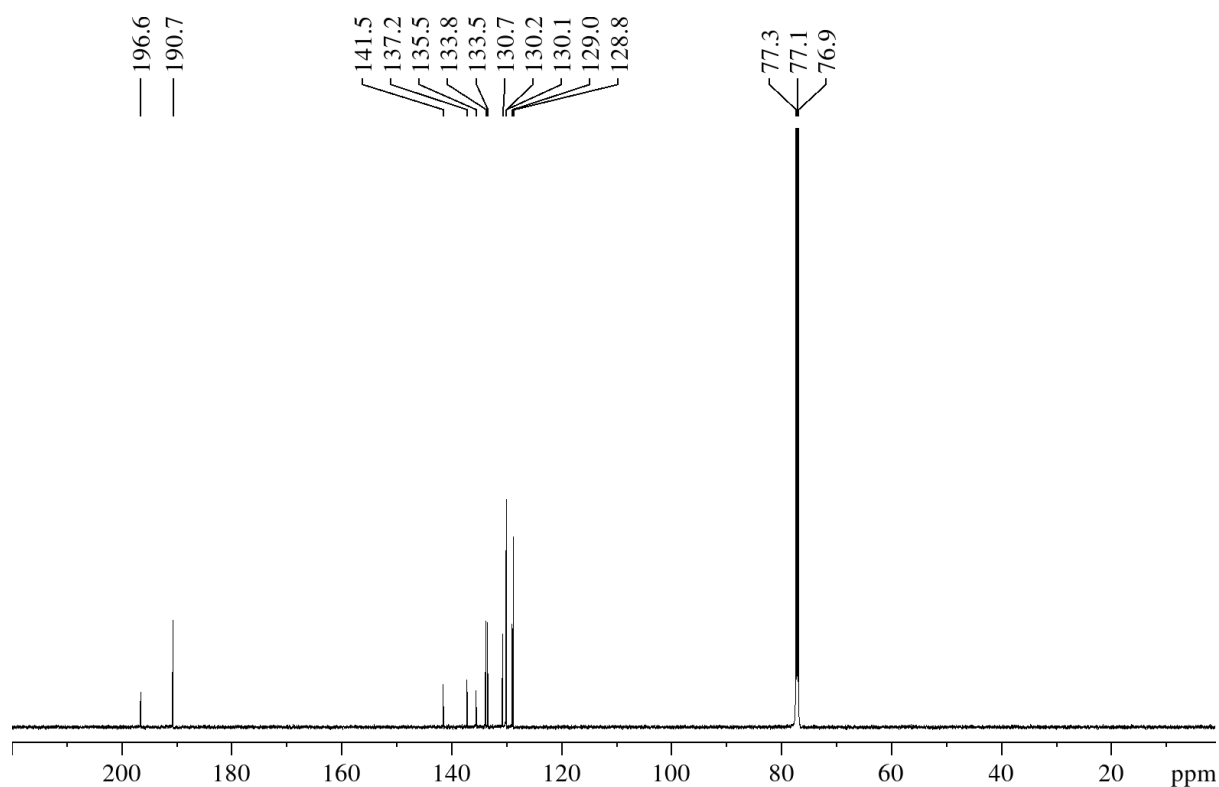


Figure 36.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2i**.

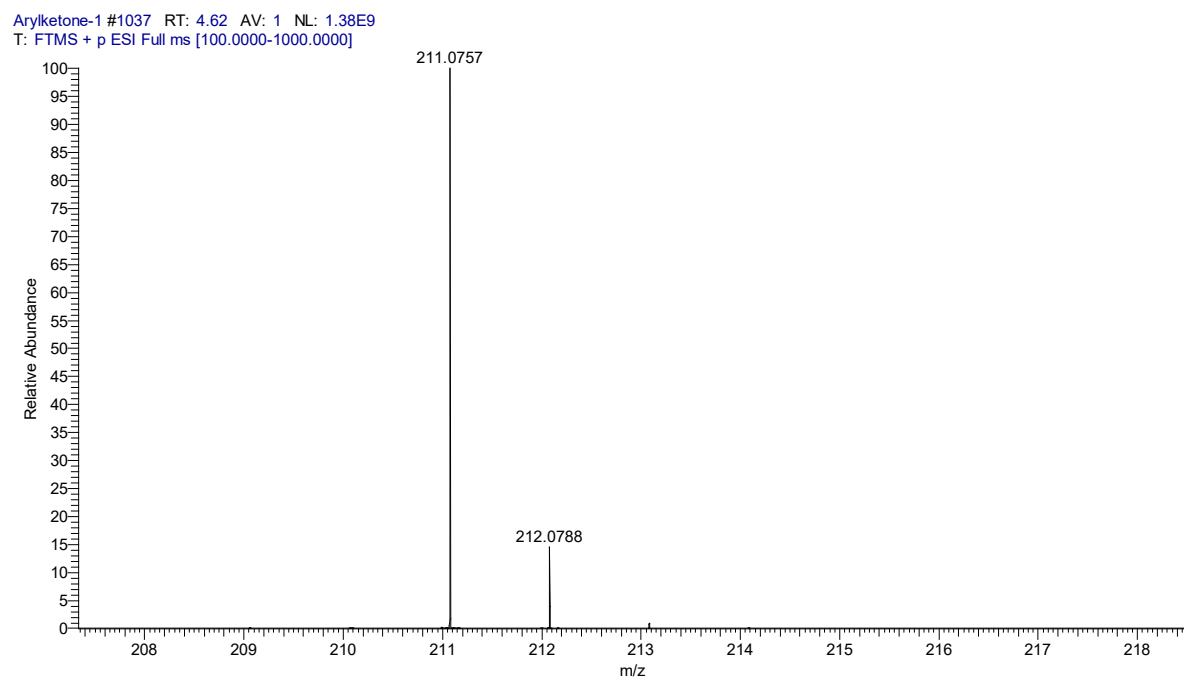
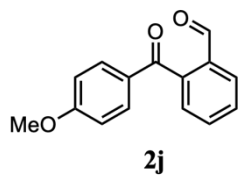


Figure 36.3 HR-ESI-MS of **2i**.



Synthesis of **2j**. To a 250 mL RB was added 4-methoxybenzoic acid (7 mmol, 1.06 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110°C for 3 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL), washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASHTM, 20-45 μm , 100 Å), 15-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 46 mg brown-red solid, 2.7%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.02 (s, 1H), 8.02 (dd, $J = 7.3, 1.2$ Hz, 1H), 7.78-7.79 (m, 2H), 7.65-7.68 (m, 2H), 7.49 (dd, $J = 7.5, 1.5$ Hz, 1H), 6.93-6.95 (m, 2H), 3.88 (s, 3H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 195.1, 190.7, 164.3, 142.2, 135.3, 133.5, 132.6, 130.4, 130.3, 129.7, 128.8, 114.1, 55.7. HR-ESI-MS (+ve): calcd. for $C_{15}H_{12}O_3$, $[M+H]^+$ $m/z = 241.0865$, found 241.0861.

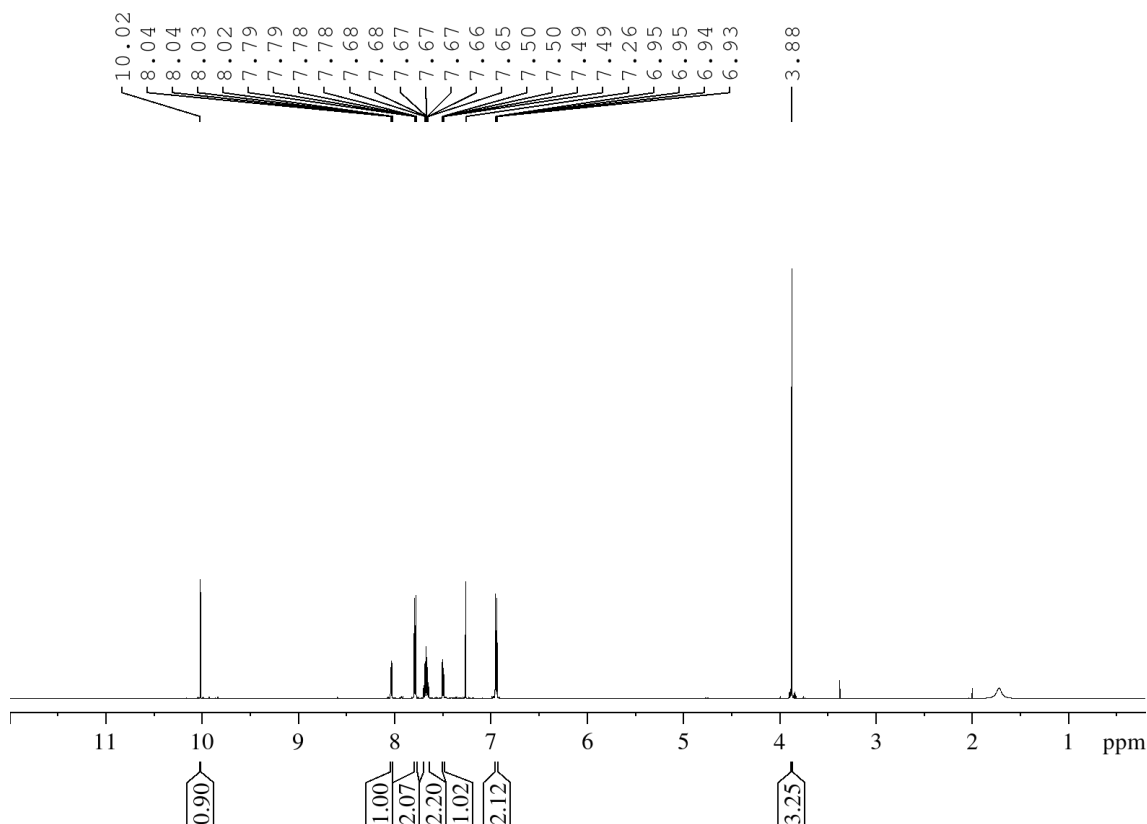


Figure 37.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2j**.

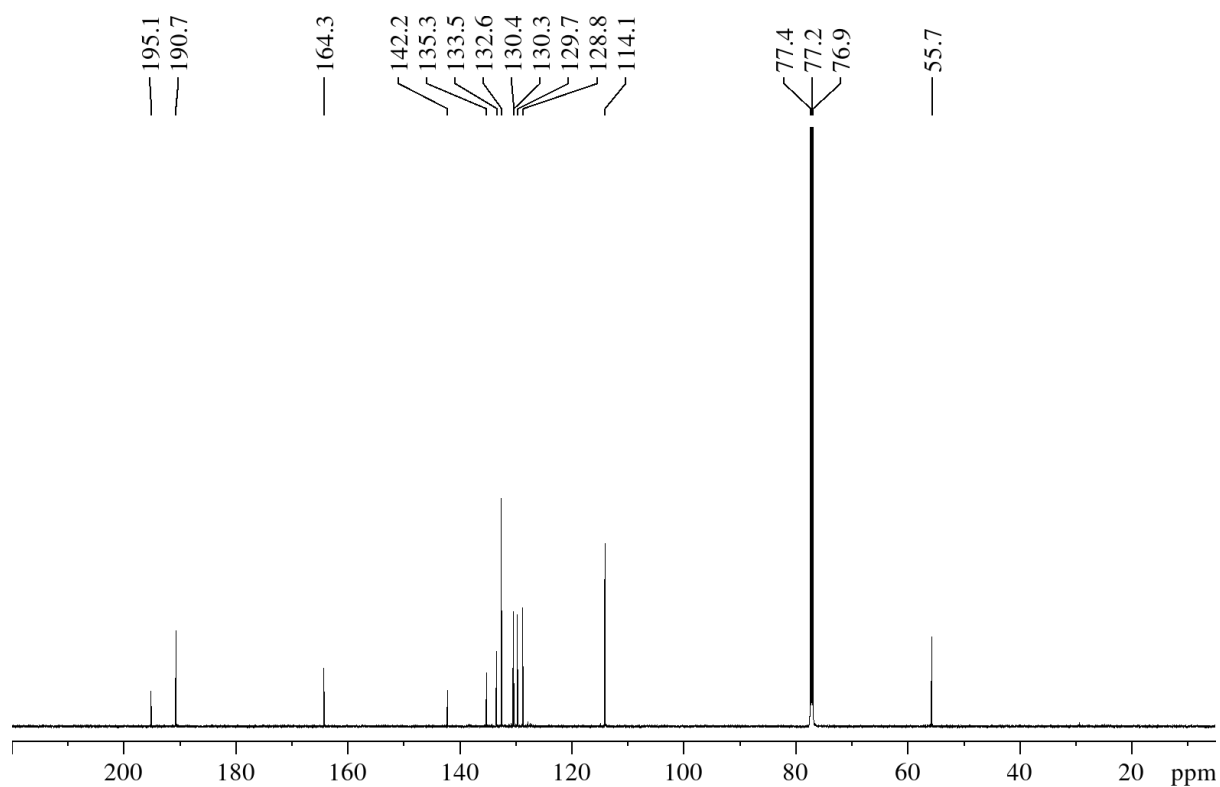


Figure 37.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2j**.

Arylketone-7-p #1082 RT: 4.82 AV: 1 NL: 1.81E9
T: FTMS + p ESI Full ms [100.0000-1000.0000]

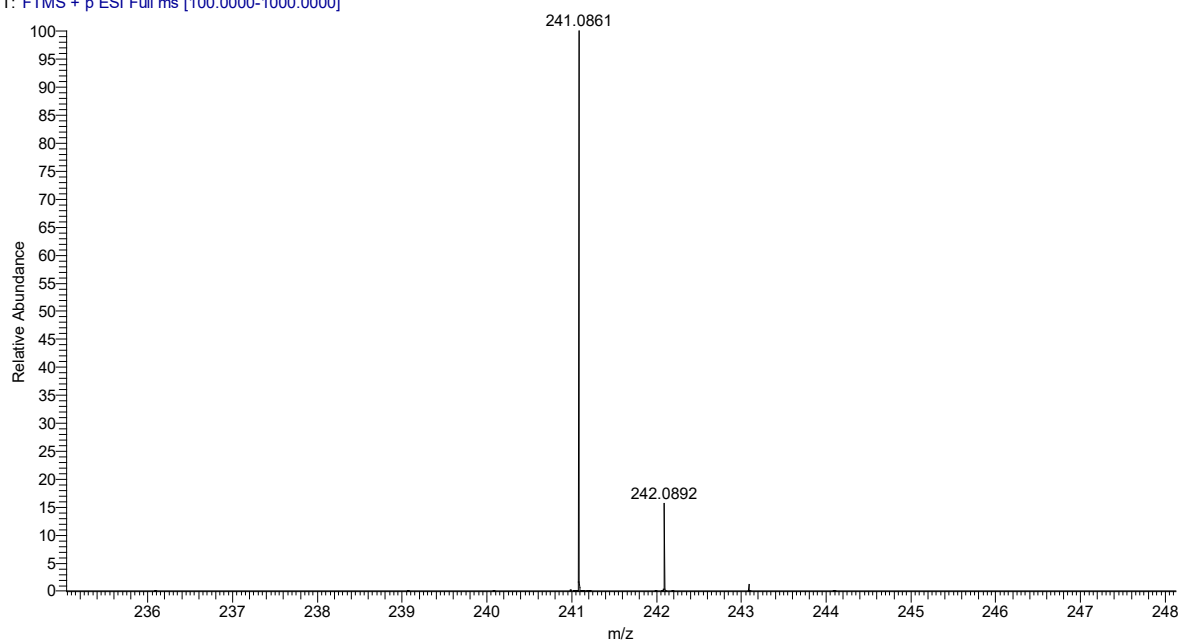
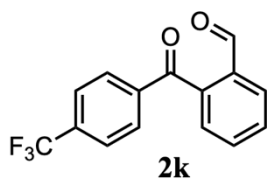


Figure 37.3 HR-ESI-MS of **2j**.



Synthesis of **2k**. To a 250 mL RB was added 4-(trifluoromethyl)benzoic acid (7 mmol, 1.33 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110 °C for 3 hr, cooled down and concentrated under a reduced pressure at 70 °C. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASH™, 20-45 μm , 100 Å), 35-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 99 mg yellow solid, 5.1%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.01 (s, 1H), 8.02 (dd, $J = 3.4, 5.6$ Hz, 1H), 7.88-7.89 (m, 2H), 7.71-7.74 (m, 4H), 7.48 (dd, 3.3, 5.4 Hz, 1H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 195.8, 190.7, 140.3, 139.8, 135.5, 134.9, 134.7, 133.9, 131.5, 131.1, 130.0, 128.8, 125.9, 125.8 (q, $J = 3.5$ Hz, 1C). HR-ESI-MS (+ve): calcd. for $C_{15}H_9F_3O_2$, $[M+H]^+$ $m/z = 279.0633$, found 279.0629.

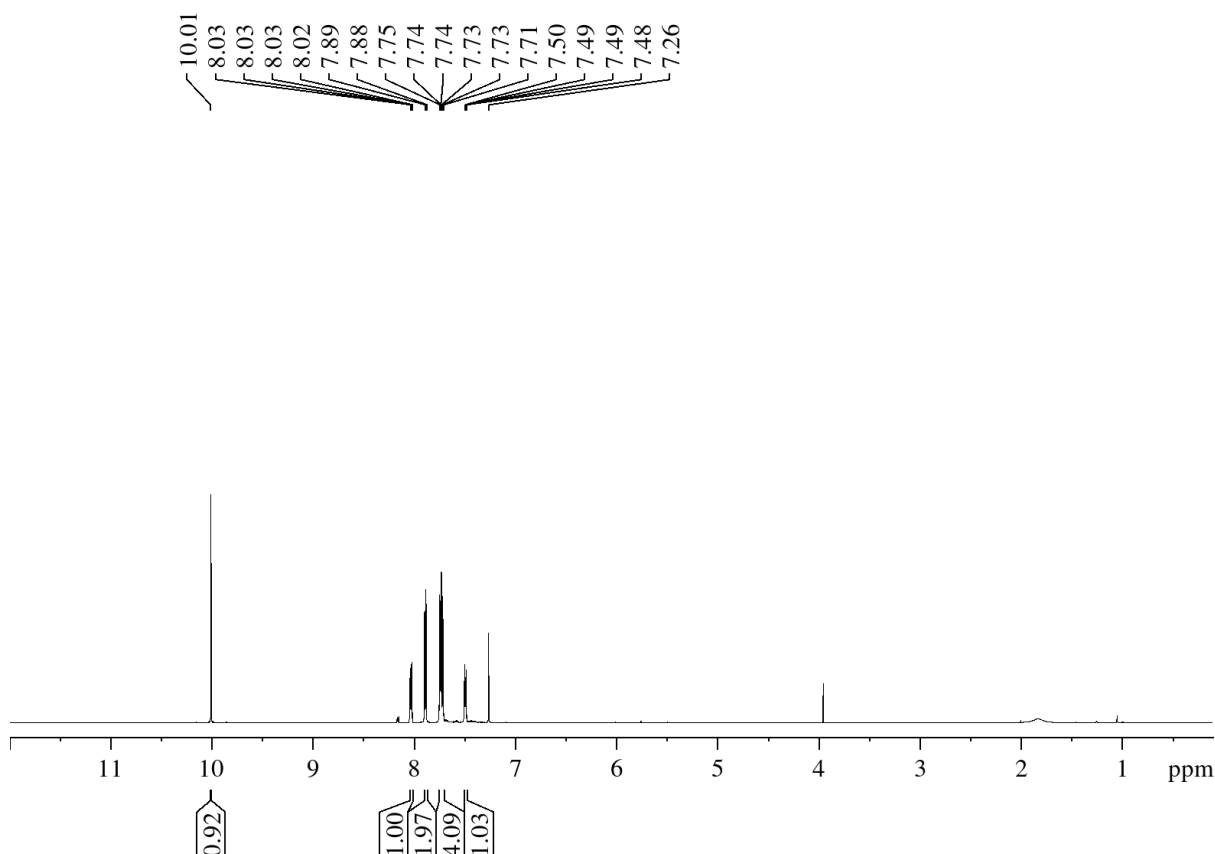


Figure 38.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2k**.

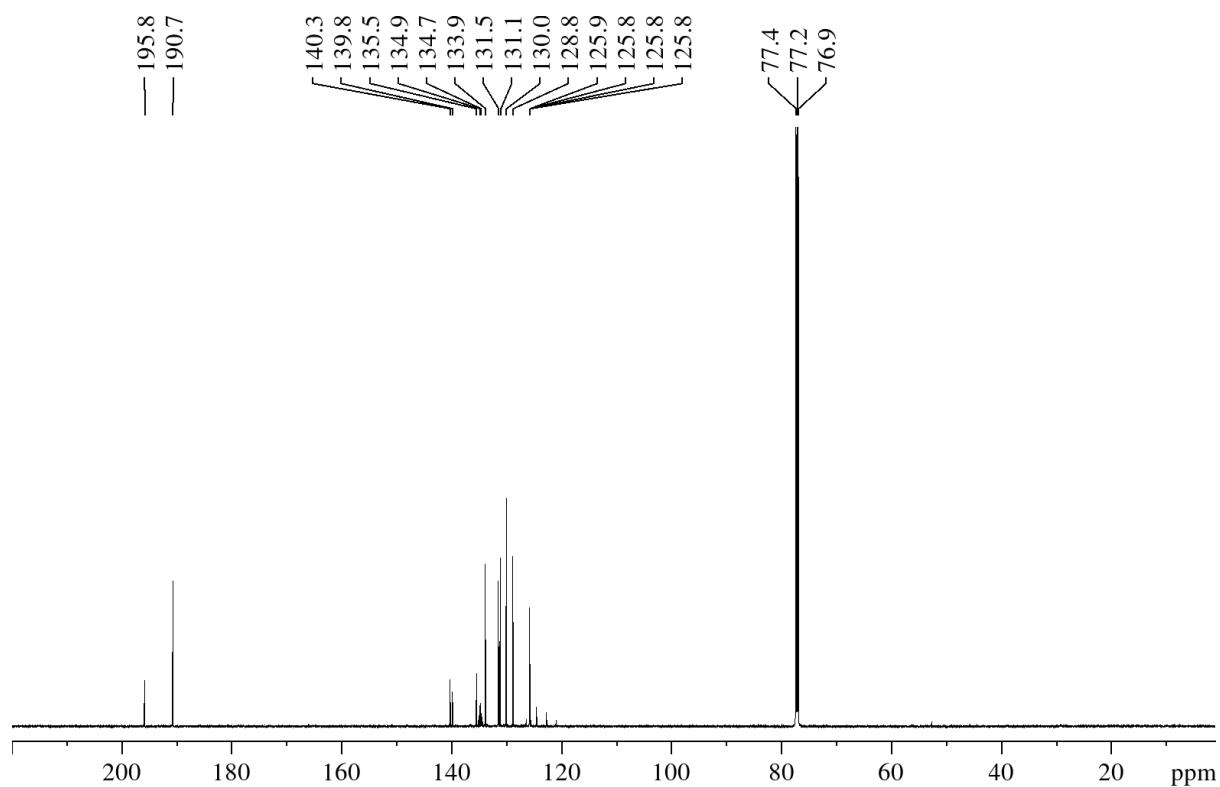


Figure 38.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2k**.

Arylketone-11 #1211 RT: 5.40 AV: 1 NL: 4.47E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]

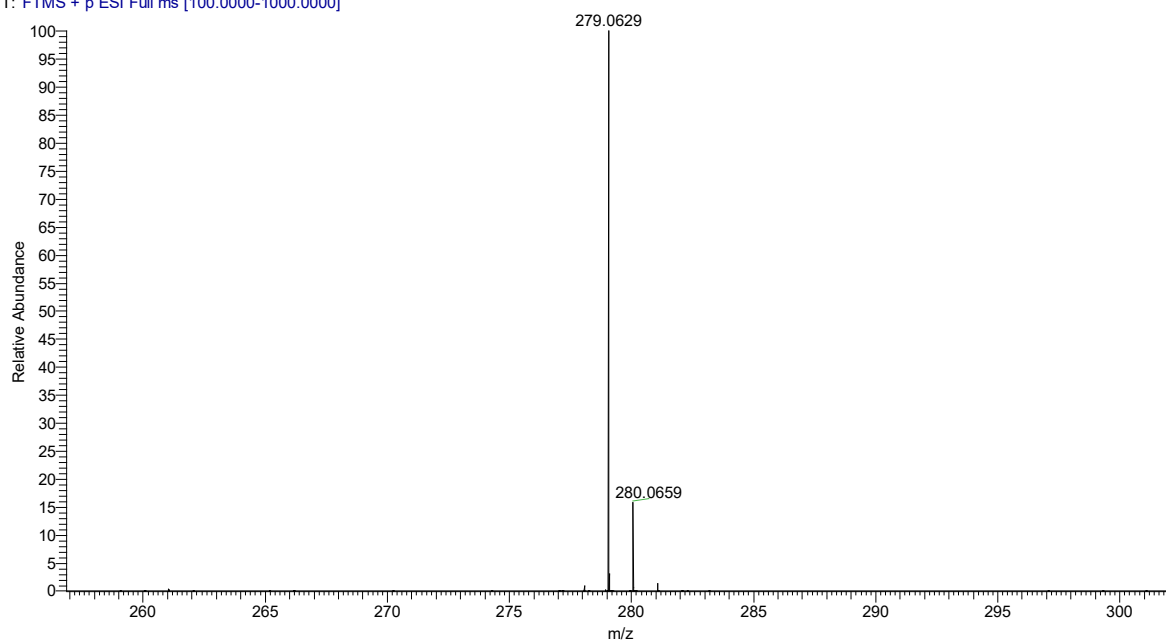


Figure 38.3 HR-ESI-MS of **2k**.

Synthesis of **21**. To a 250 mL RB was added 4-(tert-butoxycarbonyl)benzoic acid (14 mmol, 3.11 g), CDMT (14 mmol, 2.45 g), toluene (40 mL), and NMM (14 mmol, 1.4 mL). RM was sonicated, stirred at r.t. for 1 hr, followed by addition of K₃PO₄ (28 mmol, 5.94 g), (2-formylphenyl)boronic acid (14 mmol, 2.09 g) and Pd(P(Ph₃))₂Cl₂ (0.14 mmol, 98 mg). Reaction mixture was stirred at 110°C for 6 hr, cooled down and concentrated under a reduced pressure at 70 °C. Residue was added 95% TFA (20 mL) and stirred at r.t. for 1 hr. Reaction mixture was filtered, concentrated, followed by addition of EA (100 mL) and washed by HCl (1M, 100 mL). Organic layer was dried using anhydrous Na₂SO₄, filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASHTM, 20-45 μm, 100 Å), 5-100% MeCN in H₂O (0.1%TFA), 30 mL/min, 30 min. Yield: 261 mg pale yellow solid, 7.3%. ¹H NMR (600 MHz, *d*₆-DMSO, 298 K) (ppm): 9.96 (s, 1H), 8.11 (m, 1H), 8.04-8.05 (m, 2H), 7.83-7.85 (m, 2H), 7.76-7.77 (m, 2H), 7.56-7.57 (m, 1H). ¹³C{¹H} NMR (151 MHz, *d*₆-DMSO, 298 K) (ppm): 196.0, 192.4, 166.6, 139.6, 139.5, 134.8, 134.7, 134.1, 132.4, 130.8, 129.6, 129.2, 128.3. HR-ESI-MS (+ve): calcd. for C₁₅H₁₀O₄, [M+H]⁺ *m/z* = 255.0657, found 255.0655.

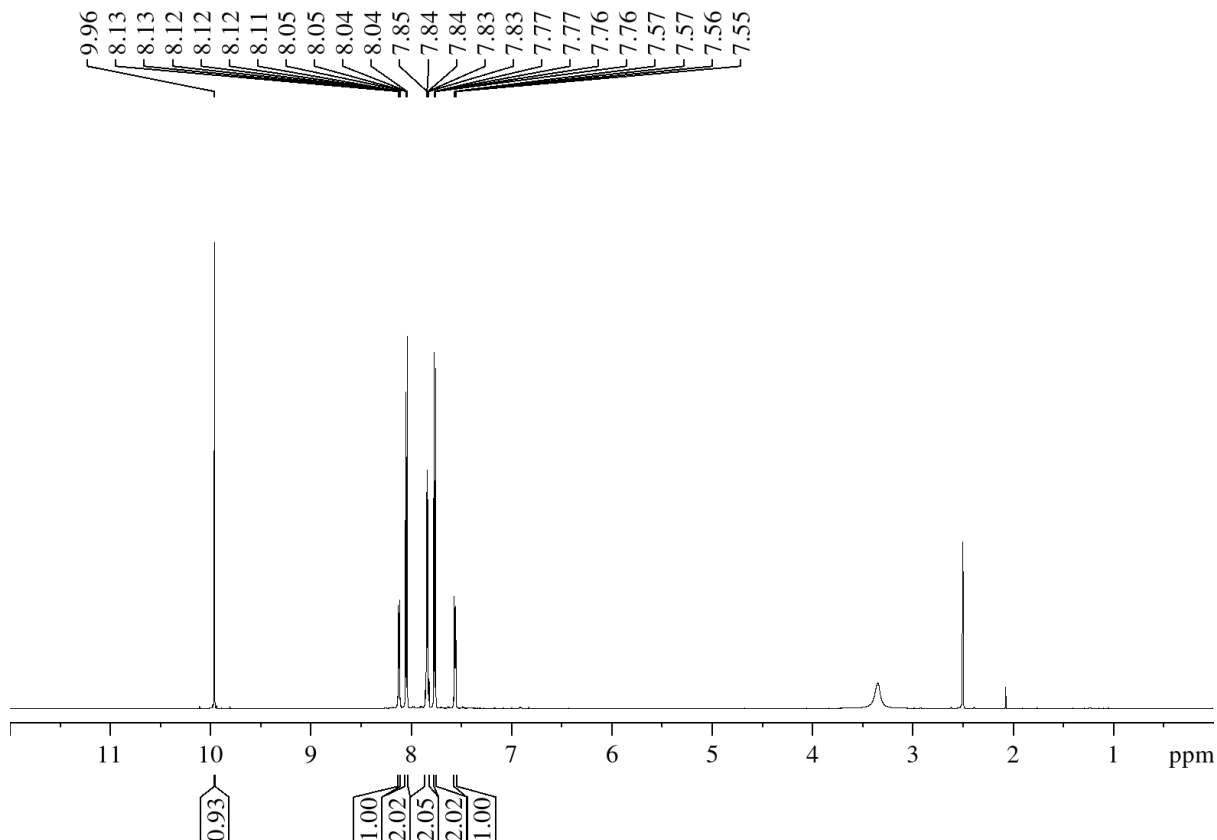


Figure 39.1 ¹H NMR (600 MHz, *d*₆-DMSO, 298 K) of **21**.

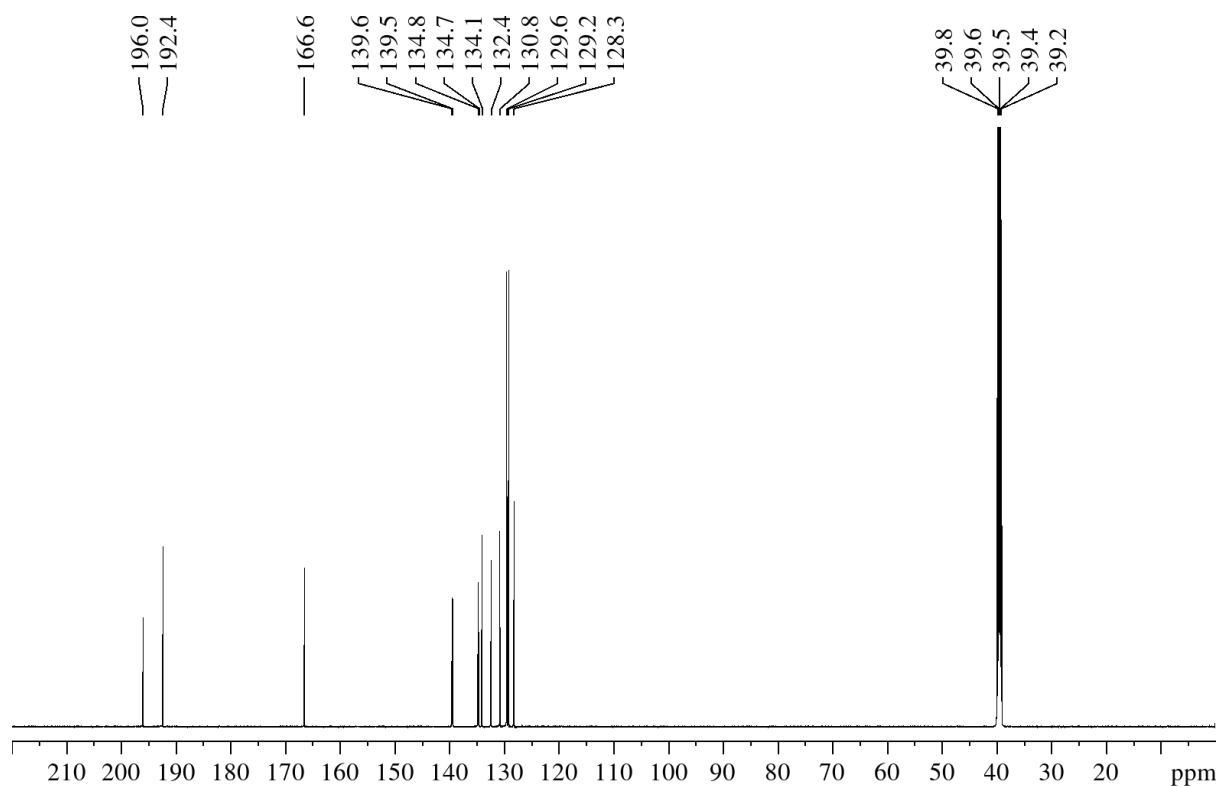


Figure 39.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, d_6 -DMSO, 298 K) of **2l**.

Arylketone-2 #917 RT: 4.09 AV: 1 NL: 3.29E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]

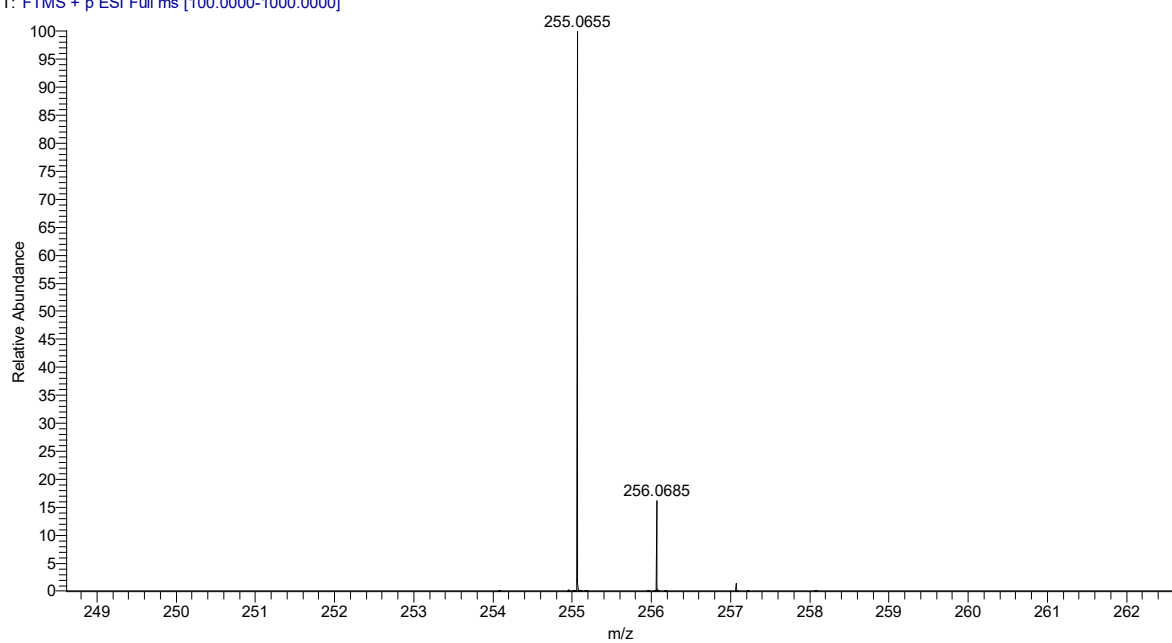
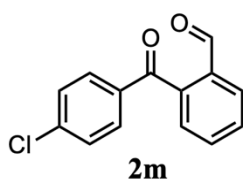


Figure 39.3 HR-ESI-MS of **2l**.



Synthesis of **2m**. To a 250 mL RB was added 4-chlorobenzoic acid (7 mmol, 1.1 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110°C for 3 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL) washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Product was isolated using a silica column (Huang Hai, 200-300 mesh) using 2-7.5% EA in PE. Yield: 66.8 mg pale- yellow solid, 3.9%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.01 (s, 1H), 8.01-8.04 (m, 1H), 7.70-7.74 (m, 4H), 7.47-7.49 (m, 1H), 7.42-7.45 (m, 2H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 195.5, 190.7, 140.9, 140.3, 135.5, 135.4, 133.7, 131.3, 130.9₁, 130.8₈, 129.2, 128.8. HR-ESI-MS (+ve): calcd. for $C_{14}H_9ClO_2$, $[M+H]^+$ m/z = 245.0369, found 245.0367.

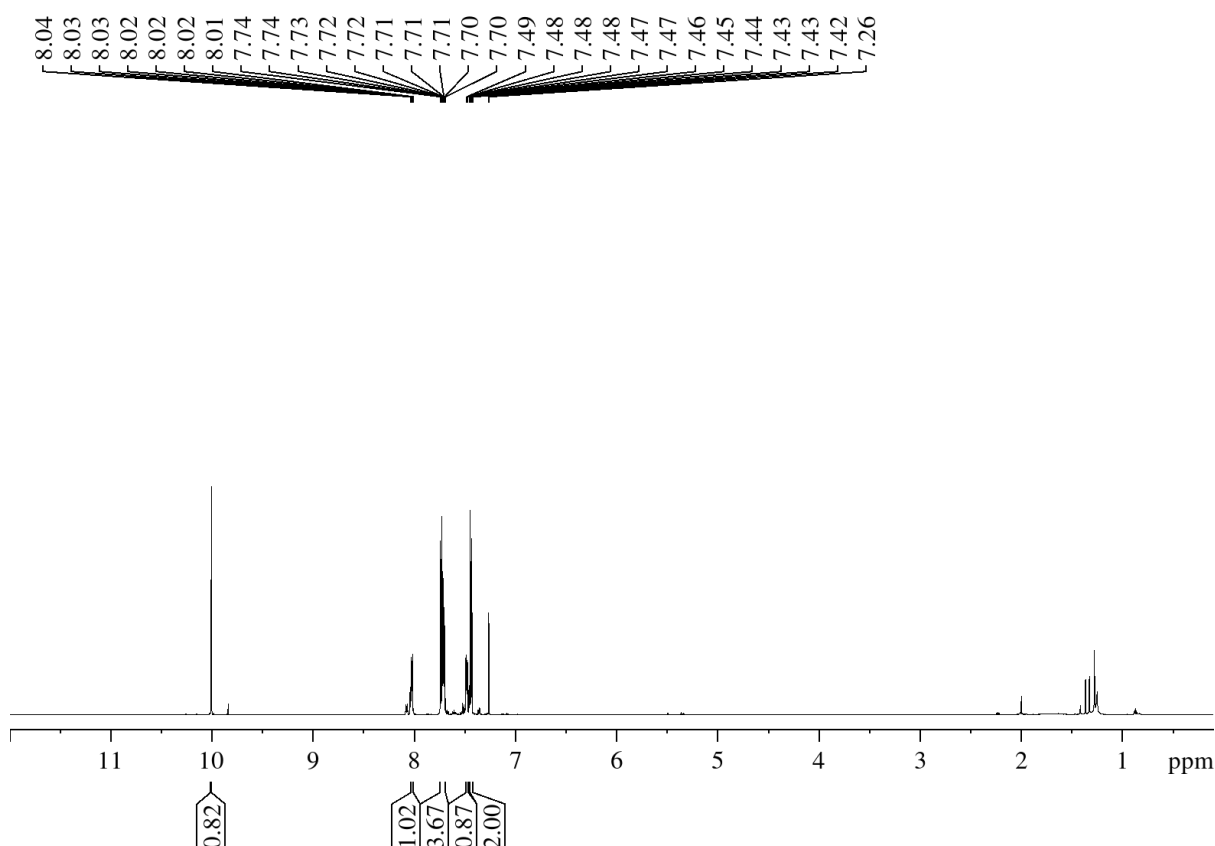


Figure 40.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2m**.

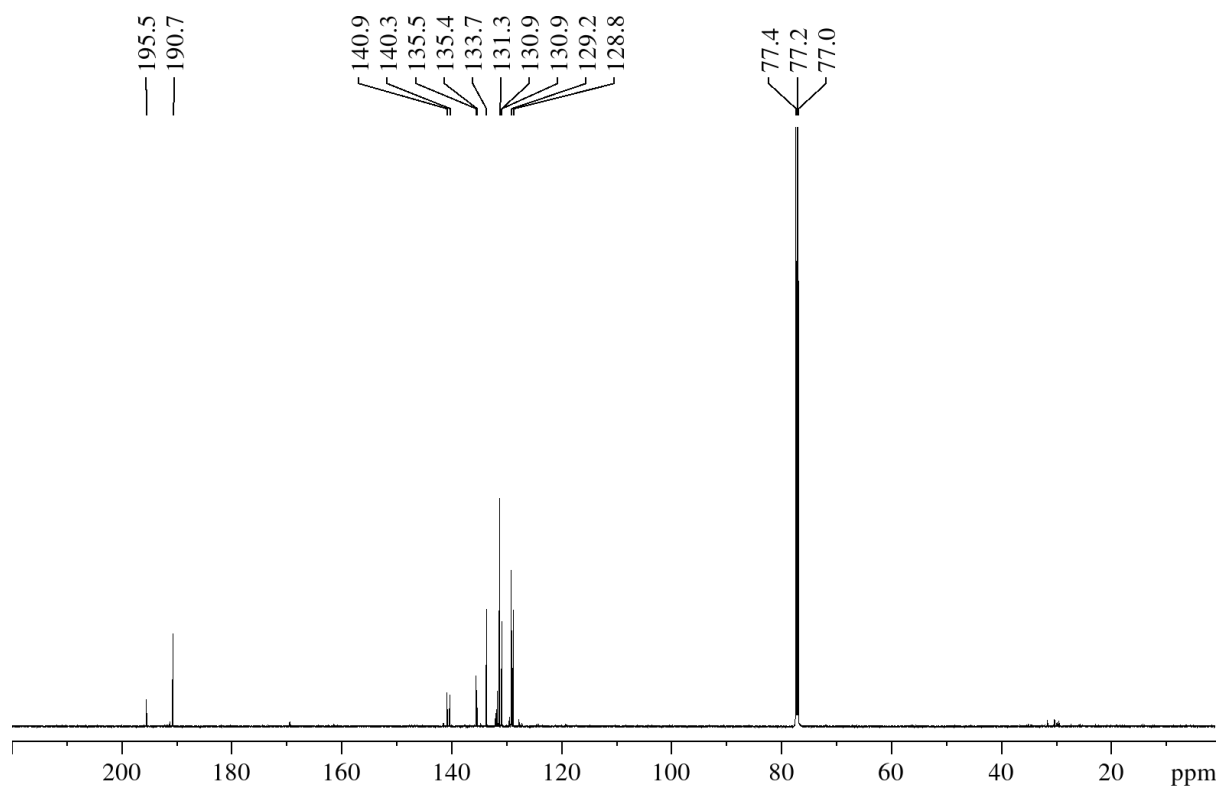


Figure 40.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2m**.

Arylketone-10 #1178 RT: 5.25 AV: 1 NL: 5.61E8
 T: FTMS + p ESI Full ms [100.0000-1000.0000]

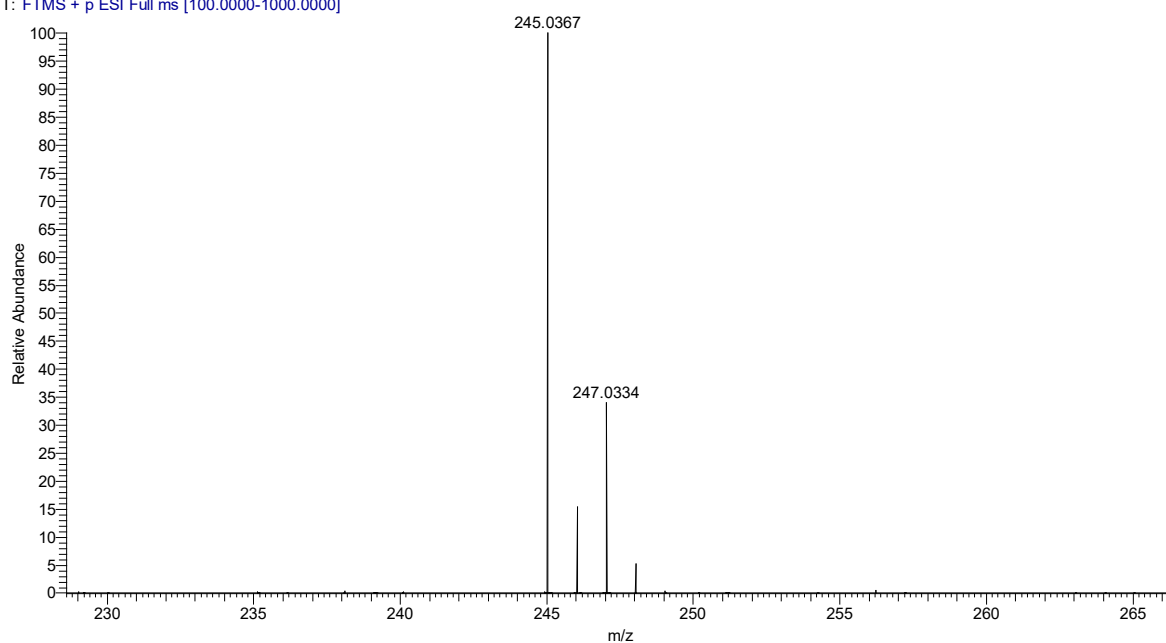
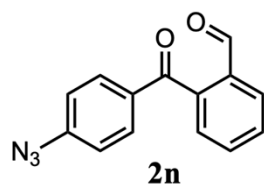


Figure 40.3 HR-ESI-MS of **2m**.



Synthesis of **2n**. To a 250 mL RB was added 4-azidobenzoic acid (7 mmol, 1.14 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110°C for 8 hr, cooled down and concentrated under a reduced pressure at 70 °C. Residue was redissolved into EA (100 mL) washed by HCl (1M, 50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASH™, 20-45 μm , 100 Å), 20-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 37 mg pale brown solid 2.1%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.00 (s, 1H), 8.01-8.03 (m, 1H), 7.78-7.80 (m, 2H), 7.68-7.70 (m, 2H), 7.47-7.49 (m, 1H), 7.07-7.09 (m, 2H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 195.1, 190.7, 145.7, 141.2, 135.3, 133.8, 133.6, 132.0, 130.7, 130.6, 128.7, 119.2. HR-ESI-MS (+ve): calcd. for $C_{14}H_9N_3O_2$, $[M+H]^+$ m/z = 252.0773, found 252.0771.

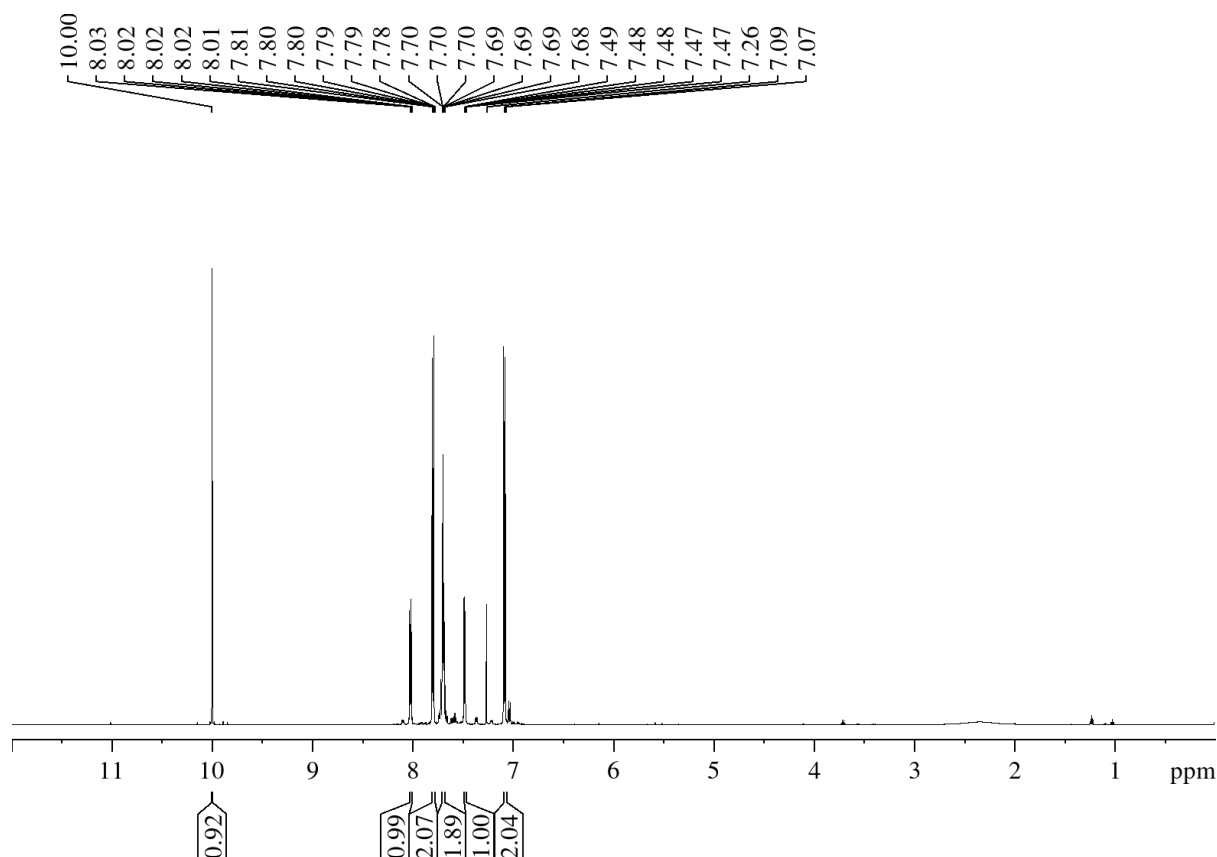


Figure 41.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2n**.

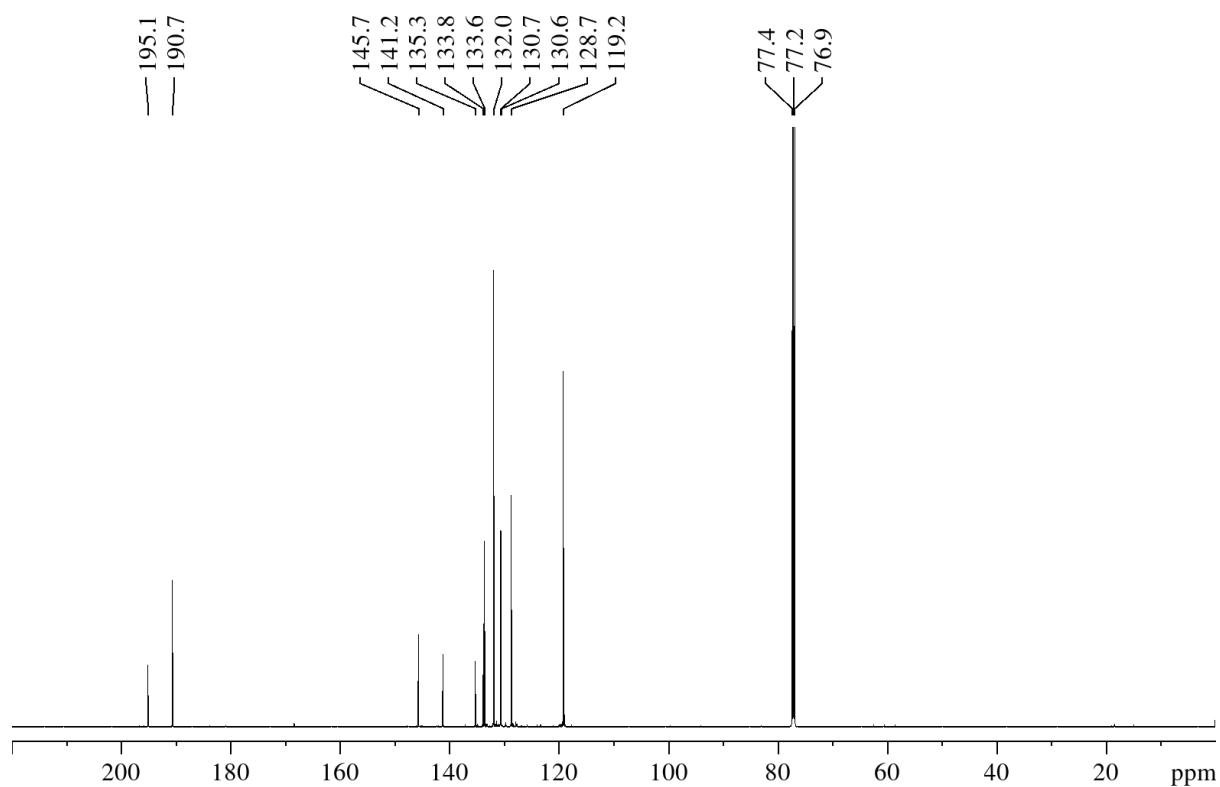


Figure 41.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2n**.

Arylketone-3 #1151 RT: 5.13 AV: 1 NL: 3.89E7
T: FTMS + p ESI Full ms [100.0000-1000.0000]

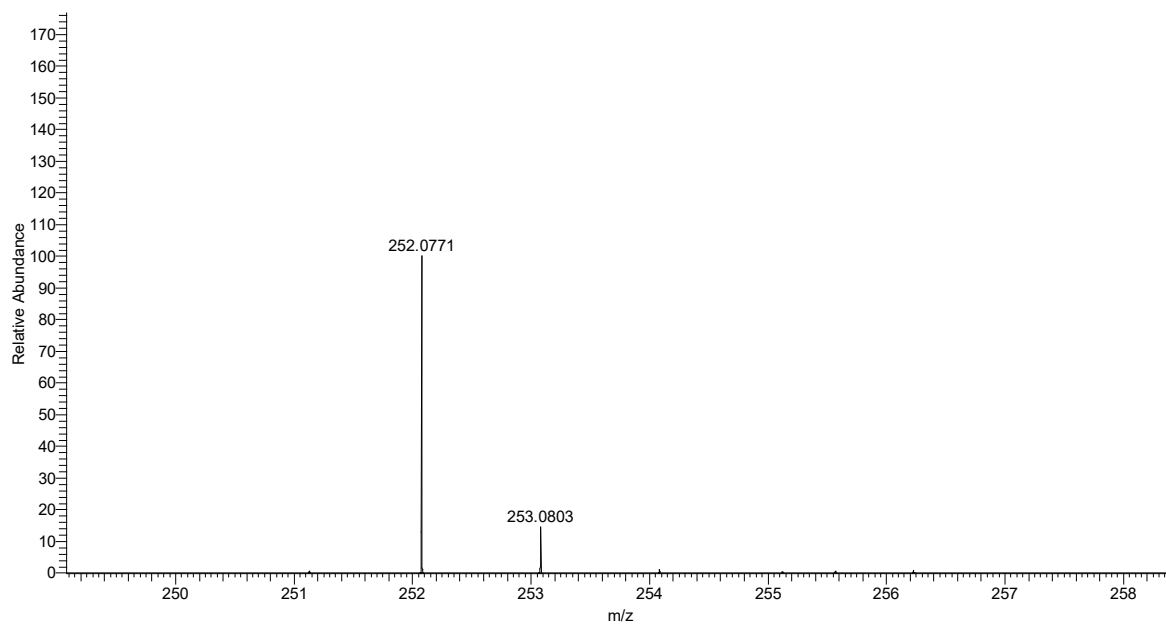
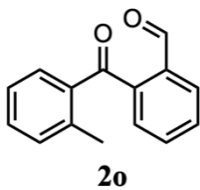


Figure 41.3 HR-ESI-MS of **2n**.



Synthesis of 2o. To a 250 mL RB was added 2-methylbenzoic acid (7 mmol, 0.95 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110°C for 3 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL) washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASH™, 20-45 μm , 100 Å), 20-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 122 mg yellow oil, 7.8%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.13 (s, 1H), 7.98-8.00 (dd, $J = 1.5, 7.4$ Hz, 1H), 7.64-7.66 (m, 2H), 7.48-7.49 (m, 1H), 7.41-7.44 (m, 1H), 7.32-7.34 (m, 1H), 7.28-7.29 (m, 1H), 7.17 (t, $J = 7.5$ Hz, 1H), 2.59 (s, 3H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 198.5, 191.2, 142.4, 139.7, 137.2, 136.2, 133.1, 132.2, 132.0, 131.3, 131.2, 129.9, 129.7, 125.5, 21.2. HR-ESI-MS (+ve): calcd. for $C_{15}H_{12}O_2$, $[M+H]^+$ m/z = 225.0916, found 225.0911.

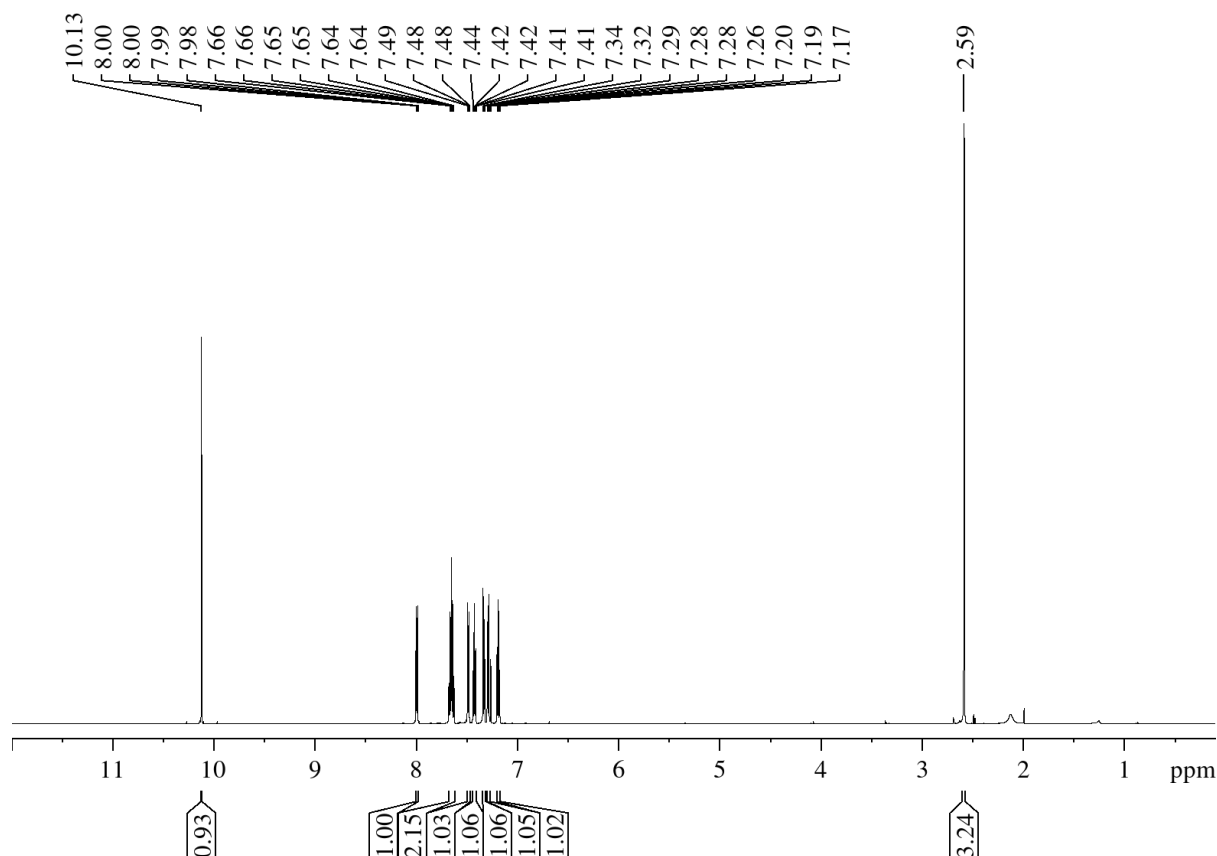


Figure 42.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2o**.

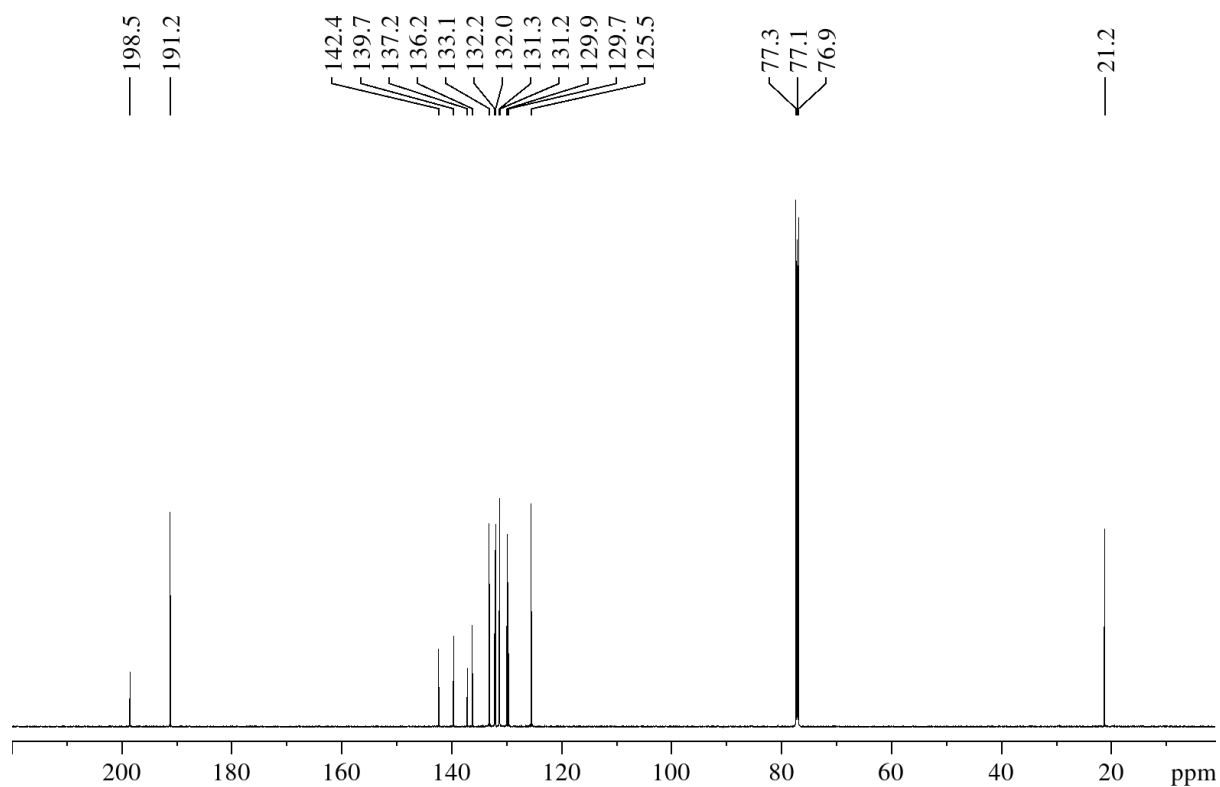


Figure 42.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2o**.

Arylketone-9 #1167 RT: 5.20 AV: 1 NL: 1.13E9
T: FTMS + p ESI Full ms [100.0000-1000.0000]

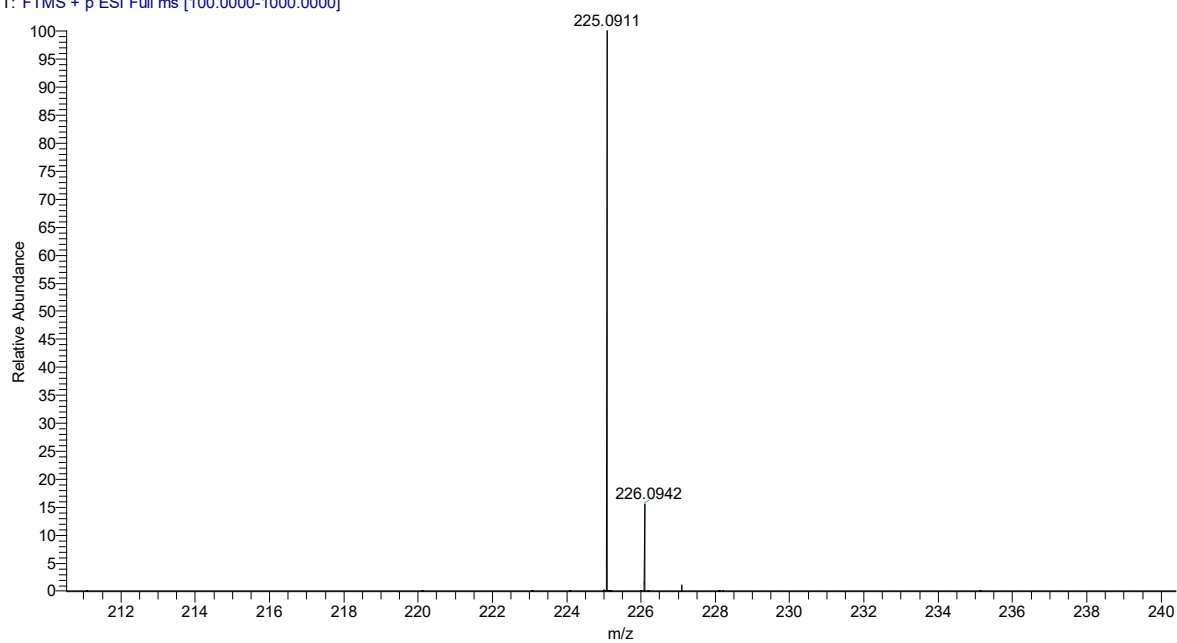
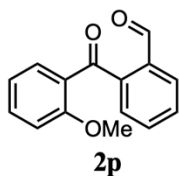


Figure 42.3 HR-ESI-MS of **2o**.



Synthesis of **2p**. To a 250 mL RB was added 2-methoxybenzoic acid (7 mmol, 1.06 g), CDMT (7 mmol, 1.22 g), toluene (20 mL), and NMM (7 mmol, 0.7 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (14 mmol, 2.97 g), (2-formylphenyl)boronic acid (7 mmol, 1.05 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.07 mmol, 49 mg). Reaction mixture was stirred at 110°C for 3 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL), washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Product was isolated using a silica column (Huang Hai, 200-300 mesh) using 5-15% EA in PE. Yield: 157 mg white solid, 9.3%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.21 (s, 1H), 7.98-7.99 (dd, $J = 7.4, 1.6$ Hz, 1H), 7.65 (dd, $J = 7.7, 1.8$ Hz, 1H), 7.56-7.61 (m, 2H), 7.52-7.55 (m, 1H), 7.44-7.45 (m, 1H), 7.72 (t, $J = 7.8$ Hz, 1H), 7.05 (t, $J = 7.5$ Hz, 1H), 6.95 (d, $J = 8.4$ Hz, 1H), 3.61 (s, 3H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 196.3, 191.6, 158.8, 143.6, 135.7, 134.4, 132.9, 131.4, 130.9, 129.1, 128.6, 127.9, 120.9, 112.1, 55.7. HR-ESI-MS (+ve): calcd. for $C_{15}H_{12}O_3$, $[M+H]^+$ $m/z = 241.0865$, found 241.0862.

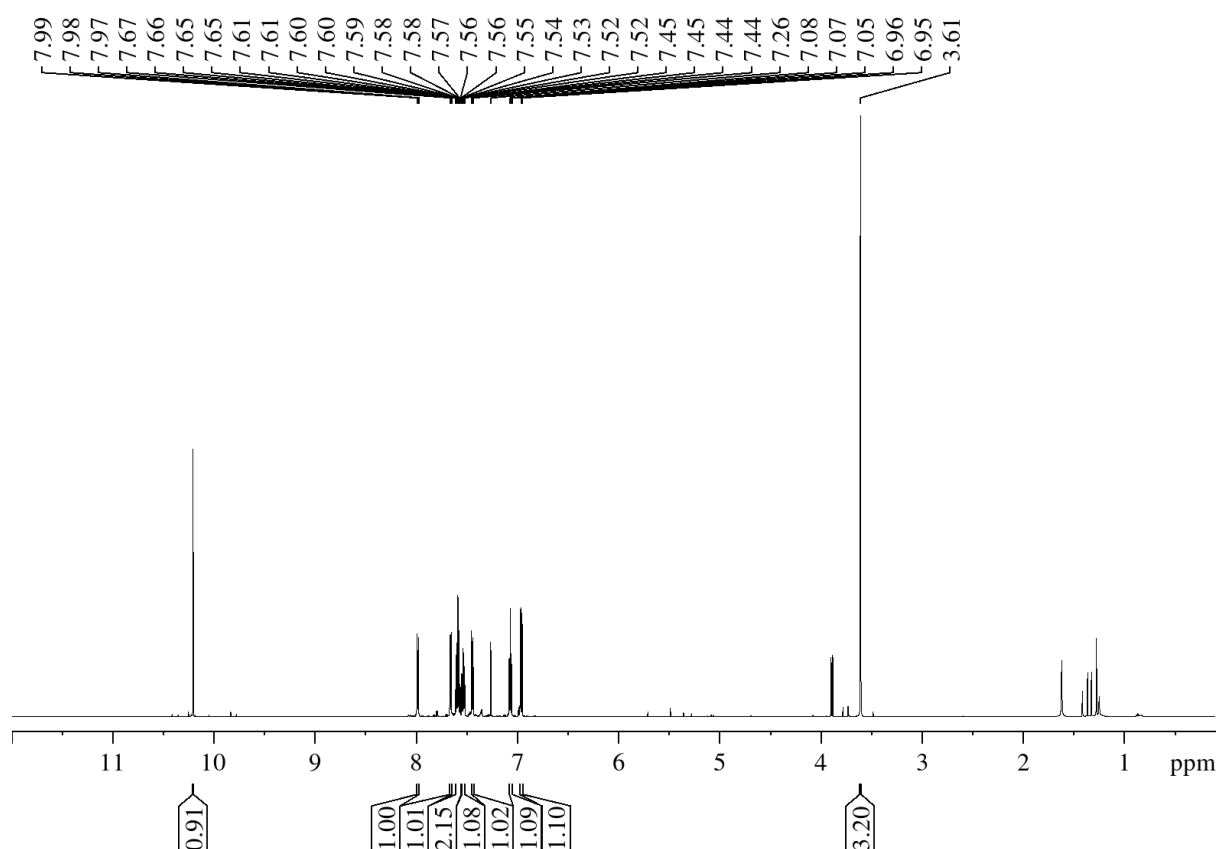


Figure 43.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2p**.

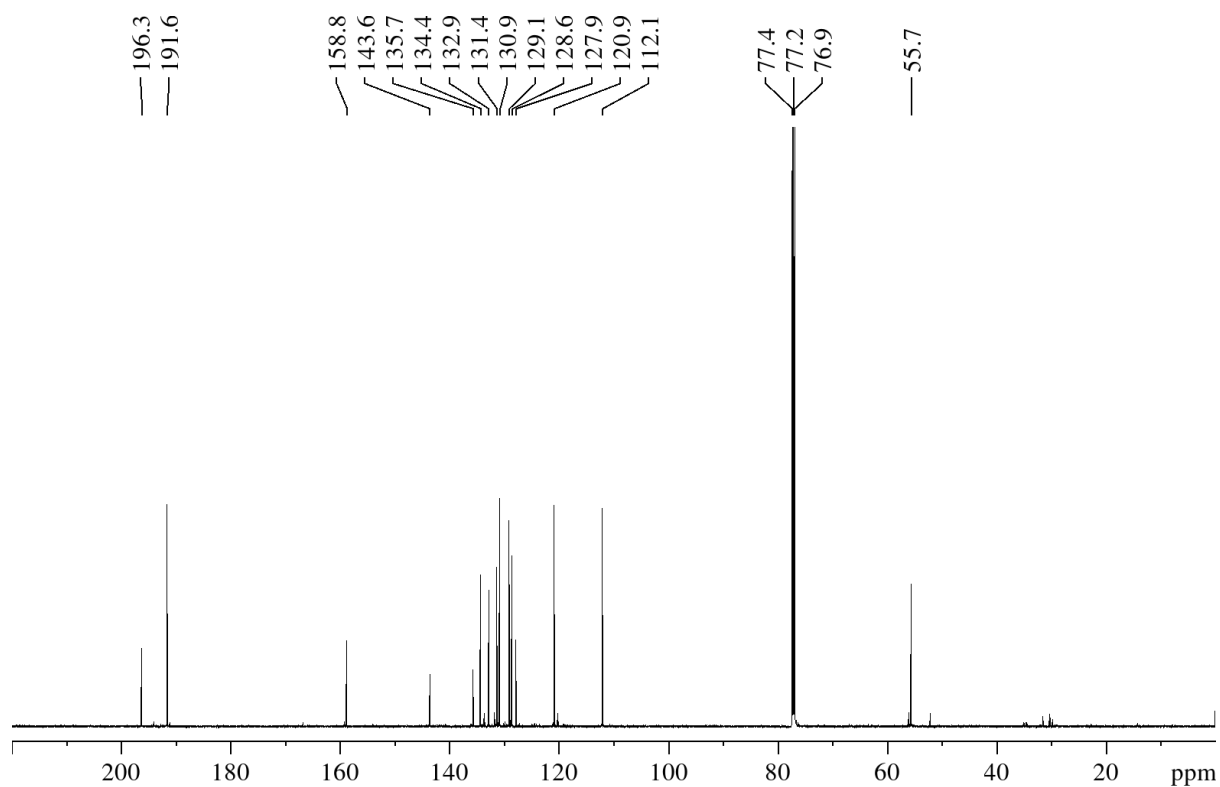


Figure 43.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2p**.

Arylketone-8-o #1093 RT: 4.87 AV: 1 NL: 9.68E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]

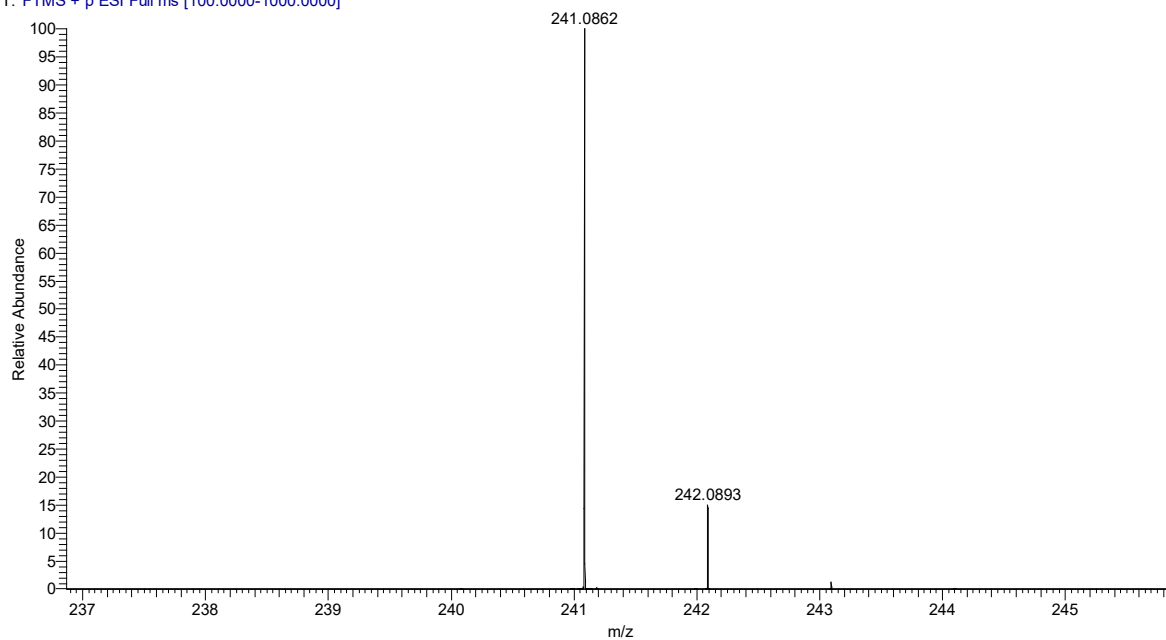
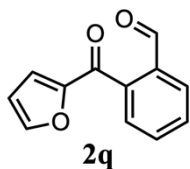


Figure 43.3 HR-ESI-MS of **2p**.



Synthesis of **2q**. To a 250 mL RB was added furan-2-carboxylic acid (14 mmol, 1.57 g), CDMT (14 mmol, 2.45 g), toluene (40 mL), and NMM (14 mmol, 1.4 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (28 mmol, 5.94 g), (2-formylphenyl)boronic acid (14 mmol, 2.09 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.14 mmol, 98 mg). Reaction mixture was stirred at 110°C for 4 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL), washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASHTM, 20-45 μm , 100 Å), 10-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 406 mg brown solid, 14%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.09 (s, 1H), 8.02-8.03 (m, 1H), 7.67-7.70 (m, 4H), 7.11 (d, $J = 3.6$ Hz, 1H), 7.11 (d, $J = 7.8$ Hz, 1H), 6.59 (dd, $J = 3.6, 1.7$ Hz, 1H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 190.6, 183.0, 152.6, 148.1, 140.2, 135.6, 133.4, 131.3, 129.7, 129.1, 121.3, 112.8. HR-ESI-MS (+ve): calcd. for $C_{12}H_8O_3$, $[M+H]^+$ m/z = 201.0552, found 201.0548.

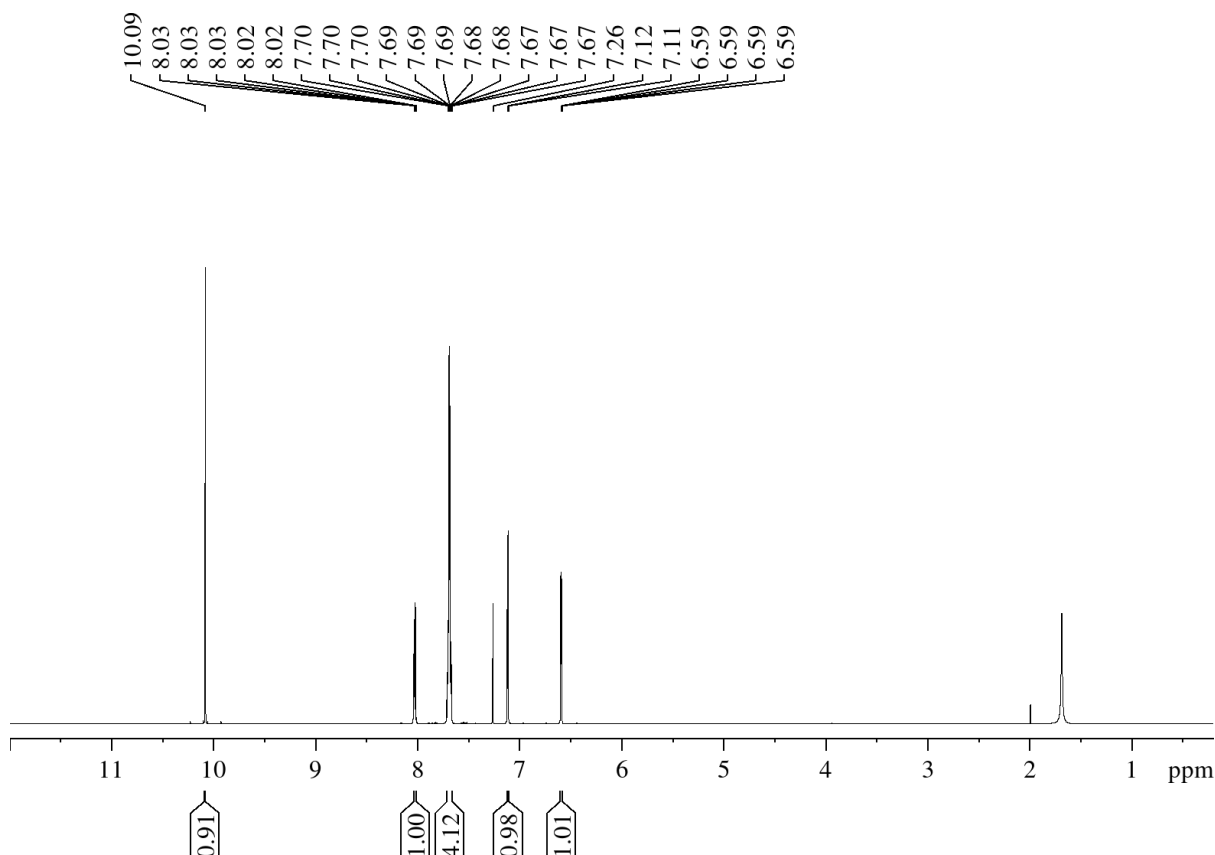


Figure 44.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2q**.

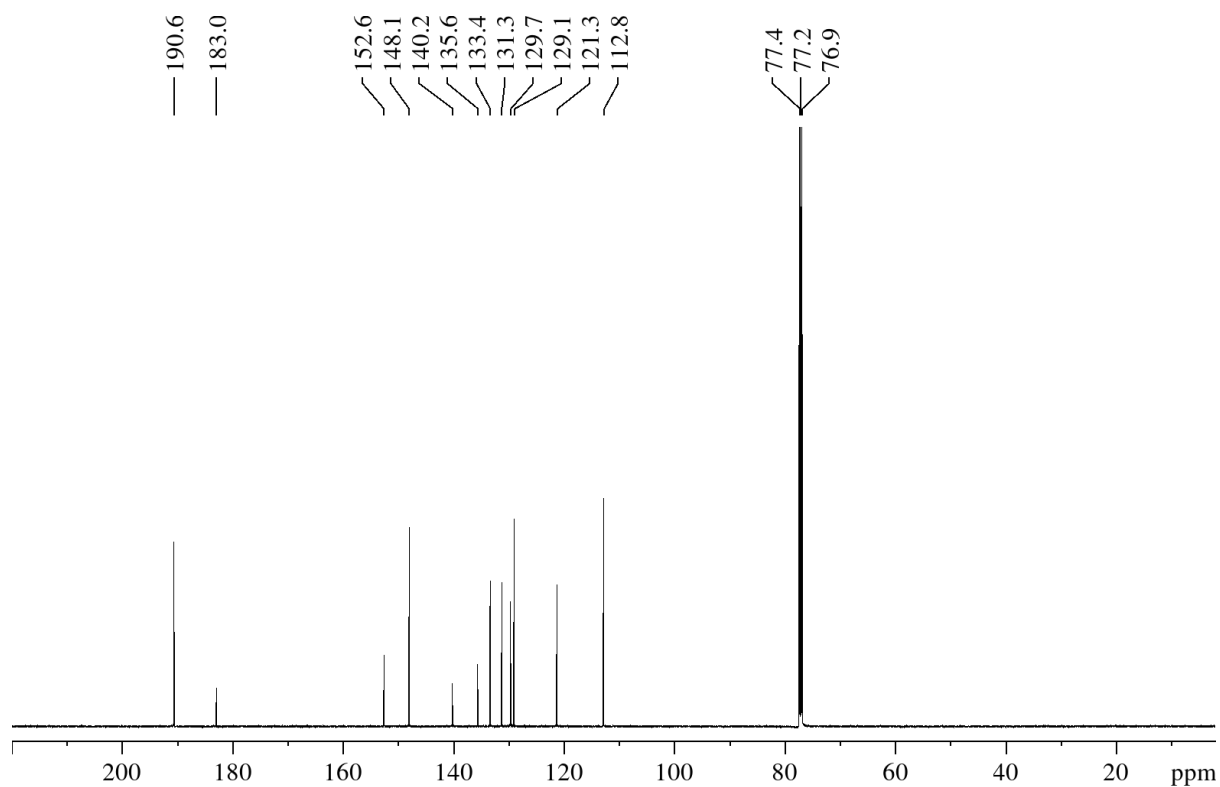


Figure 44.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2q**.

Arylketone-6 #893 RT: 3.98 AV: 1 NL: 9.72E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]

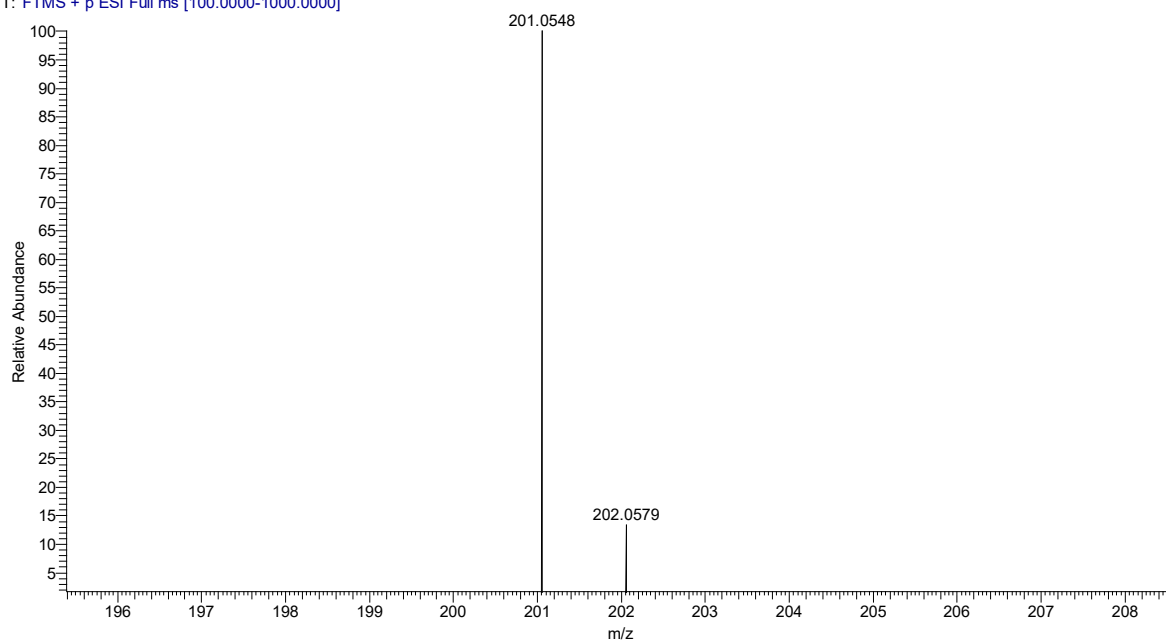
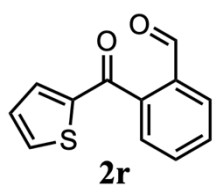


Figure 44.3 HR-ESI-MS of **2q**.



Synthesis of **2r**. To a 250 mL RB was added thiophene-2-carboxylic acid (14 mmol, 1.8 g), CDMT (14 mmol, 2.45 g), toluene (40 mL), and NMM (14 mmol, 1.4 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (28 mmol, 5.94 g), (2-formylphenyl)boronic acid (14 mmol, 2.09 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.14 mmol, 98 mg). Reaction mixture was stirred at 110°C for 7 hr, cooled down and concentrated under a reduced pressure at 70 °C. Residue was redissolved into EA (100 mL), washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried using anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolate using a C18 column (SEPAFLASH™, 20-45 μm , 100 Å), 10-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 26 mg white solid, 1%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.12 (s, 1H), 8.06-8.08 (m, 1H), 7.80 (dd, $J = 4.9, 0.8$ Hz, 1H), 7.69-7.74 (m, 2H), 7.66 (m, 1H), 7.44 (dd, $J = 3.8, 0.8$ Hz, 1H), 7.15-7.16 (dd, $J = 3.8, 0.8$ Hz, 1H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 190.8, 188.5, 144.7, 141.7, 136.20, 136.17, 135.5, 133.8, 131.3, 130.0, 129.1, 128.8. HR-ESI-MS (+ve): calcd. for $C_{12}H_8O_2S$, $[M+H]^+$ $m/z = 217.0323$, found 217.0321.

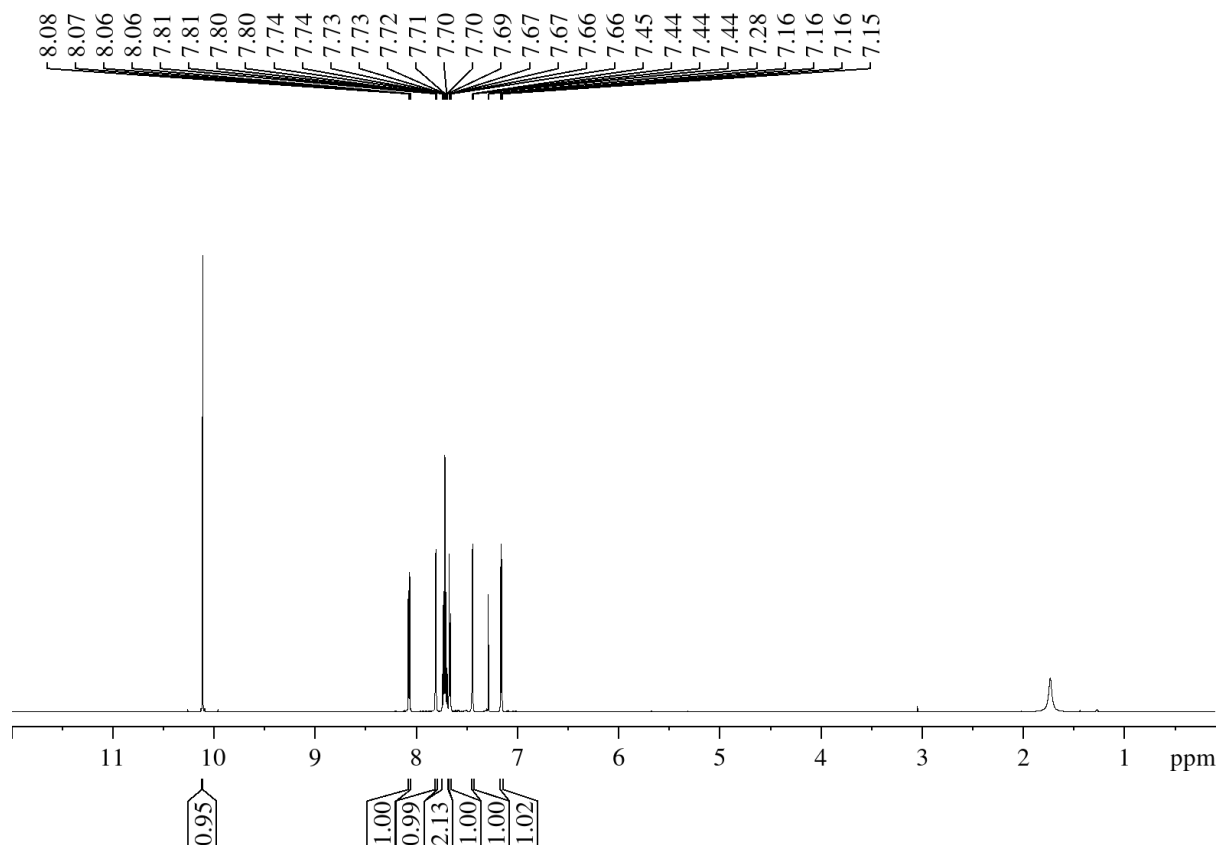


Figure 45.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2r**.

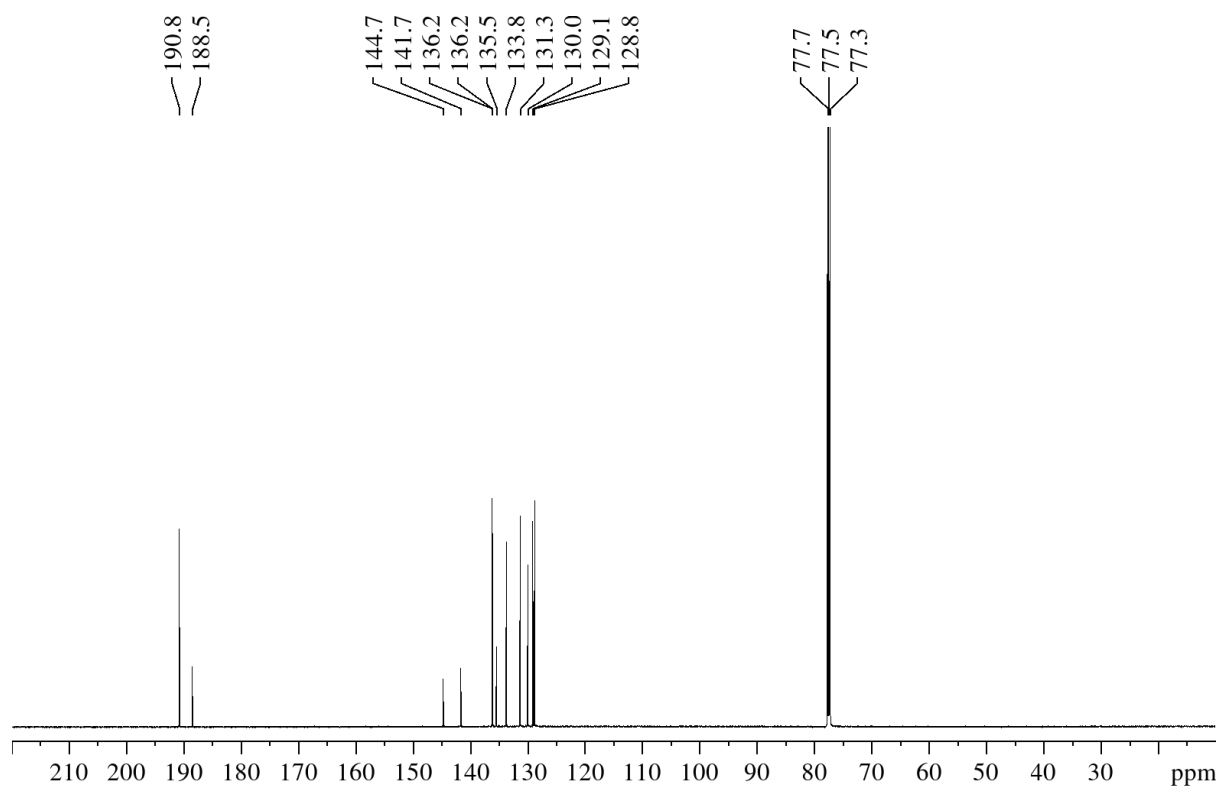


Figure 45.2 ¹³C{¹H} NMR (151 MHz, CDCl₃, 298 K) of **2r**.

Arylketone-4-o #1029 RT: 4.59 AV: 1 NL: 7.18E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]

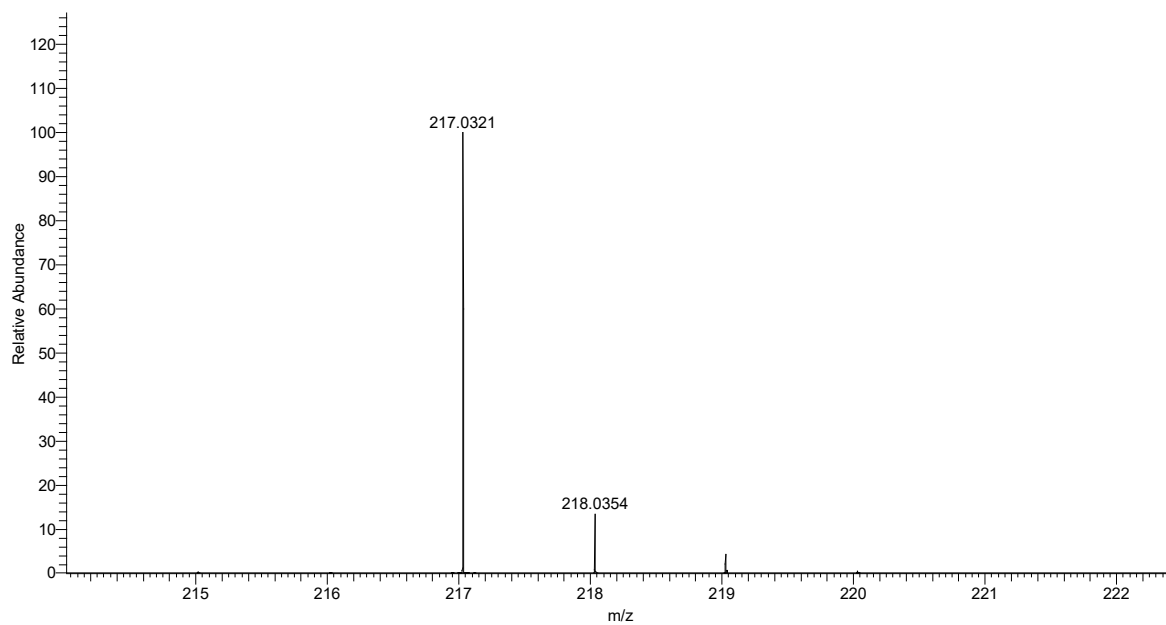
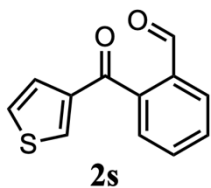


Figure 45.3 HR-ESI-MS of **2r**.



Synthesis of **2s**. To a 250 mL RB was added thiophene-3-carboxylic acid (14 mmol, 1.8 g), CDMT (14 mmol, 2.45 g), toluene (40 mL), and NMM (14 mmol, 1.4 mL). Reaction mixture was sonicated, stirred at r.t. for 1 hr, followed by addition of K_3PO_4 (28 mmol, 5.94 g), (2-formylphenyl)boronic acid (14 mmol, 2.09 g) and $Pd(P(Ph_3)_2)Cl_2$ (0.14 mmol, 98 mg). Reaction mixture was stirred at 110°C for 4 hr, cooled down and concentrated under a reduced pressure at 70°C. Residue was redissolved into EA (100 mL), washed by HCl (1M, 50 mL) and brine (50 mL). Organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Residue was redissolved into minimal amount of DMF, filtered and product was isolated using a C18 column (SEPAFLASHTM, 20-45 μm , 100 Å), 15-100% MeCN in H_2O (0.1%TFA), 30 mL/min, 30 min. Yield: 474 mg black solid, 16%. ^1H NMR (600 MHz, $CDCl_3$, 298 K) (ppm): 10.07 (s, 1H), 8.03-8.04 (m, 1H), 7.80 (d, $J = 2.5$ Hz, 1H), 7.66-7.71 (m, 2H), 7.59-7.61 (m, 2H), 7.39 (dd, $J = 2.9, 5.1$ Hz, 1H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, 298 K) (ppm): 190.7, 189.8, 142.4, 142.1, 135.7, 135.2, 133.4, 130.9, 129.7, 128.7, 127.9, 127.1. HR-ESI-MS (+ve): calcd. for $C_{12}H_8O_2S$, $[M+H]^+$ $m/z = 217.0323$, found 217.0321.

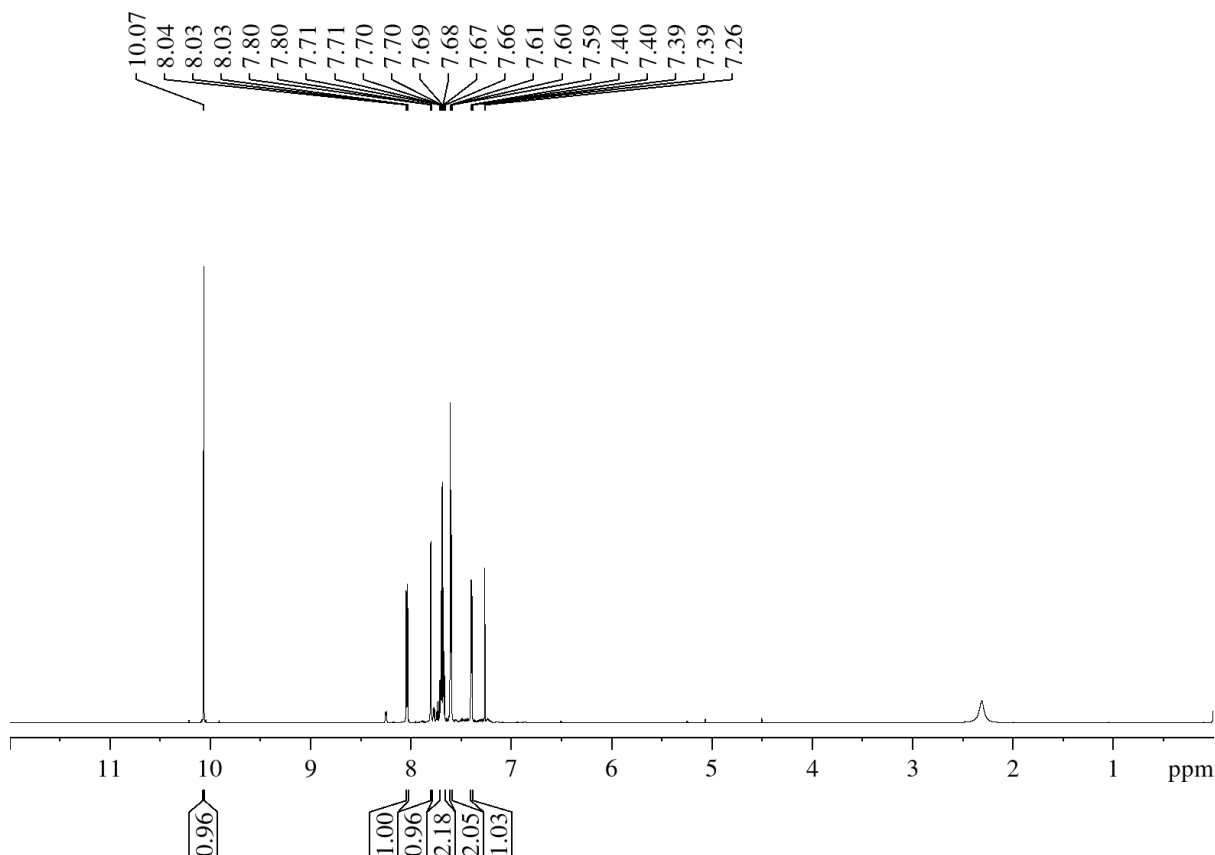


Figure 46.1 ^1H NMR (600 MHz, $CDCl_3$, 298 K) of **2s**.

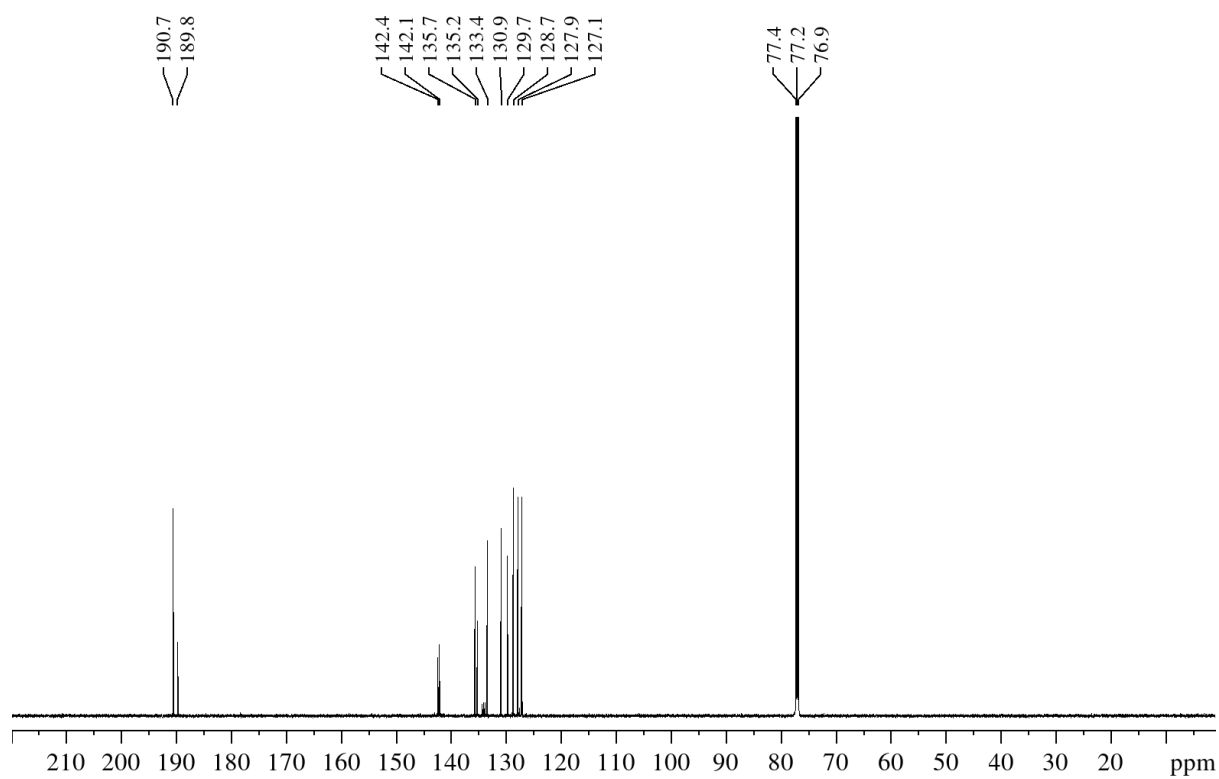


Figure 46.2 $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3 , 298 K) of **2s**.

Arylketone-5-p #1029 RT: 4.59 AV: 1 NL: 1.14E9
T: FTMS + p ESI Full ms [100.0000-1000.0000]

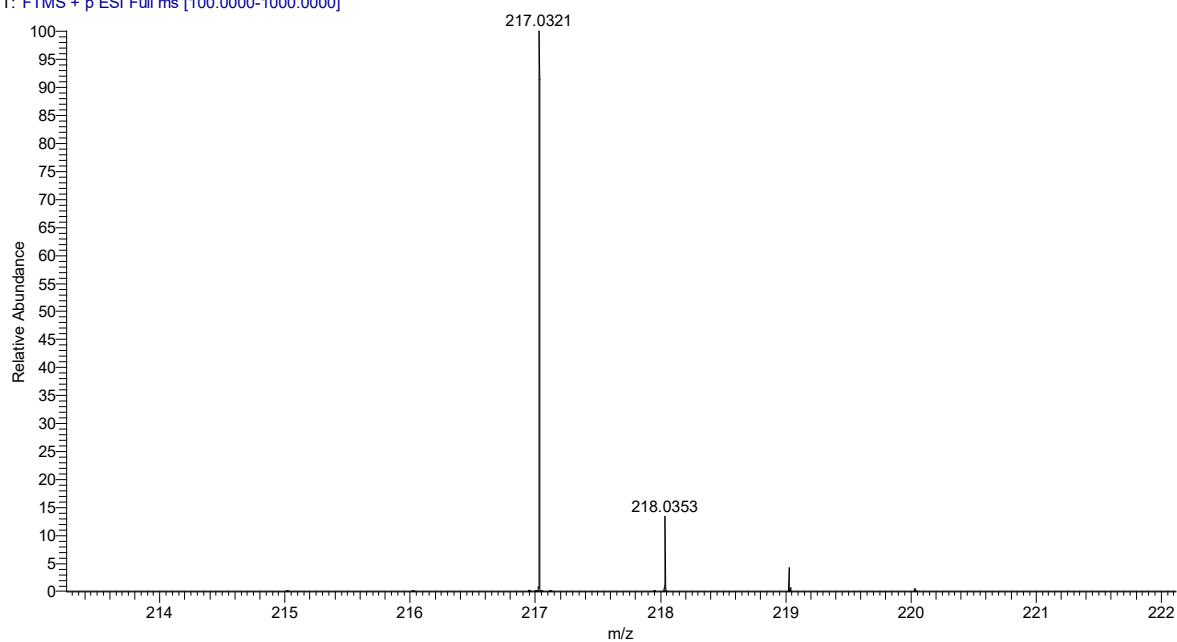


Figure 46.3 HR-ESI-MS of **2s**.

3 Synthesis of 4aa-4ah.

For all experiments, **1a** (1.0 equivalents) and **3a** (1.0 equivalents) were dissolved in PBS buffer (pH 4.0) to a final concentration of 1 mM. **2a-2h** (1.0 equivalent) was subsequently added, and the mixture was allowed to react with **1a** and **3a** at ambient temperature. The reaction progress was monitored via LC-MS.

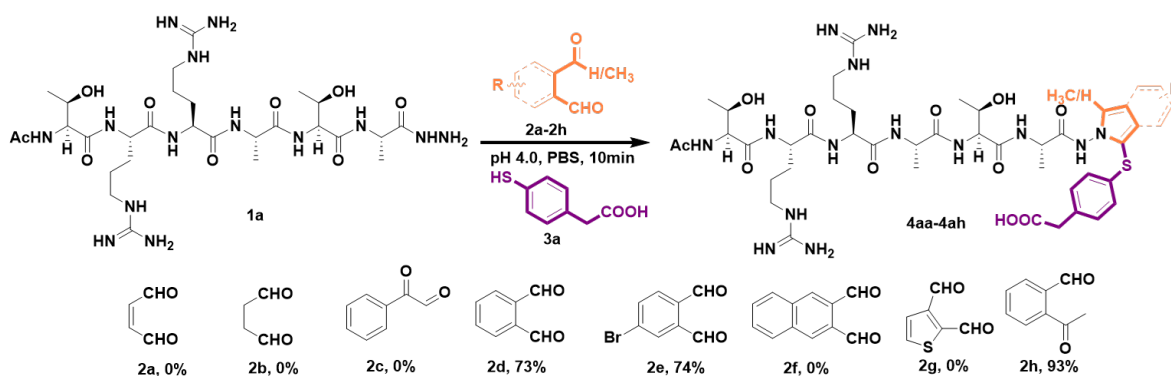


Figure 47.1 Reaction scheme of **1a** with **3a** and **2a-2h** to give **4aa-4ah**.

Synthesis of **4aa**.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2a** (0.22 mg, 1.4 μmol , 1.0 equiv, 2.2 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2a** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4aa** was not detected in the experiment.

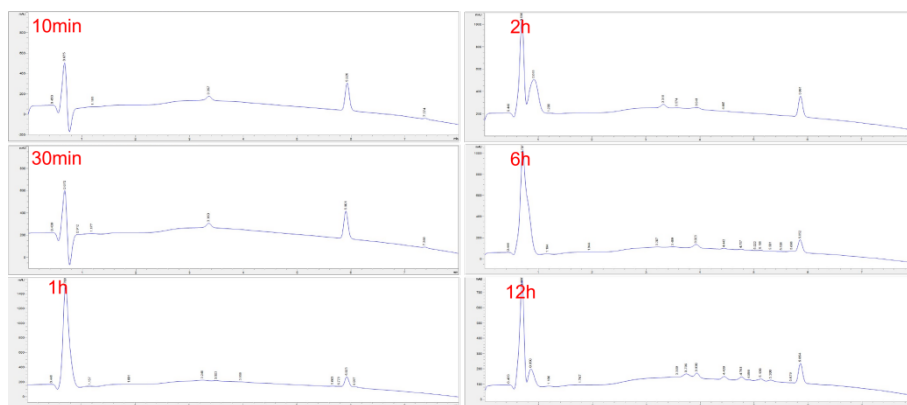


Figure 47.2 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2a**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of **4ab**.

1a (1.0 mg, 1.4 μ mol) and **3a** (0.16 mg, 1.4 μ mol, 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μ L of PBS buffer with pH 4.0, after which 10.0 μ L of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2b** (0.21 mg, 1.4 μ mol, 1.0 equiv, 2.1 mg of the sample was dissolved in 100 μ L of PBS buffer with pH 4.0, after which 10.0 μ L of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2a** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4ab** was not detected in the experiment.

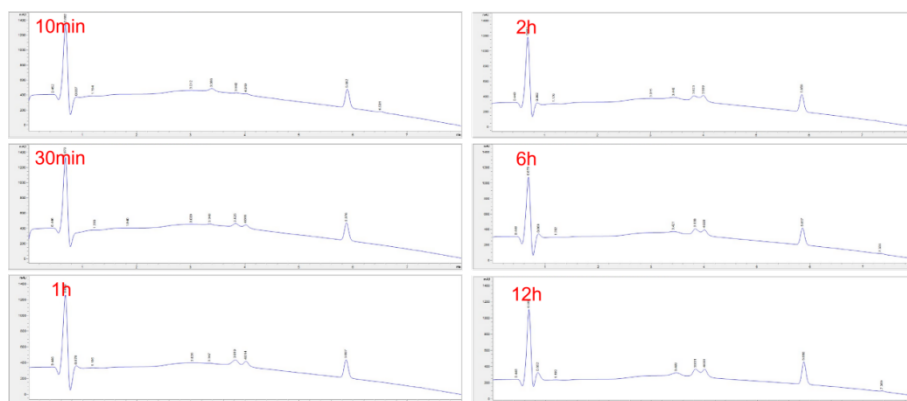


Figure 47.3 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2b**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of 4ac.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2c** (0.18 mg, 1.4 μmol , 1.0 equiv, 1.8 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2c** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4ac** was not detected in the experiment.

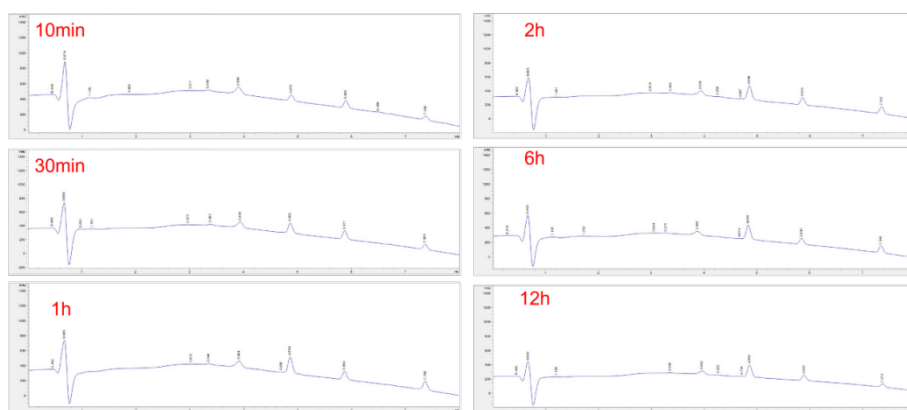


Figure 47.4 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2c**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of 4ad.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2d** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2d** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4ad** was observed in the reaction, and the corresponding conversion yields at reaction time points of 10 min, 30 min, 1 h, 2 h, 6 h and 12 h were 73%, 57%, 21%, 9%, 0% and 0%, respectively.

Figure 47.5 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2d**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of **4ae**.

1a (1.0 mg, 1.4 μ mol) and **3a** (0.16 mg, 1.4 μ mol, 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μ L of PBS buffer with pH 4.0, after which 10.0 μ L of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2e** (0.26 mg, 1.4 μ mol, 1.0 equiv, 2.6 mg of the sample was dissolved in 100 μ L of PBS buffer with pH 4.0, after which 10.0 μ L of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2e** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4ae** was observed, and the conversion yields after reaction durations of 10 min, 30 min, 1 h, 2 h, 6 h and 12 h were 74%, 70%, 54%, 20%, 0% and 0% respectively.

Figure 47.6 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2e**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of **4af**.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2f** (0.22 mg, 1.4 μmol , 1.0 equiv, 2.2 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2f** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4af** was not detected in the experiment.

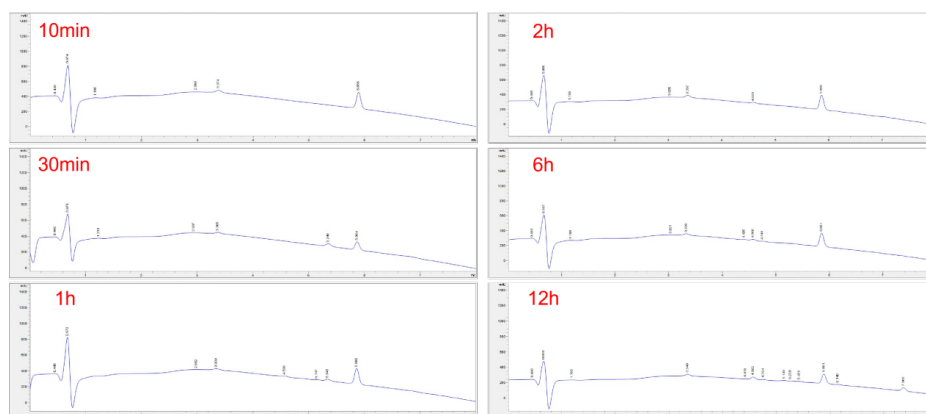


Figure 47.7 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2f**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of **4ag**.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2g** (0.17 mg, 1.4 μmol , 1.0 equiv, 1.7 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2g** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of product **4ag** was not detected in the experiment.

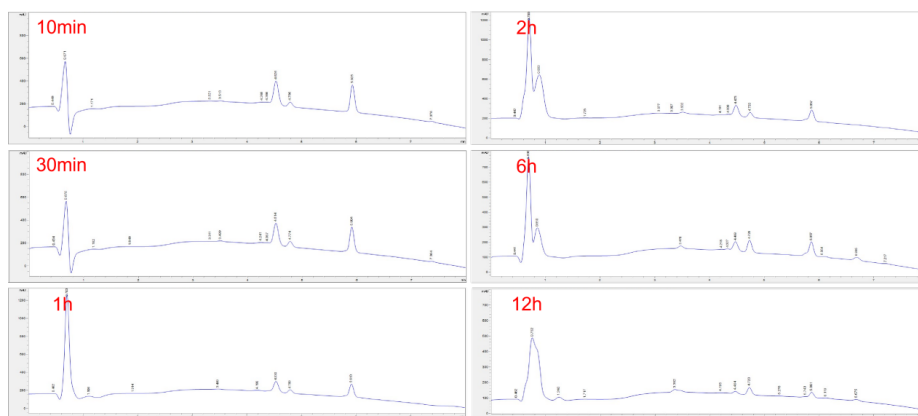


Figure 47.8 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2g**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Synthesis of **4ah**.

1a (1.0 mg, 1.4 μmol) and **3a** (0.16 mg, 1.4 μmol , 1.0 equiv, 1.6 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) were dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2** (0.18 mg, 1.4 μmol , 1.0 equiv, 1.8 mg of the sample was dissolved in 100 μL of PBS buffer with pH 4.0, after which 10.0 μL of the solution was pipetted and added.) was added and reacted at room temperature. Reaction progress was monitored by LC-MS after the addition of **2h** at 10 min, 30 min, 1 hr, 2 hr, 6 hr, 12 hr respectively. The formation of **4ah** was observed, and the conversion yields obtained at reaction time points of 10 min, 30 min, 1 hr, 2 hr, 6 hr and 12 hr were 93%, 90%, 86%, 84%, 77% and 59%, respectively.

Figure 47.9 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2h**. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

Large scale synthesis of 4ah

1a (5.0 mg, 6.8 μmol .) and **3a** (1.0 mg, 6.8 μmol , 1.0 equiv.) was dissolved in PBS buffer (pH 4.0) with a final concentration of 1 mM. **2h** (1.1 mg, 6.8 μmol , 1.0 equiv.) was added and reacted at room temperature for 10 min. Formation of **4ah** was observed and the conversion yield was 91%. Reaction mixture was then diluted by water, subjected for preparative HPLC purification (2-70% ACN/H₂O over 30 min) and lyophilization to give **4ah** (3.7 mg, 53.4 %).

Figure 47.10 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10 min, and after HPLC purification. Gradient: 2%-70% ACN/H₂O with 0.1% TFA over 8 min.

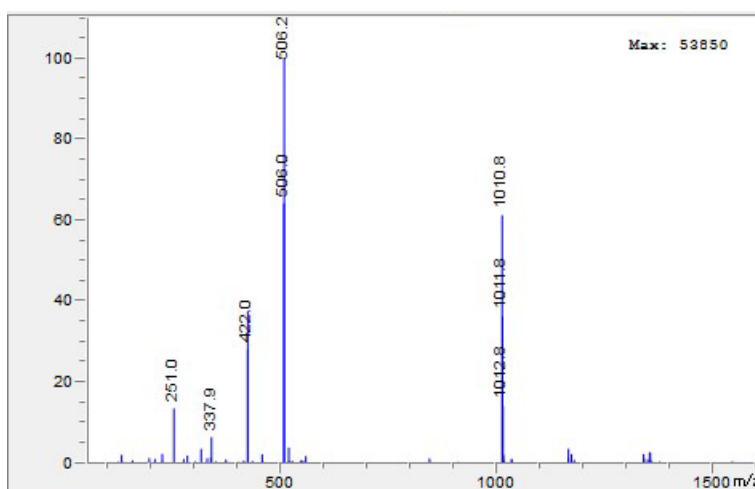


Figure 47.11 ESI-MS analysis of **4ah**. Calcd. for C₄₆H₆₇N₁₃O₁₁S [M+H]⁺ m/z = 1010.5, found 1010.8; [M+2H]²⁺ m/z = 505.7, found 506.2.

In addition to 1 mM, different reaction concentrations were also studied.

1a (1.0 equiv.) and **3a** (1.0 equiv.) were dissolved in PBS buffer (pH 4.0) with the final concentration at 500 μ M, 100 μ M 50 μ M 5 μ M. **2h** (1.0 equiv.) was added and reacted at room temperature for 10 min. In LC-MS analysis, the conversion yield of reaction concentrations at 500 μ M, 100 μ M, 50 μ M and 5 μ M were estimated as 81%, 63%, 32% and 3% respectively.

Figure 47.12 UV (205nm) trace from LC-MS analysis of reaction between peptide **1a**, MPAA (**3a**) and **2h** with the final concentrations at 5-500 μ M. Gradient: 5%-60% ACN/H₂O with 0.1% TFA over 8 min.

4 Synthesis of 1b2h3b-1b2h3f

Synthesis of 1b2h3b.

1b (5.0 mg, 3.9 μmol) and **3b** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3b**, 3.8 mg of **3b** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (4.3 μmol , 1.1 equiv) was added; for **2h**, 3.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2h3b** was observed, with a conversion yield of 97%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2h3b** (1.9 mg, 31%)

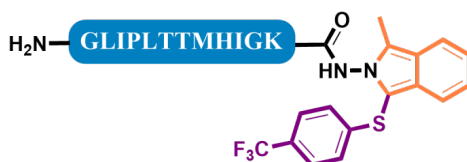


Figure 48.1 Chemical structure of 1b2h3b

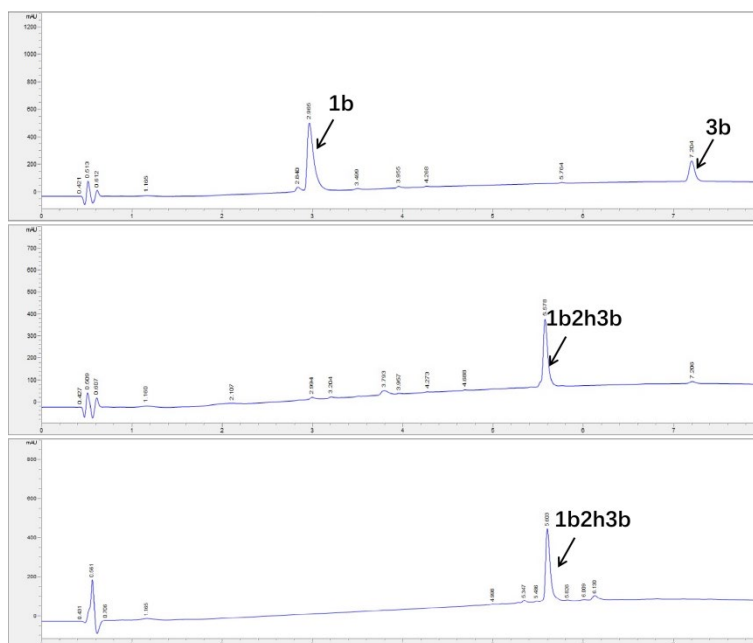


Figure 48.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

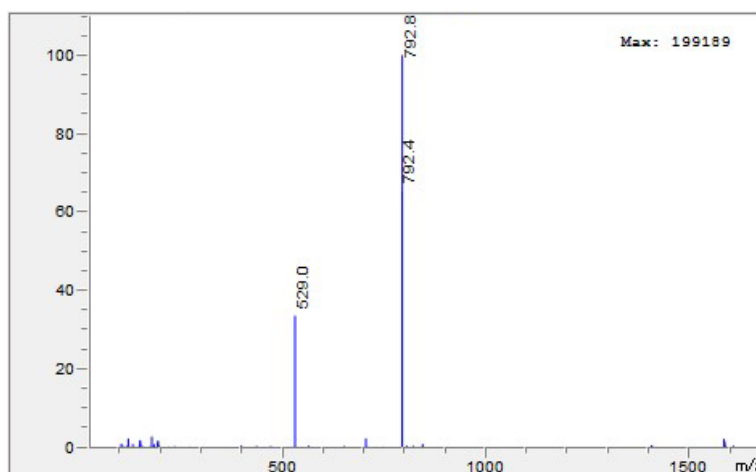


Figure 48.3 ESI-MS analysis of **1b2h3b**. Calcd. for $C_{74}H_{112}F_3N_{17}O_{14}S_2$ $[M+2H]^{2+}$ $m/z = 792.9$, found 792.8; $[M+3H]^{3+}$ $m/z = 538.9$, found 529.0.

Synthesis of **1b2h3c**.

1b (5.0 mg, 3.9 μmol) and **3c** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3c**, 2.8 mg of **3c** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (4.3 μmol , 1.1 equiv) was added; for **2h**, 3.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2h3c** was observed, with a conversion yield of 99%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2h3b** (2.7 mg, 46%)

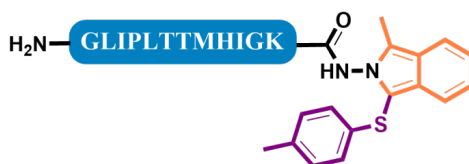


Figure 49.1 Chemical structure of **1b2h3c**

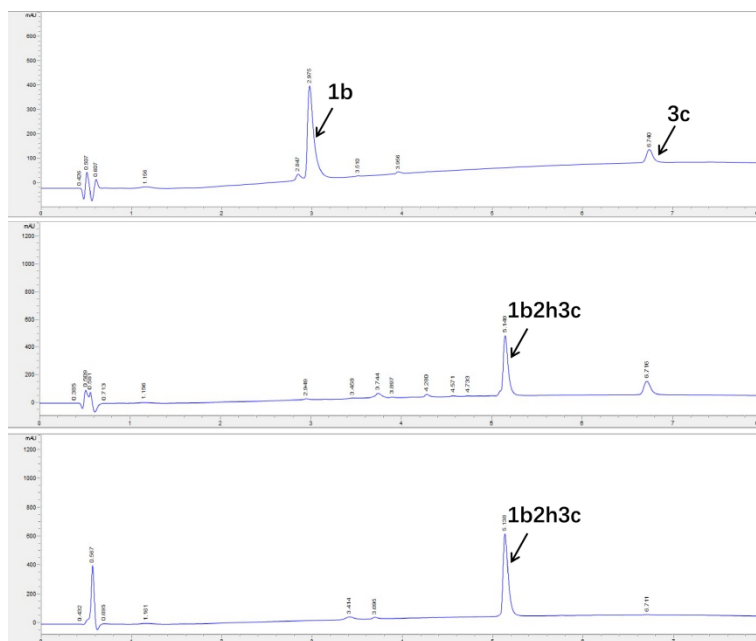


Figure 49.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

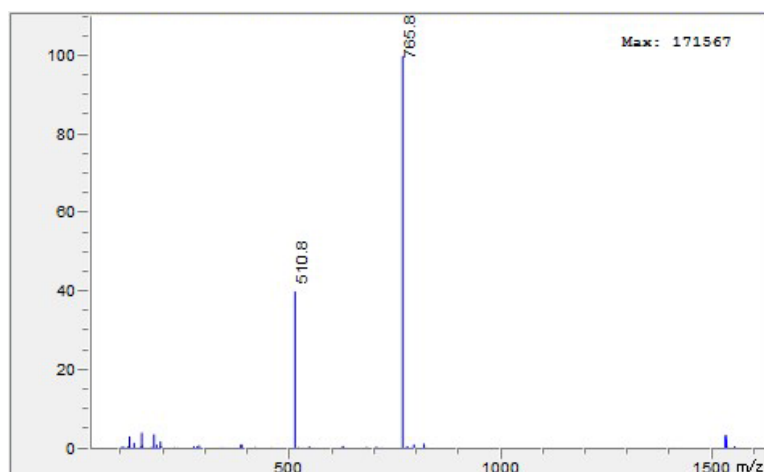


Figure 49.3 ESI-MS analysis of **1b2h3c**. Calcd. for C₇₄H₁₁₅N₁₇O₁₄S₂ [M+2H]²⁺ m/z =765.9, found 765.8; [M+3H]³⁺ m/z =510.9, found 510.8.

Synthesis of **1b2h3d**.

1b (5.0 mg, 3.9 μmol) and **3d** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3d**, 3.0 mg of **3d** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (4.3 μmol , 1.1 equiv) was added; for **2h**, 3.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2h3d** was observed, with a conversion yield of 99%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2h3d** (1.9 mg, 32%)

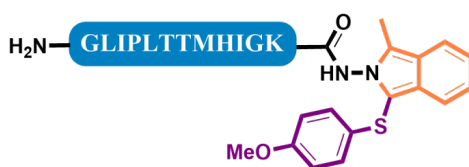


Figure 50.1 Chemical structure of **1b2h3d**

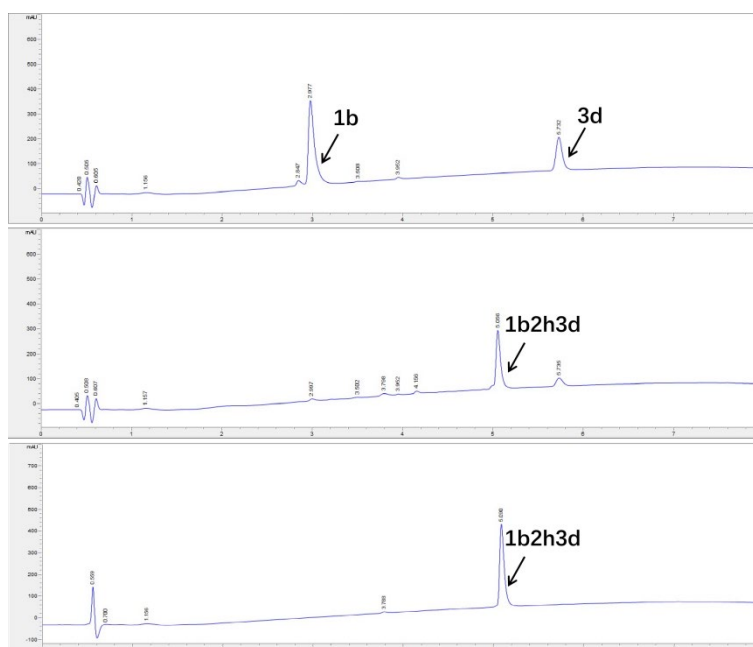


Figure 50.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

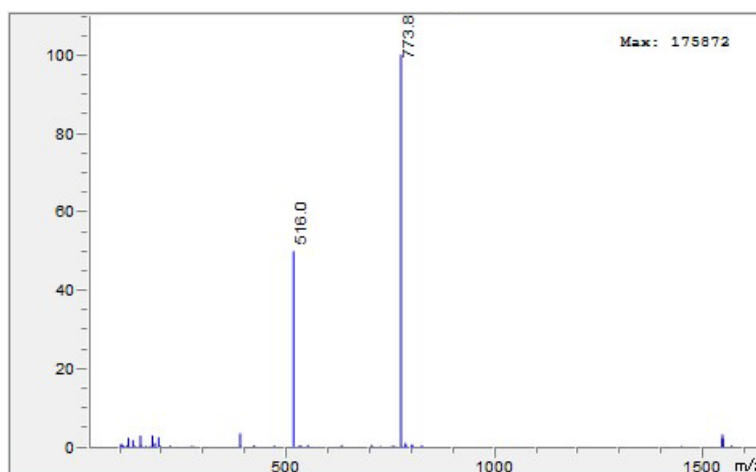


Figure 50.3 ESI-MS analysis of **1b2h3d**. Calcd. for $C_{74}H_{115}N_{17}O_{15}S_2$ $[M+2H]^{2+}$ $m/z = 773.9$, found 773.8; $[M+3H]^{3+}$ $m/z = 516.3$, found 516.0.

Synthesis of **1b2h3e**.

1b (5.0 mg, 3.9 μmol) and **3e** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3e**, 3.3 mg of **3e** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (4.3 μmol , 1.1 equiv) was added; for **2h**, 3.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2h3e** was observed, with a conversion yield of 99%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2h3e** (3.6 mg, 60%)

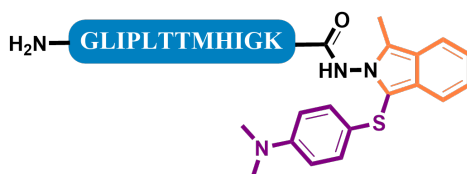


Figure 51.1 Chemical structure of **1b2h3e**

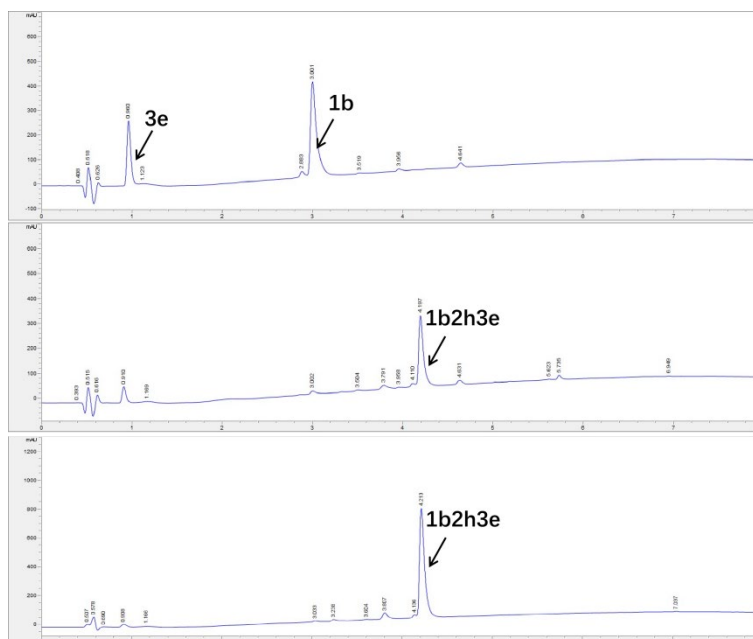


Figure 51.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

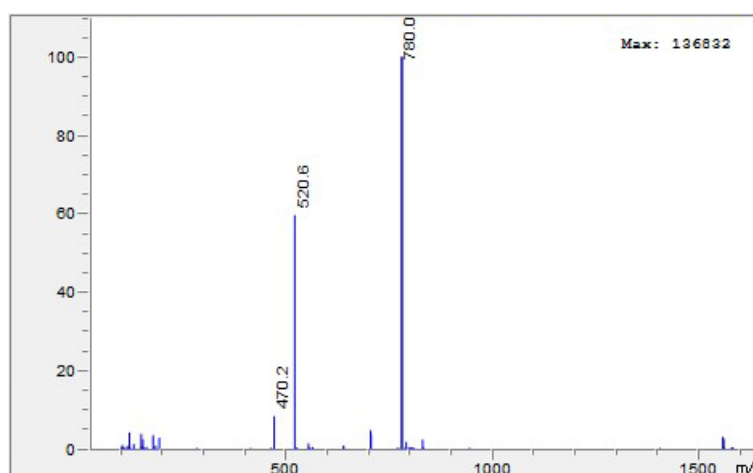


Figure 51.3 ESI-MS analysis of **1b2h3e**. Calcd. for C₇₄H₁₁₅N₁₇O₁₅S₂ [M+2H]²⁺ m/z = 780.4, found 780.0; [M+3H]³⁺ m/z = 520.6, found 520.6.

Synthesis of **1b2h3f**.

1b (5.0 mg, 3.9 μmol) and **3f** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3f**, 3.6 mg of **3f** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (4.3 μmol , 1.1 equiv) was added; for **2h**, 3.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2h3f** was observed, with a conversion yield of 78%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2h3f** (3.4 mg, 56%)

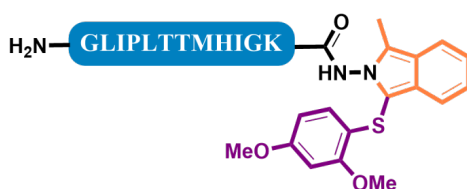


Figure 52.1 Chemical structure of **1b2h3f**

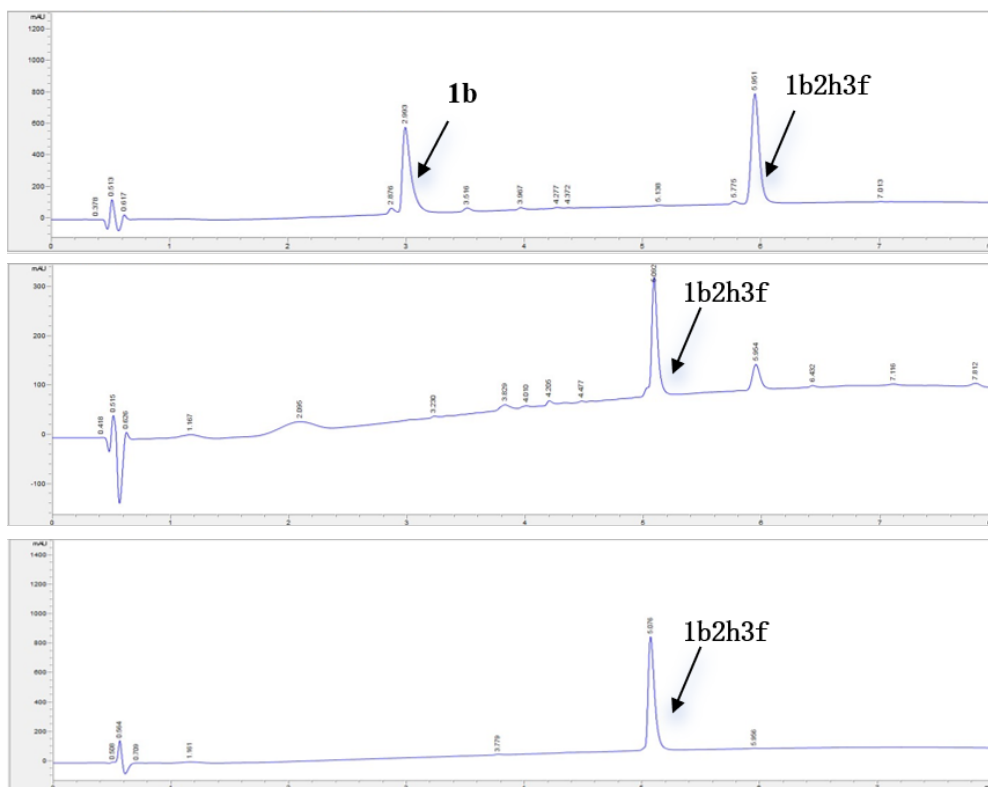


Figure 52.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** at 10

min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

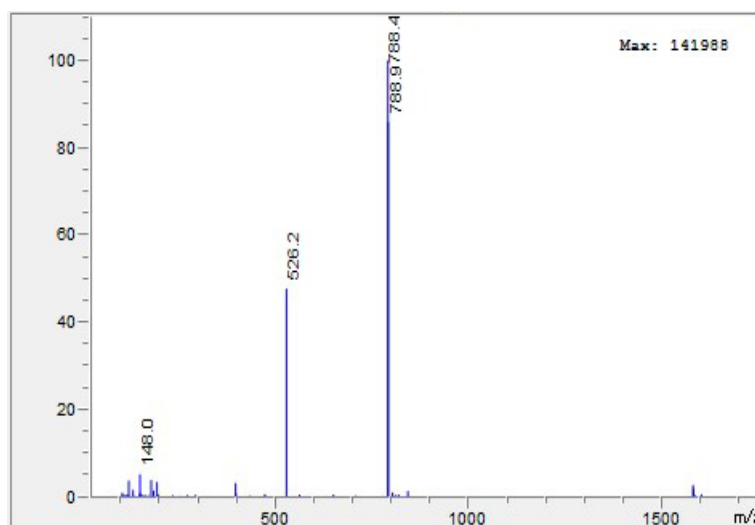


Figure 52.3 ESI-MS analysis of **1b2h3f**. Calcd. for C₇₅H₁₁₇N₁₇O₁₆S₂, [M+2H]²⁺ *m/z* = 788.9, found 788.9; [M+3H]³⁺ *m/z* = 526.3, found 526.2.

5 Synthesis of 1b2i3a-1b2s3a

Synthesis of 1b2i3a.

1b (5.0 mg, 3.9 μmol) and **3a** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2i** (4.3 μmol , 1.1 equiv) was added; for **2i**, 4.5 mg of **2i** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2i3a** was observed, with a conversion yield of 68%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2i3a** (3.9 mg, 62%)

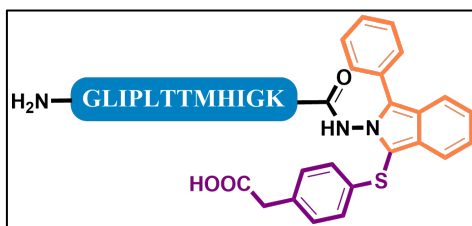


Figure 53.1 Chemical structure of **1b2i3a**

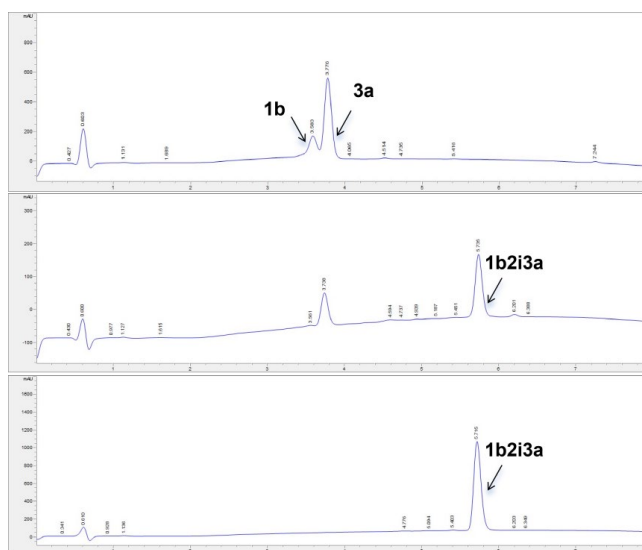


Figure 53.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2i** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

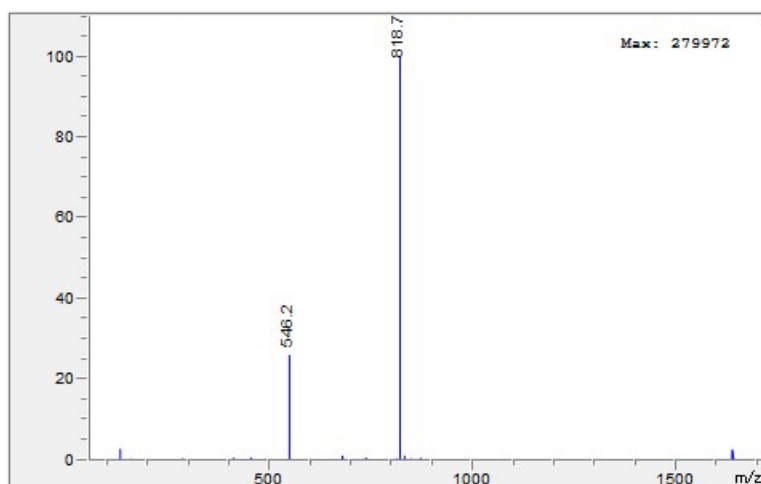


Figure 53.3 ESI-MS analysis of **1b2j3a**. Calcd. for $C_{80}H_{117}N_{17}O_{16}S_2$, $[M+2H]^{2+}$ m/z = 818.9, found 818.7; $[M+3H]^{3+}$ m/z = 546.3, found 546.2.

Synthesis of **1b2j3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2j** (4.3 μ mol, 1.1 equiv) was added; for **2j**, 5.1 mg of **2j** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2j3a** was observed, with a conversion yield of 73%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2j3a** (2.5 mg, 39%)

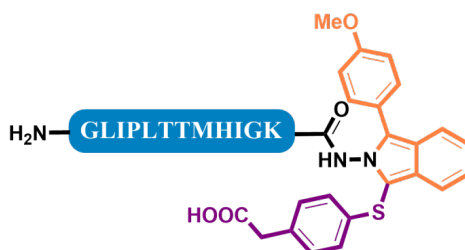


Figure 54.1 Chemical structure of **1b2j3a**

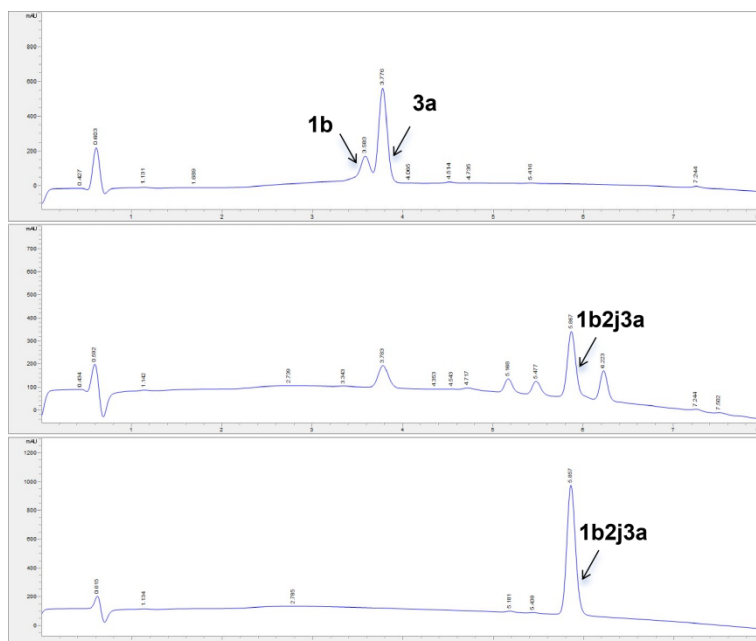


Figure 54.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2j** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

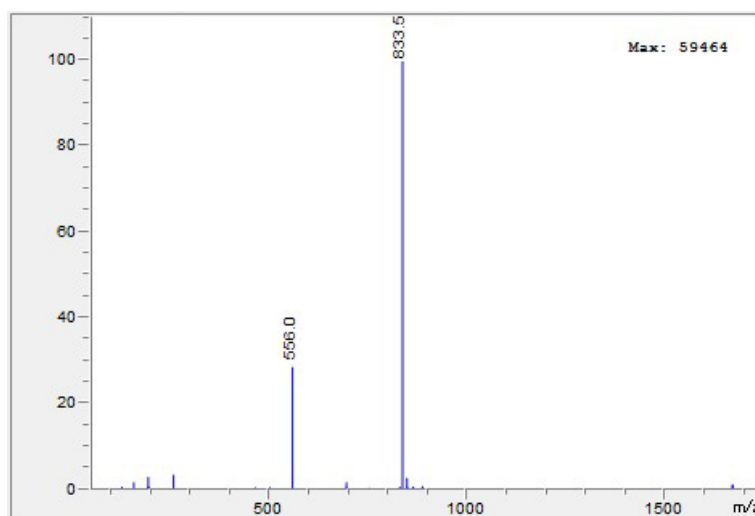


Figure 54.3 ESI-MS analysis of **1b2j3a**. Calcd. for C₈₁H₁₁₉N₁₇O₁₇S₂, [M+2H]²⁺ m/z = 833.9, found 833.5; [M+3H]³⁺ m/z = 556.3, found 556.0.

Synthesis of **1b2k3a**.

1b (5.0 mg, 3.9 μmol) and **3a** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2k** (4.3 μmol , 1.1 equiv) was added; for **2k**, 5.9 mg of **2k** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2k3a** was observed, with a conversion yield of 38%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2k3a** (2.3 mg, 35%)

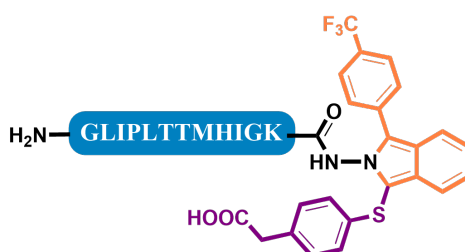


Figure 55.1 Chemical structure of **1b2k3a**

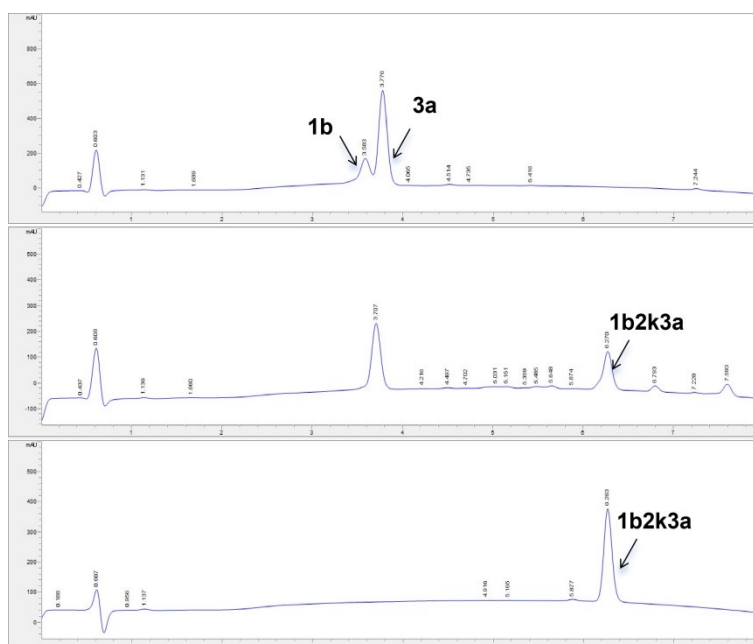


Figure 55.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2k** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

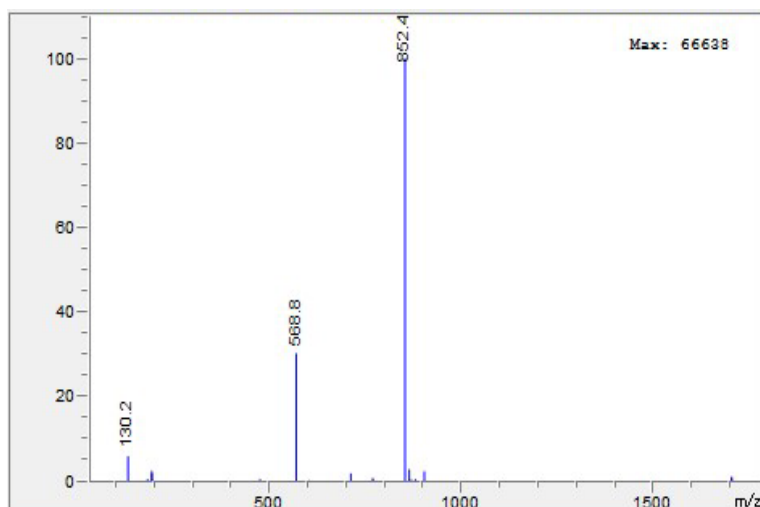


Figure 55.3 ESI-MS analysis of **1b2k3a**. Calcd. for $C_{81}H_{116}F_3N_{17}O_{16}S_2$, $[M+2H]^{2+}$ m/z = 852.9, found 852.4; $[M+3H]^{3+}$ m/z = 568.9, found 568.8.

Synthesis of **1b2l3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2l** (4.3 μ mol, 1.1 equiv) was added; for **2l**, 5.4 mg of **2l** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2l3a** was observed, with a conversion yield of 86%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2l3a** (2.7 mg, 42%)

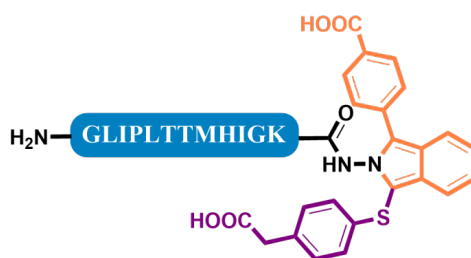


Figure 56.1 Chemical structure of **1b2l3a**

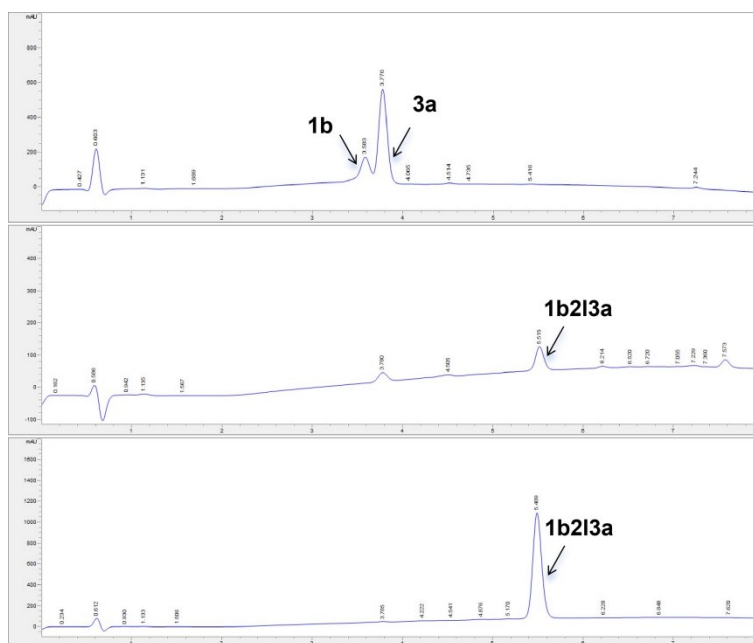


Figure 56.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2l** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

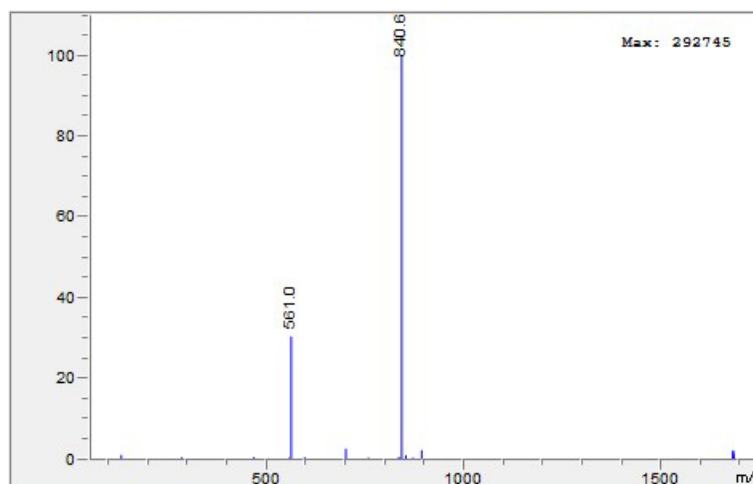


Figure 56.3 ESI-MS analysis of **1b2l3a**. Calcd for C₈₁H₁₁₇N₁₇O₁₈S₂, [M+2H]²⁺ m/z = 840.9, found 840.6; [M+3H]³⁺ m/z = 560.9, found 561.0.

Synthesis of **1b2m3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of

PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2m** (4.3 μmol , 1.1 equiv) was added; for **2m**, 5.2 mg of **2m** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2m3a** was observed, with a conversion yield of 58%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2m3a** (2.3 mg, 36%)

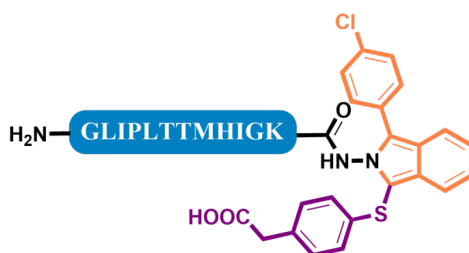


Figure 57.1 Chemical structure of **1b2m3a**

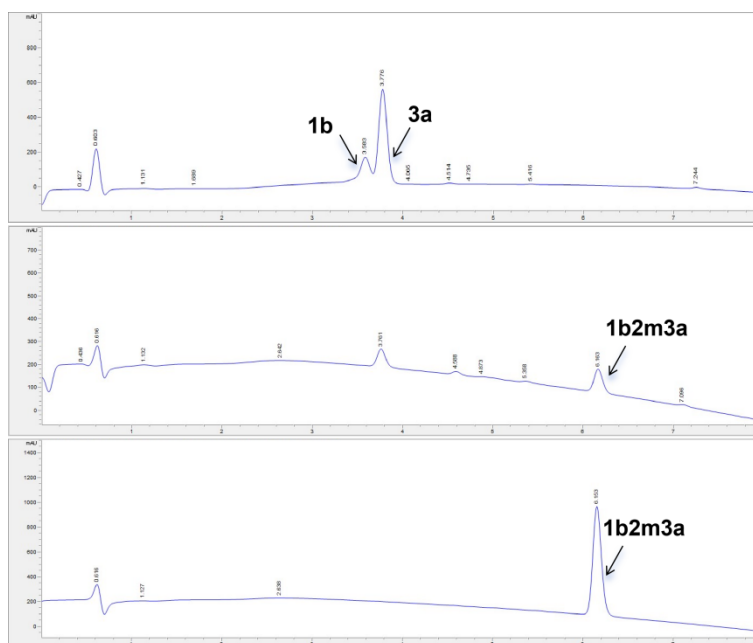


Figure 57.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2m** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/ H_2O with 0.1% TFA over 8 min.

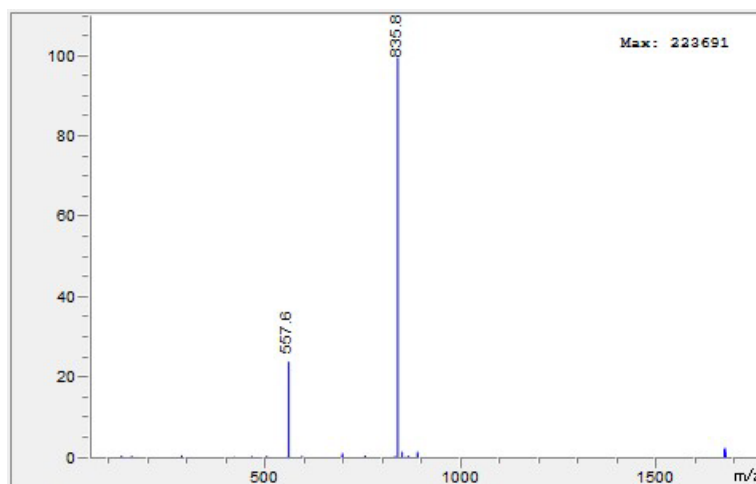


Figure 57.3 ESI-MS analysis of **1b2n3a**. Calcd. for $C_{80}H_{116}ClN_{17}O_{16}S_2$, $[M+2H]^{2+}$ m/z = 835.9, found 835.8; $[M+3H]^{3+}$ m/z = 557.6, found 557.6.

Synthesis of **1b2n3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 5.4 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2n** (4.3 μ mol, 1.1 equiv) was added; for **2n**, 5.2 mg of **2n** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2n3a** was observed, with a conversion yield of 34%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2n3a** (2.1mg, 32%)

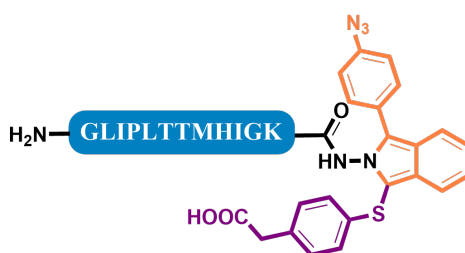


Figure 58.1 Chemical structure of **1b2n3a**

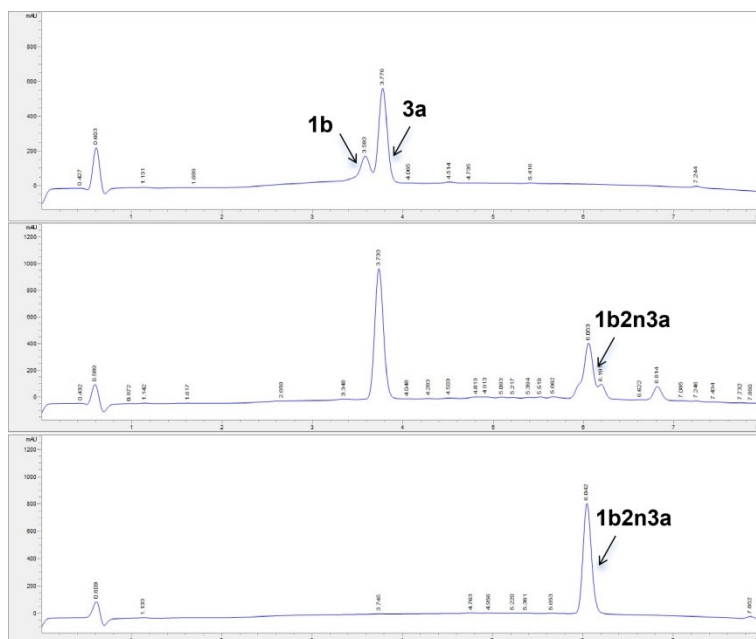


Figure 58.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2n** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

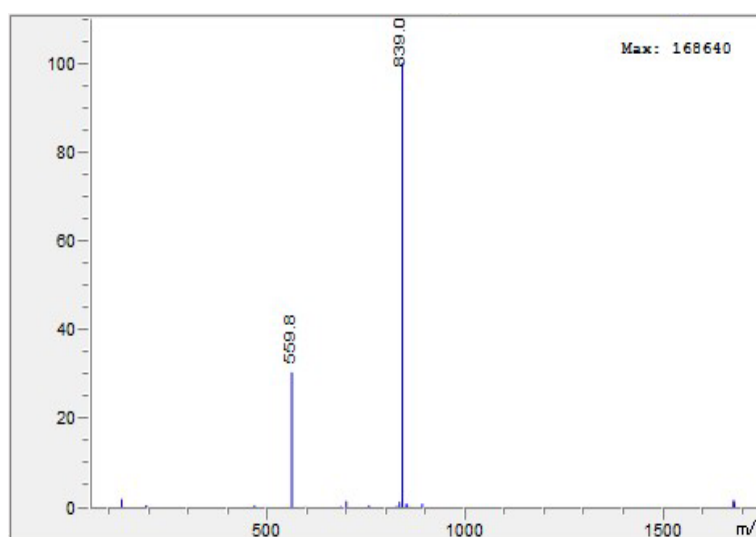


Figure 58.3 ESI-MS analysis of **1b2n3a**. Calcd. for C₈₀H₁₁₆N₂₀O₁₆S₂, [M+2H]²⁺ m/z = 839.4, found 839.0; [M+3H]³⁺ m/z = 559.9, found 559.8.

Synthesis of **1b2o3a**.

1b (5.0 mg, 3.9 μmol) and **3a** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2o** (4.3 μmol , 1.1 equiv) was added; for **2o**, 4.7 mg of **2o** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2o3a** was observed, with a conversion yield of 37%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2o3a** (1.8 mg, 28%)

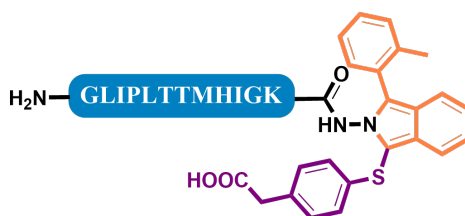


Figure 59.1 Chemical structure of **1b2o3a**

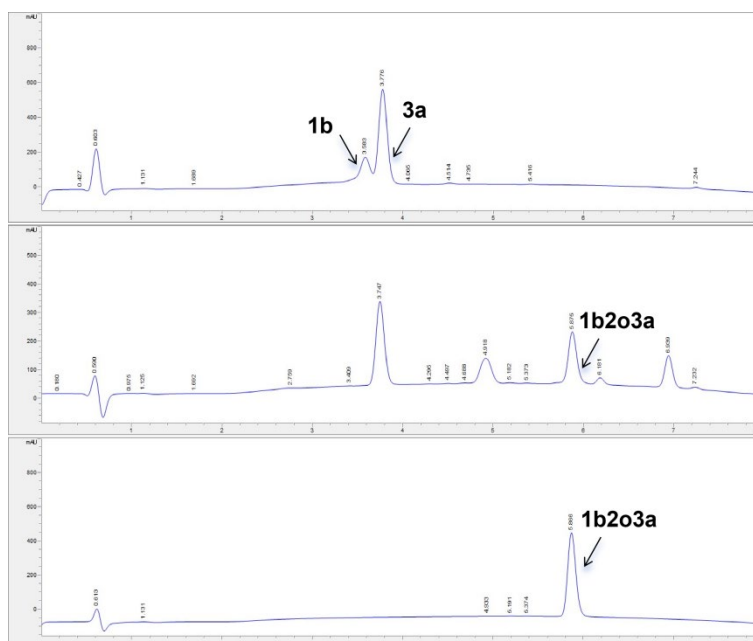


Figure 59.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2o** at 10 min, and after HPLC purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

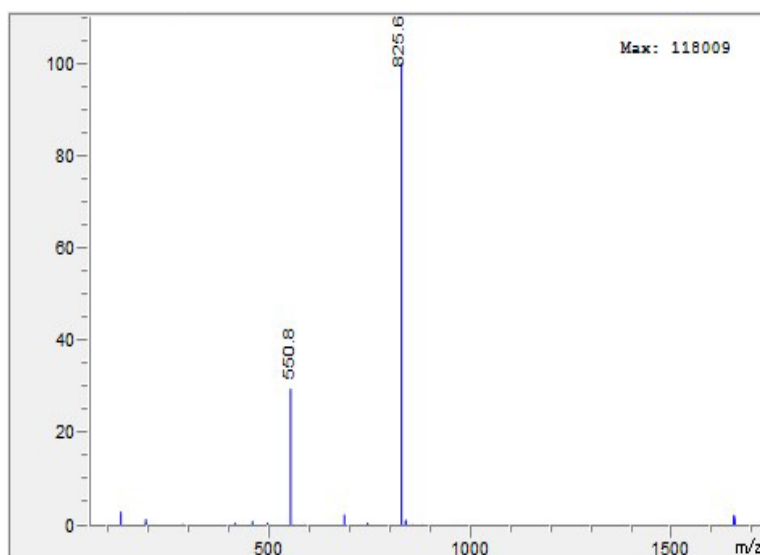


Figure 59.3 ESI-MS analysis of **1b2o3a**. Calcd. for $C_{81}H_{119}N_{17}O_{16}S_2$, $[M+2H]^{2+}$ m/z = 825.9, found 825.6; $[M+3H]^{3+}$ m/z = 550.9, found 550.8.

Synthesis of **1b2p3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2p** (4.3 μ mol, 1.1 equiv) was added; for **2p**, 5.1 mg of **2p** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2p3a** was observed, with a conversion yield of 56%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2p3a** (3.0 mg, 47%)

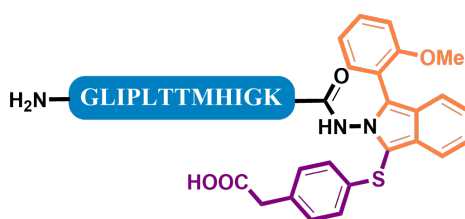


Figure 60.1 Chemical structure of **1b2p3a**

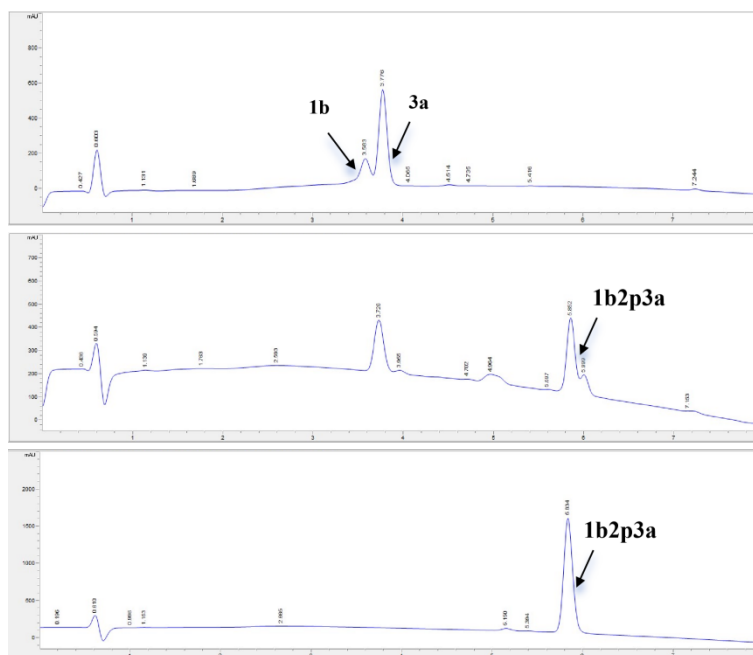


Figure 60.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2p** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

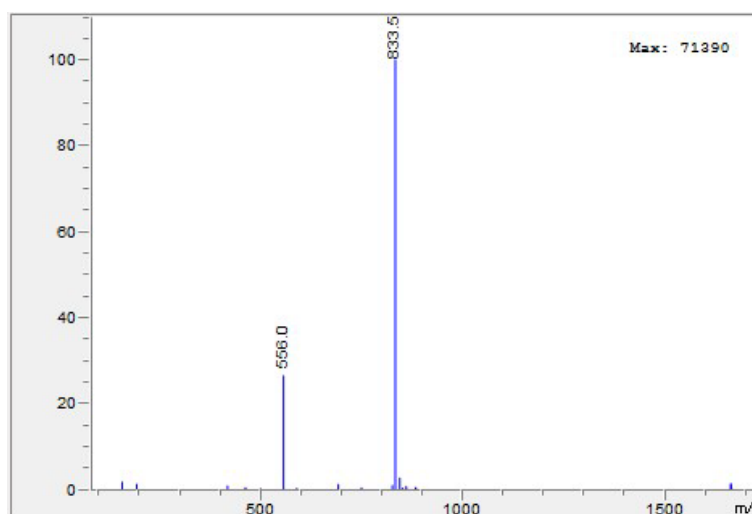


Figure 60.3 ESI-MS analysis of **1b2p3a**. calcd. for C₈₁H₁₁₉N₁₇O₁₇S₂, [M+2H]²⁺ m/z = 883.9, found 883.5; [M+3H]³⁺ m/z = 556.3, found 556.0.

Synthesis of **1b2q3a**.

1b (5.0 mg, 3.9 μmol) and **3a** (4.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2q** (4.3 μmol , 1.1 equiv) was added; for **2q**, 4.5 mg of **2q** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2q3a** was observed, with a conversion yield of 71%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2q3a** (1.8 mg, 29%)

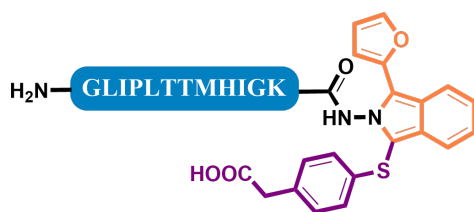


Figure 61.1 Chemical structure of **1b2q3a**

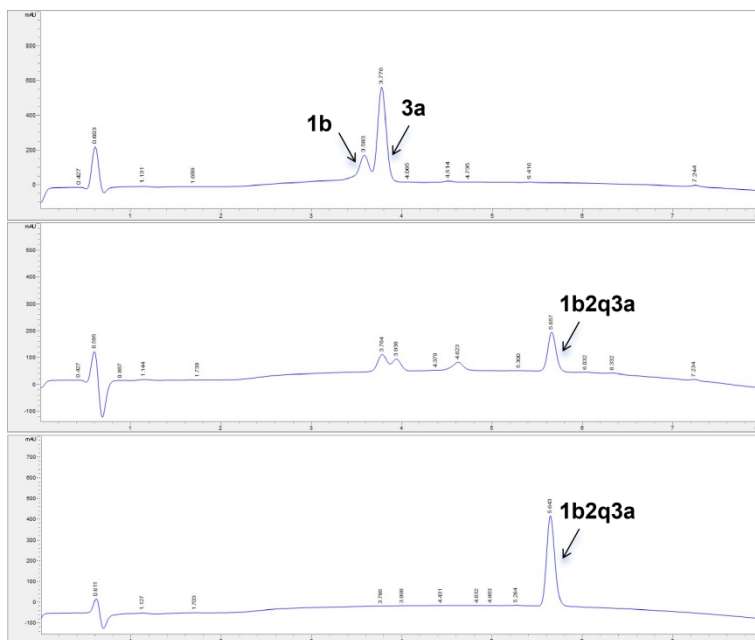


Figure 61.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2q** at 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

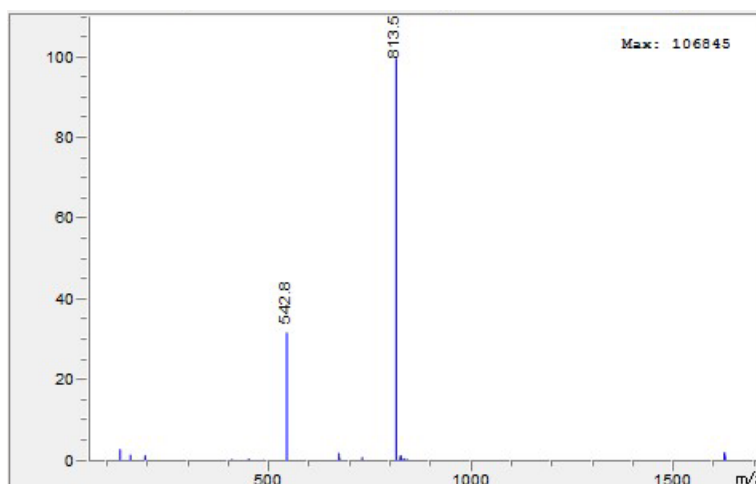


Figure 61.3 ESI-MS analysis of **1b2q3a**. Calcd. for $C_{78}H_{115}N_{17}O_{17}S_2$, $[M+2H]^{2+}$ m/z = 813.9, found 813.5; $[M+3H]^{3+}$ m/z = 542.9, found 542.8.

Synthesis of **1b2r3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2r** (4.3 μ mol, 1.1 equiv) was added; for **2r**, 4.6 mg of **2r** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2r3a** was observed, with a conversion yield of 44%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2r3a** (2.7 mg, 43%)

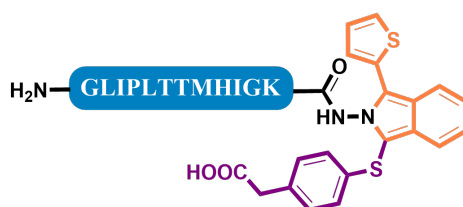


Figure 62.1 Chemical structure of **1b2r3a**

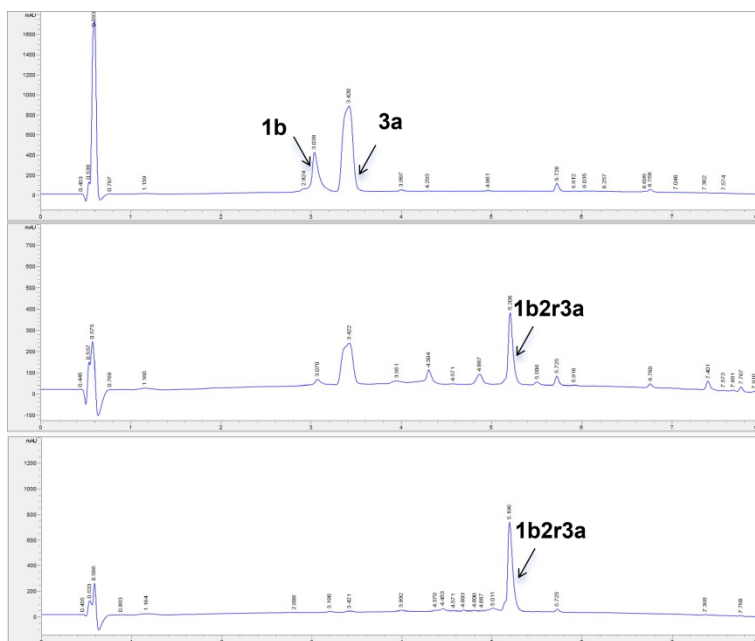


Figure 62.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2r** at 10 min, and after HPLC purification. Gradient: 30%-60% ACN/H₂O with 0.1% TFA over 8 min.

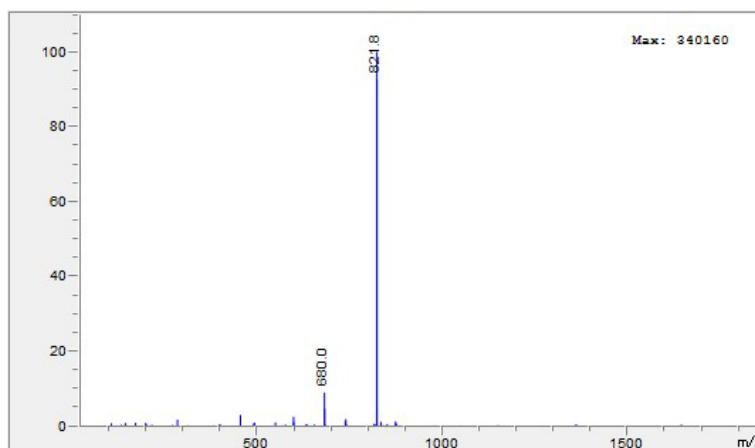


Figure 62.3 ESI-MS analysis of **1b2r3a**. Calcd. for C₇₈H₁₁₅N₁₇O₁₆S₃, [M+2H]²⁺ *m/z* = 821.9, found 821.8.

Synthesis of **1b2s3a**.

1b (5.0 mg, 3.9 μ mol) and **3a** (4.3 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.6 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2s** (4.3

μmol , 1.1 equiv) was added; for **2s**, 4.6 mg of **2s** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1b2s3a** was observed, with a conversion yield of 85%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1b2s3a** (3.9 mg, 61%)

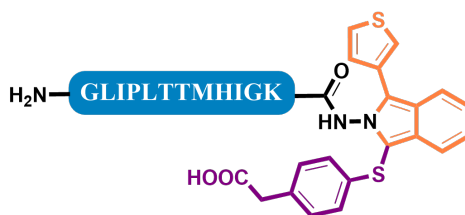


Figure 63.1 Chemical structure of **1b2s3a**

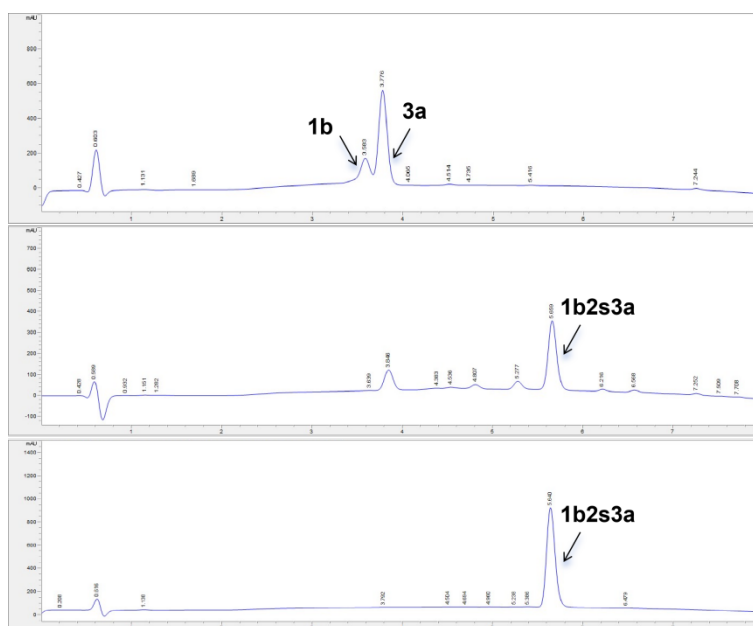


Figure 63.2 UV (205 nm) traces of the reaction: before addition of **2s**, after addition of **2s** and reacted for 10 min, and after HPLC purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

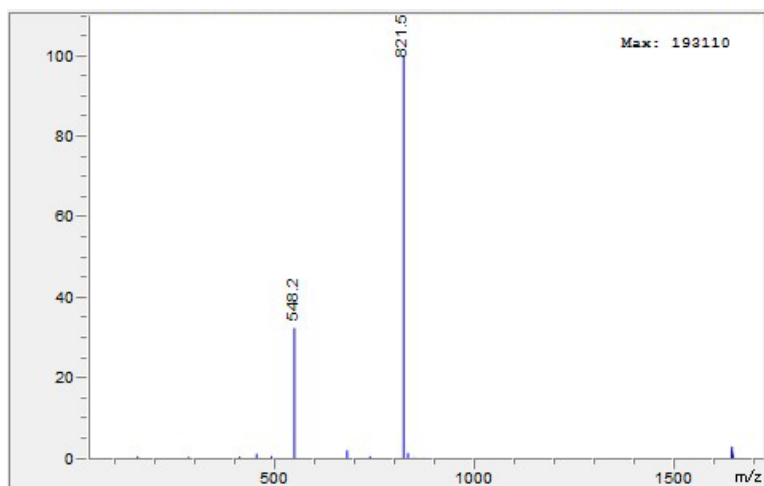


Figure 63.3 ESI-MS analysis of **1b2s3a**. Calcd. for $\text{C}_{78}\text{H}_{115}\text{N}_{17}\text{O}_{16}\text{S}_3$, $[\text{M}+2\text{H}]^{2+}$ m/z = 821.9, found 821.8; $[\text{M}+3\text{H}]^{3+}$ m/z = 548.2, found 548.2.

6 Synthesis of 1c2h3a-1s2h3a

Synthesis of 1c2h3a

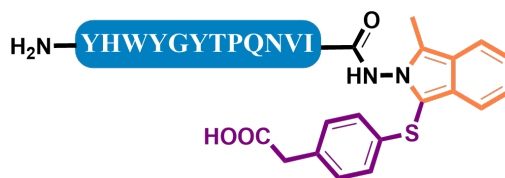


Figure 64.1 Structure of 1c2h3a

1c (5.0 mg, 3.2 μ mol) and **3a** (3.5 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.0 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (3.5 μ mol, 1.1 equiv) was added; for **2h**, 2.6 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1c2h3a** was observed, with a conversion yield of 93%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1c2h3a** (2.4 mg, 41%)

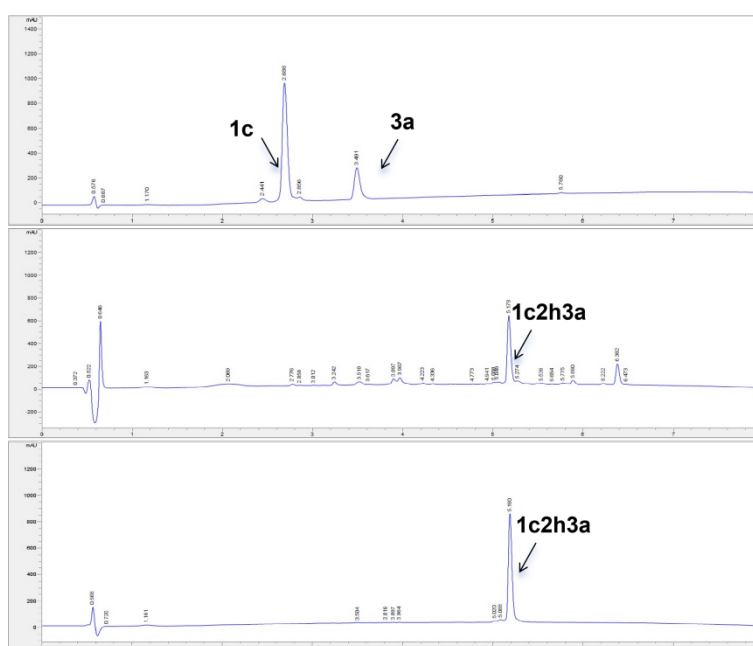


Figure 64.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and

reacted for 10 min, and after HPLC purification. Gradient: 30%-60% ACN/H₂O with 0.1% TFA over 8 min.

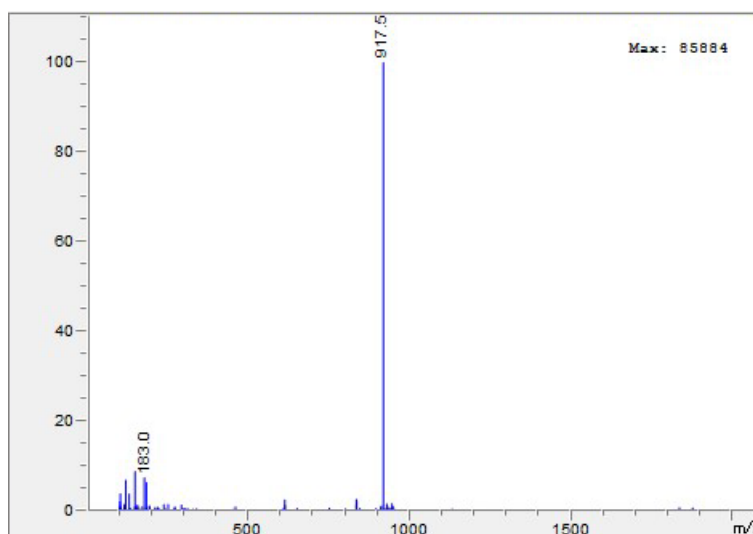


Figure 64.3 ESI-MS calcd. For C₉₂H₁₁₁N₁₉O₂₀S, [M+2H]²⁺ m/z =917.9, found 917.5.

Synthesis of **1d2h3a**

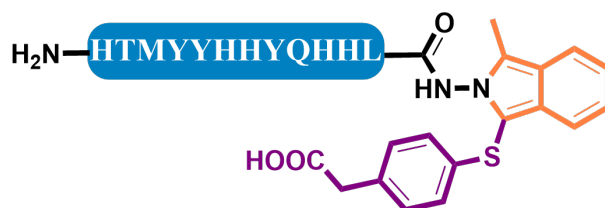


Figure 65.1 Structure of **1d2h3a**

1d (5.0 mg, 3.0 μmol) and **3a** (3.3 μmol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.8 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (3.3 μmol, 1.1 equiv) was added; for **2h**, 2.4 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1d2h3a** was observed, with a conversion yield of 85%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1d2h3a** (2.4 mg, 41%)

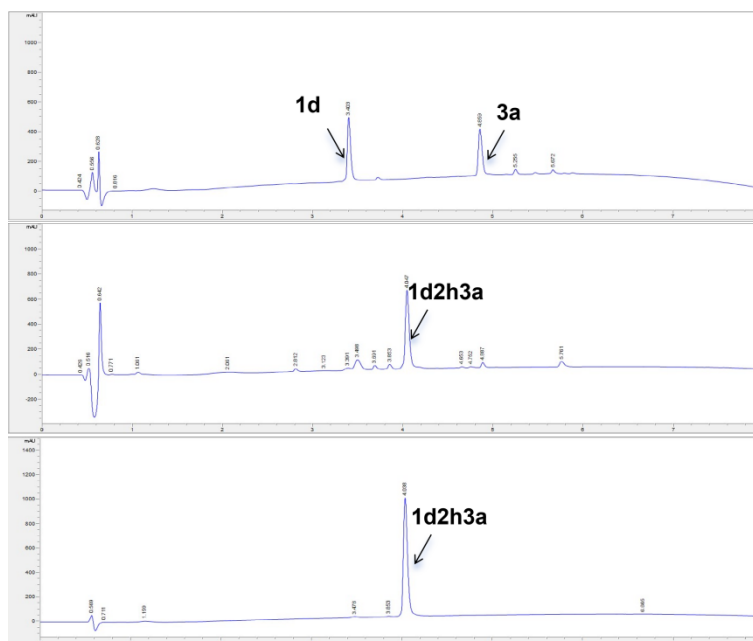


Figure 65.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

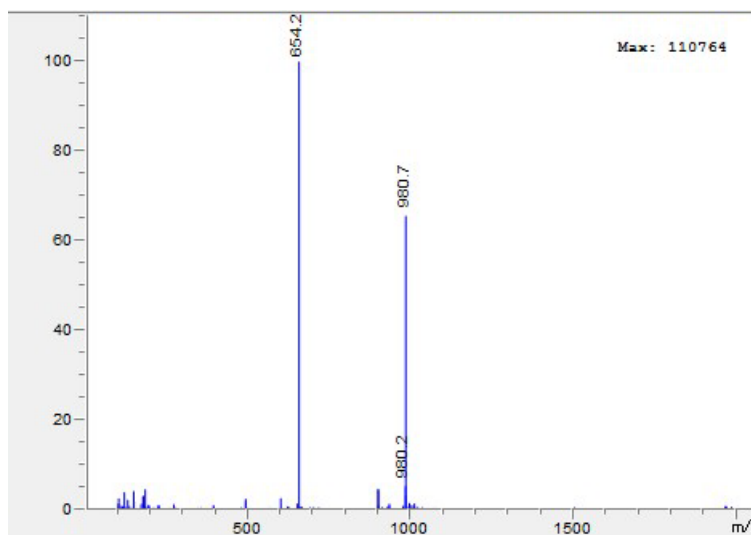


Figure 65.3 ESI-MS calcd. For C₉₄H₁₁₃N₂₅O₁₉S₂, [M+2H]²⁺ m/z =980.9, found 980.7; [M+3H]³⁺ m/z =654.3, found 654.2.

Synthesis of **1e2h3a**

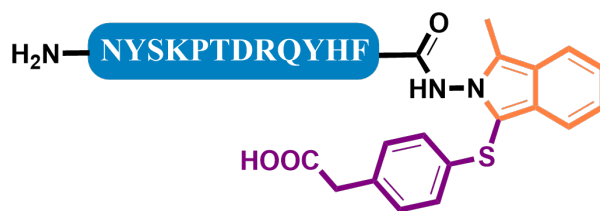


Figure 66.1 Structure of **1e2h3a**

1e (5.0 mg, 3.2 μ mol) and **3a** (3.5 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.0 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (3.5 μ mol, 1.1 equiv) was added; for **2h**, 2.6 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1e2h3a** was observed, with a conversion yield of 87%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1e2h3a** (2.9 mg, 49%)

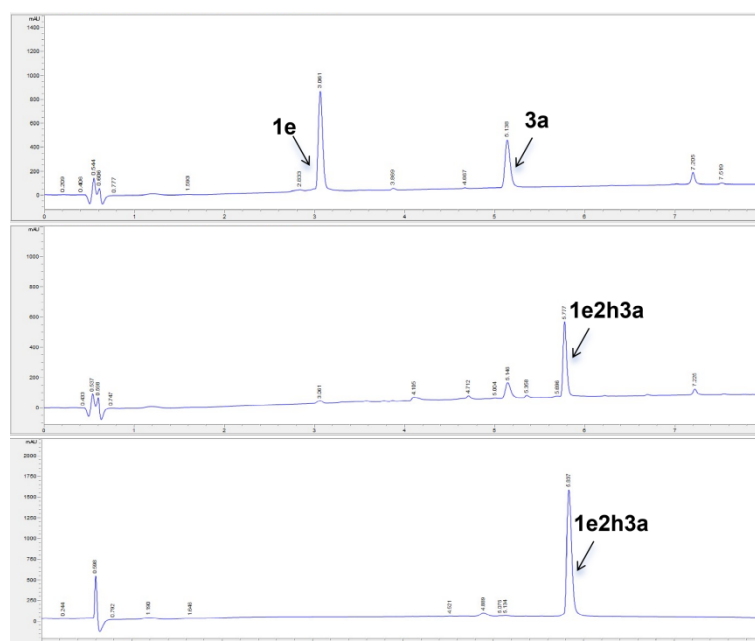


Figure 66.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

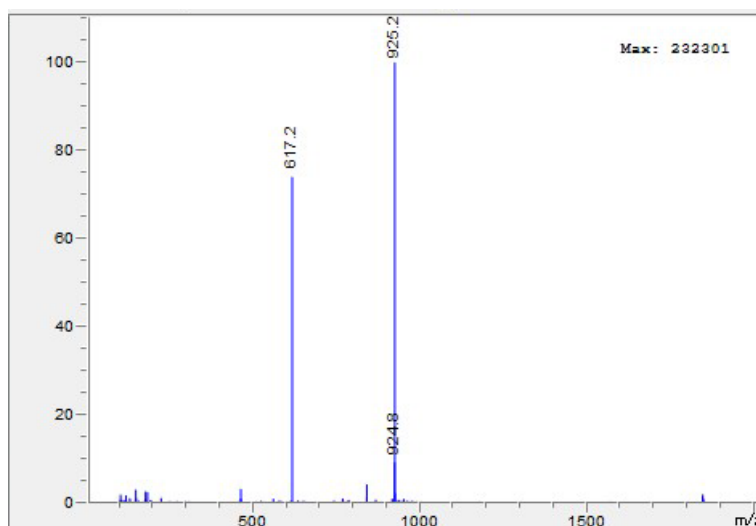


Figure 66.3 ESI-MS calcd. For $C_{87}H_{112}N_{22}O_{22}S$, $[M+2H]^{2+}$ m/z =925.4, found 925.2; $[M+3H]^{3+}$ m/z =617.3, found 617.2.

Synthesis of **1f2h3a**

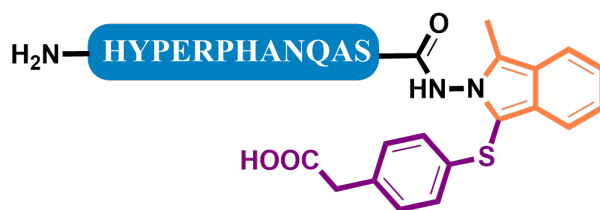


Figure 67.1 Structure of **1f2h3a**

1f (5.0 mg, 3.5 μ mol) and **3a** (3.9 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 3.3 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (3.9 μ mol, 1.1 equiv) was added; for **2h**, 2.9 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1f2h3a** was observed, with a conversion yield of 87%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1f2h3a** (2.9 mg, 48%)

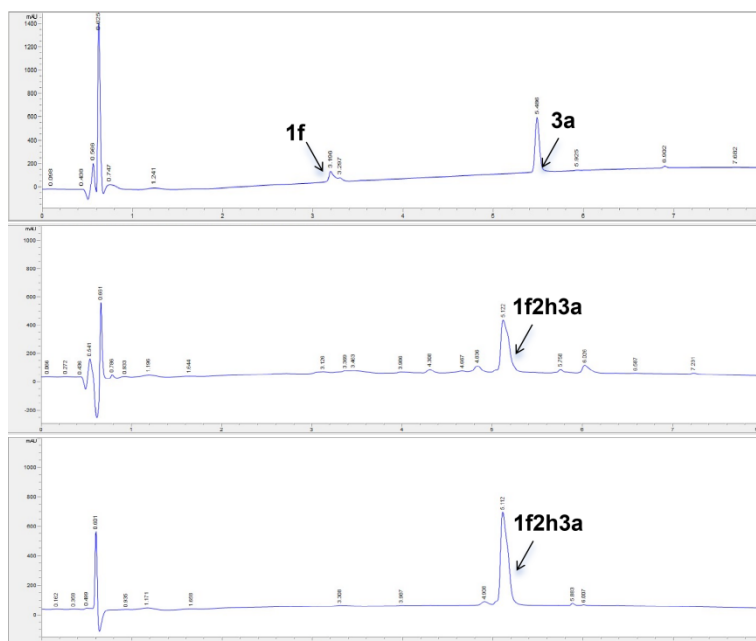


Figure 67.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

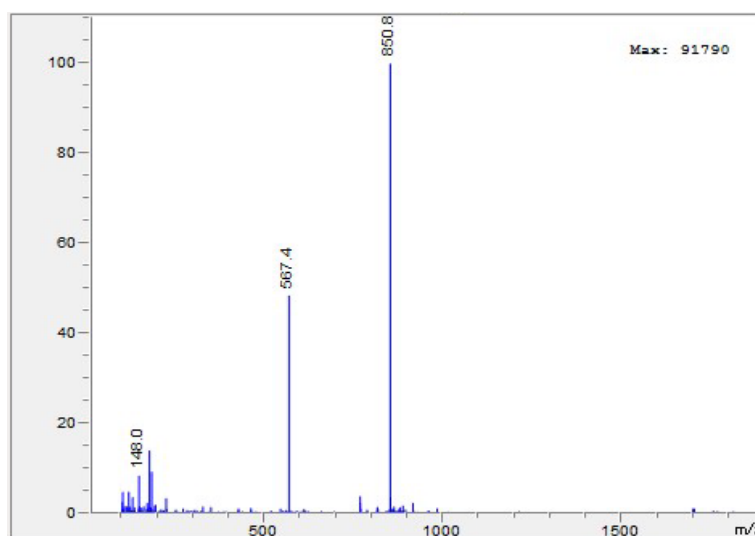


Figure 67.3 ESI-MS calcd. For C₇₇H₁₀₁N₂₃O₂₀S, [M+2H]²⁺ m/z =850.8, found 850.8; [M+3H]³⁺ m/z =567.6, found 567.4.

Synthesis of **1g2h3a**

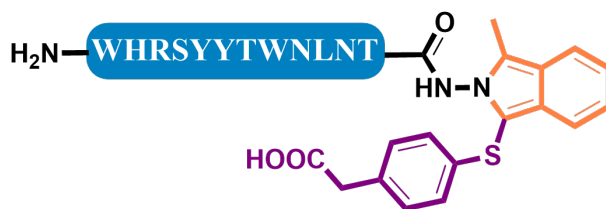


Figure 68.1 Structure of **1g2h3a**

1g (5.0 mg, 3.0 μmol) and **3a** (3.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.8 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (3.3 μmol , 1.1 equiv) was added; for **2h**, 2.5 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1g2h3a** was observed, with a conversion yield of 73%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1g2h3a** (1.7 mg, 32%)

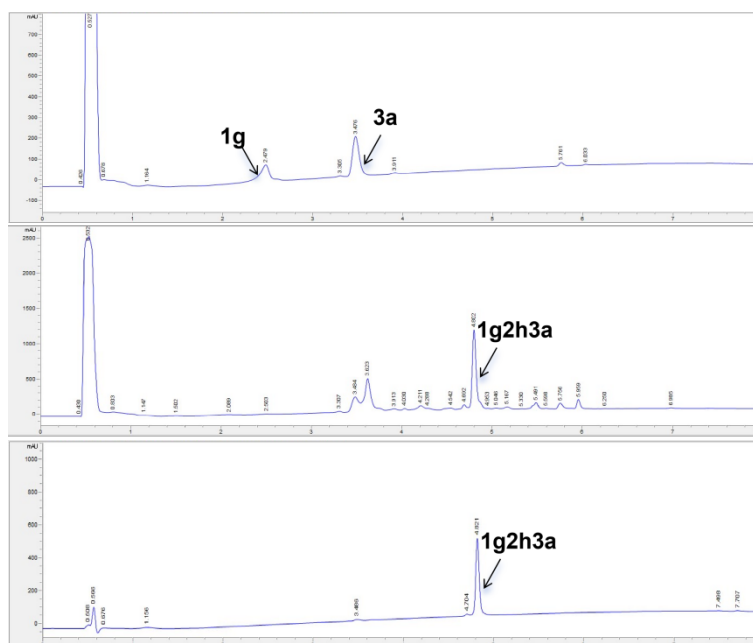


Figure 68.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

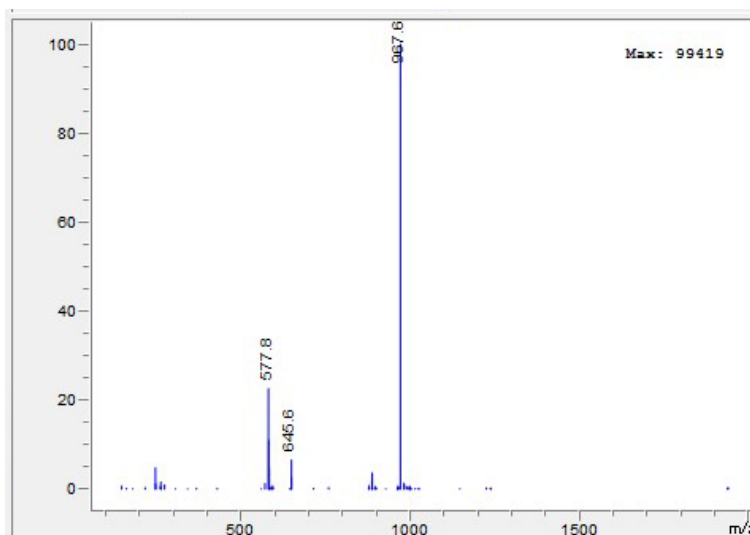


Figure 68.3 ESI-MS calcd. For $C_{94}H_{115}N_{23}O_{21}S$, $[M+2H]^{2+}$ m/z =967.9, found 967.6; $[M+3H]^{3+}$ m/z =645.6, found 645.6.

Synthesis of **1h2h3a**

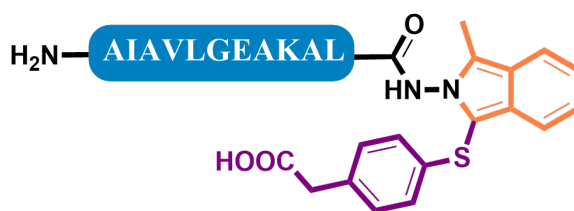


Figure 69.1 Structure of **1h2h3a**

1h (5.0 mg, 4.7 μ mol) and **3a** (5.1 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 4.3 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (5.1 μ mol, 1.1 equiv) was added; for **2h**, 3.8 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1h2h3a** was observed, with a conversion yield of 81%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1h2h3a** (3.0 mg, 48%)

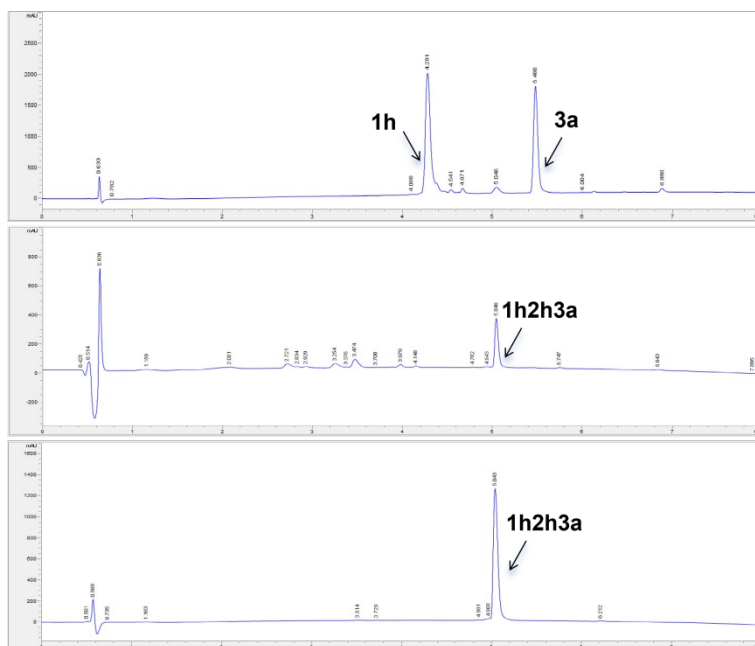


Figure 67.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

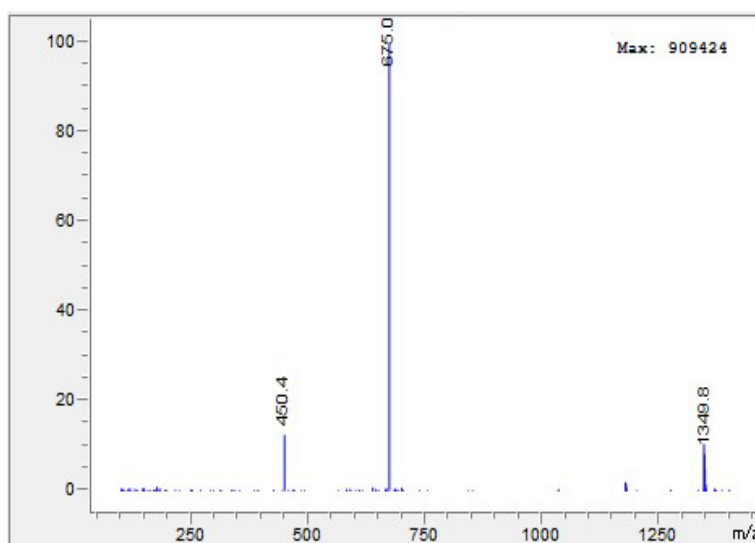


Figure 67.3 ESI-MS calcd. For C₆₅H₁₀₀N₁₄O₁₅S, [M+H]⁺ m/z =1349.7, found 1349.8; [M+2H]²⁺ m/z =675.4, found 675.0; [M+3H]³⁺ m/z =450.6, found 450.4.

Synthesis of **1i2h3a**

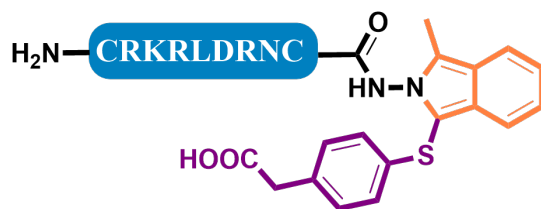


Figure 68.1 Structure of **1i2h3a**

1i (5.0 mg, 4.2 μ mol) and **3a** (4.7 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 4.0 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (4.7 μ mol, 1.1 equiv) was added; for **2h**, 3.5 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1i2h3a** was observed, with a conversion yield of 40%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1i2h3a** (1.9 mg, 31%)

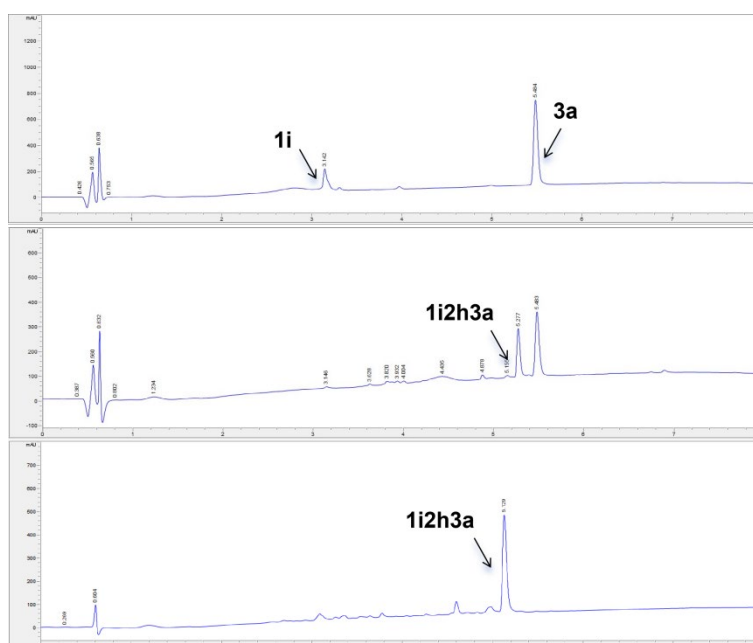


Figure 68.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

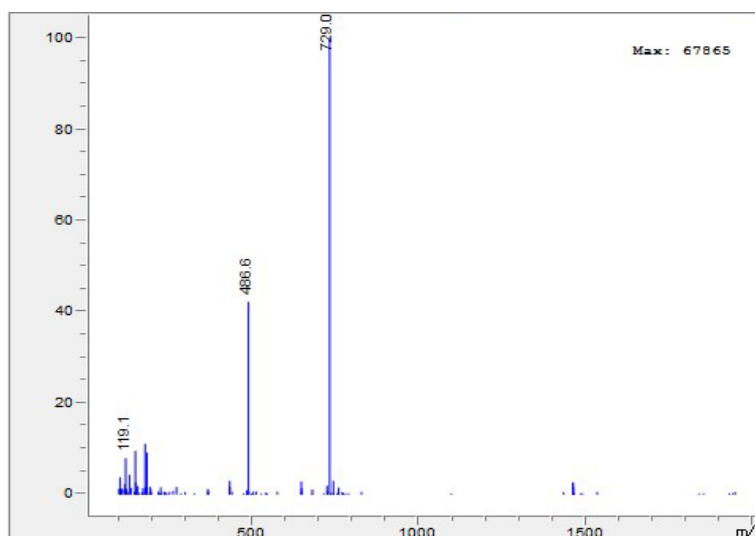


Figure 68.3 ESI-MS calcd. For $C_{61}H_{96}N_{22}O_{14}S_3$, $[M+2H]^{2+}$ $m/z = 729.4$, found 729.0; $[M+3H]^{3+}$ $m/z = 486.6$, found 486.6

Synthesis of **1j2h3a**

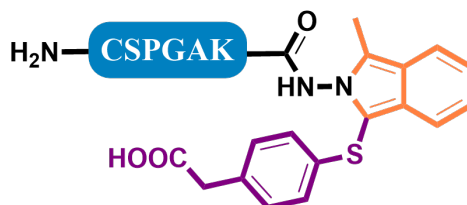


Figure 69.1 Structure of **1j2h3a**

1j (5.0 mg, 8.6 μmol) and **3a** (9.5 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 8.0 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (9.5 μmol , 1.1 equiv) was added; for **2h**, 7.1 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1j2h3a** was observed, with a conversion yield of 55%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1j2h3a** (1.8 mg, 24%)

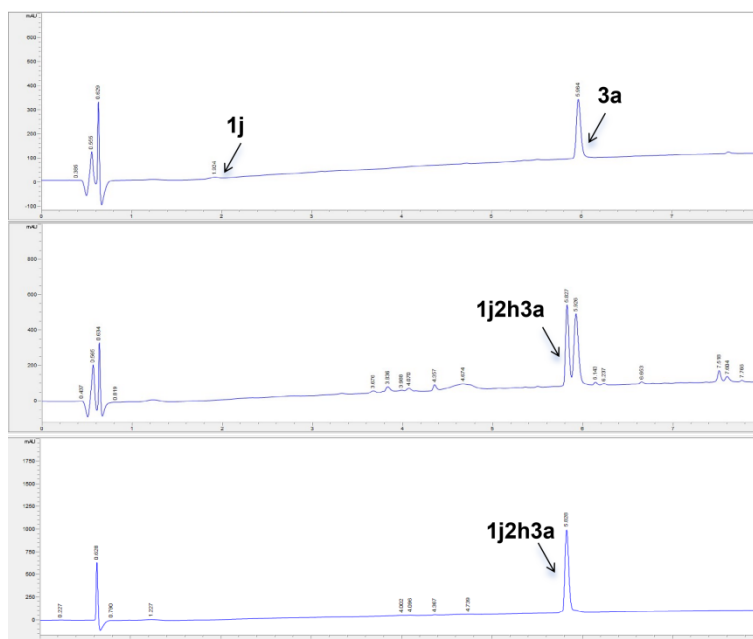


Figure 69.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

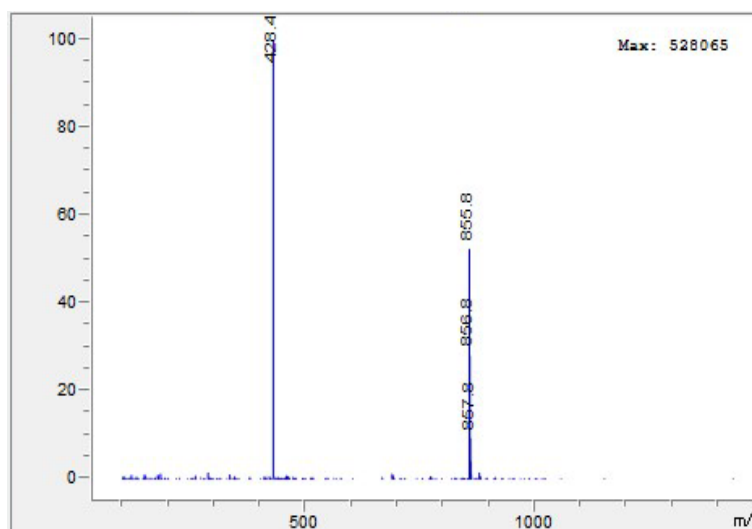


Figure 69.3 ESI-MS calcd. For C₃₉H₅₃N₉O₉S₂, [M+H]⁺ m/z =856.3, found 855.8; [M+2H]²⁺ m/z=428.7, found 428.4.

Synthesis of **1k2h3a**



Figure 70.1 Structure of **1k2h3a**

1k (5.0 mg, 2.1 μmol) and **3a** (2.4 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.0 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (2.4 μmol , 1.1 equiv) was added; for **2h**, 1.7 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1k2h3a** was observed, with a conversion yield of 74%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1k2h3a** (1.9 mg, 34%)

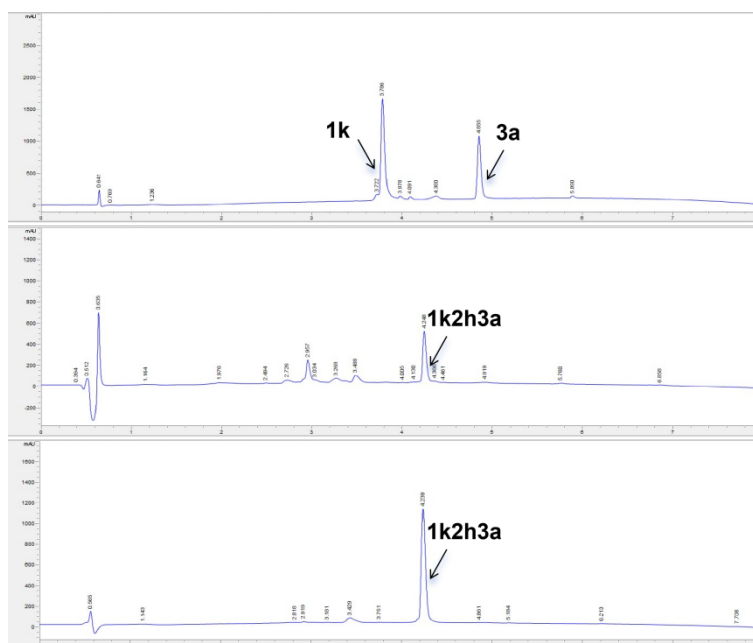


Figure 70.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

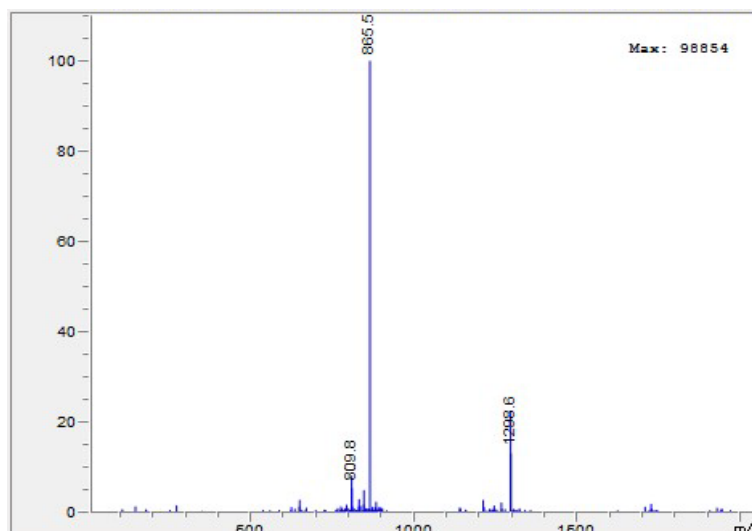


Figure 70.3 ESI-MS calcd. For $C_{121}H_{163}N_{31}O_{32}S$, $[M+2H]^{2+}$ $m/z = 1298.1$, found 1298.6; $[M+3H]^{3+}$ $m/z = 865.7$, found 865.5.

Synthesis of **112h3a**

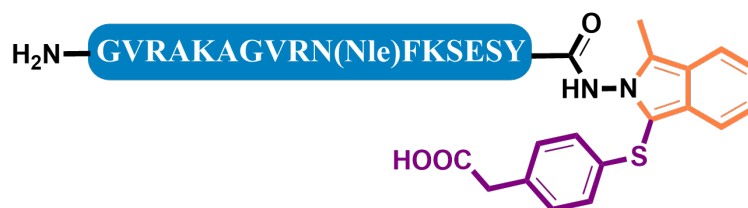


Figure 71.1 Structure of **112h3a**

11 (5.0 mg, 2.6 μmol) and **3a** (2.9 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.5 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (2.9 μmol , 1.1 equiv) was added; for **2h**, 2.2 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **112h3a** was observed, with a conversion yield of 75%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **112h3a** (2.8 mg, 48%)

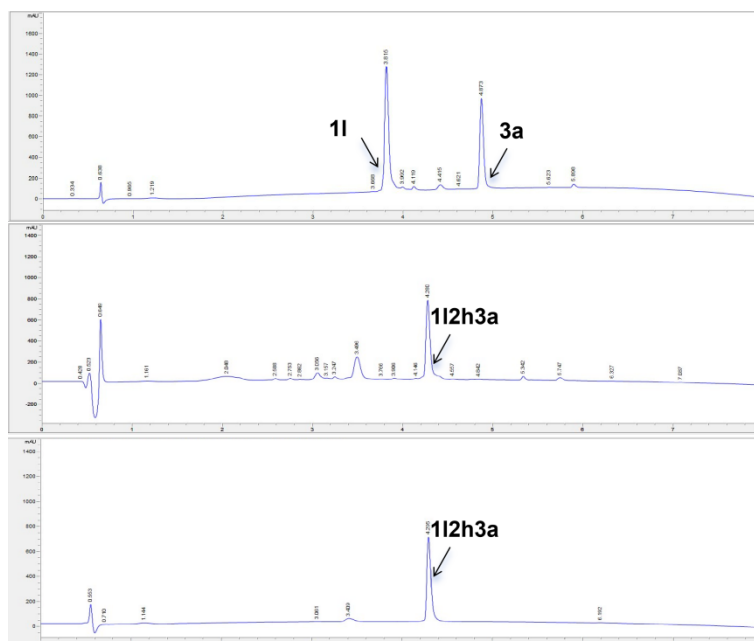


Figure 71.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

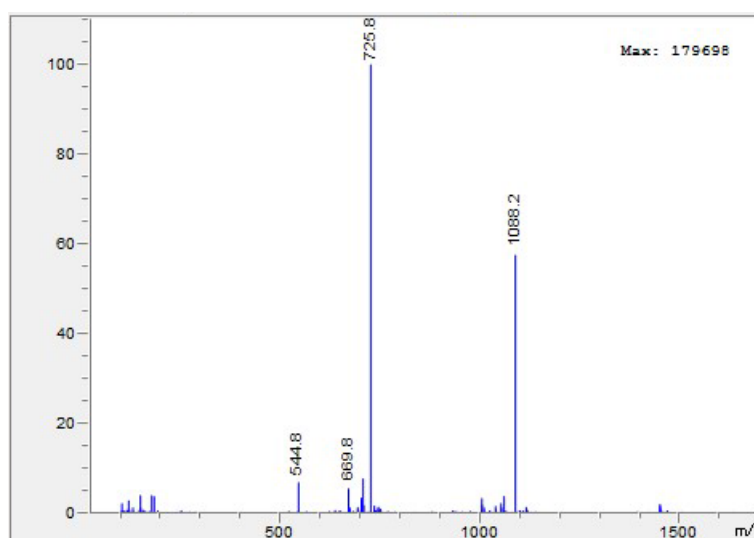


Figure 71.3 ESI-MS calcd. For C₁₀₀H₁₅₀N₂₈O₂₅S, [M+2H]²⁺ m/z =1088.6, found 1088.2; [M+3H]³⁺ m/z =726.0, found 725.8; [M+4H]⁴⁺ m/z =544.8, found 544.8.

Synthesis of **1m2h3a**



Figure 72.1 Structure of **1m2h3a**

1m (5.0 mg, 1.5 μmol) and **3a** (1.7 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 1.4 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (1.7 μmol , 1.1 equiv) was added; for **2h**, 1.3 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1m2h3a** was observed, with a conversion yield of 88%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1m2h3a** (1.8 mg, 33%)

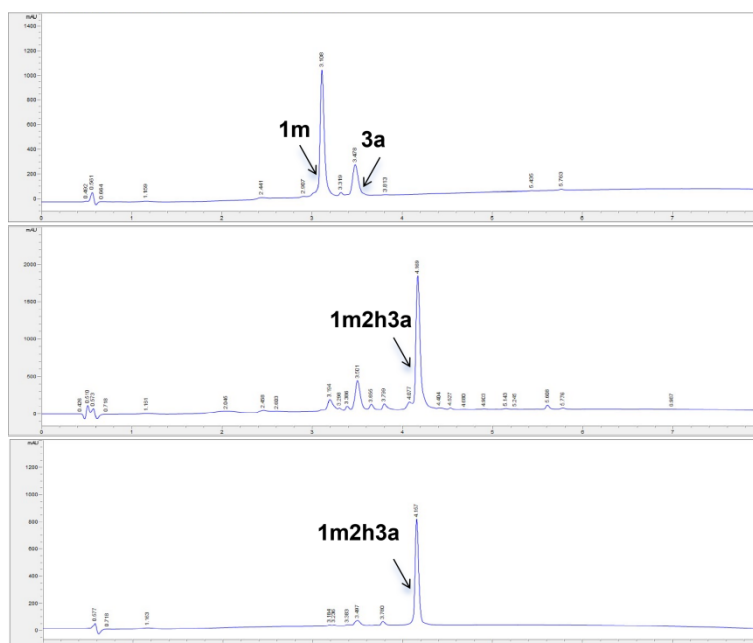


Figure 72.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

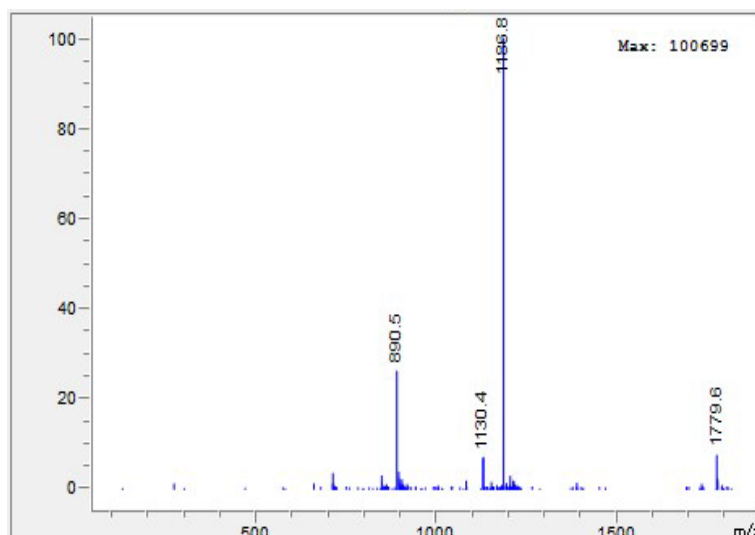


Figure 72.3 ESI-MS calcd. For $C_{158}H_{231}N_{45}O_{44}S_3$, $[M+2H]^{2+}$ m/z =1780.3, found 1779.6; $[M+3H]^{3+}$ m/z =1187.2, found 1186.8; $[M+4H]^{4+}$ m/z =890.7, found 890.5.

Synthesis of **1n2h3a**



Figure 73.1 Structure of **1n2h3a**

1n (5.0 mg, 1.8 μ mol) and **3a** (2.0 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 1.7 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (2.0 μ mol, 1.1 equiv) was added; for **2h**, 1.5 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1n2h3a** was observed, with a conversion yield of 92%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1n2h3a** (3.4 mg, 62%)

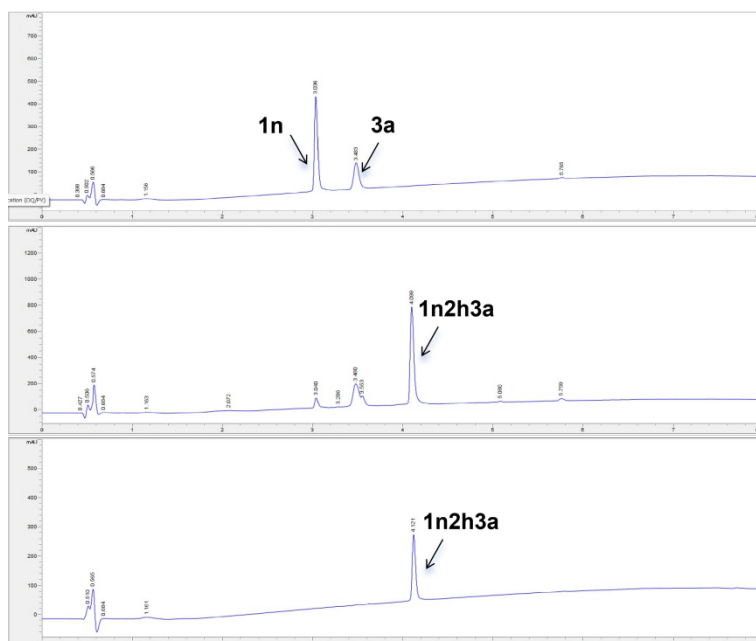


Figure 73.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

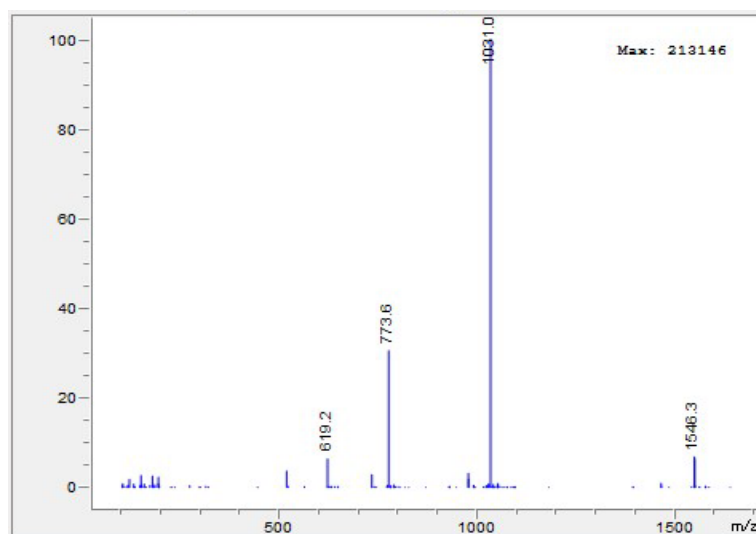


Figure 73.3 ESI-MS calcd. For C₁₄₅H₂₃₁N₄₁O₃₀S₂, [M+2H]²⁺ m/z =1546.4, found 1546.3; [M+3H]³⁺ m/z =1031.2, found 1031.0; [M+4H]⁴⁺ m/z =773.7, found 773.6; [M+5H]⁵⁺ m/z =619.1, found 619.2.

Synthesis of **1o2h3a**



Figure 74.1 Structure of **1o2h3a**

1o (3.0 mg, 1.2 μmol) and **3a** (1.3 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 1.1 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (1.3 μmol , 1.1 equiv) was added; for **2h**, 1.0 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1o2h3a** was observed, with a conversion yield of 89%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1o2h3a** (1.4 mg, 42%)

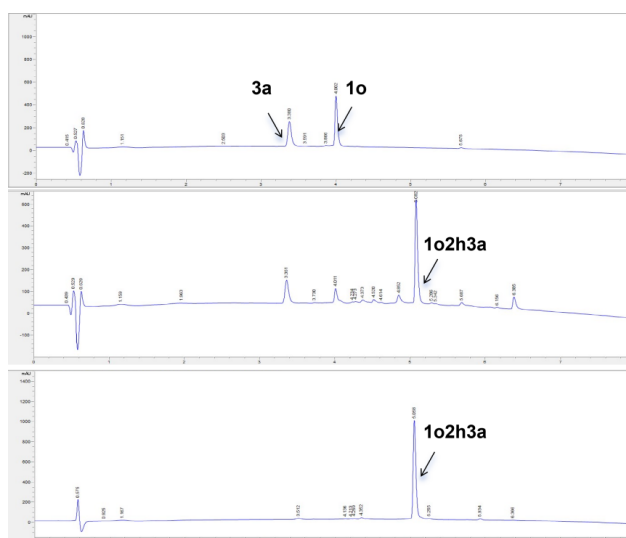


Figure 74.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

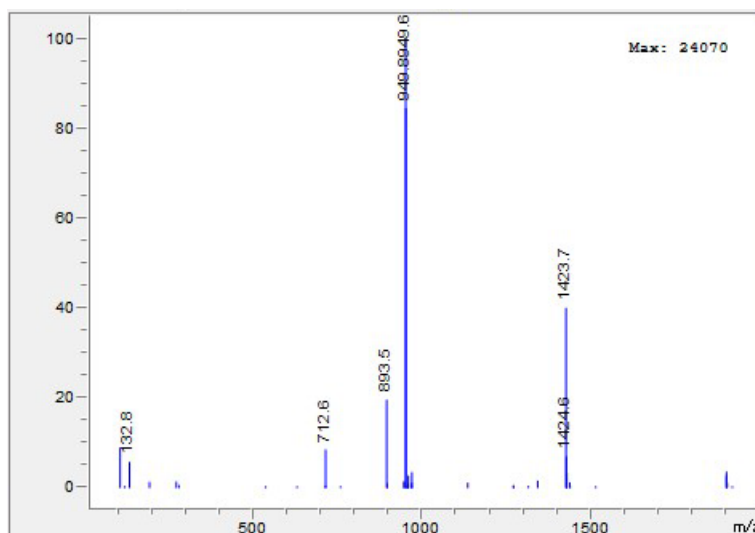


Figure 74.3 ESI-MS calcd. For $C_{134}H_{199}N_{33}O_{32}S_2$, $[M+2H]^{2+}$ m/z =1424.2, found 1423.7; $[M+3H]^{3+}$ m/z =949.8, found 949.8; $[M+4H]^{4+}$ m/z =712.6, found 712.6.

Synthesis of **1p2h3a**



Figure 75.1 Structure of **1p2h3a**

1p (5.0 mg, 2.0 μ mol) and **3a** (2.2 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 1.9 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (2.2 μ mol, 1.1 equiv) was added; for **2h**, 1.7 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1p2h3a** was observed, with a conversion yield of 78%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1p2h3a** (1.8 mg, 32%)

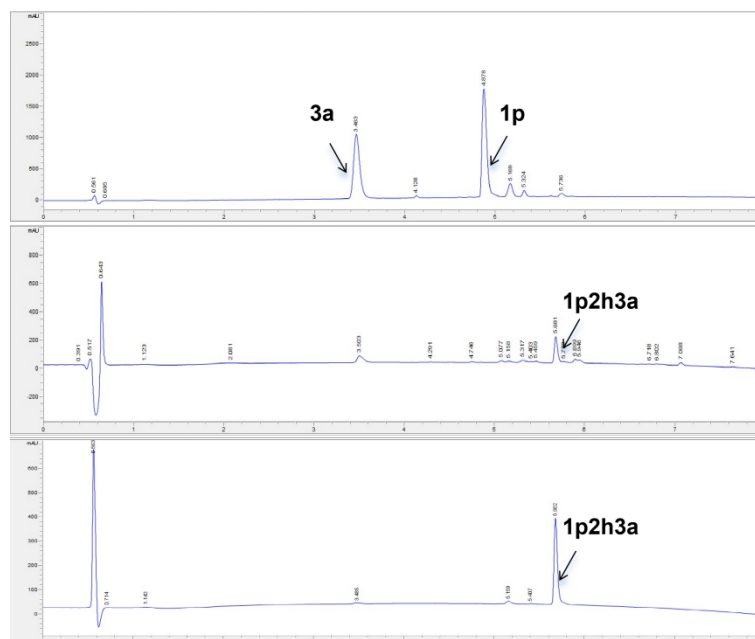


Figure 75.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

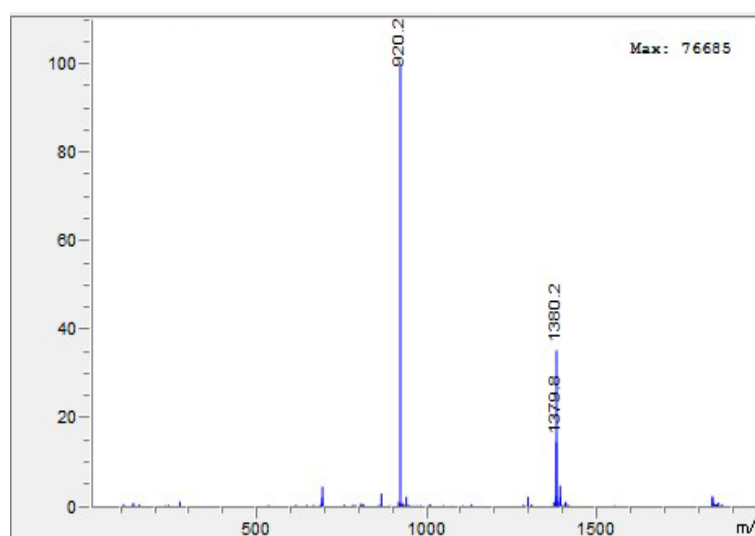


Figure 75.3 ESI-MS calcd. For C₁₂₈H₁₉₉N₃₃O₃₃S, [M+2H]²⁺ m/z = 1380.3, found 1380.2; [M+3H]³⁺ m/z = 920.5, found 920.2.

Synthesis of 1q2h3a



Figure 76.1 Structure of 1q2h3a

1q (5.0 mg, 2.2 μ mol) and **3a** (2.4 μ mol, 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.0 mg of **3a** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. Subsequently, **2h** (2.4 μ mol, 1.1 equiv) was added; for **2h**, 1.8 mg of **2h** was first dissolved in 100 μ L of PBS buffer (pH 4.0), then 20.0 μ L of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1q2h3a** was observed, with a conversion yield of 88%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1q2h3a** (3.2 mg, 57%)

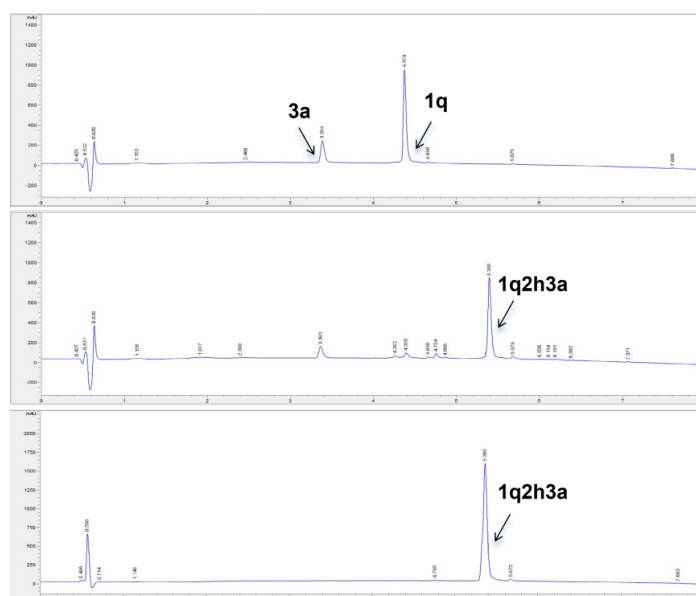


Figure 76.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

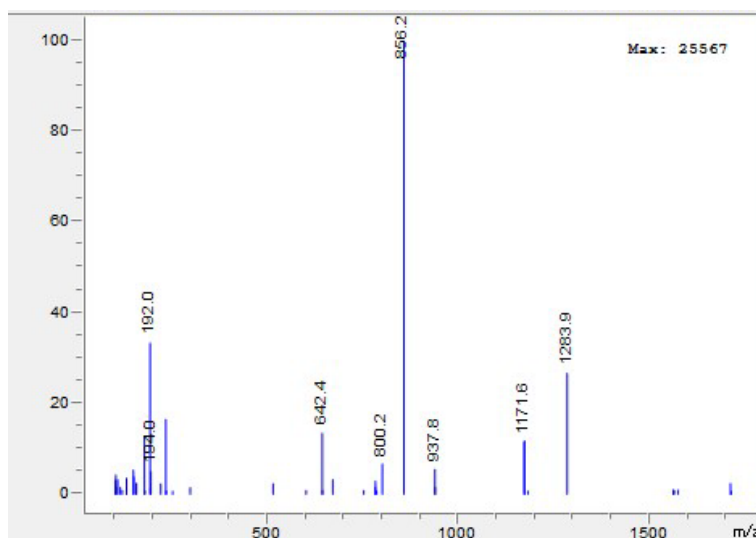


Figure 76.3 ESI-MS calcd. For $C_{126}H_{179}N_{35}O_{20}S_2$, $[M+2H]^{2+}$ $m/z = 1284.2$, found 1283.9; $[M+3H]^{3+}$ $m/z = 856.5$, found 856.2; $[M+4H]^{4+}$ $m/z = 642.6$, found 642.4.

Synthesis of **1r2h3a**



Figure 77.1 Structure of **1r2h3a**

1r (5.0 mg, 2.2 μmol) and **3a** (2.4 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 2.0 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (2.4 μmol , 1.1 equiv) was added; for **2h**, 1.8 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1r2h3a** was observed, with a conversion yield of 45%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1r2h3a** (1.4 mg, 25%)

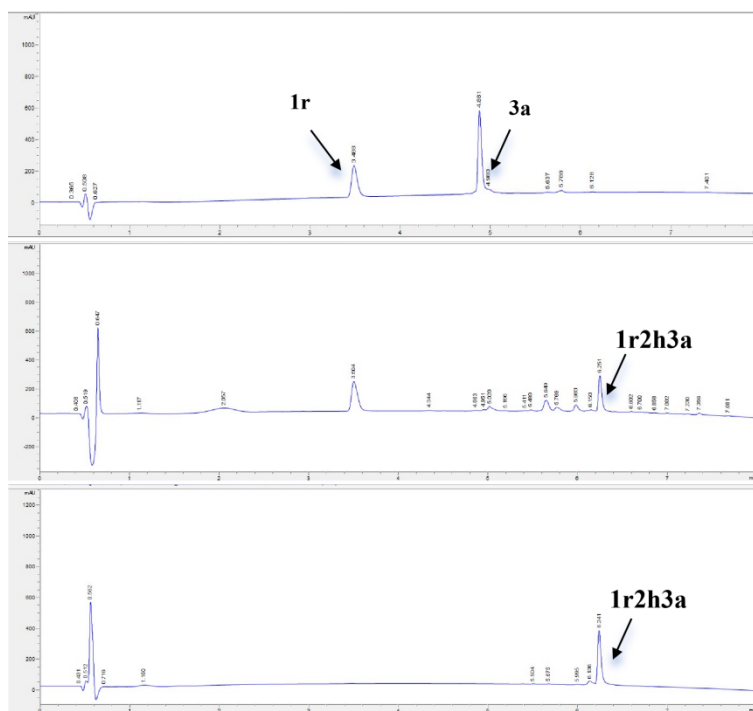


Figure 77.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%-70% ACN/H₂O with 0.1% TFA over 8 min.

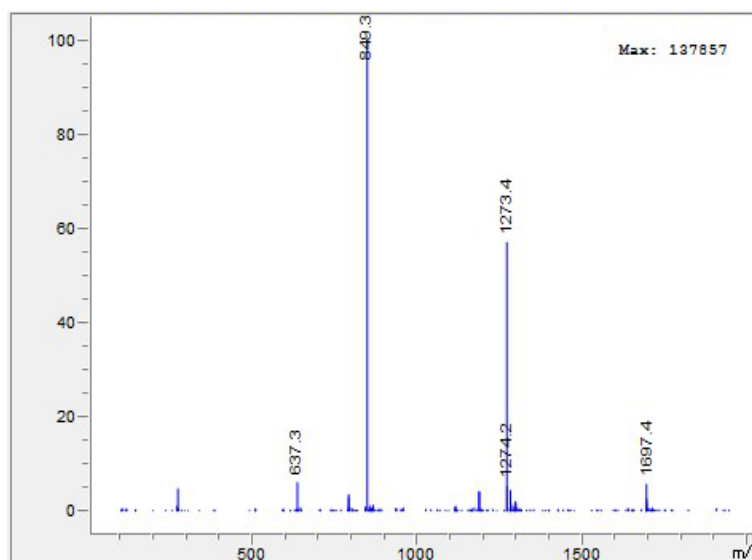


Figure 77.3 ESI-MS calcd. For C₁₂₀H₁₈₈N₃₀O₂₇S₂, [M+2H]²⁺ m/z = 1273.7, found 1273.4; [M+3H]³⁺ m/z = 849.5, found 849.3.

Synthesis of **1s2h3a**



Figure 78.1 Structure of **1s2h3a**

1s (5.0 mg, 1.9 μmol) and **3a** (2.0 μmol , 1.1 equiv) were dissolved in PBS buffer (pH 4.0) to obtain a final concentration of 1 mM. For **3a**, 1.7 mg of **3a** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. Subsequently, **2h** (2.0 μmol , 1.1 equiv) was added; for **2h**, 1.5 mg of **2h** was first dissolved in 100 μL of PBS buffer (pH 4.0), then 20.0 μL of this solution was pipetted for use. The reaction mixture was incubated at room temperature for 10 min, during which the formation of conjugate **1s2h3a** was observed, with a conversion yield of 46%. The reaction mixture was then diluted with water, purified via HPLC using a linear gradient of 30–60% acetonitrile (ACN) in water over 30 min, and lyophilized to afford **1s2h3a** (1.4 mg, 25%)

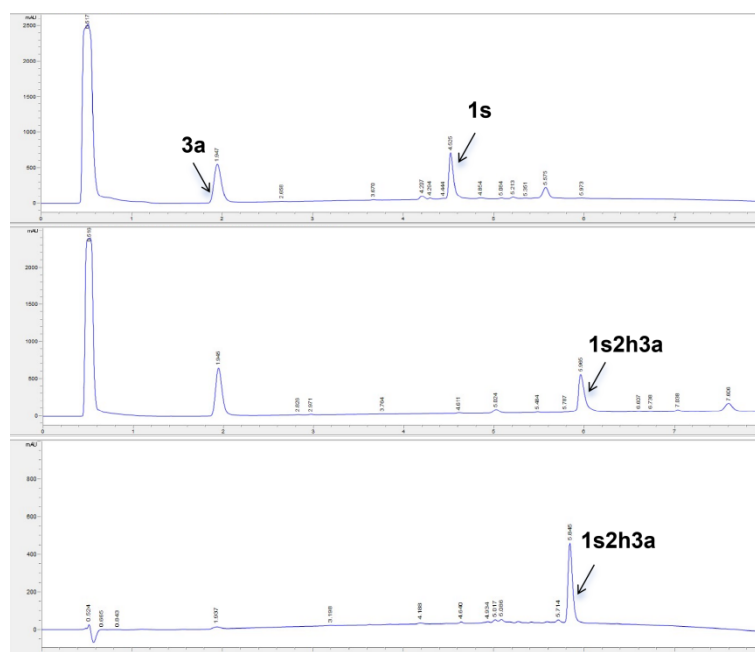


Figure 78.2 UV (205 nm) traces of the reaction: before addition of **2h**, after addition of **2h** and reacted for 10 min, and after HPLC purification. Gradient: 5%–70% ACN/H₂O with 0.1% TFA over 8 min.

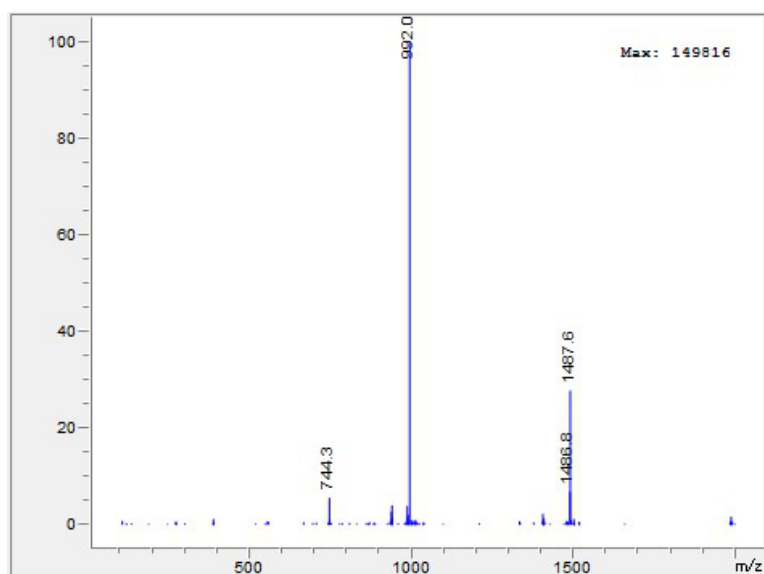


Figure 78.3 ESI-MS calcd. For $C_{140}H_{216}N_{30}O_{37}S_2$, $[M+2H]^{2+}$ $m/z = 1487.8$, found 1487.7; $[M+3H]^{3+}$ $m/z = 991.2$, found 992.0; $[M+4H]^{4+}$ $m/z = 744.4$, found 744.3.

7 Synthesis of peptide-peptide conjugates

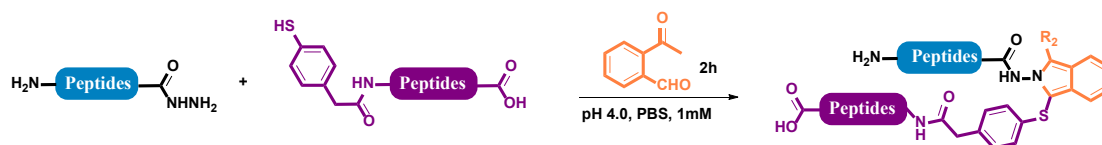


Figure 79.1 Synthetic scheme of peptide-peptide conjugates

Synthesis of 1m2h3g

1m (5.3 mg, 1.6 μ mol) and **3g** (3.7 mg, 1.3 μ mol) were dissolved in 300 μ L PBS (pH 4), followed by the addition of **2h** and thorough mixing. After the reaction proceeded at room temperature for 1 hour, LC-MS analysis confirmed the formation of conjugate **1m2h3g**, with a conversion yield of 78%. The reaction mixture was subsequently purified via HPLC (gradient elution: 20-70% CH₃CN/H₂O over 30 min), and lyophilized to afford **1m2h3g** as a pink powder (1.7 mg, 18%).

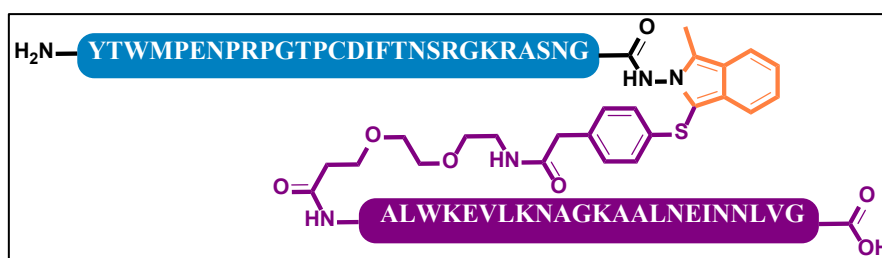


Figure 80.1 Chemical structure of **1m2h3g**.

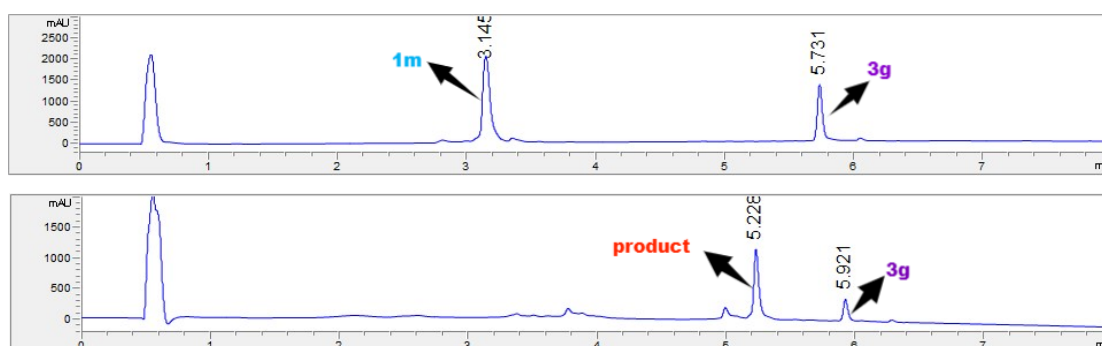


Figure 80.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 1hr. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

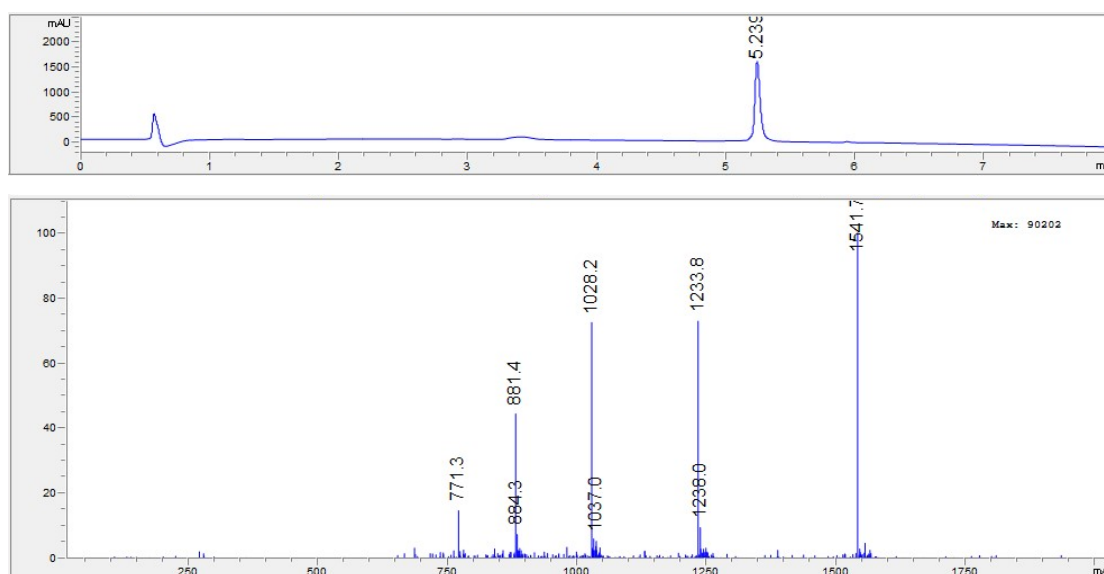


Figure 80.3 LC-MS analysis of HPLC purified 1m2h3g. UV (205 nm) and MS (100-2000 m/z). Gradient: 30%-60% ACN/H₂O with 0.1% TFA over 8 min. Calcd. for 1m2h3g C₂₇₆H₄₂₇N₇₇O₇₈S₃, [M+4H]⁴⁺ m/z = 1542.5, found 1541.7; [M+5H]⁵⁺ m/z = 1234.2, found 1233.8; [M+6H]⁶⁺ m/z = 1028.6, found 1028.2; [M+7H]⁷⁺ m/z = 881.8, found 881.4; [M+8H]⁸⁺ m/z = 771.7, found 771.3.

Synthesis of 1d2h3h

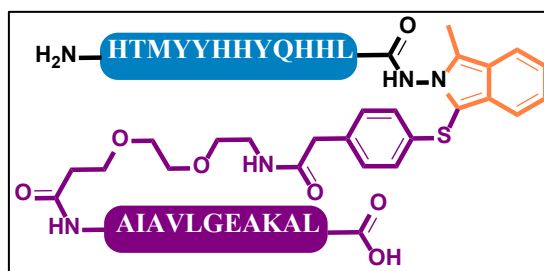
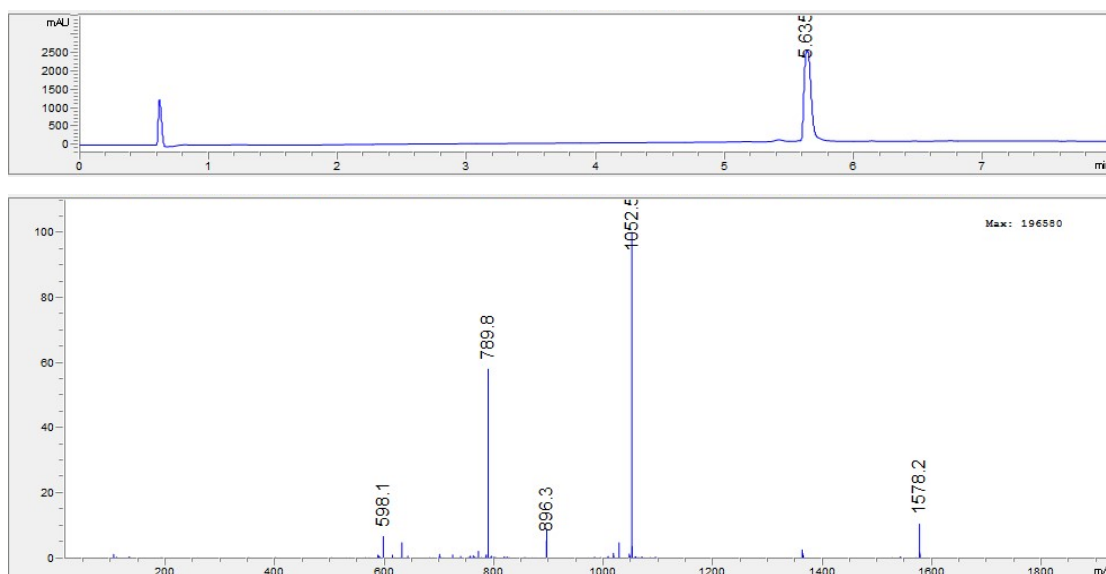
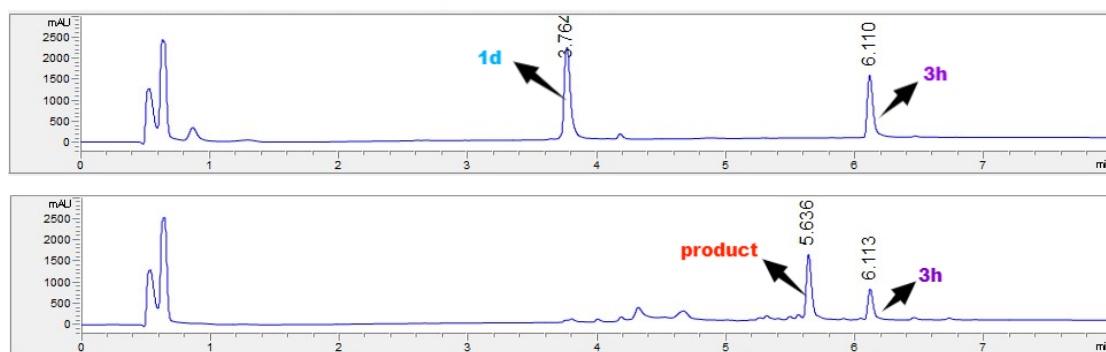


Figure 81.1 Structure of 1d2h3h

1d (5.0 mg, 2.9 μmol) and peptide **3h** (2.0 mg, 1.4 μmol) were added into 594 μL pH 4 PBS buffer containing 6M GnHCl, followed by the addition of **2h** for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 65% yield. After 10 min, the reaction was subjected to HPLC purification (2-70% CH₃CN/H₂O over 30 min, C18) and lyophilization to afford 1.5 mg (32% isolation yield) of peptide-peptide conjugate **1d2h3h** as a blue powder.



Synthesis of 1h2h3i

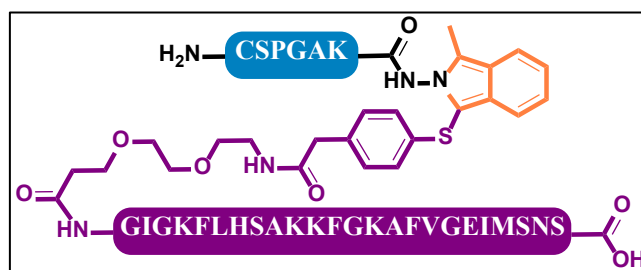


Figure 82.1 Structure of 1h2h3i

1h (5.0 mg, 8.6 μ mol) and peptide **3i** (12.0 mg, 4.2 μ mol) were added into 1735 μ L pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction

mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 72% yield. After 10 min, the reaction was subjected to HPLC purification (2-70% CH₃CN/H₂O over 30 min, C18) and lyophilization to afford 7.2 mg (48% isolation yield) of peptide-peptide conjugate **1h2h3i** as a purple powder.

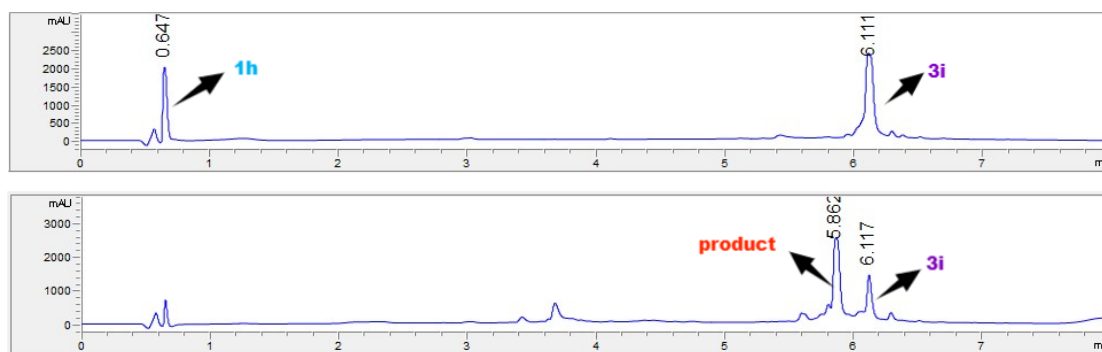


Figure 82.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

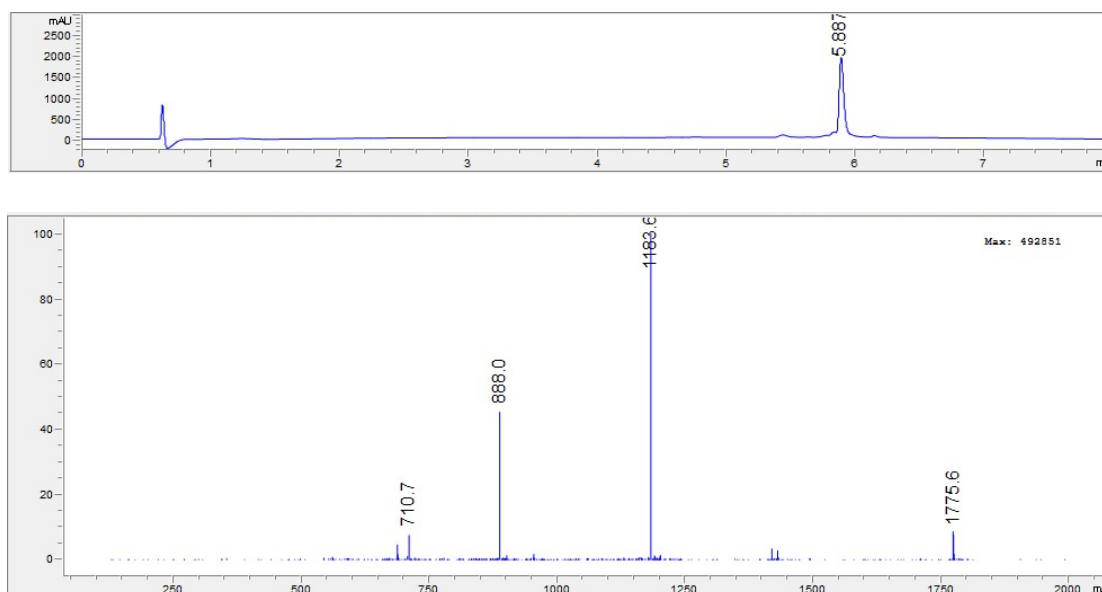


Figure 82.3 UV and MS trace from UPLC-MS analysis of **1h2h3i**. ESI-MS calcd. for C₁₆₃H₂₄₉N₄₁O₄₂S₃, [M+2H]²⁺ m/z = 1776.3, found 1775.6; [M+3H]³⁺ m/z = 1184.5, found 1183.6; [M+4H]⁴⁺ m/z = 888.6, found 888.0; [M+5H]⁵⁺ m/z = 711.1, found 710.7.

Synthesis of **1i2h3j**

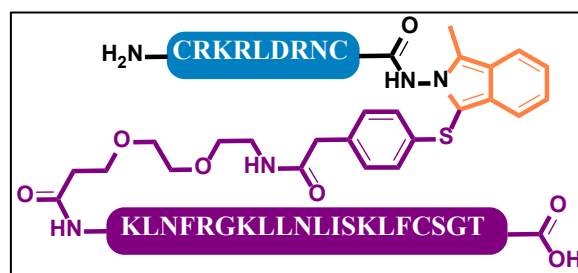


Figure 83.1 Structure of **1i2h3j**

1i (5.4 mg, 4.6 μ mol) and peptide **3j** (4.7 mg, 1.9 μ mol) were added into 845 μ L pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 55% yield. After 10 min, the reaction was subjected to HPLC purification (2-80% CH₃CN/H₂O over 30 min, C18) and lyophilization to afford 1.2 mg (15% isolation yield) of peptide-peptide conjugate **1i2h3j** as a white powder.

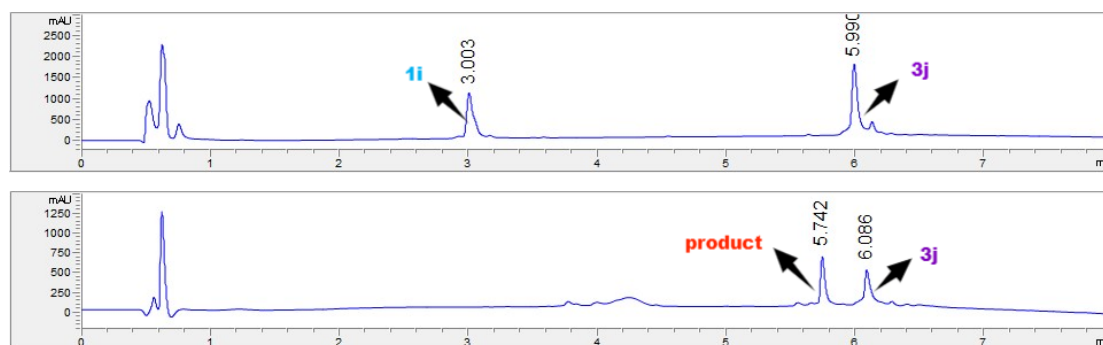


Figure 83.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

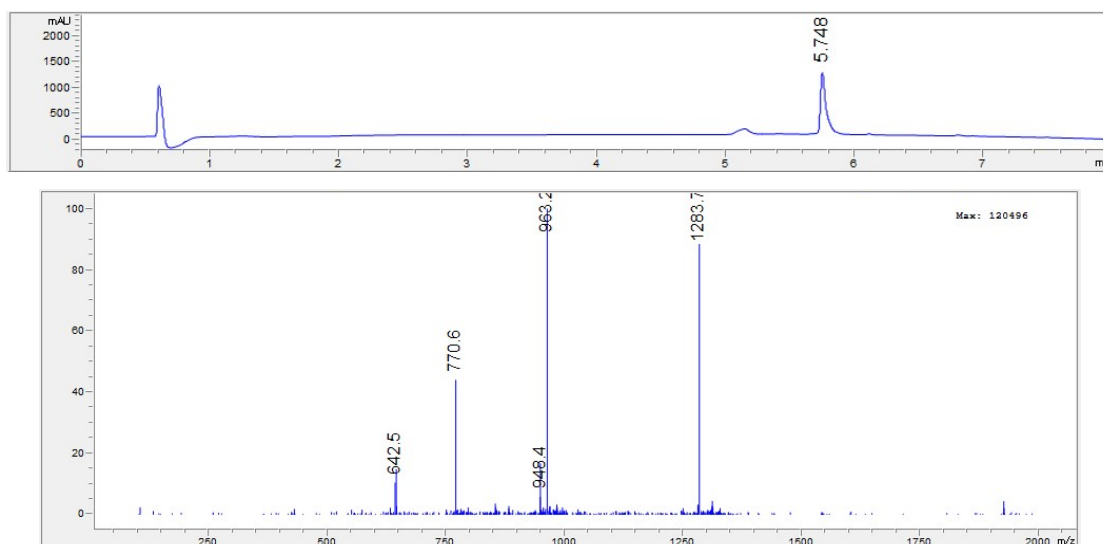


Figure 83.3 UV and MS trace from UPLC-MS analysis of **1i2h3j**. ESI-MS calcd. for $C_{171}H_{281}N_{51}O_{42}S_4$, $[M+3H]^{3+}$ $m/z = 1284.3$, found 1283.7; $[M+4H]^{4+}$ $m/z = 963.5$, found 963.2; $[M+5H]^{5+}$ $m/z = 771.0$, found 770.6; $[M+6H]^{6+}$ $m/z = 642.6$, found 642.5.

Immune-Checkpoint Blockage Bivalent Peptide Conjugates

Synthesis of 1t2h3k

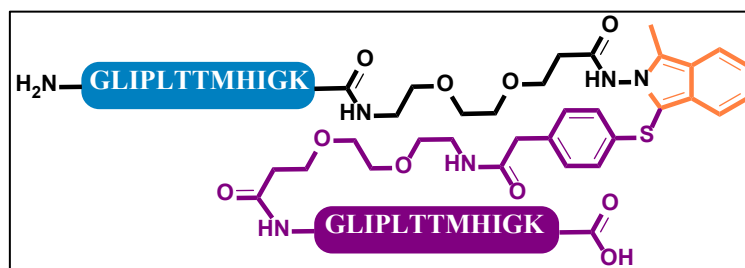


Figure 84.1 Structure of **1t2h3k**

1t (5.4 mg, 3.7 μmol) and peptide **3k** (5.0 mg, 3.1 μmol) were added into 685 μL pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 87% yield. After 10 min, the reaction was subjected to HPLC purification (15-60% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 5.1 mg (47% isolation yield) of peptide-peptide conjugate **1t2h3k** as a blue-white powder.

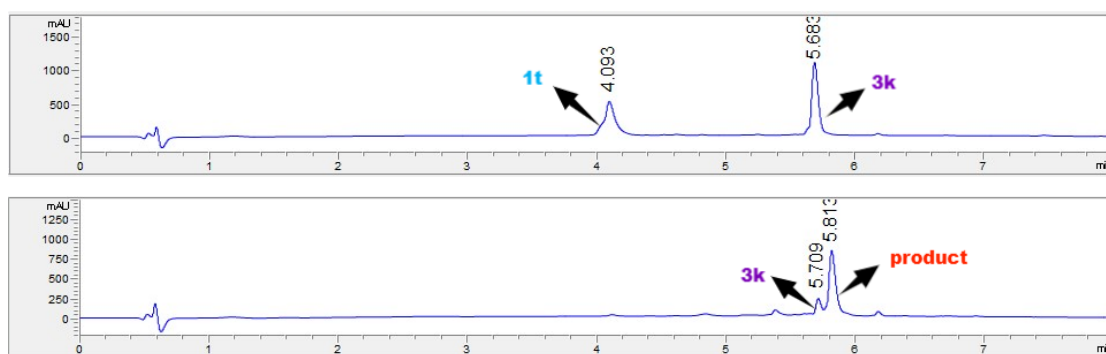


Figure 84.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

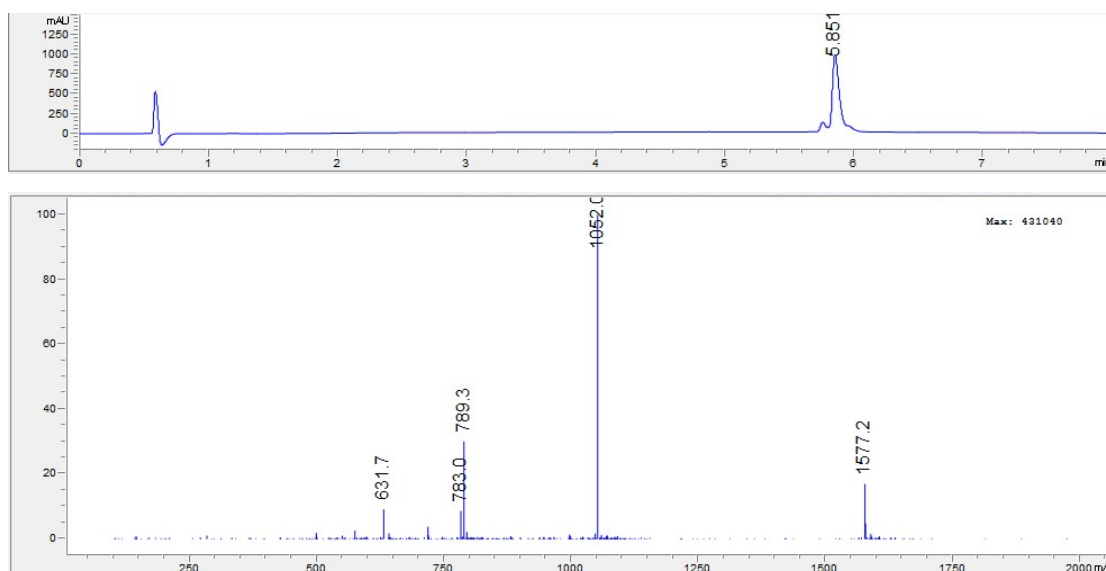


Figure 84.3 UV and MS trace from UPLC-MS analysis of **1t2h3k**. ESI-MS calcd. for C₁₄₇H₂₄₀N₃₄O₃₆S₃, [M+2H]²⁺ m/z = 1578.36, found 1577.2; [M+3H]³⁺ m/z = 1052.5, found 1052.0; [M+4H]⁴⁺ m/z = 789.6, found 789.3; [M+5H]⁵⁺ m/z = 631.9, found 631.7.

Synthesis of **1t2h3l**

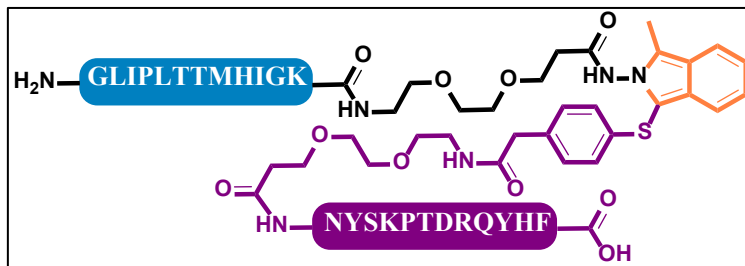


Figure 85.1 Structure of **1t2h3l**

1t (5.5 mg, 3.7 μmol) and peptide **3k** (4.8 mg, 2.6 μmol) were added into 685 μL pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 83% yield. After 10 min, the reaction was subjected to HPLC purification (2-80% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 5.0 mg (54% isolation yield) of peptide-peptide conjugate **1t2h3l** as a blue powder.

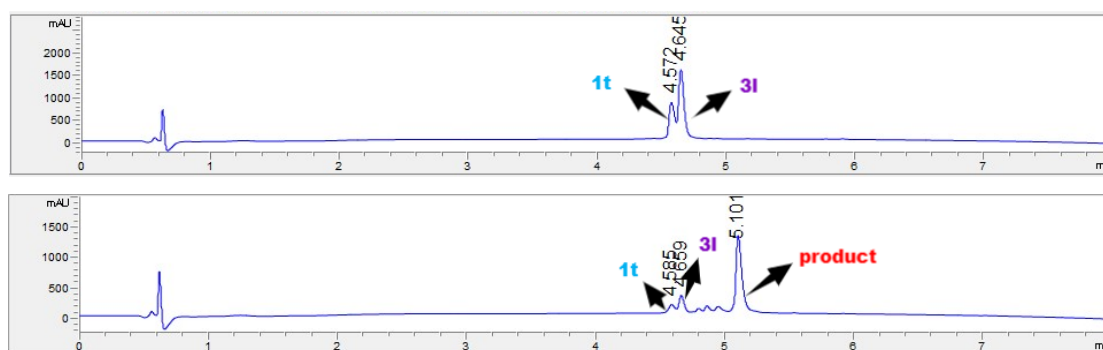


Figure 85.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/ H_2O with 0.1% TFA over 8 min.

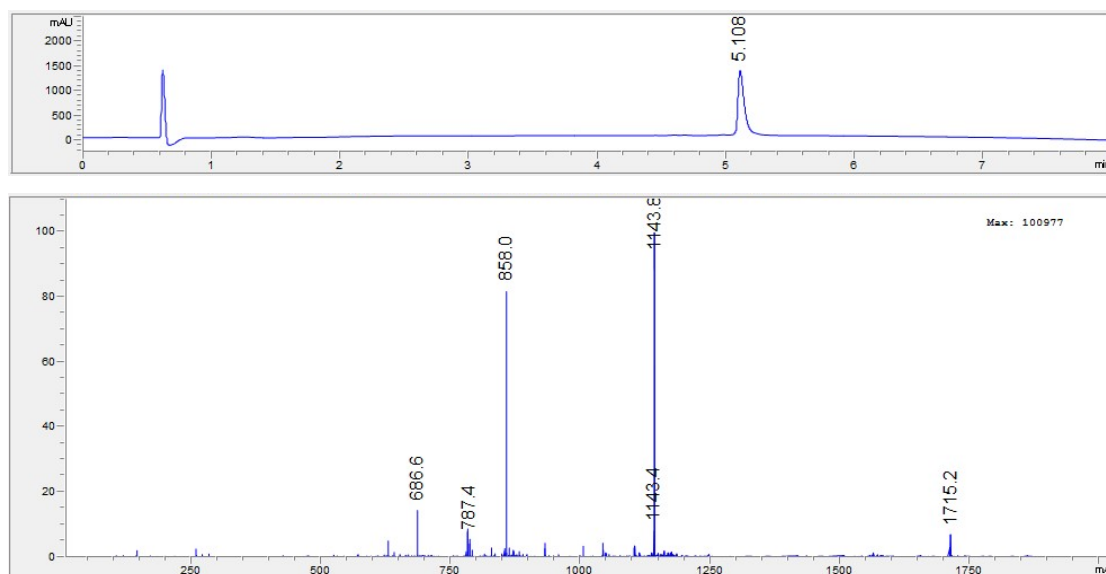


Figure 85.3 UV and MS trace from UPLC-MS analysis of **1t2h3l**. ESI-MS calcd. for $C_{159}H_{237}N_{39}O_{42}S_2$, $[M+2H]^{2+}$ $m/z = 1716.3$, found 1715.2; $[M+3H]^{3+}$ $m/z = 1144.5$, found 1143.8; $[M+4H]^{4+}$ $m/z = 858.6$, found 858.0; $[M+5H]^{5+}$ $m/z = 687.1$, found 686.6.

Synthesis of 1t2h3m

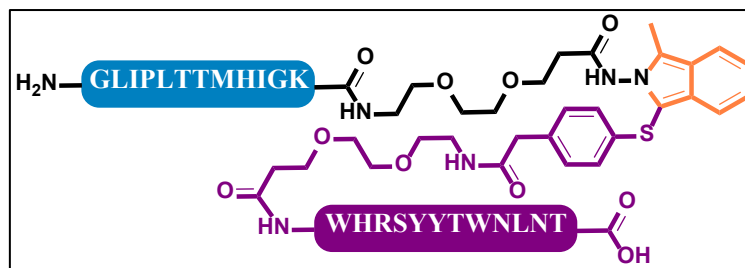


Figure 86.1 Structure of **1t2h3m**

1t (5.2 mg, 3.5 μ mol) and peptide **3m** (4.6 mg, 2.4 μ mol) were added into 480 μ L pH4 Gn-HCl buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 20 min and afforded the peptide-peptide conjugate in 50% yield. After 20 min, the reaction was subjected to HPLC purification (2-70% CH_3CN/H_2O over 30 min, C18) and lyophilization to afford 3.0 mg (25% isolation yield) of peptide-peptide conjugate **1t2h3m** as a blue powder.

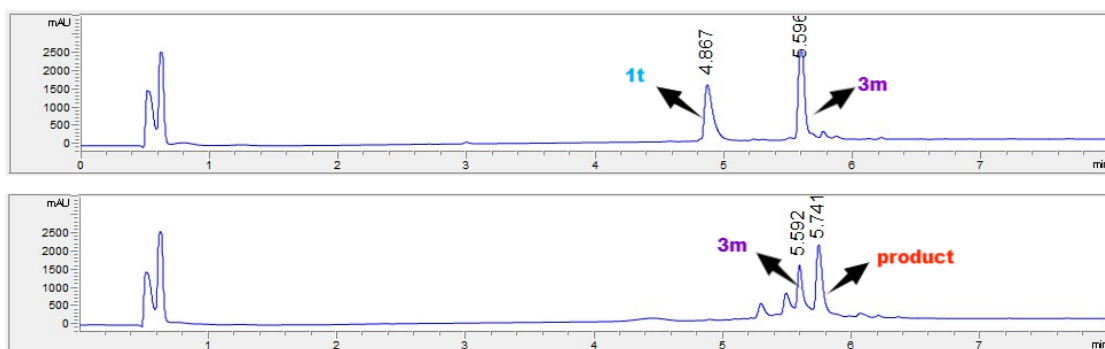


Figure 86.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

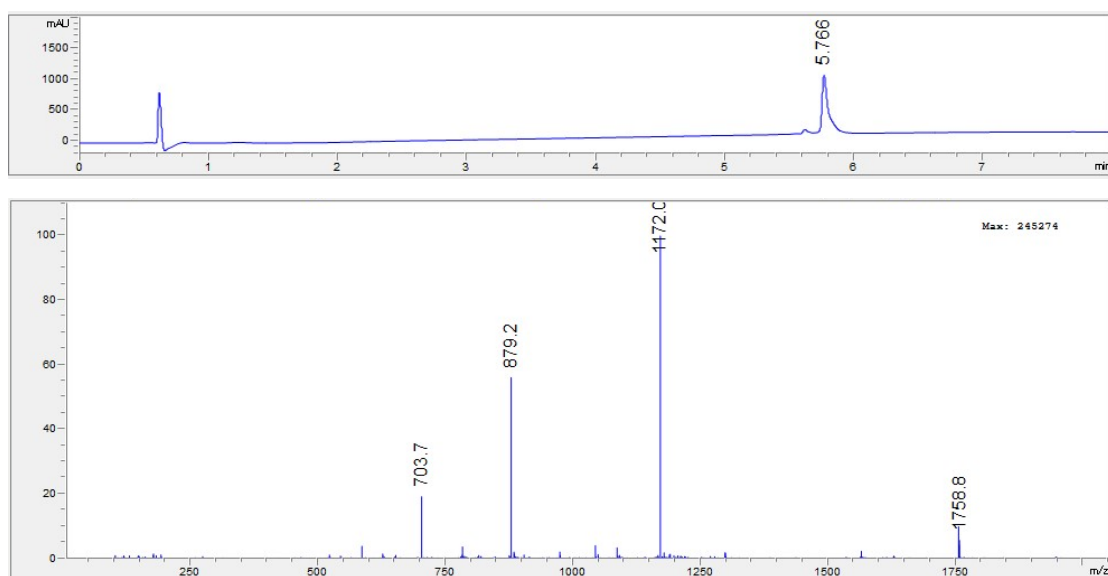


Figure 86.3 UV and MS trace from UPLC-MS analysis of **1t2h3m**. ESI-MS calcd. for C₁₆₆H₂₄₀N₄₀O₄₁S₂, [M+2H]²⁺ m/z = 1758.8, found 1758.8; [M+3H]³⁺ m/z = 1172.9, found 1172.0; [M+4H]⁴⁺ m/z = 879.9, found 879.2; [M+5H]⁵⁺ m/z = 704.1, found 703.7.

Synthesis of **1t2h3n**

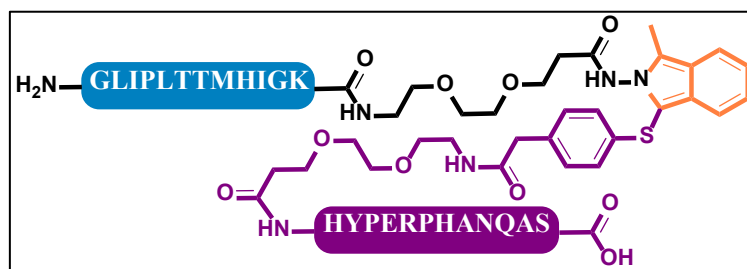


Figure 87.1 Structure of **1t2h3n**

1t (5.5 mg, 3.8 μmol) and peptide **3n** (4.8 mg, 2.8 μmol) were added into 685 μL pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 83% yield. After 10 min, the reaction was subjected to HPLC purification (10-50% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 3.7 mg (40% isolation yield) of peptide-peptide conjugate **1t2h3n** as a blue powder.

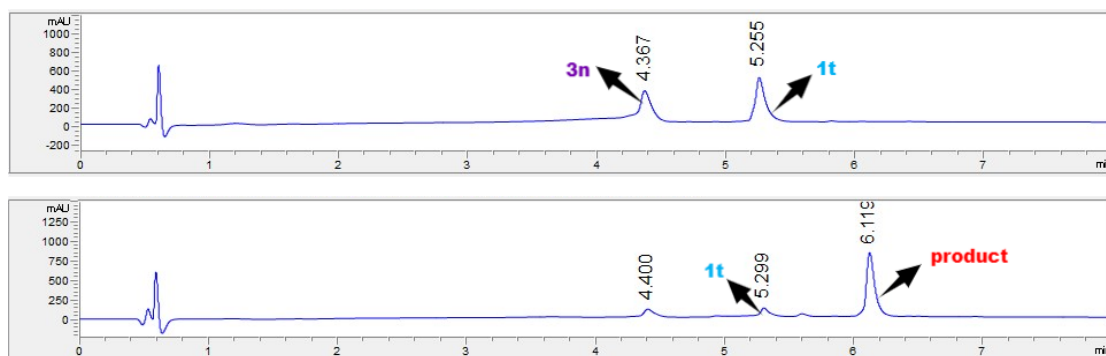


Figure 87.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/ H_2O with 0.1% TFA over 8 min.

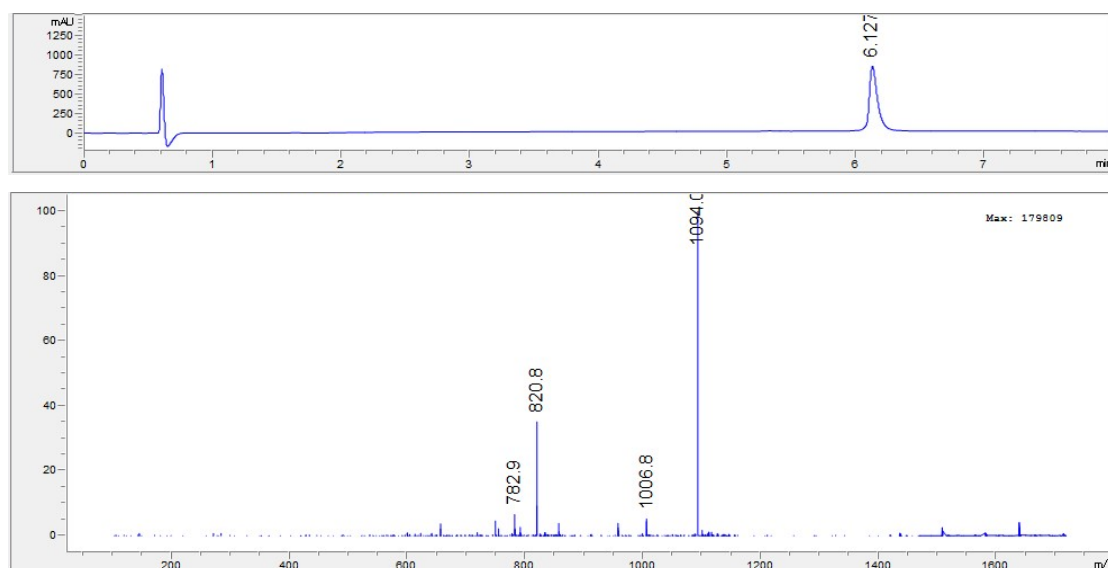


Figure 87.3 UV and MS trace from UPLC-MS analysis of **1t2h3n**. ESI-MS calcd. for $C_{149}H_{226}N_{40}O_{40}S_2$, $[M+3H]^{3+}$ $m/z = 1094.5$, found 1094.0; $[M+4H]^{4+}$ $m/z = 821.1$, found 820.8.

Synthesis of **1t2h3o**

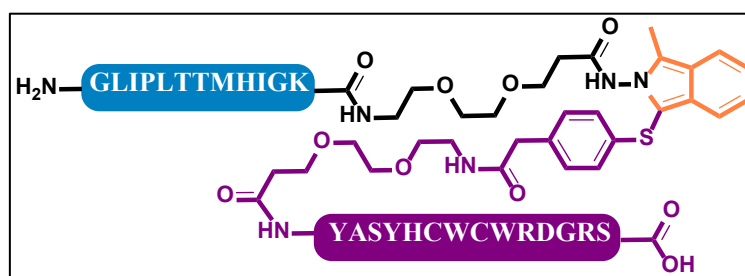


Figure 88.1 Structure of **1t2h3o**

1t (5.3 mg, 3.6 μ mol) and peptide **3o** (3.6 mg, 1.7 μ mol) were added into 685 μ L pH4 Gn-HCl buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 66% yield. After 10 min, the reaction was subjected to HPLC purification (15-60% CH_3CN/H_2O over 30 min, C18) and lyophilization to afford 2.2 mg (18% isolation yield) of peptide-peptide conjugate **1t2h3o** as a green powder.

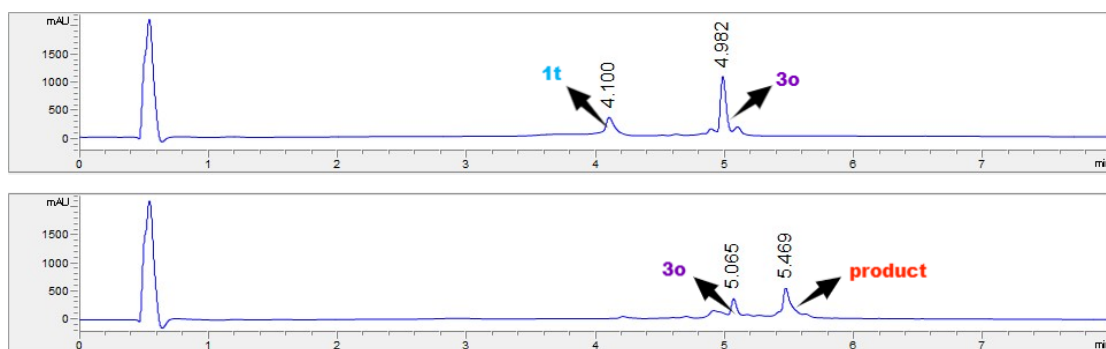


Figure 88.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

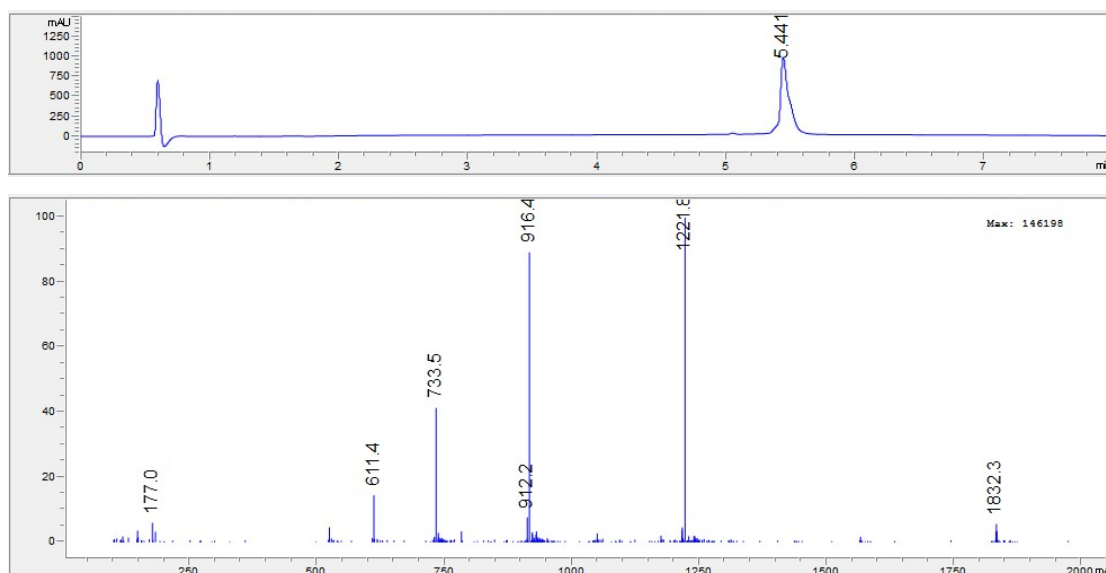


Figure 88.3 UV and MS trace from UPLC-MS analysis of **1t2h3o** ESI-MS calcd. for C₁₆₈H₂₄₃N₄₃O₄₂S₄, [M+2H]²⁺ m/z = 1832.8, found 1832.3; [M+3H]³⁺ m/z = 1222.2, found 1221.8; [M+4H]⁴⁺ m/z = 916.9, found 916.4; [M+5H]⁵⁺ m/z = 733.7, found 733.5; [M+6H]⁶⁺ m/z = 611.6, found 611.4.

Biepitotic Peptide Conjugates

Synthesis of **1e2h3l**

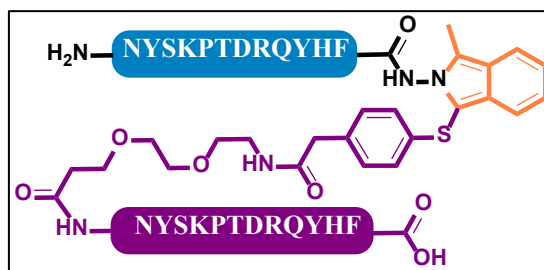


Figure 89.1 Structure of **1e2h3l**

1e (5.4 mg, 3.4 μ mol) and peptide **3l** (4.6 mg, 2.5 μ mol) were added into 505 μ L pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 80% yield. After 10 min, the reaction was subjected to HPLC purification (2-60% CH₃CN/H₂O over 30 min, C18) and lyophilization to afford 4.2 mg (37% isolation yield) of peptide-peptide conjugate **1e2h3l** as a green powder.

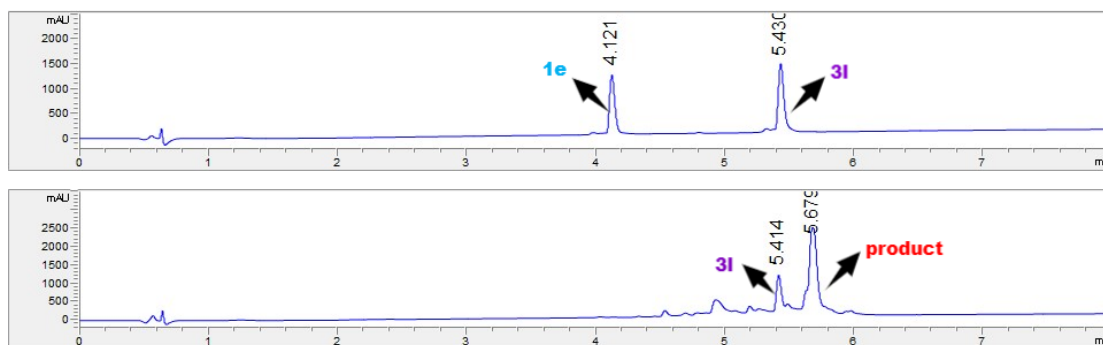


Figure 89.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

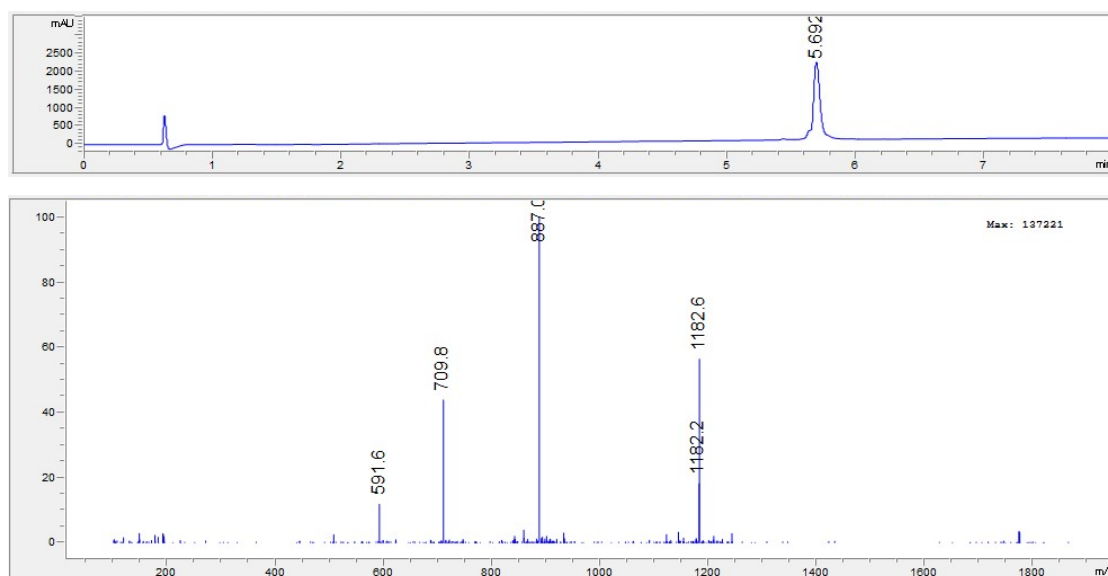


Figure 89.3 UV and MS trace from UPLC-MS analysis of **1e2h3l**. ESI-MS calcd. for $C_{164}H_{221}N_{43}O_{45}S$, $[M+3H]^{3+}$ $m/z = 1183.2$, found 1182.6; $[M+4H]^{4+}$ $m/z = 887.6$, found 887.0; $[M+5H]^{5+}$ $m/z = 710.3$, found 709.8; $[M+6H]^{6+}$ $m/z = 592.1$, found 591.6.

Synthesis of **1e2h3m**

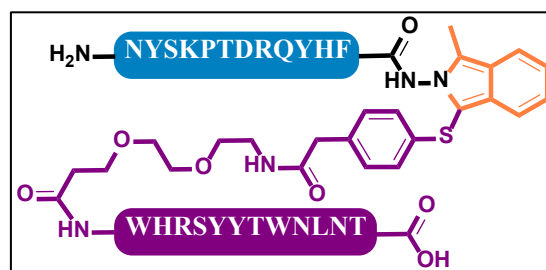


Figure 90.1 Structure of **1e2h3m**

1e (5.4 mg, 3.4 μmol) and peptide **3m** (4.8 mg, 2.5 μmol) were added into 505 μL pH4 Gn-HCl buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 52% yield. After 10 min, the reaction was subjected to HPLC purification (2-70% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 2.2 mg (19% isolation yield) of peptide-peptide conjugate **1e2h3m** as a green powder.

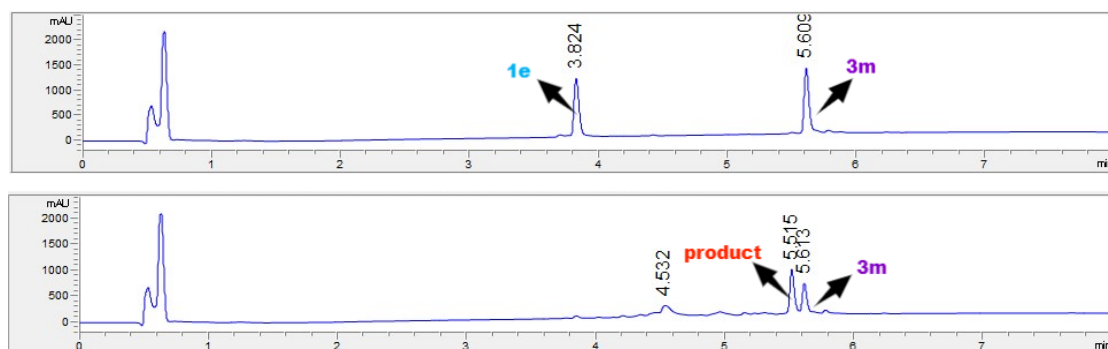


Figure 90.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

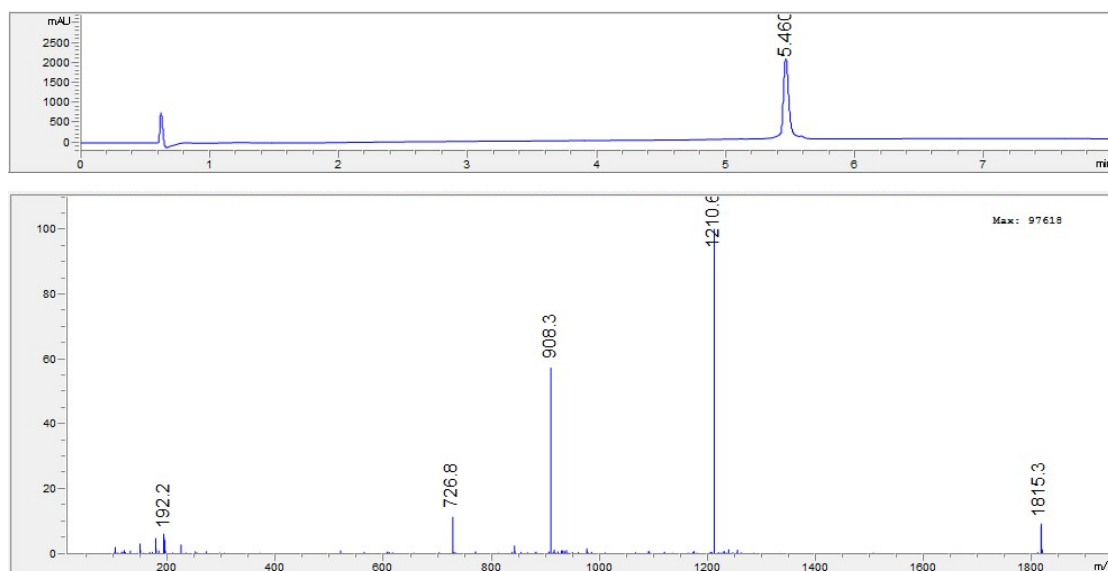


Figure 90.3 UV and MS trace from UPLC-MS analysis of **1e2h3m**. ESI-MS calcd. for C₁₇₁H₂₂₄N₄₄O₄₄S, [M+2H]²⁺ m/z = 1816.8, found 1815.3; [M+3H]³⁺ m/z = 1211.5, found 1210.6; [M+4H]⁴⁺ m/z = 908.9, found 908.3; [M+5H]⁵⁺ m/z = 727.3, found 726.8.

Synthesis of **1e2h3n**

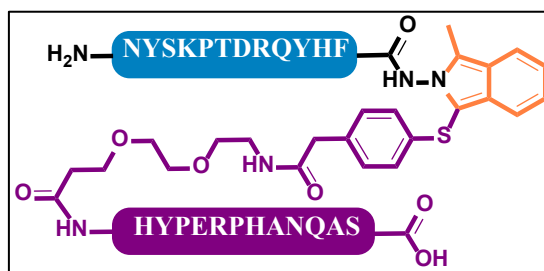


Figure 91.1 Structure of **1e2h3n**

1e (5.1 mg, 3.2 μmol) and peptide **3n** (4.9 mg, 2.8 μmol) were added into 585 μL pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 73% yield. After 10 min, the reaction was subjected to HPLC purification (2-60% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 3.6 mg (33% isolation yield) of peptide-peptide conjugate **1e2h3n** as a green powder.

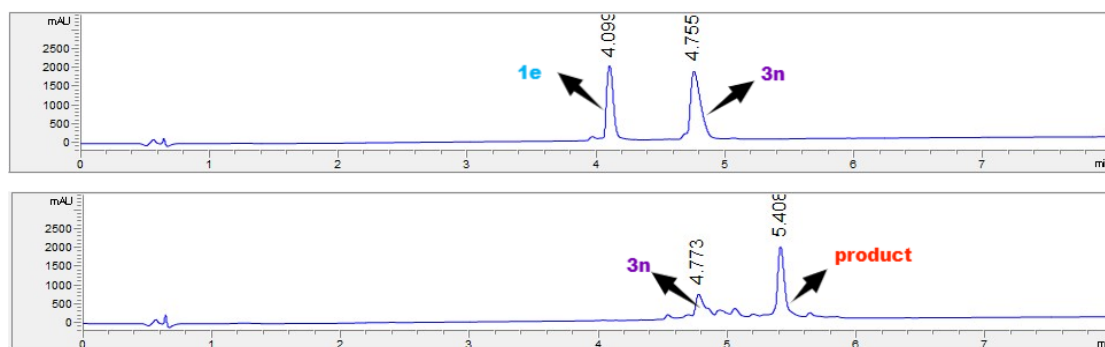


Figure 91.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% $\text{ACN}/\text{H}_2\text{O}$ with 0.1% TFA over 8 min.

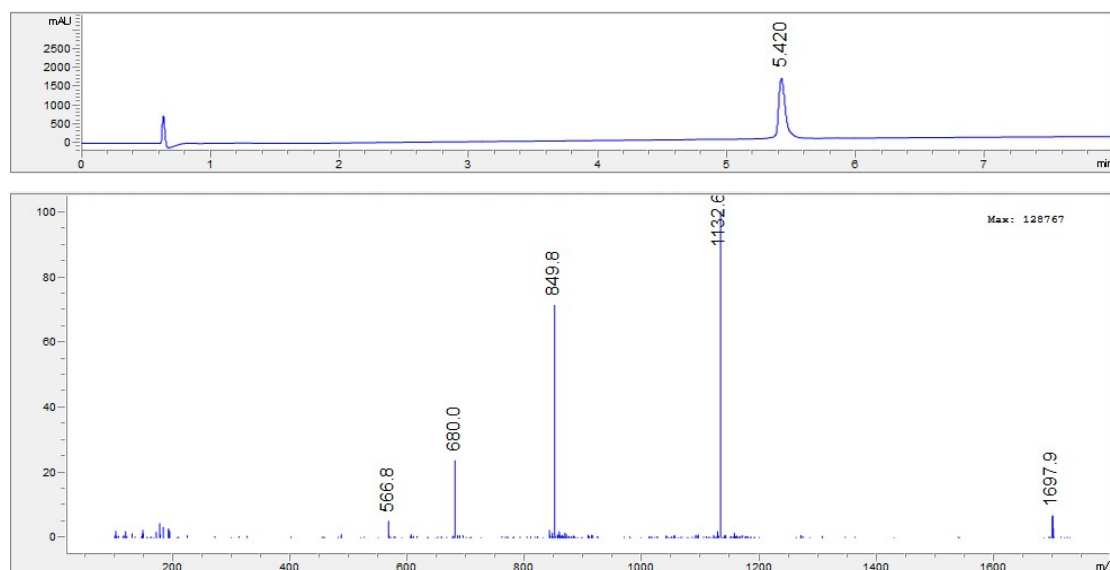


Figure 91.3 UV and MS trace from UPLC-MS analysis of **1e2h3n**. ESI-MS calcd. for $C_{154}H_{210}N_{44}O_{43}S$, $[M+2H]^{2+}$ $m/z = 1699.2$, found 1697.9; $[M+3H]^{3+}$ $m/z = 1133.1$, found 1132.6; $[M+4H]^{4+}$ $m/z = 850.1$, found 849.8; $[M+5H]^{5+}$ $m/z = 680.3$, found 680.0; $[M+6H]^{6+}$ $m/z = 567.0$, found 566.8.

Synthesis of **1e2h3o**

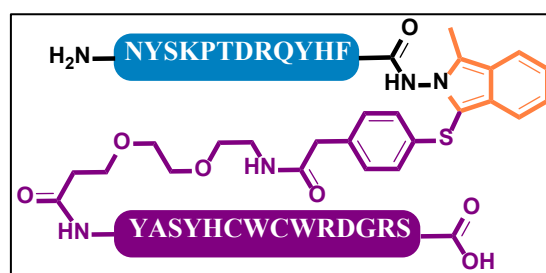


Figure 92.1 Structure of **1e2h3o**

1e (1.0 mg, 0.6 μ mol) and peptide **3o** (1.3 mg, 0.6 μ mol) were added into 125 μ L pH4 Gn-HCl buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 66% yield. After 10 min, the reaction was subjected to HPLC purification (2-80% CH_3CN/H_2O over 30 min, C18), but it was found that **3o** and the product could not be separated.

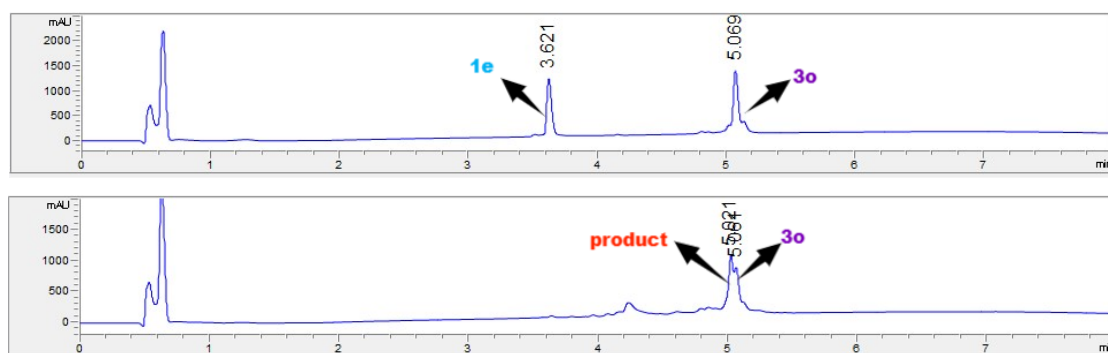


Figure 92.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

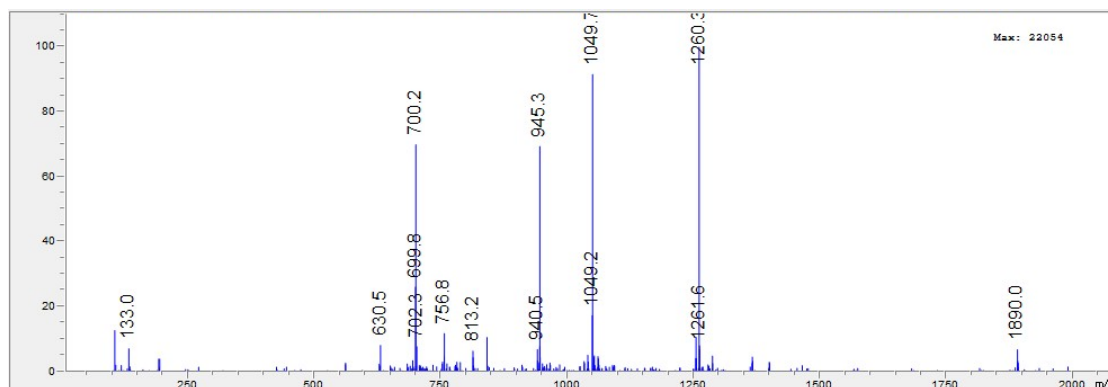


Figure 92.3 MS spectrum of **1e2h3o** ESI-MS calcd. for C₁₇₃H₂₂₇N₄₇O₄₅S₃, [M+2H]²⁺ m/z = 1891.3, found 1890.0; [M+3H]³⁺ m/z = 1261.2, found 1260.3; [M+4H]⁴⁺ m/z = 946.1, found 945.3; [M+5H]⁵⁺ m/z = 757.1, found 756.8; [M+6H]⁶⁺ m/z = 631.1, found 630.5. MS spectrum of **3o** ESI-MS calcd. for C₉₄H₁₂₃N₂₅O₂₅S₃, [M+2H]²⁺ m/z = 1050.4, found 1049.7; [M+3H]³⁺ m/z = 700.6, found 700.2.

Bitargeting Peptide Conjugates

Synthesis of **1e2h3k**

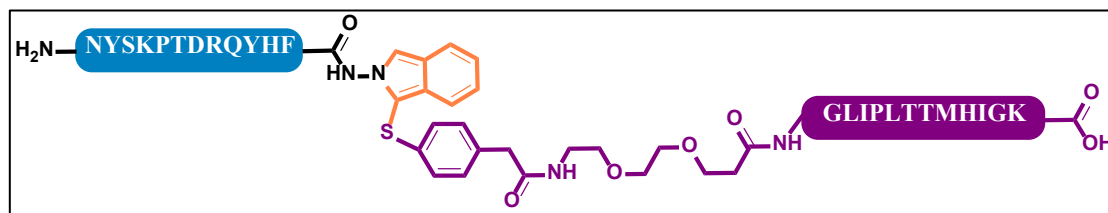


Figure 93.1 Structure of **1e2h3k**

1e (5.1 mg, 3.2 μ mol) and peptide **3k** (4.9 mg, 3.0 μ mol) were added into 625 μ L pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 76% yield. After 10 min, the reaction was subjected to HPLC purification (2-70% CH₃CN/H₂O over 30 min, C18) and lyophilization to afford 4.3 mg (41% isolation yield) of peptide-peptide conjugate **1e2h3k** as a green powder.

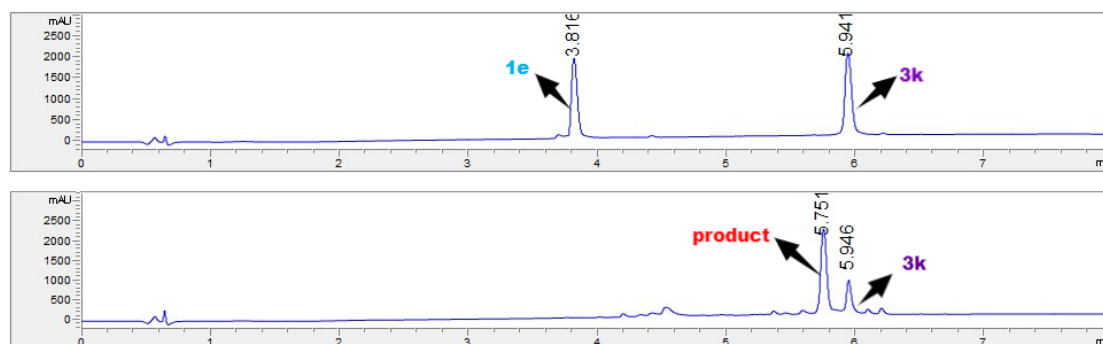


Figure 93.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

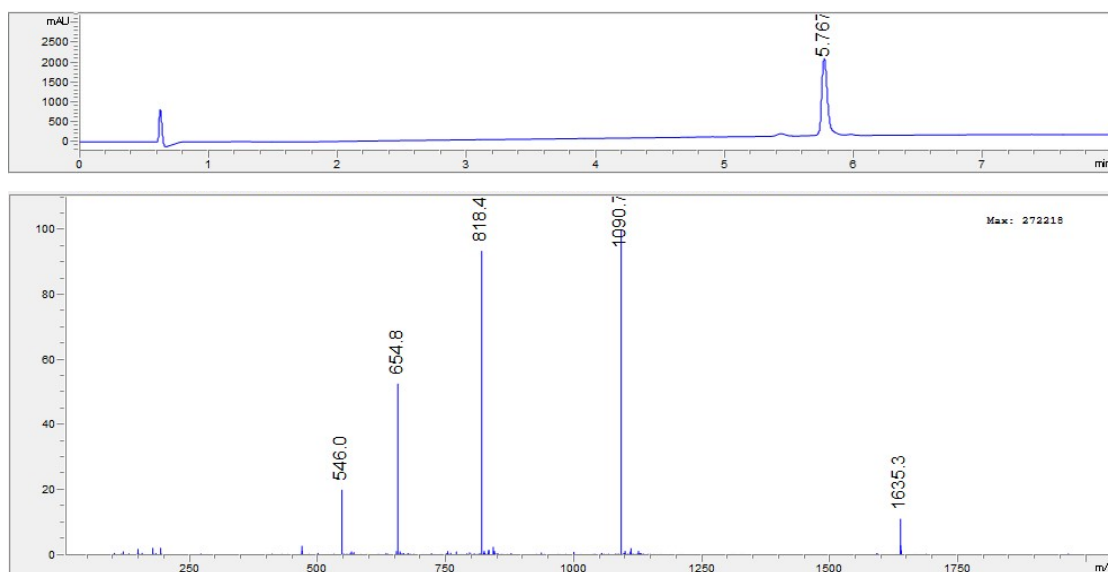


Figure 93.3 UV and MS trace from UPLC-MS analysis of **1c2h3k** ESI-MS calcd. for $C_{152}H_{224}N_{38}O_{39}S_2$, $[M+2H]^{2+}$ $m/z = 1636.8$, found 1635.3; $[M+3H]^{3+}$ $m/z = 1091.5$, found 1090.7; $[M+4H]^{4+}$ $m/z = 818.9$, found 818.4; $[M+5H]^{5+}$ $m/z = 655.3$, found 654.8; $[M+6H]^{6+}$ $m/z = 546.2$, found 546.0.

Synthesis of **1c2h3k**

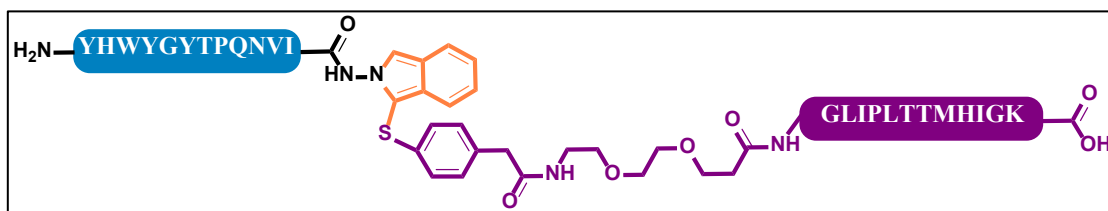


Figure 94.1 Structure of **1c2h3k**

1c (5.4 mg, 3.5 μmol) and peptide **3k** (4.8 mg, 3.0 μmol) were added into 640 μL pH4 PBS buffer, followed by the addition of 2-acetyl benzaldehyde for the reaction. The reaction mixture was stirred at r.t. for 10 min and afforded the peptide-peptide conjugate in 89% yield. After 10 min, the reaction was subjected to HPLC purification (15-70% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 30 min, C18) and lyophilization to afford 3.7 mg (35% isolation yield) of peptide-peptide conjugate **1c2h3k** as a purple powder.

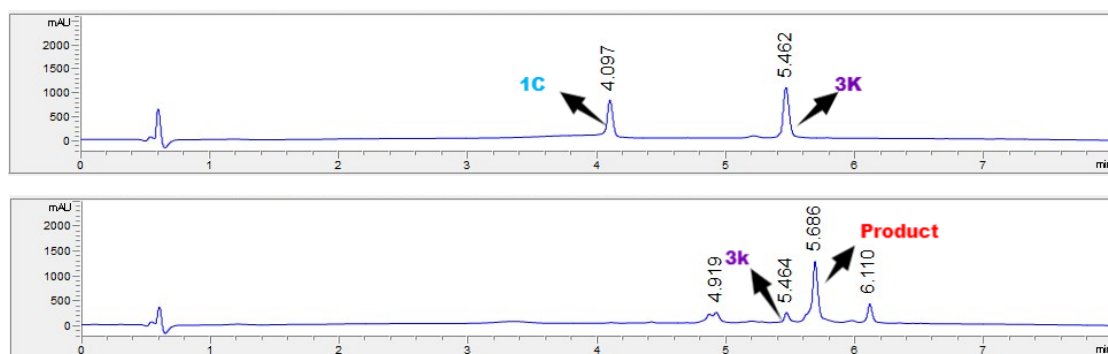


Figure 94.2 UV (205nm) trace from LC-MS analysis of before and after addition of **2h** for 10 min. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

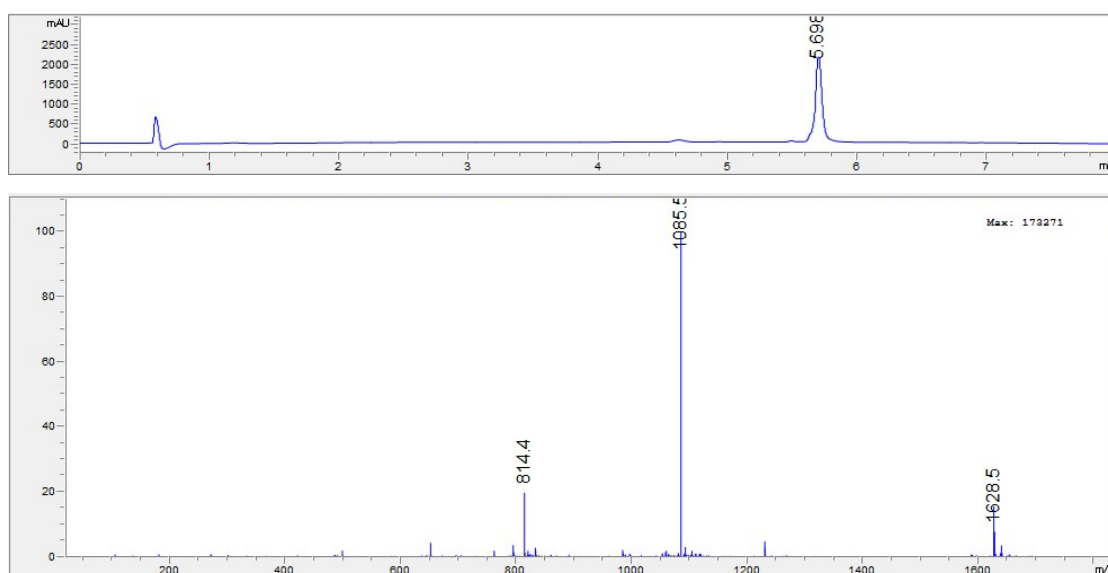


Figure 94.3 UV and MS trace from UPLC-MS analysis of **1c2h3k**. ESI-MS calcd. for C₁₅₇H₂₂₃N₃₅O₃₇S₂, [M+2H]²⁺ m/z = 1628.8, found 1628.5; [M+3H]³⁺ m/z = 1086.2, found 1085.5; [M+4H]⁴⁺ m/z = 814.9, found 814.4.

8 Synthesis of bicyclic peptides

Synthesis of **1u2h-2**

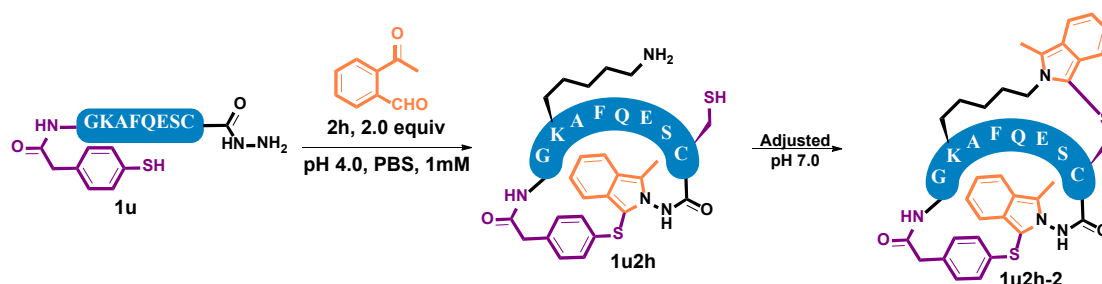


Figure 95.1 Synthetic scheme of bicyclic peptide **1u2h-2**

1u (2.5 mg, 2.4 μmol) was dissolved in PBS buffer (pH 4.0) to obtain a solution with a final concentration of 1 mM. Then, 2.0 equivalents of **2h** (0.7 mg, 4.8 μmol) was added to the above solution, and the reaction mixture was allowed to stir at room temperature for 10 minutes. The pH of the mixture was subsequently slowly adjusted to 7.0 using 1 M aqueous sodium hydroxide (NaOH) solution. After dilution with deionized water and filtration, the crude product was purified via preparative high-performance liquid chromatography (HPLC) with a gradient of 30%-60% acetonitrile/water (ACN/H₂O) over 30 minutes, followed by lyophilization to afford the product **1u2h-2** (0.8 mg, 26% yield).

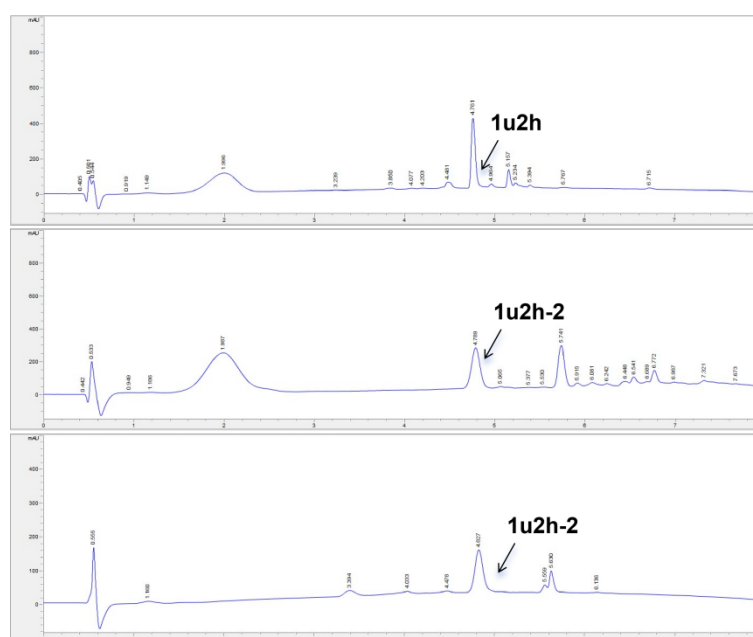


Figure 95.2 UV (205nm) trace from LC-MS analysis of first step, second step and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

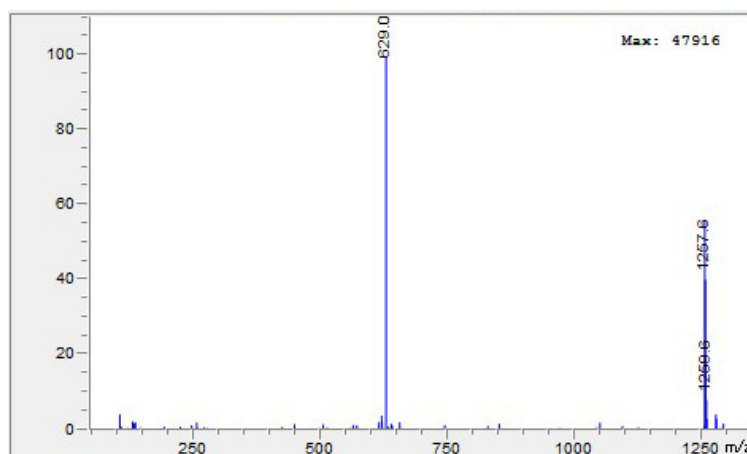


Figure 95.3 **1u2h-2** ESI-MS calcd. For $C_{62}H_{72}N_{12}O_{13}S_2$, $[M+H]^+$ $m/z = 1257.5$, found 1257.6; $[M+2H]^{2+}$ $m/z = 629.7$, found 629.0.

Synthesis of **1v2h-2**

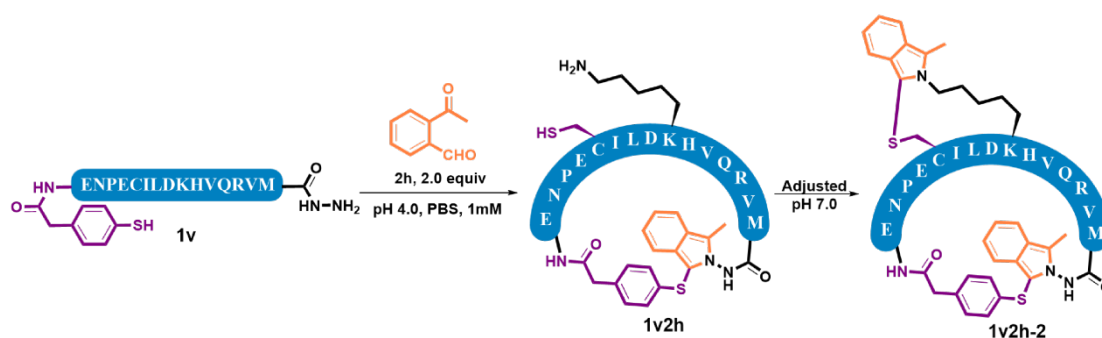


Figure 96.1 Synthetic scheme of bicyclic peptide **1v2h-2**

1v (2.5 mg, 1.2 μmol) was dissolved in PBS buffer (pH 4) to obtain a final concentration of 1 mM. 2.0 equivalents of compound **2h** (0.4 mg, 2.5 μmol) was added to the solution, and the reaction mixture was allowed to stand at room temperature for 10 min. The pH of the mixture was then slowly adjusted to 7.0 using 1 M NaOH solution. After dilution with ultrapure water and filtration, the crude product was purified via preparative HPLC with a gradient elution of 30%–60% acetonitrile/water (ACN/ H_2O) over 30 min. The purified fractions were lyophilized to afford **1v2h-2** (0.6 mg, 22% yield).

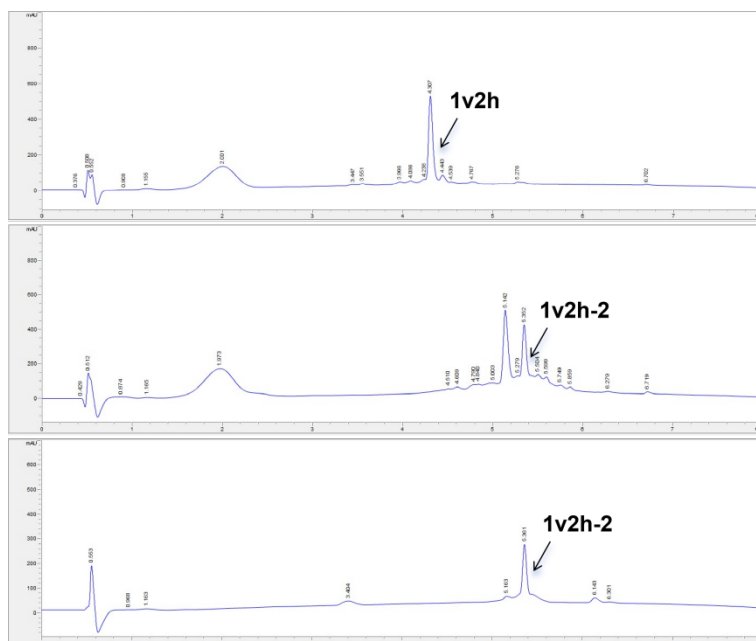


Figure 96.2 UV(205nm) trace from LC-MS analysis of first step, second step and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 10 min.

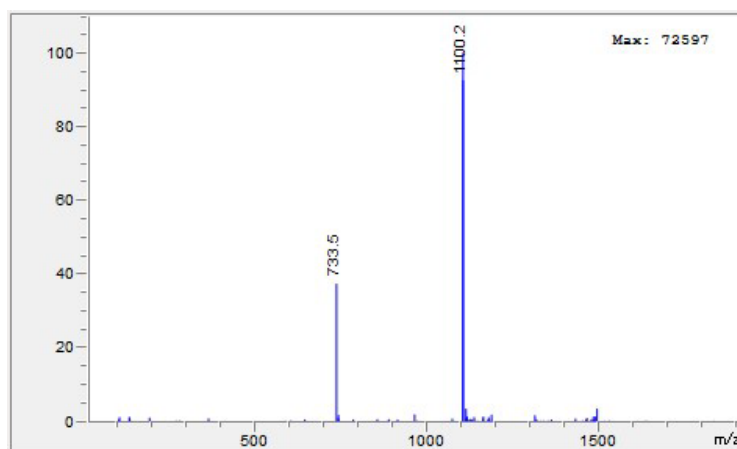


Figure 96.3 ESI-MS calcd. For C₁₀₂H₁₄₃N₂₅O₂₄S₃, [M+2H]²⁺ m/z =1100.0, found 1100.2; [M+3H]³⁺ m/z =733.6, found 733.5.

Synthesis of 1w2h-2

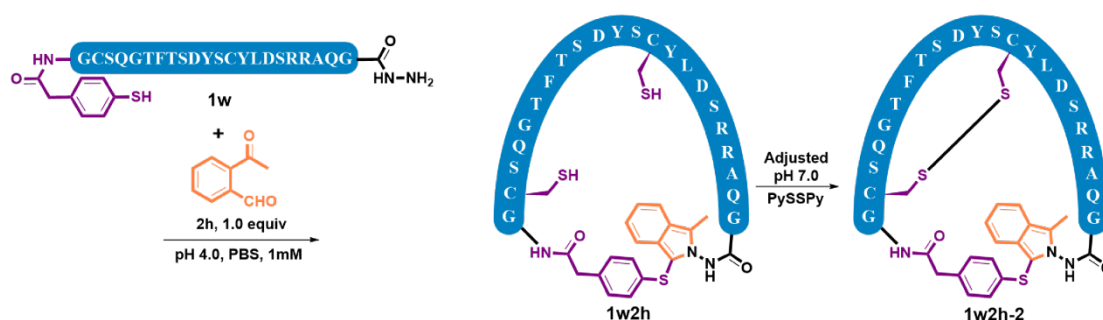


Figure 97.1 Synthetic scheme of bicyclic peptide **1w2h-2**

1w (10 mg, 3.9 μmol) was dissolved in phosphate-buffered saline (PBS buffer, pH 4.0) to prepare a solution with a final concentration of 1 mM. **2h** (0.6 mg, 3.9 μmol , 1 equivalent) was added to the resulting solution, and the reaction mixture was left standing at room temperature for 10 min. Next, PySSPy (2.1 mg, 9.7 μmol , 2.5 equivalents) was added to the mixture, and the pH of the system was slowly adjusted to 7.0 using 1 M aqueous sodium hydroxide (NaOH) solution. After the resulting solution was diluted with ultrapure water and filtered, the crude product was purified via preparative high-performance liquid chromatography (HPLC, 30–60% acetonitrile/water gradient over 30 min) followed by lyophilization to afford **1w2h-2** (3.1 mg, 30% yield).

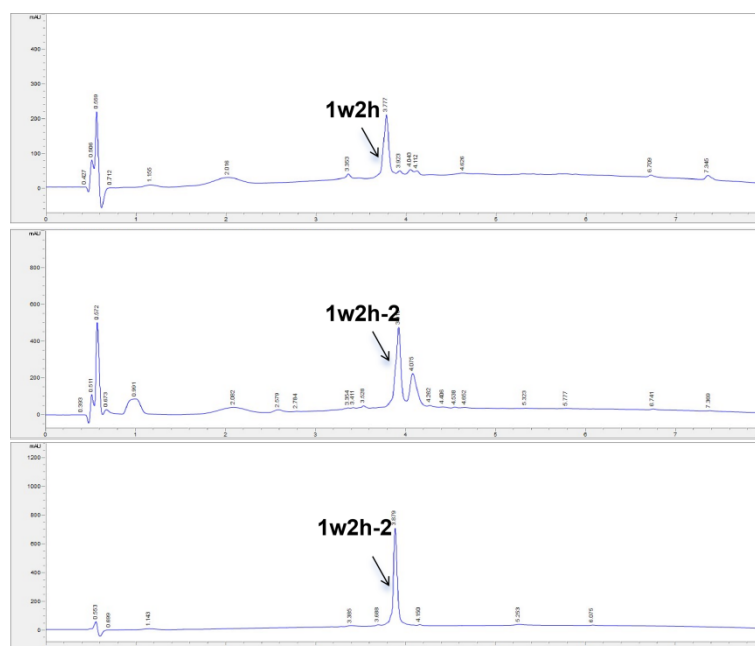


Figure 97.2 UV (205nm) trace from LC-MS analysis of first step, second step and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

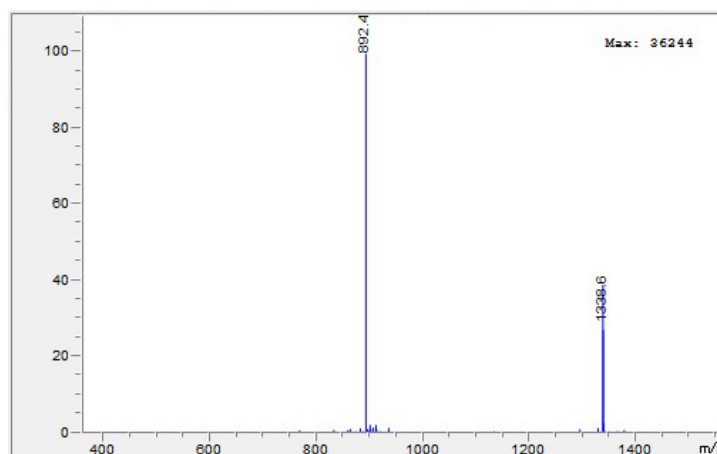


Figure 97.3 ESI-MS calcd. For $C_{115}H_{158}N_{32}O_3S_3$, $[M+2H]^{2+}$ $m/z = 1338.5$, found 1338.6; $[M+3H]^{3+}$ $m/z = 892.6$, found 892.4.

Synthesis of 1x-1a

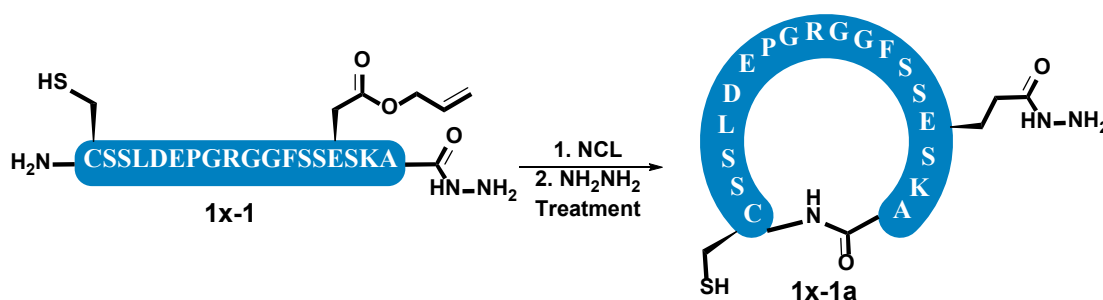


Figure 98.1 Synthetic scheme of cyclic peptide 1x-1a

Peptide **1x-1** (10 mg, 5.3 μmol) was dissolved in 2.0 mL aqueous buffer containing Saturated GuHCl and (pH = 3.0), Acetylacetone (AcAc) (0.5 μL , 5 μmol , 0.95 equiv.) and 4-mercaptophenylacetic acid (MPAA, 4.5 mg, 26.8 μmol , 5 equiv.) was added to the solution. The reaction was stirred for 6 h. Subsequently, Then the pH value of reaction mixture was then adjusted to 7.0 slowly with aqueous NaOH solution (1 M) to initiate the NCL at room temperature. The reaction mixture was stirred for 8 h to afford peptide. Add hydrazine hydrate (5% volume ratio), the reaction was stirred for 2 h. This solution was diluted by water and filtrated, then it was subjected to preparative HPLC purification (20-70% ACN/ H_2O over 30 min) and lyophilization to give **1x-1a** (3.8 mg, 39 %).

Peptide **1x-1** (10 mg, 5.3 μmol) was dissolved in 2.0 mL of aqueous buffer (pH = 3.0) containing saturated guanidine hydrochloride (GuHCl). Acetylacetone (AcAc) (0.5 μL , 5 μmol , 0.95 equiv.) and 4-mercaptophenylacetic acid (MPAA, 4.5 mg, 26.8 μmol , 5 equiv.) were added to the resulting solution, and the reaction mixture was stirred for 6 hours. Subsequently, the pH of the reaction mixture was slowly adjusted to 7.0 using 1 M aqueous sodium hydroxide (NaOH) solution to initiate native chemical ligation (NCL) at room temperature, followed by another 8 hours of stirring to yield the crude peptide product. After that, hydrazine hydrate was added at a 5% volume ratio, and the reaction was stirred for an additional 2 hours. The resulting solution was diluted with water, filtered, and then purified via preparative high-performance liquid chromatography (HPLC) using a 20–70% acetonitrile/water (ACN/H₂O) gradient over 30 minutes. After lyophilization, the target product **1x-1a** was obtained with a yield of 39% (3.8 mg)

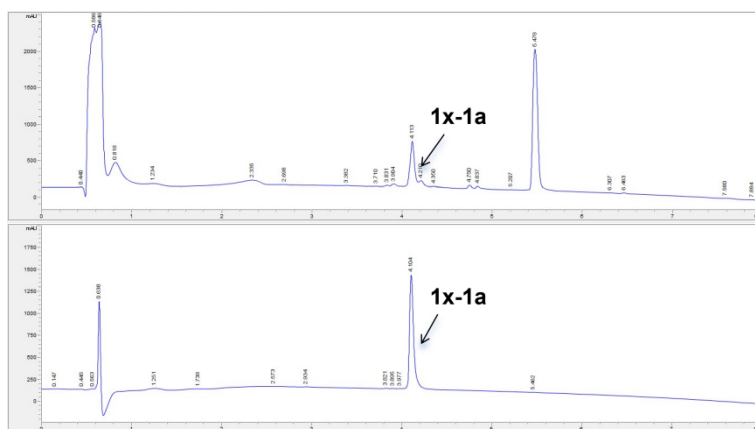


Figure 98.2 UV (205nm) traces from LC-MS analysis of the reaction and after purification. Gradient: 20%–70% ACN/H₂O with 0.1% TFA over 8 min.

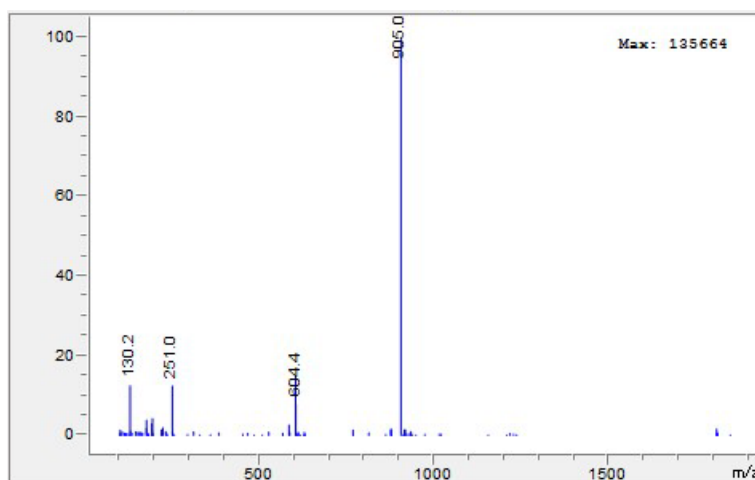


Figure 98.3 ESI-MS calcd. For C₇₃H₁₁₆N₂₄O₂₈S, [M+2H]²⁺ m/z = 905.4, found 905.0; [M+3H]³⁺ m/z = 603.9, found 604.4.

Synthesis of 1x-2a

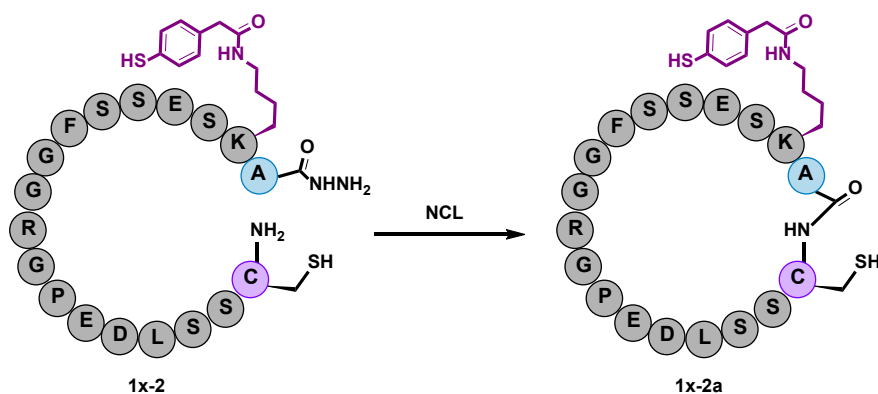


Figure 99.1 Synthetic scheme of cyclic peptide **1x-2a**

Peptide **1x-2** (30 mg, 15.2 μmol) was dissolved in 2.0 mL of aqueous buffer containing saturated guanidine hydrochloride (GuHCl) at pH 3.0. Acetylacetone (AcAc, 1.4 μL , 1.4 μmol , 0.95 equiv.) and 4-mercaptophenylacetic acid (MPAA, 12.7 mg, 76.0 μmol , 5 equiv.) were then added to the solution, and the reaction mixture was stirred for 6 hours. Subsequently, the pH of the reaction mixture was slowly adjusted to 7.0 using 1 M aqueous sodium hydroxide (NaOH) solution to initiate native chemical ligation (NCL) at room temperature, followed by another 8 hours of stirring to yield the crude target peptide. The crude mixture was diluted with water, filtered, and purified via preparative high-performance liquid chromatography (HPLC) with a gradient of 20%–70% acetonitrile (ACN) in water over 30 minutes. After lyophilization, the target product **1x-2a** was obtained as a lyophilized powder with a yield of 30% (10.6 mg).

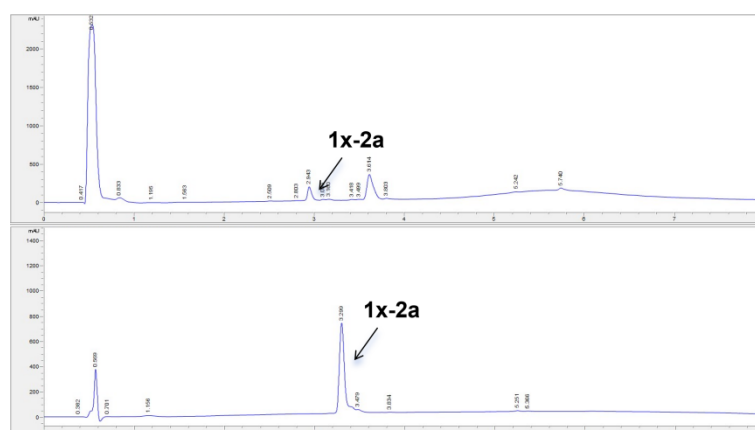


Figure 99.2 UV (205nm) trace from LC-MS analysis of the reaction and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 10 min.

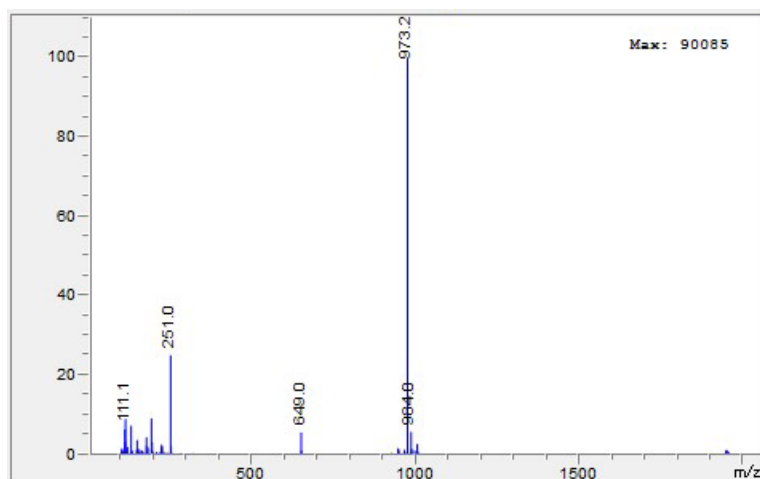


Figure 99.3 ESI-MS calcd. For $C_{81}H_{121}N_{23}O_{29}S_2$, $[M+2H]^{2+}$ $m/z = 972.9$, found 973.2; $[M+3H]^{3+}$ $m/z = 648.9$, found 649.0

Synthesis of 1x-3a

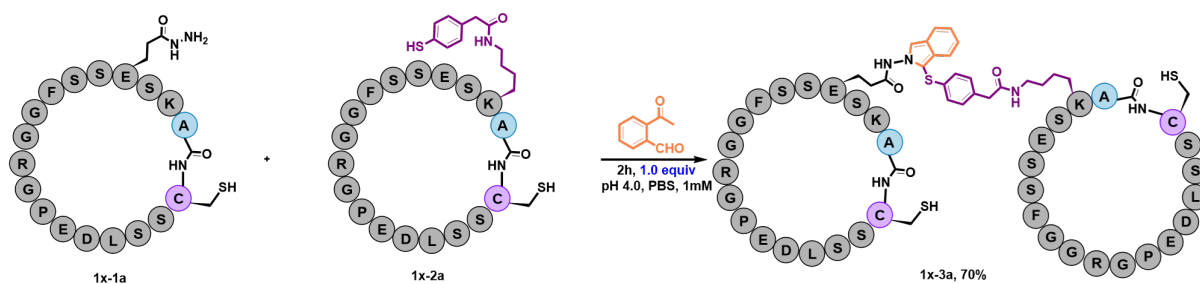


Figure 100.1 Synthetic scheme of cyclic peptide **1x-3a**

1x-1a (3.0 mg, 1.7 μmol , 1.0 equiv) and **1x-2a** (3.2 mg, 1.7 μmol , 1.0 equiv.) were dissolved in PBS buffer (pH 4) to a final concentration of 1 mM. **2h** (0.5 mg, 3.3 μmol , 2.0 equiv.) was added to the solution, and the reaction was allowed to stand at room temperature for 10 min. Reaction conversion was monitored via LC-MS, and the conversion yield was determined to be 70%. The crude reaction solution was diluted with water, filtered, purified by preparative HPLC (20-70% acetonitrile/water gradient over 30 min), and then lyophilized to afford **1x-3a** (3.2 mg, 50% yield).

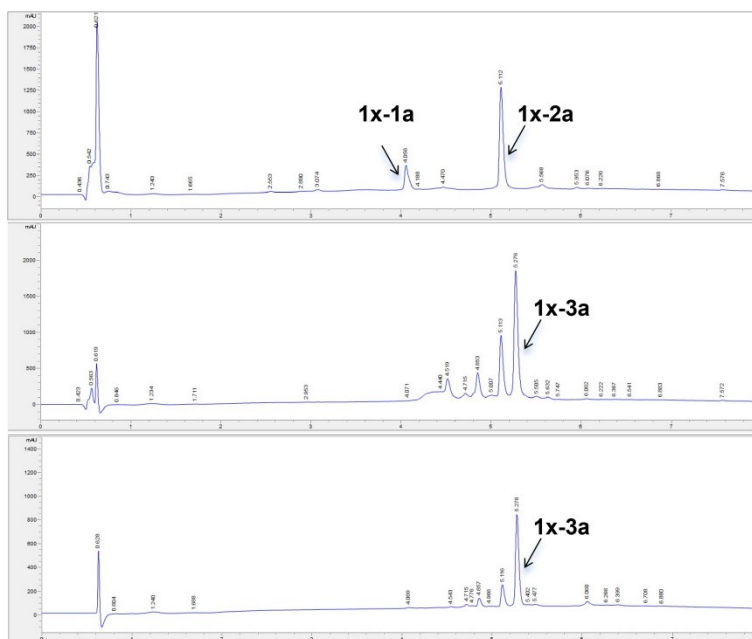


Figure 100.2 UV (205nm) trace from LC-MS analysis of start materials (**1x-1a** and **1x-2a**), labeling reaction after 10 min and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

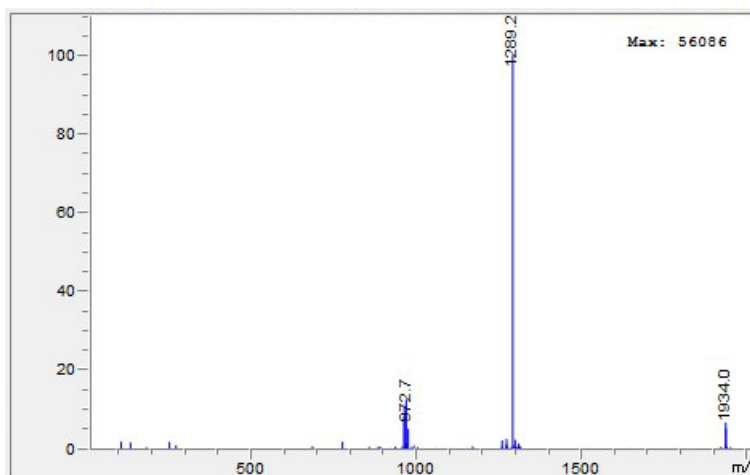


Figure 100.3 ESI-MS calcd. For C₁₆₃H₂₄₁N₄₇O₅₇S₃, [M+2H]²⁺ m/z =1933.3, found 1934.0; [M+3H]³⁺ m/z =1289.2, found 1289.2.

Synthesis of **1z2h3o**

1z (5 mg, 9.1 μ mol) and **3o** (12.8 mg, 9.1 μ mol, 1.0 equiv.) were dissolved in PBS buffer (pH 4.0) to a final concentration of 1 mM. **2h** (1.5 mg, 9.1 μ mol, 1.0 equiv.) was added to the resulting solution, and the reaction mixture was stored at room temperature for 1 hour

(conversion yield 43%). Subsequently, PySSPy (5.0 mg, 22.5 μmol , 2.5 equiv.) was added, and the pH of the mixture was slowly adjusted to 7.0 with 1 M aqueous NaOH solution. After dilution with water and filtration, the crude product was purified via preparative HPLC (20–70% ACN/H₂O gradient over 30 min) followed by lyophilization to afford **1z2h3o** (1.7 mg, 9% yield).

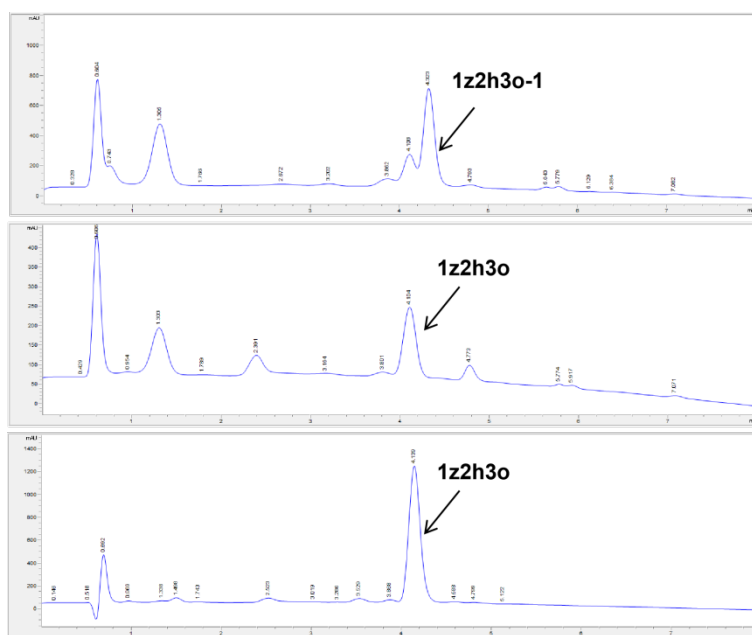


Figure 101.1 UV (205nm) traces of reaction and purification of **1z2h3o**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 10 min.

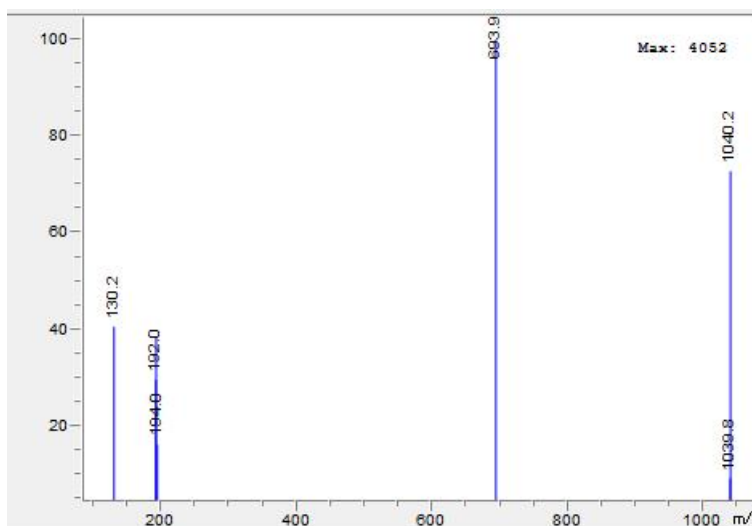


Figure 101.2 ESI-MS calcd. For $\text{C}_{93}\text{H}_{122}\text{N}_{20}\text{O}_{29}\text{S}_3$, $[\text{M}+2\text{H}]^{2+}$ $m/z = 1040.4$, found 1040.2; $[\text{M}+3\text{H}]^{3+}$ $m/z = 693.9$, found 693.9.

Synthesis of **1z2n3o-1a**

1z (5.0 mg, 9.1 μmol) and **3o** (12.8 mg, 9.1 μmol , 1.0 equiv.) were dissolved in PBS buffer (pH 4.0) to obtain a solution with a final concentration of 1 mM. **2n** (2.3 mg, 9.1 μmol , 1.0 equiv.) was added to the solution, and the reaction was allowed to stand at room temperature for 1 hour (conversion yield 51%). Subsequently, PySSPy (5.0 mg, 22.5 μmol , 2.5 equiv.) was added, and the pH of the mixture was slowly adjusted to 7.0 with 1 M aqueous sodium hydroxide (NaOH) solution. The resulting solution was diluted with water, filtered, and further purified via preparative HPLC with a gradient of 20%–70% acetonitrile/water (ACN/H₂O) over 30 minutes. The target fraction was lyophilized to afford **1z2n3o-1a** as a solid (1.4 mg, 7% yield).

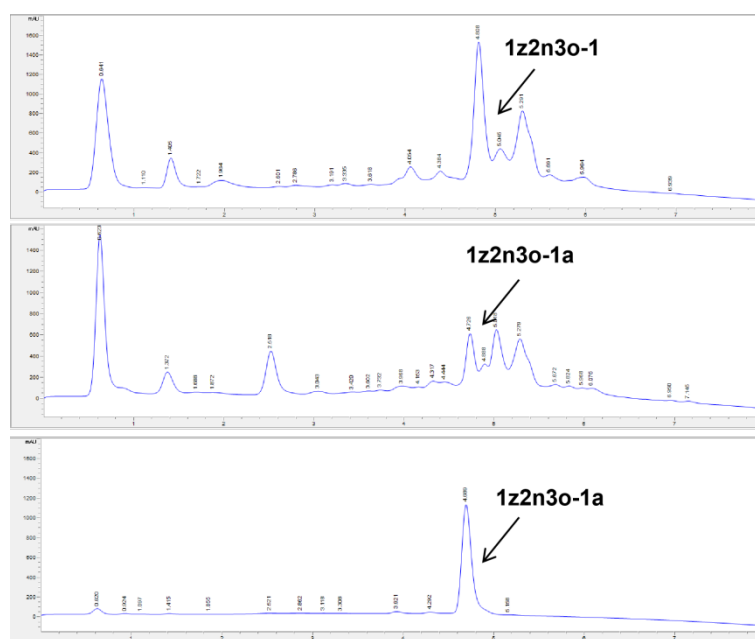


Figure 102.1 UV (205nm) trace from LC-MS analysis of labeling reaction and after purification. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

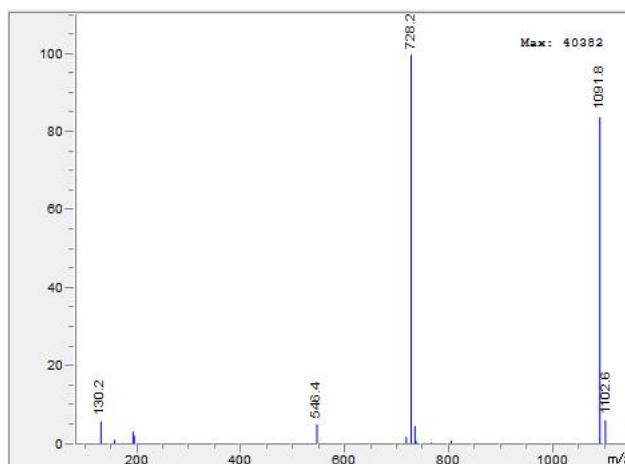


Figure 102.2 ESI-MS calcd. For $C_{98}H_{122}N_{22}O_{30}S_3$, $[M+2H]^{2+}$ $m/z = 1092.0$, found 1091.8; $[M+3H]^{3+}$ $m/z = 728.3$, found 728.2.

Synthesis of **1z2n3o**

1z-1 (2.3 mg, 1.4 μ mol) and **1z2n3o-1a** (3.3 mg, 1.4 μ mol, 1.0 equiv.) were dissolved in Tris buffer (pH 8.0) to reach a final concentration of 1 mM. The reaction mixture was allowed to stirred at room temperature for 1 hour (conversion yield 85%). The resulting solution was diluted with water, filtered, then purified via preparative HPLC (gradient elution 30–80% acetonitrile/water over 30 minutes), followed by lyophilization to afford product **1z2n3o** (1.5 mg, 26.7% yield).

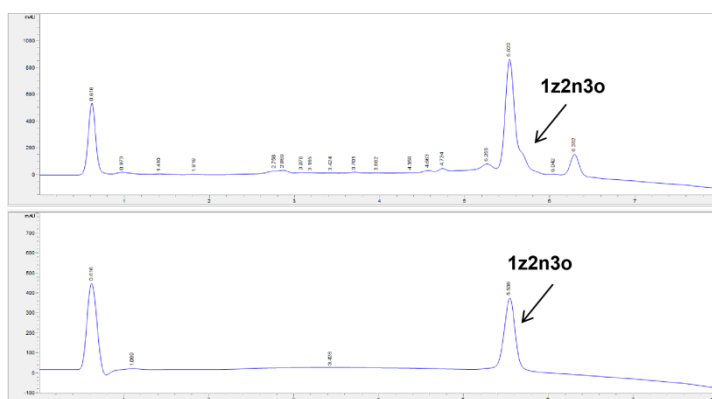


Figure 103.1 UV (205nm) traces of reaction and purification of **1z2n3o**. Gradient: 30%–80% ACN/H₂O with 0.1% TFA over 8 min.

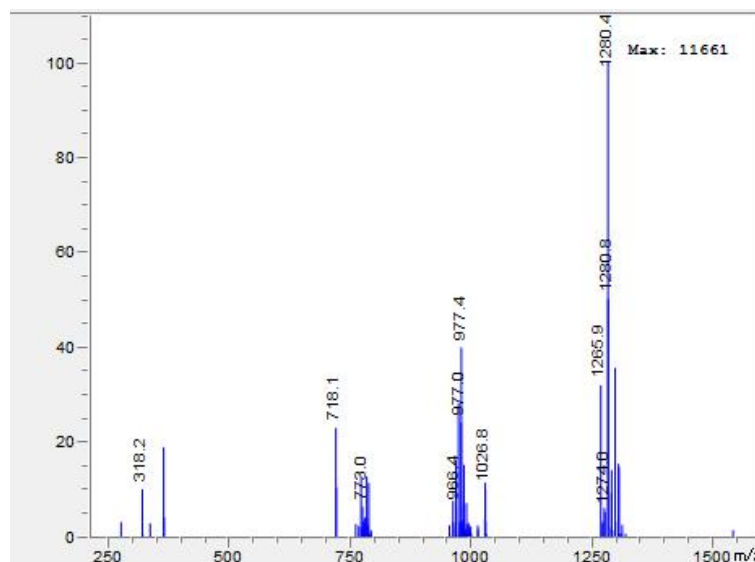


Figure 103.2 ESI-MS calcd, For $C_{186}H_{252}N_{34}O_{49}S_3$, $[M+2H]^{2+}$ $m/z = 1280.0$, found 1280.4. $[M+3H]^{3+}$ $m/z = 977.7$, found 977.4,

Synthesis of **3p-1**

Peptide **3p** (40.0 mg, 32.0 μmol) was dissolved in 2.0 mL aqueous buffer (pH = 3.0) containing saturated guanidine hydrochloride (GuHCl). Acetylacetone (AcAc) (3.0 μL , 30.4 μmol , 0.95 equiv.) and 4-mercaptophenylacetic acid (MPAA, 26.0 mg, 160 μmol , 5 equiv.) were added to the solution, and the reaction mixture was stirred for 6 h. Subsequently, the pH of the reaction mixture was slowly adjusted to 7.0 with 1 M aqueous sodium hydroxide (NaOH) solution to initiate native chemical ligation (NCL) at room temperature, followed by stirring for 8 h to obtain the crude peptide. After that, the mixture was diluted with water, filtered, purified via preparative high-performance liquid chromatography (HPLC) with a linear gradient of 20–70% acetonitrile (ACN) in water over 30 min, and lyophilized to afford product **3p-1** (10.5 mg, 27% yield).

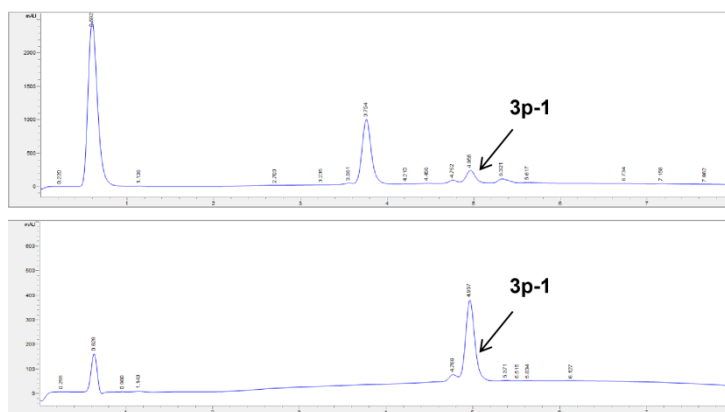


Figure 104.1 UV (205nm) traces of reaction and after purification of **3p-1**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

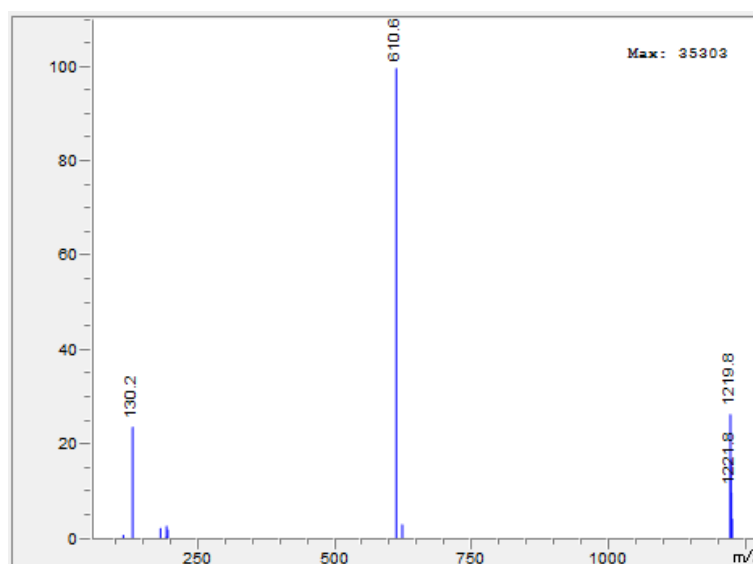


Figure 104.2 ESI-MS calcd, For C₅₃H₈₁N₁₃O₁₄S₃, [M+1H]¹⁺ m/z =1219.5, found 1219.8. [M+2H]²⁺ m/z = 610.3, found 610.6.

Synthesis of 1z2h3p

1z (3.0 mg, 5.4 μ mol) and **3p-1** (6.6 mg, 5.4 μ mol, 1.0 equiv.) were dissolved in PBS buffer (pH 4.0) to reach a final concentration of 1 mM. **2h** (0.8 mg, 9.1 μ mol, 1.7 equiv.) was then added to the solution, and the reaction mixture was allowed to stirred at room temperature for 1 hour (conversion yield 25%). The resulting solution was diluted with deionized water, filtered,

purified via preparative (HPLC) with a linear gradient of 20–70% acetonitrile/water over 30 minutes, and finally lyophilized to afford product **1z2h3p** (1.5 mg, 9% yield).

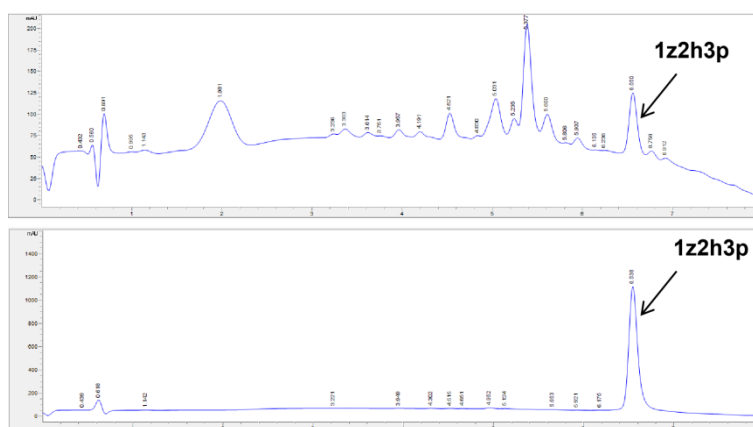


Figure 105.1 UV (205nm) traces of reaction and purification of **1z2h3p**. Gradient: 20%-70% ACN/H₂O with 0.1% TFA over 8 min.

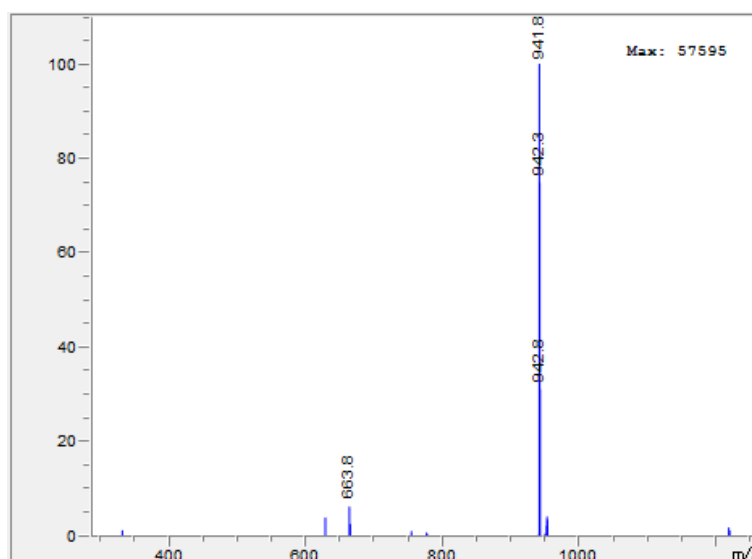


Figure 105.2 ESI-MS calcd, For C₈₉H₁₁₄N₁₈O₂₂S₃, [M+2H]²⁺ m/z = 942.4, found 941.8.

9 In vitro cytotoxicity assay

HeLa cells were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 μ L of MEM medium (10% FBS, 1% P/S) and allowed to adhere overnight. The next day, cells were treated with serial dilutions of CPT, PDC1, or PDC2 for 48 h. Each compound was first dissolved in DMSO at a concentration of 10 mM, and then serially diluted in culture medium to achieve final concentrations ranging from 500 nM to 256 μ M. After the treatments, 10 μ L of CCK-8 solution was added to each well and the plates were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 1 h. Absorbance at 450 nm was measured using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific). Cell viability was calculated as $(OD_{\text{treated}} - OD_{\text{blank}}) / (OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$. All experiments were performed in triplicate and independently repeated three times. Data were analyzed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

Caco-2 cells were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 μ L of MEM containing nonessential amino acids, supplemented with 20% FBS and 1% P/S, and allowed to adhere overnight. The next day, cells were treated with serial dilutions of CPT or PDC3 for 48 h. Each compound was first dissolved in DMSO at a concentration of 10 mM, and then serially diluted in culture medium to achieve final concentrations ranging from 500 nM to 256 μ M. After the treatments, 10 μ L of CCK-8 solution was added to each well and the plates were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 1 h. Absorbance at 450 nm was measured using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific). Cell viability was calculated as $(OD_{\text{treated}} - OD_{\text{blank}}) / (OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$. All experiments were performed in triplicate and independently repeated three times. Data were analyzed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

References

1. A. G. Cheetham, Y.-C. Ou, P. Zhang, H. Cui, "Linker-determined drug release mechanism of free camptothecin from self-assembling drug amphiphiles" *Chemical Communications* **50**, (2014): 6039-6042.