Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

A Water Soluble Cu^{I}–NHC for CuAAC ligation of unprotected peptides under open air conditions.

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SI 1- Materials and chemicals.

SI 2- Experimental procedures.

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NMR spectra were recorded in Fourier Transform mode with a Bruker AVANCE 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz), at 298K. Data are reported as chemical shifts (δ) in ppm. Residual solvent signals were used as internal references (^{1}H, ^{13}C). Kinetic surveys of CuAAC reactions were performed using a Bruker AVANCE 500 spectrometer (^{1}H at 500 MHz).

Electrospray (positive mode) high–resolution mass spectra were recorded on a Q-TOF micro spectrometer (Waters), using an internal lock mass (H_3PO_4) and an external lock mass (leucine-enkephalin $[M+H]^+$: m/z = 556.2766).

HPLC conditions: C18: 95:5 [H_2O (formic acid: 1/1000): MeCN] to 5: 95 during 15 minutes then 5:95 during 10 minutes for peptides **14a,b**, **16a,b** and **17a,b**.

For **15a,b** the previous analytic system is not suitable because variable amount of *S*–oxidation – taking place in the ionisation chamber of the mass spectrometer – is detected while analysing both the starting material and the product HPLC peaks. However, an Hydrophilic Interaction Liquid Chromatographic column (HILIC) with a buffered eluent allows an unbiased detection of peptides **15a,b**: [80 : 20 MeCN : H_2O containing 10 mM $AcONH_4$ pH 7.0), isocratic condition].

IR spectra were recorded on a Shimadzu Fourier Transform Infrared Spectrophotometer FTIR-8400S, equipped with a PIKE MIRacle Attenuated Total Reflectance (ATR) accessory (ATR crystal plate: germanium).

Elemental analyses were performed at the Service de Microanalyse, Université Henry Poincaré,Vandoeuvre-les-Nancy, France.

Fmoc-protected amino acids, Fmoc-Gly-Wang resin, Fmoc-Rink polystyrene and HBTU were purchased from Merck Biosciences (Nottingham, UK). Other reagents and solvents were purchased from Sigma-Aldrich, Acros and Fisher Scientific and used without further purification.

(*S*)-2-azido 3-phenylpropanoic acid was synthesised according to Goddard-Borger.[1] (*S*)-2-azido 3-(4-hydroxyphenyl)propanoic acid was synthesised following the same procedure; analytical data were identical to literature.[2]

SI 2- Experimental procedures

7: Azadiene **6**[3] (49.90 g, 96.7 mmol, 1.0 equiv.) was dissolved in 500 mL of THF. $NaBH_4$ (14.63 g, 386.8 mmol, 4.0 equiv.) was added in portions separately in 100 mL of ice-cold MeOH. The latter solution was added to the former *via* an addition funnel while the temperature was kept close to RT with a water bath. The temperature was subsequently risen to 50°C. After 2h, two extra equivalents of $NaBH_4$ were added (7.32 g, 193.4 mmol), followed by one equivalent after 4h. After 5h, TLC analysis (ethyl acetate/ cyclohexane *v*/*v* 1:1) indicated the total disappearance of **6** and the formation of a single product. The excess of $NaBH_4$ was quenched by addition of saturated aqueous NH_4Cl (caution: exothermic reaction, the temperature was kept at RT with a cool water bath). Organic solvents were evaporated and the resulting aqueous slurry was extracted with 3×150 mL Et_2O. The joint organic phases were dried over $MgSO_4$ and evaporated yielding 46.84g of **6** as an off-white crystalline solid. Yield: 93%.
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.32 (s, 4H, H_{arom}), 3.17 (s, 4H, CH_2-NH), 2.24 (s, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) : δ (ppm) = 145.6 (C_{arom}-N), 137.4 (C_{arom}-H), 131.9 (C_{arom}-CH_3), 85.3 (C_{arom}-I), 48.6 (*C*H_2-NH), 18.3 (*C*H_3); IR ν (cm^{-1}): 3330, 2940, 2850, 1470, 1450, 1260, 1220, 1110, 1030, 1010. HRMS (ESI+) : Calculated for $C_{18}H_{23}N_2I_2^+$ $[M+H]^+$ 520.9951. Found: 520.9924.

8: Diamine **7** (24.53 g, 47.1 mmol, 1.0 equiv.), NaN_3 (11.08 g, 170.2 mmol, 3.6 equiv.), *N*,*N*'-dimethylethylenediamine (1.38 mL, 1.130g, 0.30 equiv., 12.8 mmol), ascorbic acid (1.65 g, 9.4 mmol, 0.2 equiv.) and NaOH (376 mg, 9.4 mmol, 0.2 equiv.) were added to a mixture of 400 mL of DMSO and 40 mL of H_2O which was subsequently heated to 70° with stirring in the dark and deoxygenated by bubbling argon during 20 minutes. Copper(I) iodide (1.79 g, 9.4 mmol, 0.2 equiv.) was then added and the mixture was stirred until TLC analysis (cyclohexane/EtOAc *v*/*v* 4:1) indicated a complete consumption of starting material and a clean conversion to a single product (∼2h). The reaction mixture was cooled to room temperature and poured on 1 L of ice-water. After 1 hour of vigorous stirring, **7** separated as a solid which was recovered by filtration, washed with water and dried *in vacuo* to constant mass to give 13.283 g of a light brown solid. Yield: 81%.
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 6.70 (s, 4H, H_{arom}), 3.97 (s, 2H, N*H*-CH_2), 3.01 (s, 4H, CH_2-NH), 2.20 (s, 12H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 143.1 (C_{arom}-N), 133.4 (C_{arom}-N_3), 131.7 (C_{arom}-CH_3), 119.2 (C_{arom}-H), 49.0 (*C*H_2-NH), 18.6 (*C*H_3). IR ν (cm^{-1}): 2944, 2110 (N_3), 1603, 1477, 1427, 1379, 1322, 1304, 1291, 1259, 1239, 1206, 1118, 1030, 1005, 961, 940, 862, 841, 825. HRMS (ESI+) : Calculated for $C_{18}H_{23}N_8^+$ $[M+H]^+$ 351.2046. Found: 351.2030.

***N*-(2-hydroxyethyl)-*N*,*N*-dimethylprop-2-yn-1-aminium chloride:** To a solution of propargyl chloride (70% w/w in toluene, 25 mL, 0.2 mol) in toluene (30 mL) was added dropwise a solution of *N*,*N*-dimethylaminoethanol (17 mL, 0.17 mol) in toluene (30 mL). The reaction was maintained at room temperature with a water bath during the addition and the reaction mixture was stirred for 3 days. The precipitate was filtered off and washed with pentane to give 23.8 g of an off-white hygroscopic solid (0.15 mol, 86%);
^{1}H NMR (400 MHz, CD_3OD): 3.28 (6H, s), 3.55 (1H, s), 3.61 (2H, br s), 4.03 (2H, br s), 4.46 (2H, s); ^{13}C NMR (100 MHz, CD_3OD): 52.0 ($2CH_3$), 56.5, 57.0, 66.6 (CH_2), 72.7 (CH), 83.0 (C); IR (ATR): 3254, 3216, 2124, 1458, 1441, 1376, 1149, 1085, 1026, 1002, 995, 917, 874 cm^{-1} HRMS (ESI+) calcd for $C_7H_{14}NO$ (M^+) 128.1075, found 128.1078.

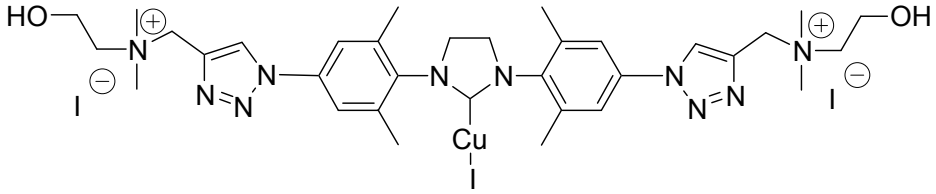
9: **8** (20.00 g, 56.4 mmol, 1 equiv), *N*-(2-hydroxyethyl)-*N,N*-dimethylprop-2-yn-1-aminium chloride (17.96 g, 141.1 mmol, 2.5 equiv) and **3b** (0.184 g, 0.28 mmol, 0.5 mol%)[4] were dissolved in 400 mL CH_2Cl_2/methanol (*v/v* 1:1). The mixture was stirred at room temperature during 24h after what additional 36 mg (0.06 mmol, 0.1mol %) of catalyst were added. After stirring 18h, the solvent was evaporated to yield a brownish oil. An ethanolic solution of HCl (prepared by dropwise addition over 10 minutes with stirring of 50 mL (5 equiv.) of acetyl chloride to 400 mL anhydrous ethanol) was added on the crude mixture and the solution was triturated until a beige solid was obtained. **7** was recovered by filtration and washed with ice-cold ethanol until a colourless filtrate was obtained that was the washed with 2x100 mL Et_2O and dried *in vacuo* to give 39.99 g. Yield: 81%.
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.08 (s, 2H, $H_{triazole}$), 7.69 (s, 4H, H_{arom}), 4.83 (s, 4H, $C_{triazole}$-CH_2), 3.94 (m, 4H, CH_2-OH), 3.48 (s, 4H, N-CH_2-CH_2-N), 3.45 (m, 4H, N^+-CH_2-CH_2-OH), 3.14 (s, 12H, CH_3-N^+), 2.51 (s, 12H, C_{arom}-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = [139.1, 136.3, 133.3, 126.7 (C_{arom}-N_{amine}, C_{arom}-$N_{triazole}$, C_{arom}-CH_3, $C_{triazole}$-CH_2)], 132.9 ($C_{triazole}$-H), 121.0 (C_{arom}-H), 64.5 (*C*H_2-OH), 58.4 ($C_{triazole}$-*C*H_2), 54.9 (CH_2-N^+), 50.5 (N^+-*C*H_3), 46.7 (CH_2-N_{amine}), 18.6 (C_{arom}-*C*H_3). IR ν (cm^{-1}): 3350 (broad), 1568, 1496, 1466, 1442, 1077, 1050, 1015, 1005, 904, 868. MS (ESI+): *m/z* = 303.2 [**9**-2HCl]$^{2+}$ (100). EA calcd for $C_{32}H_{50}Cl_2N_{10}O_2$, 4HCl, 3$H_2O$: C 43.79, H 6.89, N 15.96, found: C 43.56, H 6.87, N 15.34.

10a: **9** (10.00 g, 11.4 mmol) was dissolved in 220 mL anhydrous ethanol. Then, 110 mL of triethyl orthoformate and 3 drops of formic acid were added. The resulting suspension was refluxed overnight after what the solvent was distilled to one third. The reaction mixture was cooled to 0°C and a solid separated. Crude imidazoliniun salt was recovered by filtration and washed with 2x50 mL of cold EtOH. The crude salt was taken up with 200 mL H_2O and resulting solution was heated to ebullition in an open flask for 2.5h. Activated charcoal was added until a colourless supernatant was obtained. Charcoal was removed by vacuum filtration over a silica plug and water was rotary evaporated. Finally, the resulting white solid was dissolved in 20 mL methanol, the solution was filtered (0.2 µm nylon membrane) and the product was precipitated, under stirring, by the dropwise addition of 80 mL of acetone. The resulting white solid was recovered by filtration to give 6.52 g. Yield: 74%.
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.45 (s, 1H, NC*H*N), 9.36 (s, 2H, $H_{triazole}$), 8.01 (s, 4H, H_{arom}), 5.82 (t, *J* = 4.7 Hz, 2H, O*H*), 4.90 (s, 4H, $C_{triazole}$-CH_2), 4.60 (s, 4H, N-CH_2-CH_2-N), 3.95 (m, 4H, CH_2-OH), 3.46 (t, *J* = 4,9 Hz, 4H, N^+-CH_2-CH_2-OH), 3.17 (s, 12H, CH_3-N^+), 2.56 (s, 12H, C_{arom}-CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) = [139.9, 138.8, 138.0, 134.9, 128.1 (C_{arom}-N_{amine}, C_{arom}-$N_{triazole}$, C_{arom}-CH_3, $C_{triazole}$-CH_2, $C_{triazole}$-H)], 122.0 (C_{arom}-H), 66.7 (*C*H_2-OH), 60.5 ($C_{triazole}$-*C*H_2), 57.0 (CH_2-N^+), [52.7, 52,0 (N^+-*C*H_3, CH_{2Im})], 18.4 (C_{arom}-*C*H_3). IR ν (cm^{-1}): 3355 (broad), 1627, 1494, 1293, 1265, 1215, 1073, 1053, 1015, 984, 880. MS (ESI+) : *m/z* = 205.8 [M-3Cl+H]$^{3+}$ (100). EA calcd for $C_{33}H_{49}Cl_3N_{10}O_2$, 2.5$H_2O$: C 51.53, H 7.08, N 18.21, found: C 51.18, H 6.41, N 18.32.

10b: To 2.00 g of **10a** dissolved in 13 mL of methanol was added a concentrated solution of NaI (1.54 g in 65 mL of acetone) until precipitation stopped. After filtration the resulting white solid was washed with acetone and dried on air to afford 2.64 g (96% yield).
^{1}H NMR(400 MHz, DMSO-d_6) : δ (ppm) = 9.34 (s, 1H, NC*H*N), 9.28 (s, 2H, $H_{triazole}$), 7.99 (s, 4H, H_{arom}), 5.65 (t, *J* = 4,7 Hz, 2H, O*H*), 4.88 (s, 4H, $C_{triazole}$-CH_2), 4.60 (s, 4H, N-CH_2-CH_2-N), 3.96 (m, 4H, CH_2-OH), 3.46 (t, *J* = 4,9 Hz, 4H, N^+-CH_2-CH_2-OH), 3.16 (s, 12H, CH_3-N^+), 2.56 (s, 12H, C_{arom}-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) : δ (ppm) = 160.6 (N*C*HN^+), [138.1, 136.6, 136.5, 133.4, (C_{arom}-N_{amine}, C_{arom}-$N_{triazole}$, C_{arom}-CH_3, $C_{triazole}$-CH_2,)], 126.9

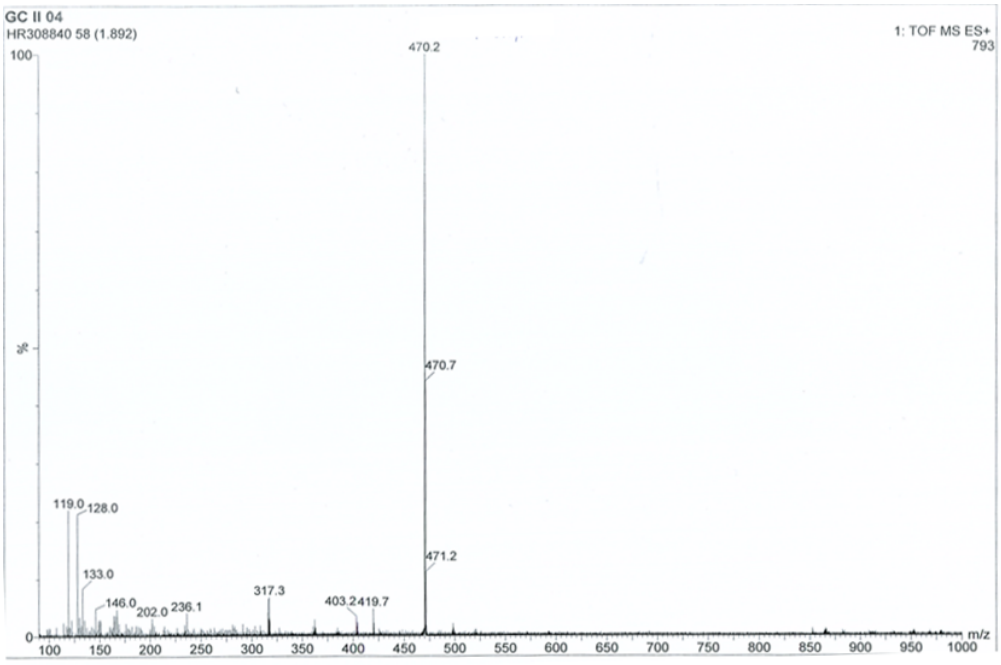
($C_{triazole}$-H), 120.4 (C_{arom}-H), 64.3 (CH_2-OH), 58.2 ($C_{triazole}$-CH_2), 54.8 (CH_2-N^+), [51.0, 50.4 (N^+-CH_3, CH_{2Im})], 17.6 (C_{arom}-CH_3). IR ν (cm^{-1}): 3350, 1653, 1627, 1558, 1539, 1495, 1265, 1050, 1071, 1012, 980, 910, 878. HRMS (ESI+): Calculated for $C_{33}H_{49}N_{10}O_2^+$ $[M]^{3+}$ 205.8013. Found: 205.7992.

Preparation of copper(I) complex 5:



1.00 g of **10b** (1.00 mmol, 1.0 eq.), 191 mg of CuI (1.00 mmol, 1.0 eq.), and 40.0 mg of NaOH (1.00 mmol, 1.0 eq.) were dissolved in 40 mL of methanol. After 3h under stirring at reflux, a white precipitate appeared and the reaction mixture was cooled to 0°C. The resulting solid was filtered, washed with cold methanol then methanol/acetone (1:1) and finally acetone to afford 1.11 g of a white solid (98% yield).

^{1}H-NMR (400 MHz, DMSO-d6): δ (ppm) = 9.11 (s, 2H, $H_{triazole}$), 7.83 (s, 4H, H_{arom}), 5.34 (t , , J = 4.8 Hz, 2H, O*H*), 4,82 (s, 4H, $C_{triazole}$-CH_2), 4.08 (s, 4H, N-CH_2-CH_2-N), 3,95 (broad, 4H, CH_2-OH), 3.44 (broad, 4H, N+-CH_2-CH_2-OH), 3.12 (s, 12H, CH_3-N^+), 2,51 (s, 12H, C_{arom}-CH_3, partly overlapped with solvent signal). RMN-^{13}C (100 MHz, DMSO-d_6): δ (ppm) = 207.1 (C-Cu), [138.8, 138.7, 136.2, 135.3, 126.5 (C_{arom}-N_{amine}, C_{arom}-$N_{triazole}$, C_{arom}-CH_3, $C_{triazole}$-CH_2, $C_{triazole}$-H)], 119.8 (C_{arom}-H), 64.5 (CH_2-OH), 58.5 ($C_{triazole}$-CH_2), 55.0 (CH_2-N^+), [50.3 (CH_{2Im}, N^+-CH_3, overlapped signals)], 18.1 (C_{arom}-CH_3). IR ν (cm^{-1}) : 3400 (broad), 2358, 2332, 1600, 1495, 1484, 1436, 1410, 1310, 1280, 1263, 1181, 1091, 1072, 1047, 1013, 980, 914, 875, 857. MS (ESI+) : Calculated for $C_{33}H_{48}CuIN_{10}O_2$ $[M-2I]^{2+}$: 470.2; found 470.2. EA Calcd for $C_{33}H_{48}CuI_3N_{10}O_2$, 0.5$H_2O$: C 37,04, H 4,62; N 13,09; Found: C 36.72; H 4.18; N 13.15.



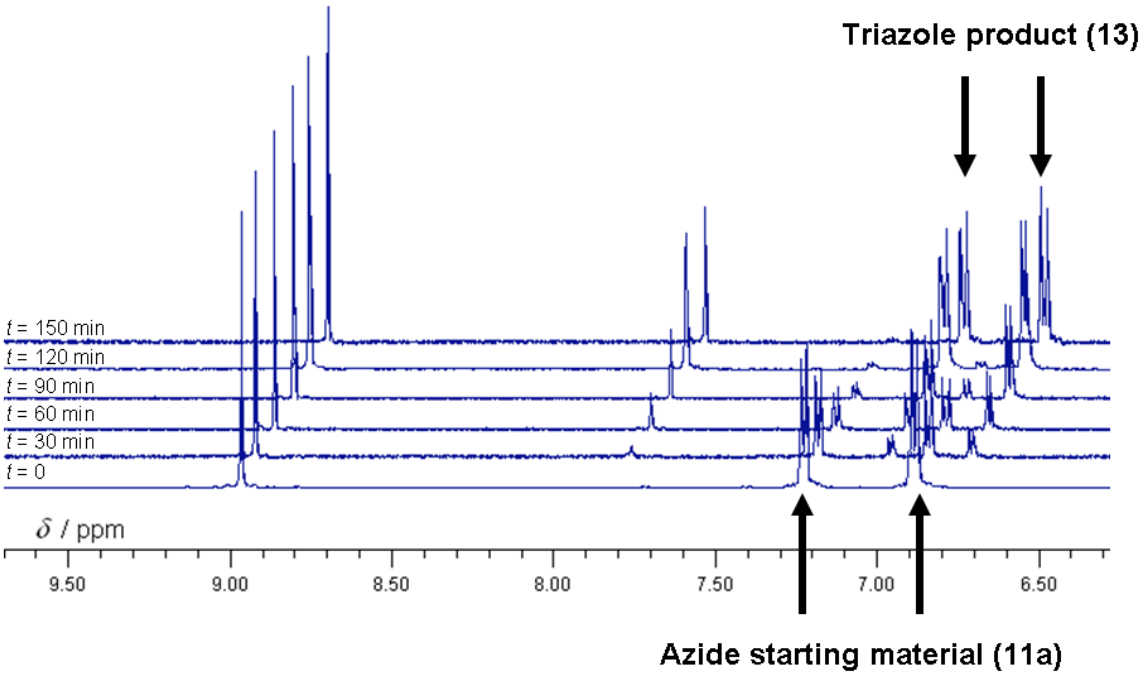
Starting from **10a** the application of the same method with CuCl instead of CuI resulted in a complex isolated as very oxidation sensitive solid.

Solid phase peptide synthesis of 14-17

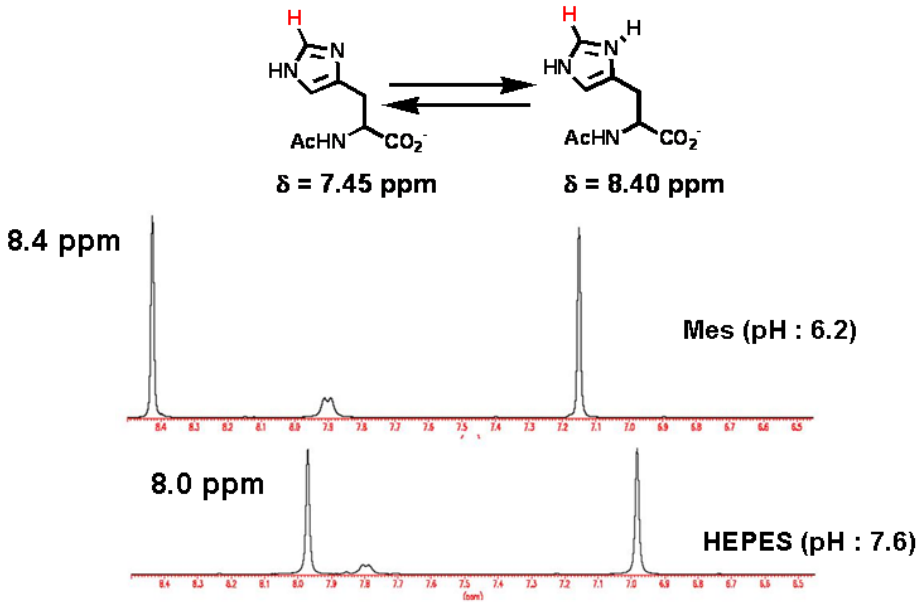
Automated solid-phase peptide synthesis (SPPS) was run on a 433A synthesizer from Applied Biosystems using standard Fmoc/*t*Bu chemistry at a 0.1 mmol scale with HBTU/HOBt as coupling reagents and 20% piperidine in NMP as deprotection reagent. The elongation was carried out automatically using a 10-fold excess of protected amino acids and coupling reagents, starting from Fmoc-Gly-Wang resin for peptides **14-16** and Fmoc-Rink polystyrene resin for peptide **17**. The 0.1 mmol scale Fastmoc program purchased from the manufacturer was used followed by capping with acetic anhydride after each amino acid coupling. The side-chain protecting groups used were Arg(Pbf), Cys(Trt), Gln(Trt), His(Trt), Trp(Boc), Tyr(*t*Bu).

Peptides were cleaved from the resin and deprotected by a standard 2h treatment with a mixture of TFA/H_2O/*i*Pr_3SiH/PhOH (87.5:5:2.5:5), followed by precipitation by pouring onto ice-cold diethyl ether. The solid was recovered by centrifugation then washed twice by suspending in diethyl ether followed by centrifugation. Solvent traces were removed under reduced pressure and the precipitate was finally dissolved in ultrapure water then lyophilized.

Kinetics of the formation of 13 at pH 6.2 and 7.6: 10 mg of **11a** (0.048mmol, 1.0 equiv.), 5.7 µL of propargylic alcohol (0.096 mmol, 2.0 equiv.) and 6.0 mg of sodium picrate (0.024 mmol, 1.0 equiv.) as internal standard were dissolved in 1.6 mL of the appropriate buffer (HEPES, pH= 7.6 or MES, pH = 6.2) and 0.4 mL of D_2O are added. The reaction was started with the addition of 200µL of a stock solution ($4.8.10^{-3}$ mol L^{-1}) of catalyst **5** ($9.6.10^{-4}$ mmol, 2 mol-%). Spectra were recorded automatically every 10 minutes with tube rotation (20 Hz) using a presaturation solvent suppression program. The conversion (relative to internal standard) was determined by the average of both tyrosines aromatic protons integrations (7.24 ppm, 6.89 ppm for azide **11a** and 7.01 ppm, 6.76 ppm for **13**). Typical spectra observed during the course of the reaction (pH = 7.6, HEPES buffer) are shown below:



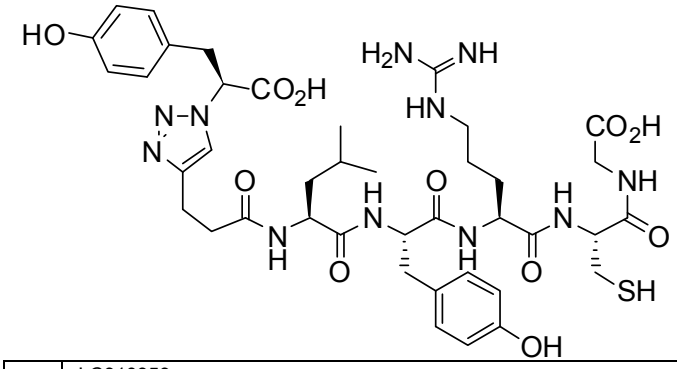
^{1}H NMR spectra on N^{α}-acetyl histidine in aqueous 0.2 M HEPES (pH 7.6) and 0.2 M MES (pH 6.2) buffers.

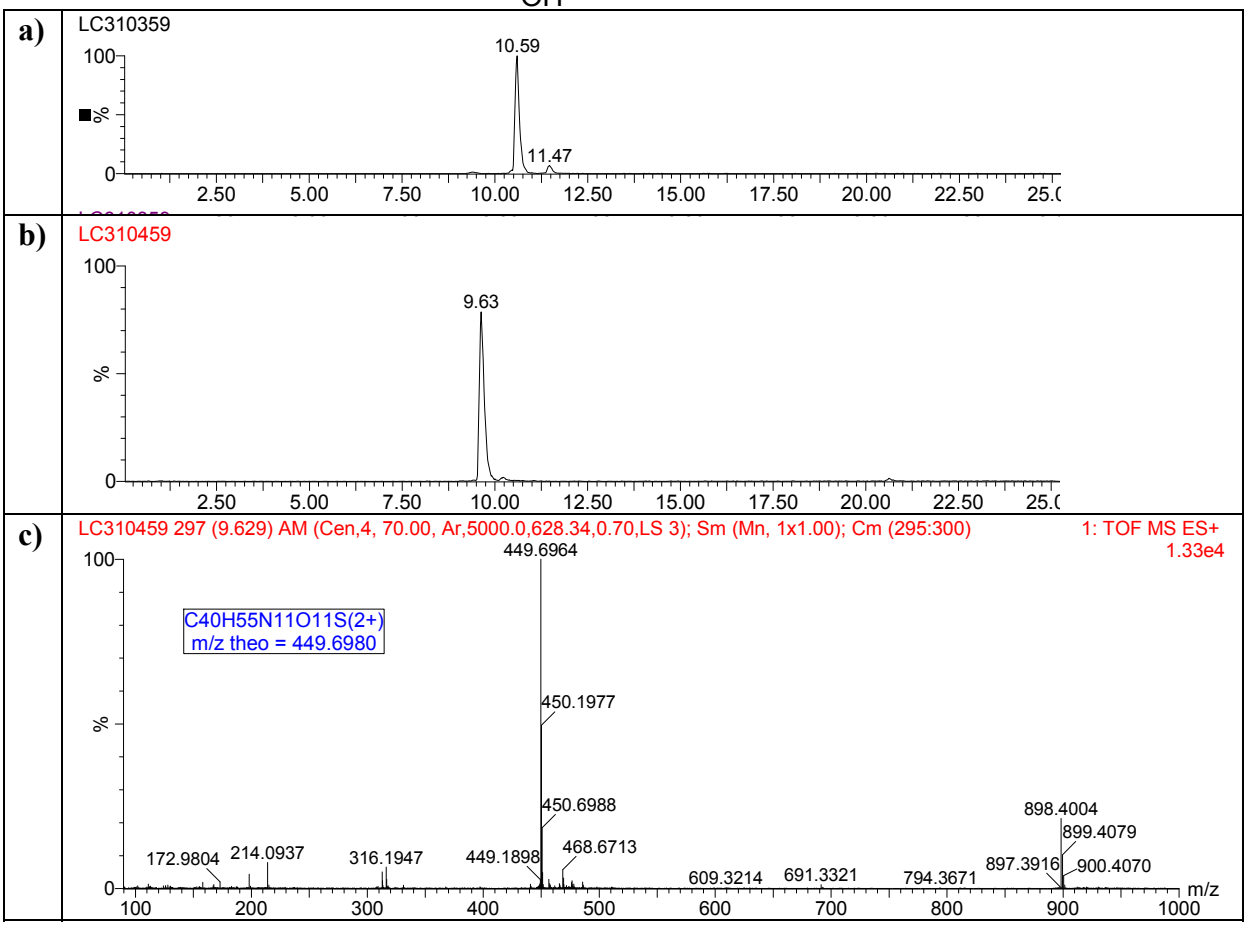


Typical example for CuAAC reaction with peptides.
Peptide (1.38 10^{-3}mmol, 1.0 equiv.) was dissolved in 0.5 mL of HEPES buffer (0.2M, pH = 7.6). In a second vial, catalyst **5** (4.9 mg, 4.6 10^{-3}mmol, one equivalent – peptides **16** and **17** – or 2.0 mg, 1.8 10^{-3}mmol for 40 mol% – peptides **14** and **15**), azidotyrosine (4.7 mg, 22.7 10^{-3} mmol) were added under stirring to a mixture of 2.4 mL HEPES (0.2M, pH = 7.6) and 0.6 mL of HFIP. The solution turned limpid after 2-3 minutes. 1 mL of the latter solution (1.5 10^{-3} mmol, 1.1 equiv. of **5** and 7.6 10^{-3}mmol, 5.5 equiv.of azidotyrosine) was added to the former. The solution was shaken gently overnight (18h). Before LCMS analysis, the buffer and the excess of azidotyrosine were removed using SPE C18 cartridges. The column was conditioned with 1 mL MeOH and washed with 1 mL water. Then 1mL of the reaction mixture was deposed, passed through, washed with 1 mL water and the compound was eluted with 1 mL MeCN. 100 µL of this solution were diluted in 900 µL of water and injected in LCMS.

HPLC chromatograms and high resolution mass spectra of compounds 14a-17b
a) HPLC of starting material
b) HPLC after reaction
c) HRMS (ESI+) after reaction

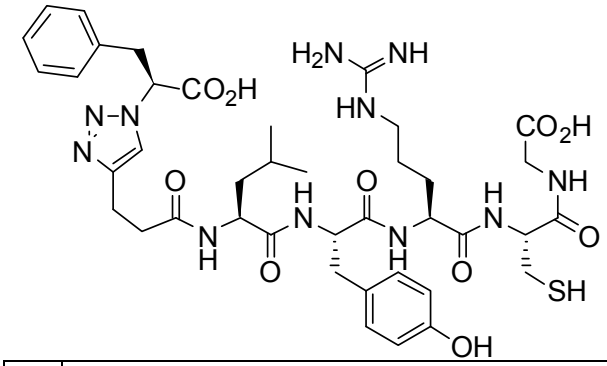
14a:

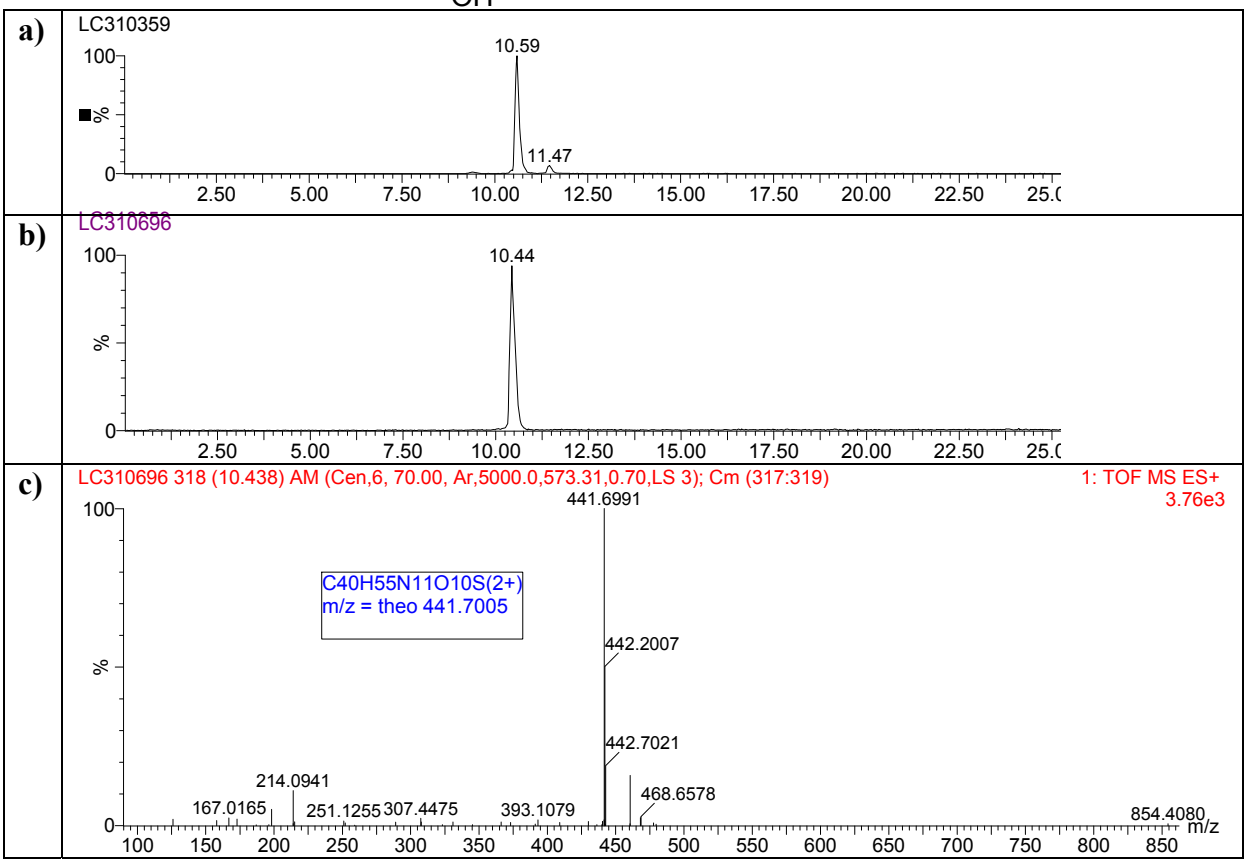




$C_{40}H_{55}N_{11}O_{11}S$ (M)
***m/z* theo (2+) = 449.6980**

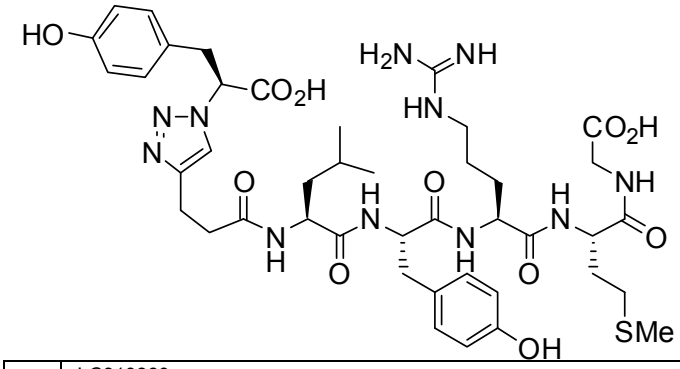
14b:

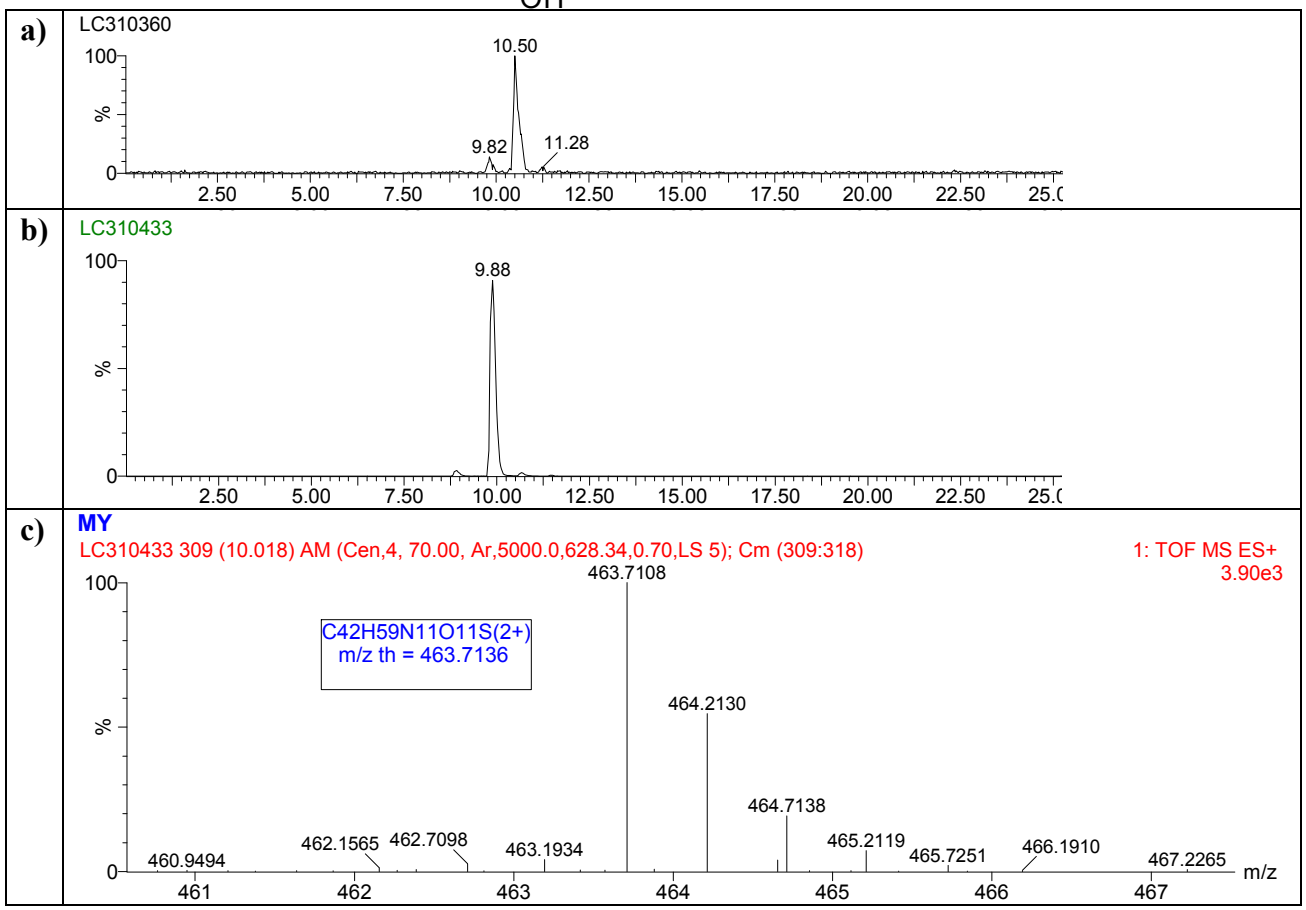




$C_{40}H_{55}N_{11}O_{10}S$ (M)
***m/z* theo (2+) = 441.7005**

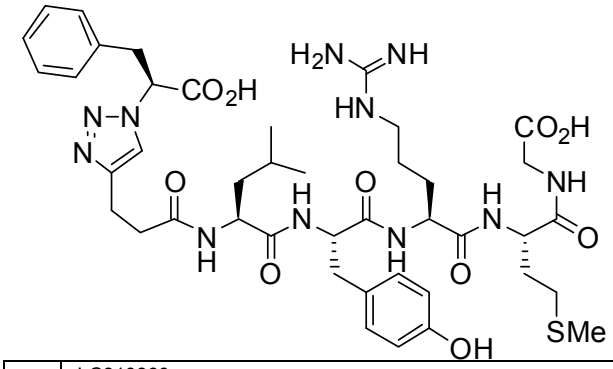
15a:

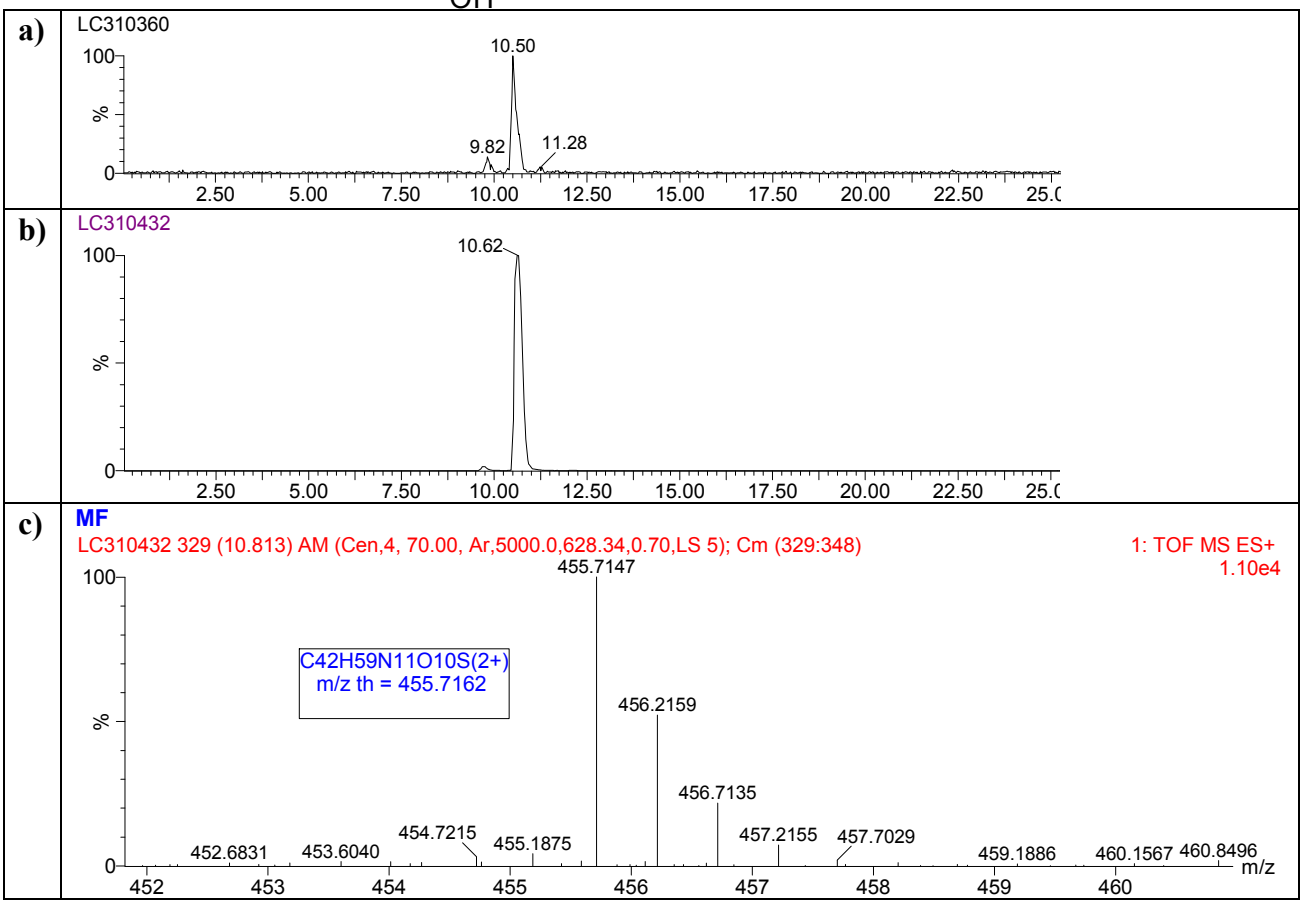




$C_{42}H_{59}N_{11}O_{11}S$ (M)
***m/z* theo (2+) = 463.7136**

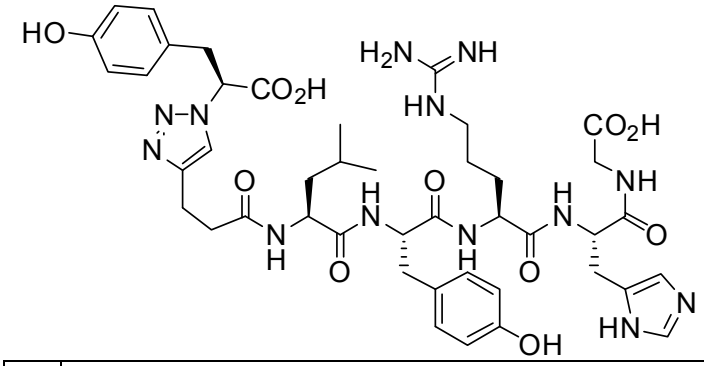
15b:

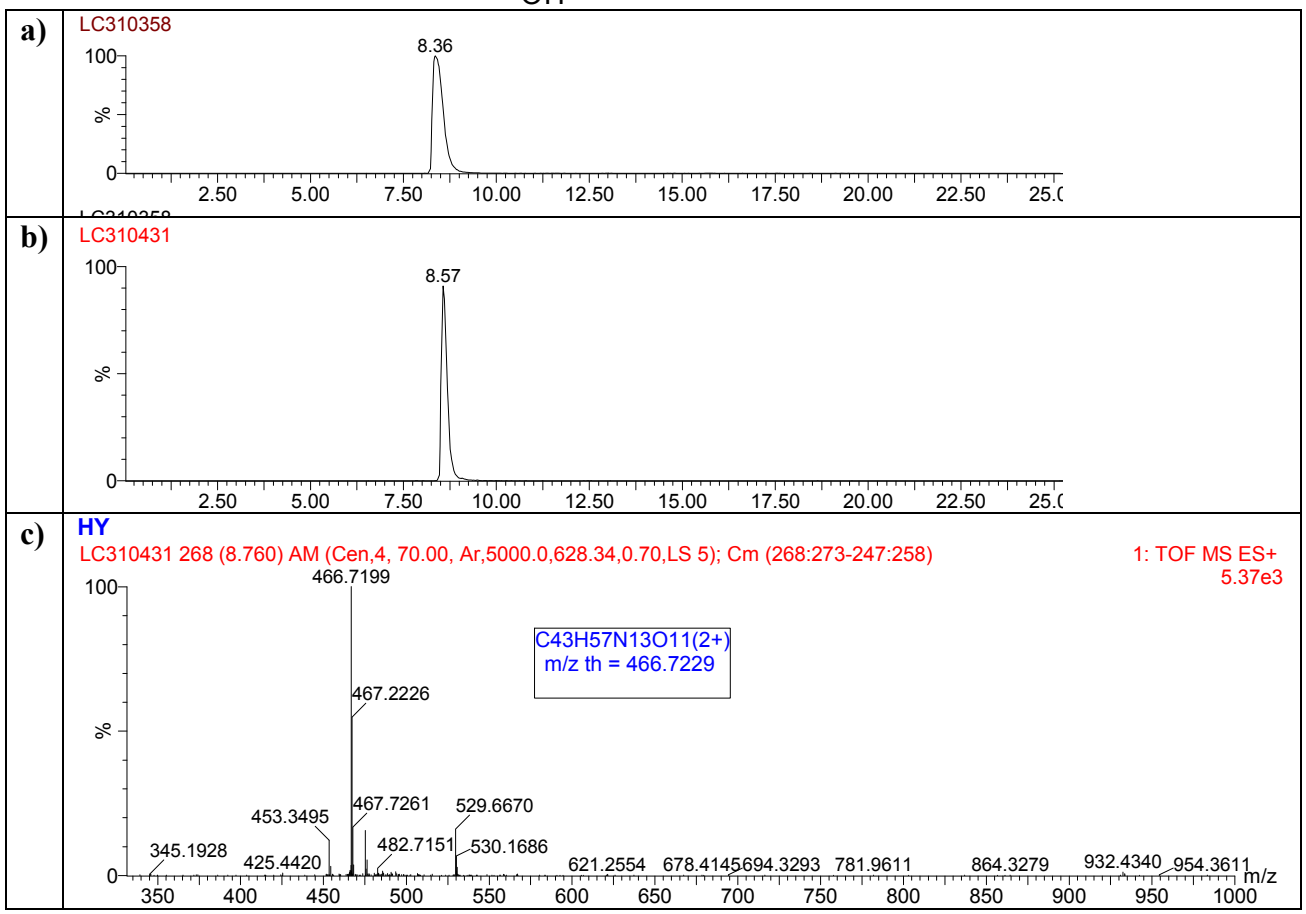




$C_{42}H_{59}N_{11}O_{10}S$ (M)
***m/z* theo (2+) = 455.7162**

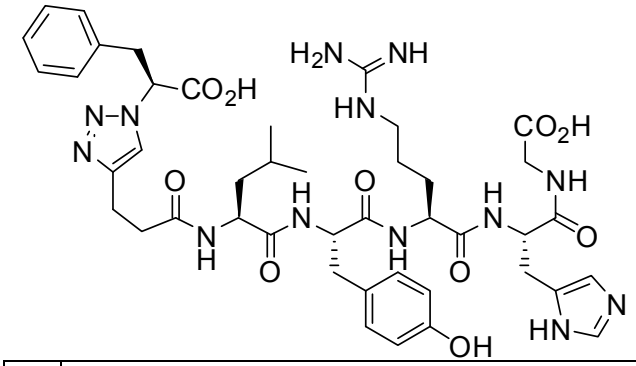
16a:

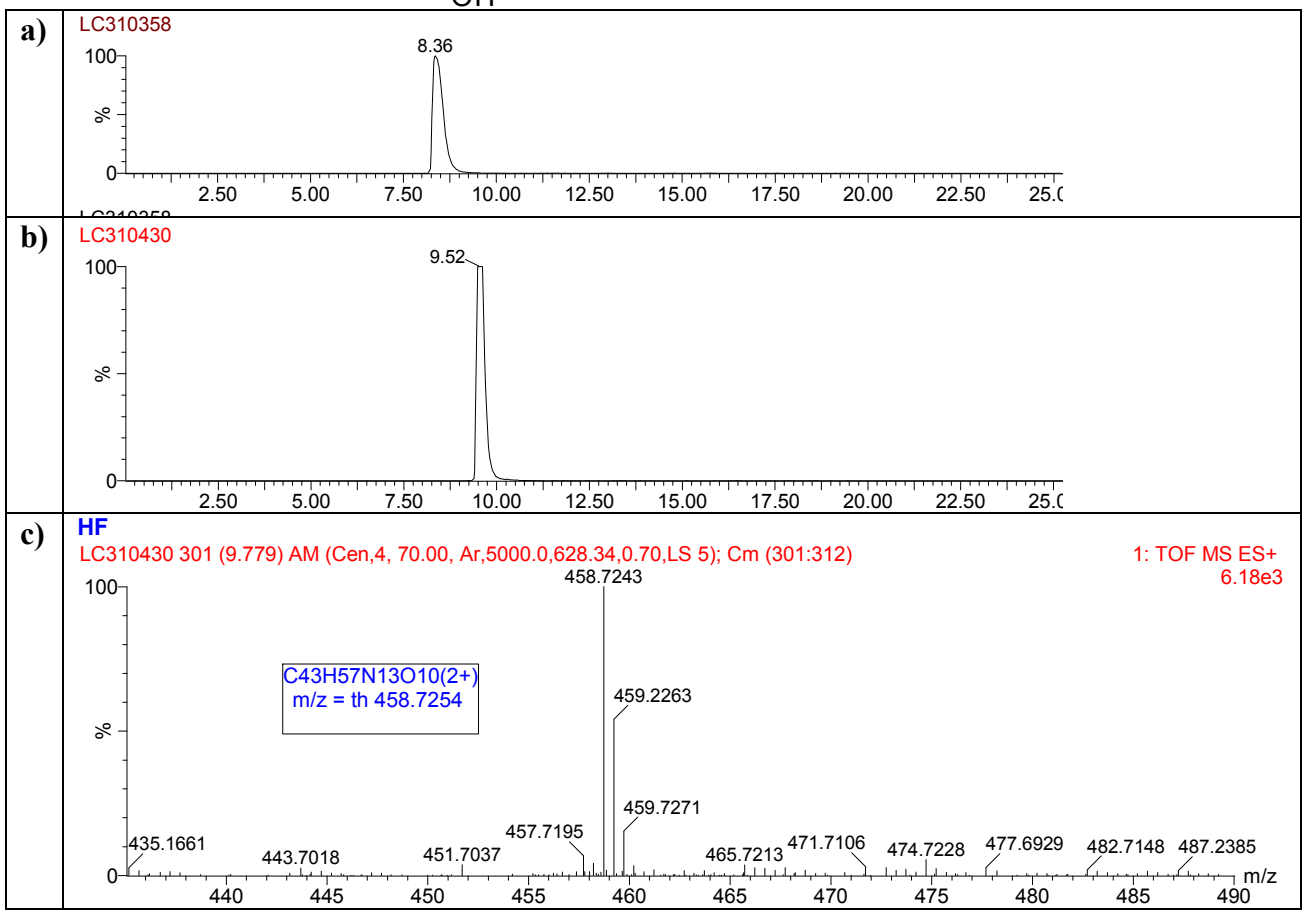




$C_{43}H_{57}N_{13}O_{11}$ (M)
***m/z* theo (2+) = 466.7229**

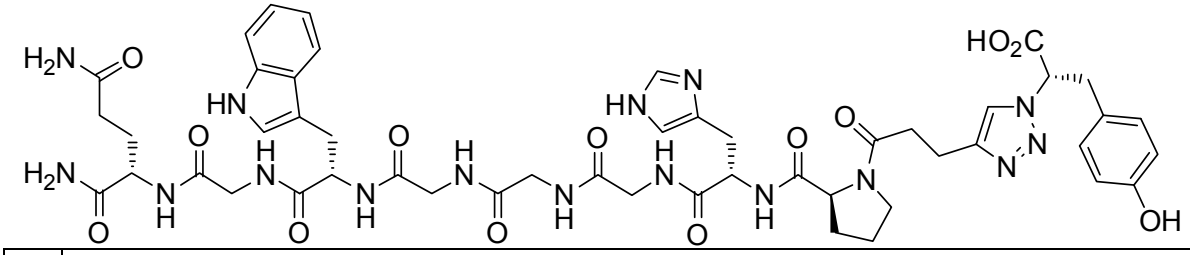
16b:

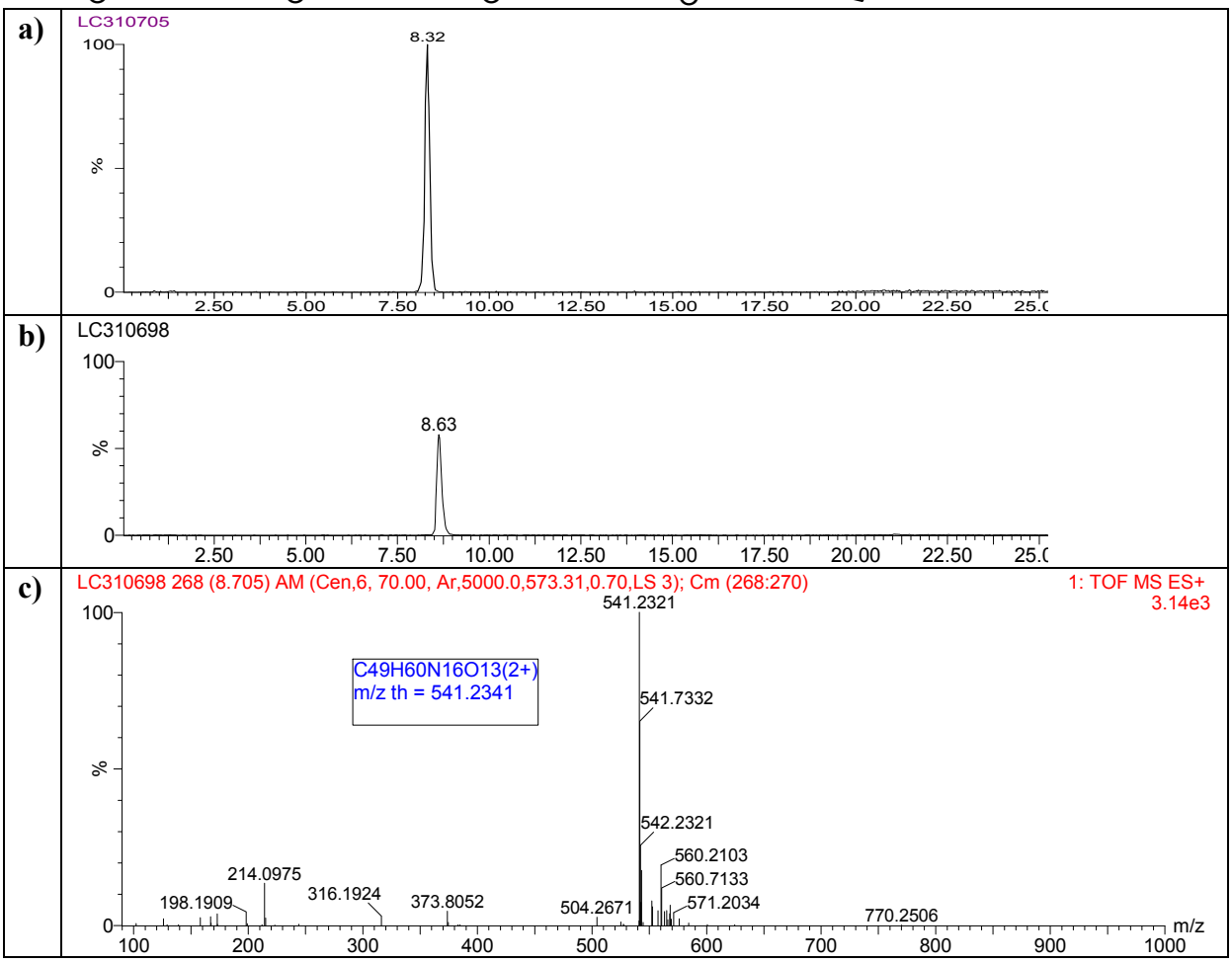




$C_{43}H_{57}N_{13}O_{10}$ (M)
***m/z* theo (2+) = 458.7245**

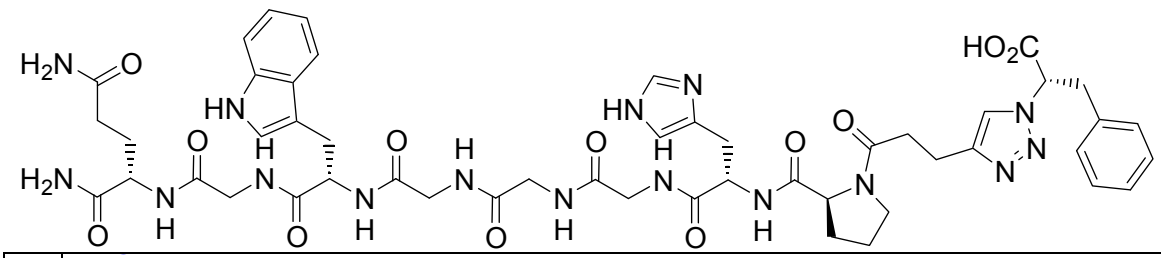
17a:

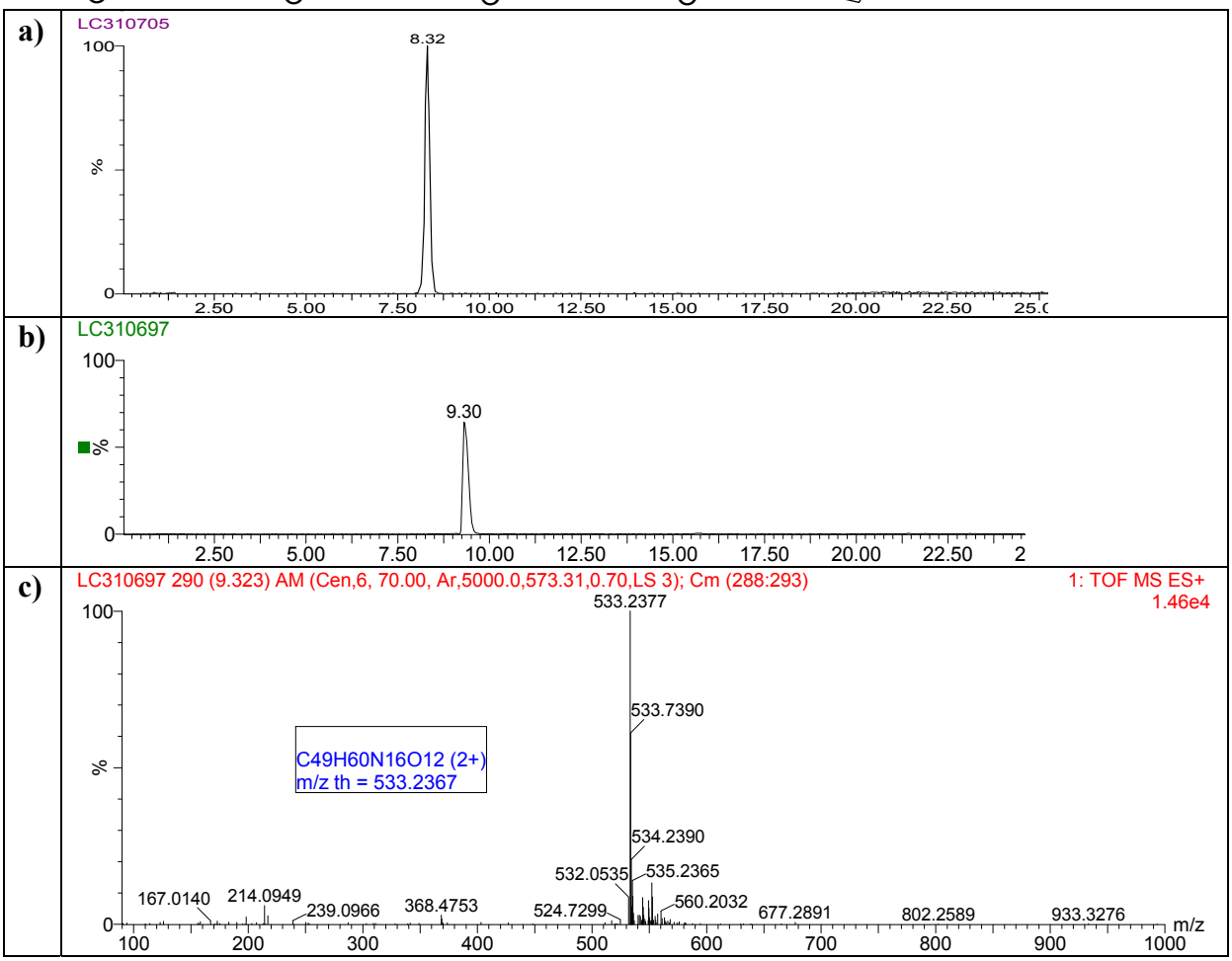




$C_{49}H_{60}N_{16}O_{13}$ (M)
***m/z* theo (2+) = 541.2341**

17b:





$C_{49}H_{60}N_{16}O_{12}$ (M)
***m/z* theo (2+) = 533.2367**

References:

[1] With the HCl adduct of imidazole-1-sulfonyl azide: E. D. Goddard-Borger and R. V. Stick *Org. Lett.* **2007**, *9,* 3797 – 3800. After submission of this work, a subsequent publication highlighted the safer-to-use H_2SO_4 adduct of salt of imidazole-1-sulfonyl azide. Fischer, E. D. Goddard–Borger, R. Greiner, T. M. Klapötke, B. W. Skelton and J. Stierstorfer, *J. Org. Chem.* **2012**, *77***,** 1760–1764.

[2] V. D. Bock, D. Speijer, H. Hiemstra and J. H. van Maarseveen *Org. Biomol. Chem.*, **2007**, *5*, 971–975

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