Electronic Supplementary Material (ESI) for ChemComm.

Scalable and practical synthesis of clickable Cu-chelating azides

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Supplementary Information

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I. General Information

Organic solvents (Aldrich) were used without further purification. Purifications of reactions products were carried out by flash chromatography using Merck silica gel (40-63 µm). FT-ATR-IR spectra were recorded on a Perkin-Elmer UAR Two Spectrum spectrometer and are reported as wavenumber (cm^{-1}). ^{1}H NMR (400 MHz),^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Electrospray mass spectra were obtained using an ESI-Quadripole autopurify, Waters (pump 2545, mass: ZQ2000) mass Spectrometer. Unless otherwise noted, all other commercially available reagents and solvents were used without further purification.

MATERIALS

The Myoglobin from equine heart (1882), $CuSO_4$, AscNa, Coumarin 343 were from Sigma Aldrich. Alkyne-peg_4NHS (2,5-dioxopyrrolidin-1-yl 4,7,10,13,16-pentaoxanonadec-18-ynoate, ref TA-103) and TAMRA-Peg3-N3 (5-((2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate, ref. AZ109) were purchased at Click Chemistry Tools (US). The Mini-Protean III electrophoresis system, 4-20% gradient mini-gels, Running buffer and Precision Plus Molecular marker were from Bio-Rad Laboratories (Germany). THPTA was synthesized according to literature procedures.[1]

METHODS

SDS-PAGE

Proteins samples in loading buffer were boiled at 95 °C for 5 min and subsequently loaded into wells of a 4-20% SDS-**PAGE** gel.

FLUORESCENCE IMAGING

Fluorescent measurements were performed with VersaDoc MP 4000 Molecular Digital Imaging System (Bio-Rad) allowing visualization of bands relative to fluorescent proteins. Green LED was used as light source and the choosen filter for emission collection was at 605 nm.

LC-ESI-MS

LC-ESI-MS experiments were carried out using an Esquire HCT Electrospray ion trap mass spectrometer (Bruker Daltonik GmbH, Germany) in the positive ion mode coupled on-line to an Agilent 1100 HPLC. Nebulization and desolvation ESI conditions were optimized to obtain maximum sensitivity. Acidified samples were injected onto a monolith reverse-phase micro-column (ProSwift RP-4H, 1.0 x 50 mm, Thermo-Scientific) equilibrated in 0.1% TFA/water and elution was carried out at 200µL/min with a fast linear gradient of acetonitrile in 0.1% TFA. During elution, the flow was split with 10% directed to the electrospray mass spectrometer and 90% to the diode array UV-Vis detector. HyStar/EsquireControl softwares were used for full scan MS acquisitions. DataAnalysis software was used for data processing and obtention of deconvoluted spectra.

MALDI-MS

Proteins sample were analysed using a 4800 spectrometer MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA). Proteins samples diluted with sinapinic acid matrix solution prepared at 10 mg/mL in H_2O/CH_3CN/TFA (70/30/0.1) and acidified solutions were manually spotted on MALDI plate. MS spectra were recorded from crystallized samples using linear mode.

II - CHELAZIDE SYNTHESIS AND CHARACTERISATION

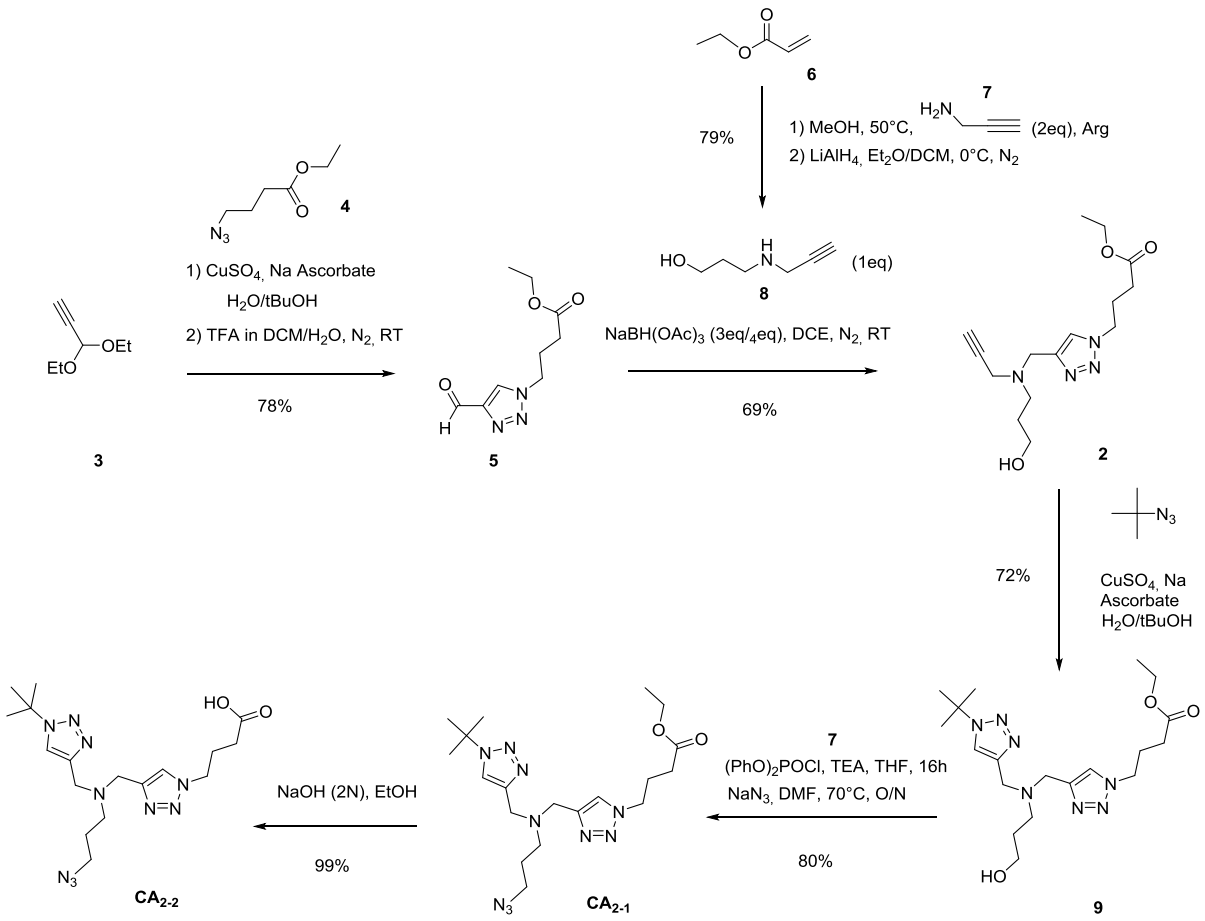
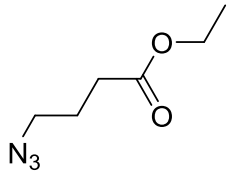


Figure 1: Synthon 9 synthesis Scheme

- **Ethyl 4-azidobutanoate (4)**



Ethyl-4-bromobutyrate (5 g, 25.6 mmol) and sodium azide (2.5 g, 38.5 mmol) were dissolved in 20 ml of DMSO. The mixture was stirred at 45 °C for 24 hrs. The reaction was then cooled down to room temperature, 20 ml of water were slowly added and the desired compound was

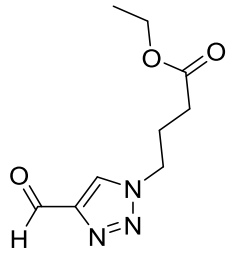
extracted with Et_2O (3 x 10 ml), dried over $MgSO_4$, filtered and concentrated to afford the desired product **4** as a colourless oil (4 g, 25.45 mmol, yield 100 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.1 Hz, 2H), 3.34 (t, *J* = 6.7 Hz, 2H), 2.39 (t, *J* = 7.2 Hz, 2H), 1.90 (q, *J* = 6.9 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H).

IR (cm^{-1}) 2982, 2939, 2094, 1730, 1374, 1251, 1162, 1028, 862.

Data in agreement with literature.[2]

- **Ethyl 4-(4-formyl-1H-1,2,3-triazol-1-yl)butanoate (5)**



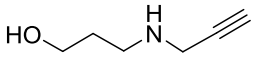
To a solution of 3,3-diethoxypropyne (163 mg, 1.27 mmol) and **4** (200 mg, 1.27 mmol) in an equimolar solution of H_2O/*t*-BuOH (6 ml), copper sulfate pentahydrate (32 mg, 0.127 mmol) and sodium ascorbate (252 mg, 1.27 mmol) were added and the reaction mixture was stirred for 12h at room temperature. The solvent was removed by vacuum and the crude was solubilized in DMC and washed with water. The aqueous phase was extracted three times with DCM and the resulting organic phases were combined and dried over MgSO4, filtered and concentrated. The residue was then solubilized in a mixture of DCM (6ml) and water (3ml) to which was added TFA (0.972 ml, 12.7 mmol). The reaction mixture was stirred for 2h at room temperature under argon. After completion, the reaction was quenched with NaOH to pH 7, extracted with DCM, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography (DCM:MeOH 95:5) to afford the product as a yellowish oil (211 mg, 0.99 mmol, yield 78%).

1H NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1H), 8.20 (s, 1H), 4.44 (t, *J* = 6.9 Hz, 2H), 3.97 (dt, *J* = 7.2, 5.4 Hz, 2H), 2.25 (q, *J* = 6.8 Hz, 2H), 2.20 – 2.11 (m, 2H), 1.10 (t, *J* = 7.1 Hz, 3H).

IR (cm^{-1}) 3119, 2965, 1722, 1683, 1540, 1434, 1370, 1354, 1271, 1231, 1179, 1066, 1044, 1023, 887, 784, 743, 559.

Data in agreement with literature.[3]

- **3-(prop-2-yn-1-ylamino)propan-1-ol (8)**



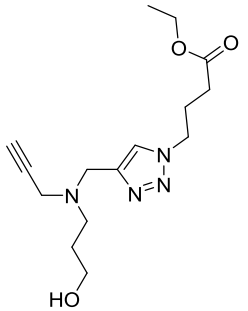
To a solution of propargylamine (5.81 ml, 90.78 mmol) in methanol (80 ml) was added ethyl acrylate (4.95 ml, 45.38 mmol) and the solution was stirred 24 h at 50 °C under argon. After evaporation of the solvent, the residue was solubilized in 30 ml of a DCM/Et_2O mixture (10 ml DCM + 20 ml Et_2O). This solution was added carefully to a solution of lithium aluminium hydride (3.445 g, 90.77 mmol) in Et_2O (188 ml) at 0 °C and the mixture was stirred overnight under argon. After completion, the reaction was quenched with water at 0 °C, and dried with $MgSO_4$. The precipitates were filtered through celite and rinsed with DCM. After concentration, the crude was then purified by silica gel chromatography (DCM:MeOH 95:5) to afford the product as a yellow-orange oil (4.907 g, 43.36 mmol, yield 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76 (t, *J* = 5.5 Hz, 2H), 3.42 (d, *J* = 2.3 Hz, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 2.23 (t, *J* = 2.2 Hz, 1H), 1.75 – 1.65 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 81.48, 71.75, 63.29, 47.79, 37.88, 30.90.

IR (cm^{-1}) 3287, 2930, 2845, 1452, 1333, 1106, 1059, 1029, 908, 636, 506.

- **Ethyl 5-(4-(((3-Hydroxypropyl)(prop-2-yn-1-yl)amino)methyl)-1H-1,2,3-triazol-1-yl) pentanoate (2)**



To a mixture of 3-(prop-2-yn-1-ylamino)propan-1-ol **8** (2.645 g, 23.37 mmol) and **e**thyl 4-(4-formyl-1H-1,2,3-triazol-1-yl)butanoate **5** (4.88 g, 23.14 mmol) in 4 ml of 1,2-dichloroethane, was added $NaBH(OAc)_3$ (12.262 g, 57.85 mmol). The mixture was stirred overnight at room temperature under argon. The reaction was then diluted with DCM, water was added (30 ml) and the mixture was basified to pH 14 with NaOH (6N). The solution was then extracted with DCM, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography

(DCM:MeOH 95:5) to afford the product as an yellowish oil (5.139 g, 16.66 mmol, yield 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 4.44 (t, *J* = 6.8 Hz, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 2H), 3.79 – 3.73 (m, 2H), 3.46 (d, *J* = 2.1 Hz, 2H), 2.84 (t, *J* = 5.9 Hz, 2H), 2.35 (t, *J* = 6.9 Hz, 2H), 2.29 (t, *J* = 2.2 Hz, 1H), 2.28 – 2.19 (m, 2H), 1.76 (dt, *J* = 10.9, 5.6 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 4H).

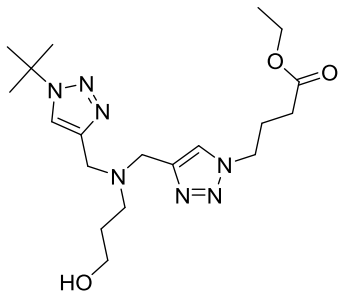
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.38, 144.64, 122.86, 77.89, 73.96, 63.61, 60.85, 52.49, 49.40, 48.66, 42.08, 30.84, 28.28, 25.57, 14.29.

IR (cm^{-1}) 3282, 2935, 2850, 1727, 1444, 1376, 1330, 1258, 1189, 1128, 1048, 803, 658.

LC-MS (ESI+), *m/z:* 308.9 $[M]^+$, 309.2 $[M+H]^+$, 310.3 $[M+2H]^+$.

HRMS (ESI) calculated for $C_{15}H_{24}N_4O_3$ [M+H]+, 309.192117, found 309.192095; calculated for $C_{15}H_{24}N_4NaO_3$ [M+Na]+ 331.174061, found 331.173702.

- **Ethyl 5-(4-((((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) (3-hydroxypropyl)amino) methyl)-1H-1,2,3-triazol-1 -yl)pentanoate (9)**



To a solution of ethyl 5-(4-(((3-Hydroxypropyl)(prop-2-yn-1-yl)amino)methyl)-1H 1,2,3-triazol-1-yl) pentanoate **2** (5.139 g, 16.66 mmol) and *t*-butylazide (3.3 g, 33.33 mmol; ATTENTION: *t*-butylazide should be handled with care and used in presence of a protective shield)[4] in an equimolar solution of H_2O/*t*-BuOH (70 ml), copper sulfate pentahydrate (4.16 g, 16.66 mmol) and sodium ascorbate (6.6 g, 33.33 mmol) were added and the reaction mixture was stirred overnight at room temperature. After completion, 120 ml of DCM was added to the mixture directly followed by NH_4OH (6 ml). The mixture was extracted with DCM (3 x 120 ml). The aqueous phases were collected and basified to pH 14 and extracted. The organic phases were collected, dried over $MgSO_4$, filtered, concentrated and the crude was purified by silica gel chromatography (EtOAc:MeOH 85:15) to afford the product as an yellowish oil (4.92 g, 12.07 mmol, yield 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.70 (s, 1H), 4.45 (t, *J* = 6.8 Hz, 2H), 4.14 (p, *J* = 7.3 Hz, 3H), 3.82 (s, 2H), 3.79-3.76 (m, 4H), 2.81 (t, *J* = 5.5 Hz, 2H), 2.36 (t, *J* = 7.1 Hz, 2H), 2.30 – 2.21 (m, 2H), 2.06 (s, 2H), 1.87 – 1.79 (m, 2H), 1.69 (s, 9H), 1.27 (t, *J* = 7.1 Hz, 3H).

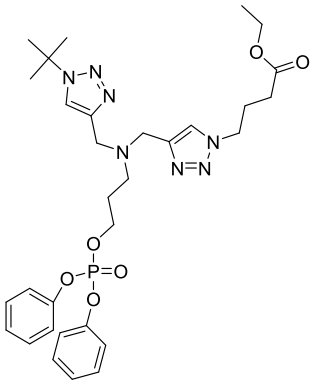
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.27, 143.41, 142.89, 123.60, 120.38, 63.61, 60.72, 59.42, 53.05, 49.29, 47.47, 47.32, 30.74, 29.95 (3C), 27.90, 25.41, 14.15.

IR (cm^{-1}) 3373, 2979, 2939, 1728, 1462, 1372, 1329, 1205, 1045, 825, 667.

LC-MS (ESI+), *m/z:* 408.3 $[M+H]^+$.

HRMS (ESI) calculated for $C_{19}H_{34}N_7O_3$ [M+H]+, 408.271764 found 408.271429; calculated for $C_{19}H_{33}N_7NaO_3$ [M+Na]+ 430.253709, found 430.253276.

- **Ethyl 4-(4-((((1-(tert-butyl)-1H-1,2,3-triazol-4-yl) methyl) (3 ((diphenylphosphoryl) oxy)propyl)amino)methyl)-1H-1,2,3-triazol-1-yl)butanoate (10)**



To a mixture of ethyl 5-(4-((((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) (3-hydroxypropyl)amino) methyl)-1H-1,2,3-triazol-1 -yl)pentanoate **9** (1.5 g, 3.68 mmol) and trimethylamine (0.994 ml, 7.362 mmol) in 10 ml of THF, was added diphenylchlorophosphate (1.144 ml, 5.52 mmol) dropwise at 0 °C under argon. After 20h, the solvent was evaporated and the residue was purified by silica gel chromatography (EtOAc:MeOH 95:5) to afford the product as an yellowish oil (1.994 g, 3.12 mmol, yield 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (t, *J* = 7.8 Hz, 4H), 7.12 (dd, *J* = 16.2, 7.9 Hz, 6H), 4.31 (dt, *J* = 8.8, 4.9 Hz, 4H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.66 (s, 2H), 3.63 (s, 2H), 2.54 (t, *J* = 6.7 Hz, 2H), 2.25 (t, *J* = 7.0 Hz, 2H), 2.19 – 2.08 (m, 2H), 1.95 (dd, *J* = 12.5, 6.2 Hz, 2H), 1.58 (s, 9H), 1.17 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.17, 150.49, 150.42, 143.89, 143.29, 129.75 (3C), 125.29, 123.48, 120.28, 119.99 (2C), 119.95 (2C), 67.47, 67.41, 60.58, 59.14, 49.12, 49.03, 47.44, 47.30, 30.73, 29.91 (3C), 27.87, 27.81, 25.39, 14.13.

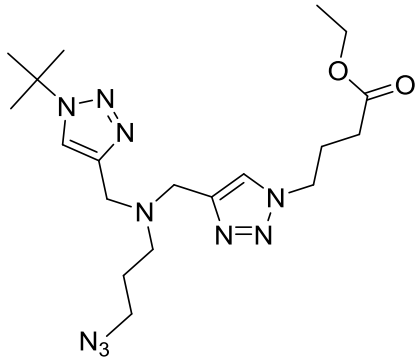
^{31}P NMR (162 MHz, $CDCl_3$) δ -11.79 (t, *J* = 7.2 Hz).

IR (cm^{-1}) 2980, 1730, 1590, 1488, 1372, 1283, 1188, 1162, 1022, 946, 772, 727, 689, 523.

LC-MS (ESI+), *m/z:* 640.4 $[M+H]^+$, (isotope) 641.7 $[M+H]^+$.

HRMS (ESI) calculated for $C_{31}H_{43}N_7O_6P$ [M+H]+, 640.300695 found 640.300055; calculated for $C_{31}H_{42}N_7NaO_6P$, [M+Na]+ 662.282639, found 662.281976.

- **Ethyl 5-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) amino) methyl)-1H-1,2,3-triazol-1-yl)pentanoate ($CA_{2\text{-}1}$)**



To a solution of ethyl 4-(4-((((1-(tert-butyl)-1H-1,2,3-triazol-4-yl) methyl) (3-((diphenylphosphoryl) oxy)propyl)amino)methyl)-1H-1,2,3-triazol-1-yl)butanoate **10** (1.031 g, 1.61 mmol) in DMF (15 ml) was added sodium azide (262 mg, 4.02 mmol) under argon. The mixture was heated to 80 °C for 2 days under argon. The solvent was then evaporated and the residue was purified by silica gel chromatography (EtOAc:MeOH 95:5) to afford the product as an yellowish oil (656 mg, 1.52 mmol, yield 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.68 (s, 1H), 4.44 (t, *J* = 6.8 Hz, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.75 (d, *J* = 11.4 Hz, 4H), 3.37 (t, *J* = 6.6 Hz, 2H), 2.59 (t, *J* = 6.9 Hz, 2H), 2.36 (t, *J* = 6.9 Hz, 2H), 2.30 – 2.20 (m, 2H), 1.94 – 1.84 (m, 2H), 1.74 – 1.65 (m, 9H), 1.27 (t, *J* = 7.1 Hz, 3H).

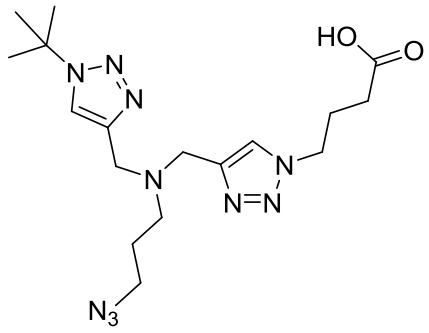
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.29, 162.52, 123.53, 120.31, 60.75, 59.29, 50.14, 49.33, 49.25, 47.58, 47.32, 36.50, 31.44, 30.78 (3C), 26.53, 25.48, 14.20.

IR (cm^{-1}) 3338, 2977, 2094, 1698, 1516, 1453, 1366, 1250, 1167, 1046, 800, 730.

LC-MS (ESI+), *m/z:* 433.6 $[M+H]^+$.

HRMS (ESI) calculated for $C_{19}H_{33}N_{10}O_2$ [M+H]+, 433.278247 found 433.278092; calculated for $C_{19}H_{32}N_{10}NaO_2$, [M+Na]+ 455.260191, found 455.260211.

- **5-(4-(((3-Azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)pentanoic acid ($CA_{2\text{-}2}$)**



To a solution of ethyl 5-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) amino) methyl)-1H-1,2,3-triazol-1-yl)pentanoate **$CA_{2\text{-}1}$** (500 mg, 1.156 mmol) in EtOH (5 ml) was added NaOH (69 mg, 1.734 mmol) under argon. The solvent was then evaporated and the residue was triturated in MeOH to precipitate the salts to afford the product as an yellowish oil (463 mg, 1.14 mmol, yield 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.65 (s, 1H), 4.29 (s, 2H), 3.67 (d, *J* = 5.3 Hz, 4H), 3.28 (t, *J* = 6.5 Hz, 2H), 2.47 (t, *J* = 6.5 Hz, 2H), 2.06 (s, 2H), 1.99 (s, 2H), 1.84 – 1.71 (m, 2H), 1.63 (s, 9H).

^{13}C NMR (101 MHz, MeOD) δ 179.56, 169.28, 143.93, 143.55, 124.01, 121.22, 59.49, 49.86, 49.50, 48.90, 47.85, 34.12, 28.86 (3C), 27.05, 26.08.

IR (cm^{-1}) 3385, 2939, 2095, 1570, 1414, 1370, 1305, 1208, 1046, 768.

LC-MS (ESI+), *m/z:* 405.3 $[M+H]^+$, 406.3 $[M+2H]^+$.

HRMS (ESI) calculated for $C_{17}H_{29}N_{10}O_2$ [M+H]+, 405.246947 found 405.246625; calculated for $C_{17}H_{28}N_{10}NaO_2$, [M+Na]+ 427.228891, found 427.228580.

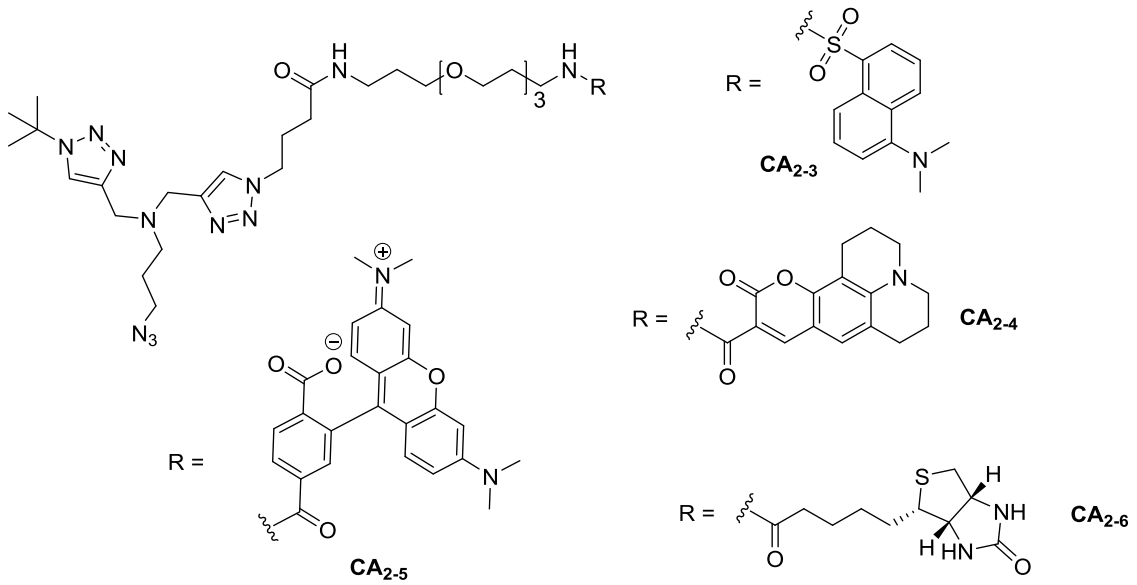


Figure 2: CA_2 probes

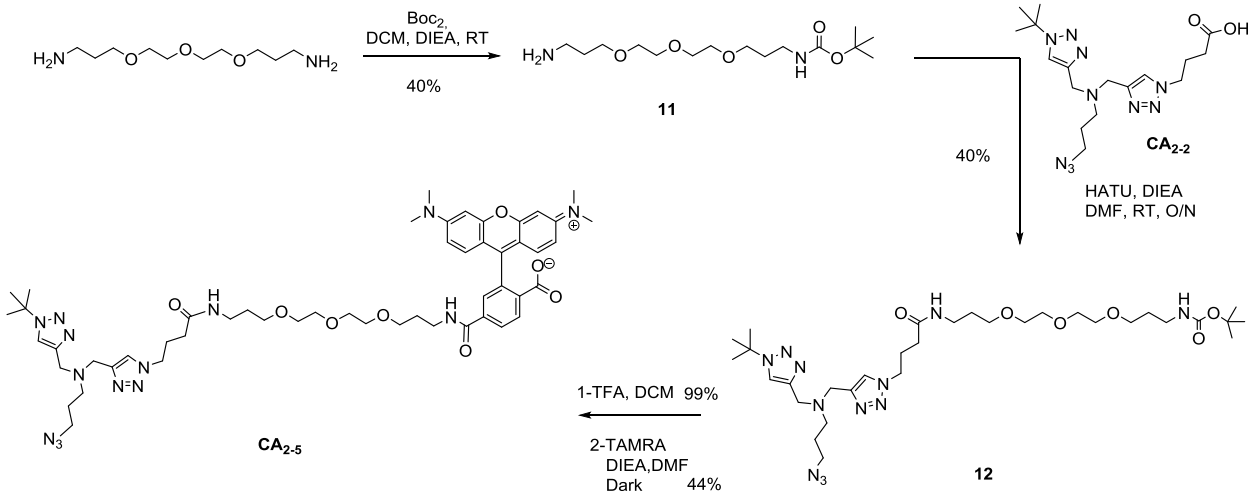
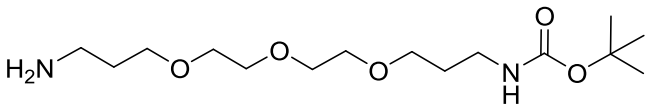


Figure 3: Chelazide-TAMRA synthesis

- ***tert*-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (11)**



To a solution of 3,3'-((oxybis(ethane-2,1-diyl))bis(oxy))bis(propan-1-amine) (500 mg, 2.27 mmol) and DIEA (0.395 µl, 2.27 mmol) in DCM (10 ml) was drop-wisely (addition rate: 1.2 ml/min) added di-*tert*-butyl dicarbonate (262 mg, 4.02 mmol) overnight at room temperature under argon. The solvent was then evaporated and the residue was solubilized in DCM and washed with Na_2CO_3, the organic phases were collected, dried over $MgSO_4$, filtered, concentrated and the crude was purified by silica gel chromatography (DCM:MeOH:TEA 93:7:1) to afford the product as an yellowish oil (296 mg, 0.92 mmol, yield 40%).

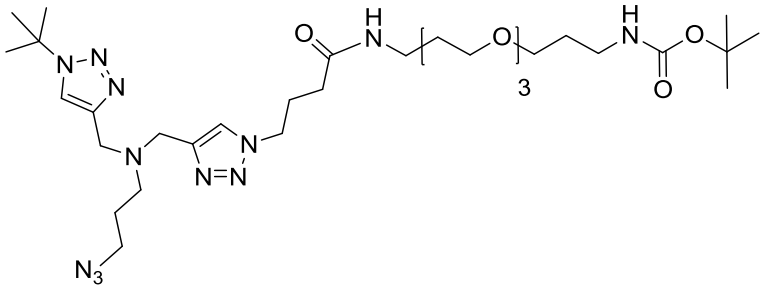
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 1H), 3.70 – 3.52 (m, 12H), 3.24 (dd, *J* = 11.7, 5.7 Hz, 2H), 2.82 (t, *J* = 6.7 Hz, 2H), 1.82 – 1.70 (m, 4H), 1.45 (s, 9H).

IR (cm^{-1}) 3358, 2928, 2865, 1704, 1519, 1390, 1364, 1271, 1249, 1170, 1104, 1039, 945, 860, 778.

LC-MS (ESI+), *m/z:* 321.3 $[M+H]^+$.

Data in agreement with literature.[5]

- **tert-butyl (3-(3-(4-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) amino) methyl) -1H-1,2,3-triazol-1-yl)butanamido)propoxy)propyl)carbamate (12)**



To a solution of 5-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl) methyl) amino) methyl)-1H-1,2,3-triazol-1-yl)pentanoic acid **$CA_{2\text{-}2}$** (200 mg, 0.494 mmol) in DMF (3 ml) is added successively HATU (207 mg, 0.544 mmol) and *tert*-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate **11** (158 mg, 0.494 mmol) solubilized in DMF (5 mL) overnight at room temperature under argon. After 10 min, diispropylethylamine

(344µl, 0.256 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was then evaporated and the residue was purified by silica gel chromatography (DCM:MeOH 99:1 to 95:5) to afford the product as an yellowish oil (202 mg, yield 57%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.68 (s, 1H), 6.69 (s, 1H), 5.04 (s, 1H), 4.44 (t, *J* = 6.4 Hz, 2H), 3.78 (s, 2H), 3.74 (s, 2H), 3.60 (ddt, *J* = 14.7, 12.0, 5.4 Hz, 12H), 3.39 (q, *J* = 6.3 Hz, 4H), 3.27 – 3.18 (m, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.30 – 2.14 (m, 4H), 1.94 – 1.85 (m, 4H), 1.78 (ddd, *J* = 18.8, 12.4, 6.2 Hz, 4H), 1.70 (s, 9H), 1.44 (s, 9H).

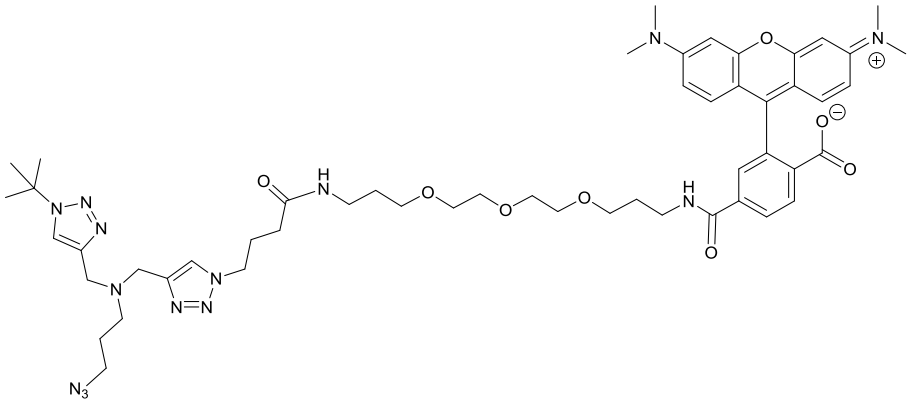
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.43, 156.10, 143.88, 143.49, 123.79, 120.24, 78.97, 70.41, 70.11, 70.00, 69.77, 69.44, 59.32, 50.31, 49.30, 49.26, 47.78, 47.52, 38.36, 37.71, 32.54, 30.01, 29.67, 29.06, 28.45, 26.57, 26.19.

IR (cm^{-1}) 3328, 2929, 2868, 2094, 1704, 1658, 1530, 1454, 1365, 1269, 1249, 1169, 1104, 844, 778, 666, 557.

LC-MS (ESI+), *m/z:* 707.5 $[M+H]^+$.

HRMS (ESI) calculated for $C_{32}H_{59}N_{12}NaO_6$ [M+H+Na]2+ 365.228362, found 365.228552; calculated for $C_{32}H_{59}N_{12}O_6$ [M+H]+, 707.467504 found 707.467759; calculated for $C_{32}H_{58}N_{12}NaO_6$, [M+Na]+ 729.449448, found 729.449785.

- **4-((18-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-15-oxo-4,7,10-trioxa-14-azaoctadecyl)carbamoyl)-2-(6-(diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)benzoate ($CA_{2\text{-}5}$)**



To a solution of tert-butyl (3-(3-(4-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) amino) methyl) -1H-1,2,3-triazol-1-yl)butanamido)propoxy)propyl)carbamate **12** (60 mg, 0.0849 mmol) in DCM (1 ml) was added TFA (32 µl, 0.425mmol). The reaction mixture was stirred for 2h at room temperature under argon. After completion, the mixture

was evaporated and directly used for coupling. The residue was solubilized in DMF (1ml). To this solution were added successively HATU (32 mg, 0.0849 mmol), TAMRA (33.2 mg, 0.0771 mmol) and diispropylethylamine (67µl, 0.385 mmol) under argon. The reaction was stirred overnight at room temperature in the dark. The solvent was then evaporated and the residue purified via HPLC (with gradient H_2O:acetronitrile from 95:5 to 55:65, flux 21.2 ml/min, Column Interchim Uptisphere HDO C18, 150 x 21.2 mm, 5 µm) to afford the product as a bright pink oil (37.5 mg, 0.036 mmol, yield 44%).

^{1}H NMR (400 MHz, MeOD) δ 8.17 (d, *J* = 8.1 Hz, 1H), 8.10 (dd, *J* = 8.1, 1.6 Hz, 1H), 8.05 (s, 1H), 7.97 (s, 1H), 7.73 (d, *J* = 1.5 Hz, 1H), 7.29 (s, 1H), 7.26 (s, 1H), 7.04 (dd, *J* = 9.5, 2.4 Hz, 2H), 6.95 (d, *J* = 2.4 Hz, 2H), 4.42 (t, *J* = 6.3 Hz, 2H), 3.74 (d, *J* = 5.6 Hz, 4H), 3.59 (dd, *J* = 8.5, 3.3 Hz, 8H), 3.54 – 3.44 (m, 7H), 3.38 (d, *J* = 7.5 Hz, 2H), 3.31 (s, 12H), 3.23 (dd, *J* = 12.4, 5.5 Hz, 4H), 2.53 (t, *J* = 6.9 Hz, 2H), 2.22 – 2.15 (m, 4H), 1.93 – 1.85 (m, 2H), 1.83 – 1.76 (m, 2H), 1.73 (dd, *J* = 13.4, 6.9 Hz, 3H), 1.69 (s, 9H).

^{13}C NMR (151 MHz, H_2O+D_2O) δ 176.13, 174.88, 170.04, 159.77, 158.57, 158.53, 144.64, 136.04, 132.80, 132.62, 130.90, 130.61, 129.87, 127.53, 125.00, 115.55, 114.48, 97.85, 71.34, 71.17, 71.08, 70.44, 70.09, 62.22, 51.42, 51.37, 50.38, 48.90, 48.86, 41.74, 39.20, 38.15, 34.12, 30.65, 30.15, 29.91, 27.33, 26.34.

IR (cm^{-1}) 3328, 2927, 2098, 1647, 1596, 1492, 1408, 1366, 1349, 1189, 1124, 844.

LC-MS (ESI+), *m/z:* 1020.0 $[M+H]^+$.

HRMS (ESI) calculated for $C_{52}H_{72}N_{14}O_8$ [M+2H]2+, 510.282329 found 510.281950; calculated for $C_{32}H_{58}N_{12}NaO_6$, [M+Na]+ 1041.539326, found 1041.539572.

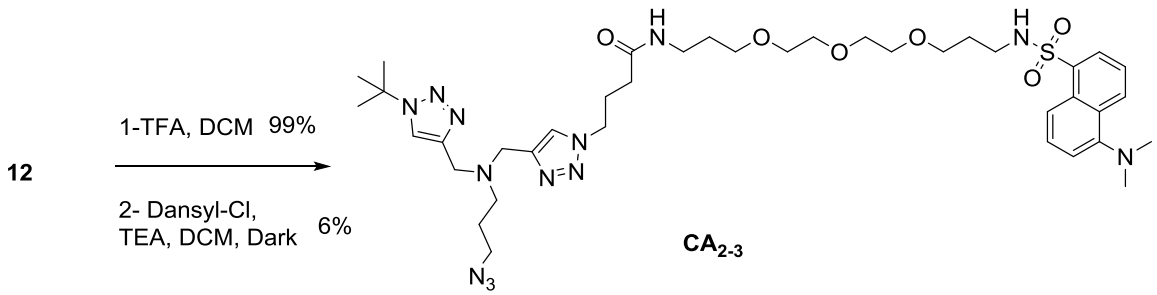
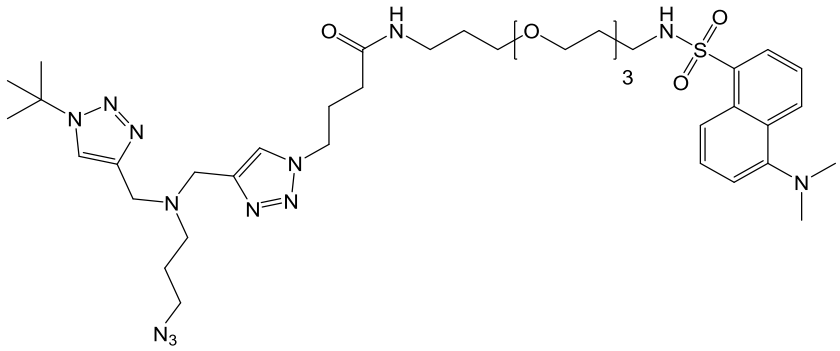


Figure 4: Chelazide-Dansyl synthesis

- **4-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-(3-((5-(dimethylamino)naphthalene)-1-sulfonamido)propoxy)propyl)butanamide (CA_{2-3})**



To a solution of tert-butyl (3-(3-(4-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl) amino) methyl) -1H-1,2,3-triazol-1-yl)butanamido)propoxy) propyl)carbamate **12** (30 mg, 0.0416 mmol) in DCM (1 ml) was added TFA (16 µl, 0.212 mmol). The reaction mixture was stirred for 2h at room temperature under argon. After completion, the mixture was evaporated and directly used for coupling. The residue was solubilized in DCM (1ml). To this solution were added successively triethylamine (28 µl, 0.208 mmol) and dansyl chloride (23 mg, 0.083 mmol) under argon. The reaction is stirred for two days. The solvent was then evaporated and the residue was purified via HPLC (with gradient H_2O:acetronitrile from 95:5 to 40:60, flux 17 ml/min, Column Bridge Waters C18, 19 x 150 mm, 5 µm) to afford the product as a yellowish oil (2 mg, 0.002 mmol, yield 6%).

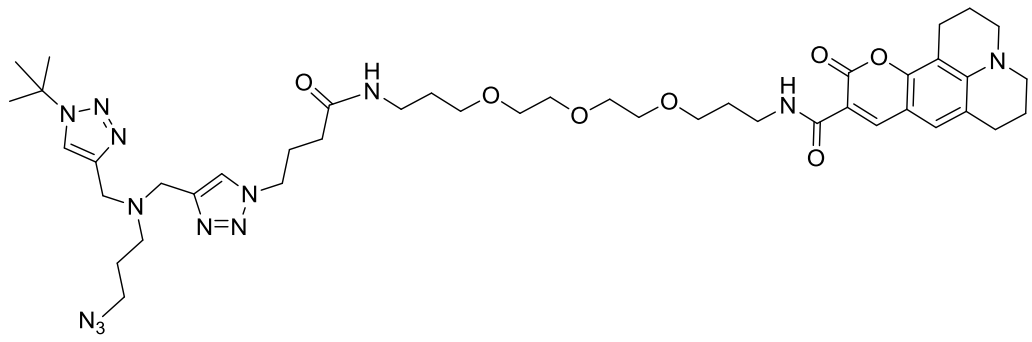
^{1}H NMR (400 MHz, MeOD) δ 8.58 (d, *J* = 8.5 Hz, 1H), 8.36 (d, *J* = 8.7 Hz, 1H), 8.20 (dd, *J* = 7.3, 0.9 Hz, 1H), 8.05 (s, 1H), 7.98 (s, 1H), 7.60 (td, *J* = 8.1, 3.0 Hz, 2H), 7.29 (d, *J* = 7.5 Hz, 1H), 4.44 (t, *J* = 6.5 Hz, 2H), 3.75 (d, *J* = 5.1 Hz, 4H), 3.61 – 3.53 (m, 4H), 3.50 (dd, *J* = 7.8, 4.5 Hz, 4H), 3.36 (d, *J* = 7.6 Hz, 6H), 3.25 (t, *J* = 6.8 Hz, 3H), 2.95 (t, *J* = 6.6 Hz, 2H),

2.90 (s, 6H), 2.53 (t, *J* = 6.8 Hz, 2H), 2.20 (t, *J* = 5.9 Hz, 4H), 1.85 – 1.71 (m, 4H), 1.69 (s, 9H), 1.60 (dt, *J* = 12.6, 6.3 Hz, 3H).

LC-MS (ESI+), *m/z:* 841.2 $[M+H]^+$.

HRMS (ESI) calculated for $C_{39}H_{63}N_{13}O_6S$ [M+2H]2+, 420.736700 found 420.736748; calculated for $C_{39}H_{61}N_{13}NaO_6S$, [M+Na]+ 862.448068, found 862.448755.

- **N-(18-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-15-oxo-4,7,10-trioxa-14-azaoctadecyl)-11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide ($CA_{2\text{-}4}$)**



To a solution of Coumarin 343 (12.5 mg, 0.0438 mmol) in DMF (1 ml) were added successively HATU (18.5 mg, 0.0482 mmol), *tert*-butyl (3-(3-aminopropoxy)propyl)carbamate **11** (14 mg, 0.0438 mmol) and diisopropylethylamine (31 µl, 0.175 mmol) under argon. The reaction was stirred for two days at room temperature in the dark. After evaporation of the solvent and purification of the crude by silica gel chromatography (AcOEt:MeOH:TEA 93:7:0 to 80:20:2) a solution of the resulting tert-butyl (1-oxo-1-(11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-10-yl)-6,9,12-trioxa-2-azapentadecan-15-yl)carbamate (25 mg, 0.0425 mmol) in DCM (1 ml) was made and TFA (0.5 ml, 13.3 mmol) was added. The reaction mixture was stirred for 3h at room temperature under argon in the dark. After completion, the mixture was evaporated and directly used for coupling. The residue was solubilized in DMF (1ml). To this solution were added successively 5-(4-(((3-Azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-pentanoic acid **$CA_{2\text{-}2}$** (17 mg, 0.0419 mmol)

were added successively HATU (18 mg, 0.0461 mmol) and diisopropylethylamine (51 µl, 0.293 mmol) under argon. The reaction was stirred two nights at room temperature in the dark. The solvent was then evaporated and the residue purified via HPLC (with gradient H_2O:acetronitrile from 95:5 to 40:60, flux 21.2 ml/min, column Phenomenex Synergi Fusion-RP 150 x 21.2 mm, 5 µm) to afford the product as a bright yellow oil (12.7 mg, 0.0145 mmol, yield 35%).

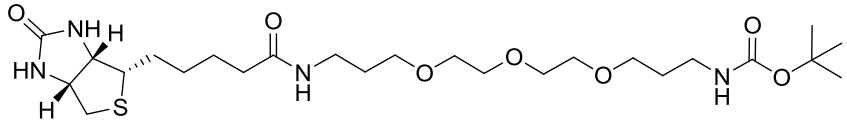
^{1}H NMR (400 MHz, MeOD) δ 9.18 (s, 1H), 8.52 (s, 1H), 8.06 (s, 1H), 7.98 (s, 1H), 7.94 (s, 1H), 7.15 (s, 1H), 4.45 (t, *J* = 6.4 Hz, 1H), 3.76 (d, *J* = 4.4 Hz, 1H), 3.71 – 3.55 (m, 1H), 3.52 (dd, *J* = 11.6, 5.8 Hz, 1H), 3.44 – 3.36 (m, 1H), 3.26 (dd, *J* = 12.0, 6.2 Hz, 1H), 3.15 (s, 1H), 2.86 (t, *J* = 6.4 Hz, 1H), 2.83 – 2.77 (m, 1H), 2.54 (t, *J* = 6.8 Hz, 1H), 2.22 (d, *J* = 3.4 Hz, 1H), 2.04 – 1.94 (m, 1H), 1.88 (dd, *J* = 12.6, 6.2 Hz, 1H), 1.84 – 1.70 (m, 1H), 1.69 (s, 1H).

IR (cm^{-1}) 3333, 2933, 2872, 2095, 1700, 1650, 1616, 1538, 1447, 1369, 1286, 1210, 1110, 794.

LC-MS (ESI+), *m/z:* 874.7 $[M+H]^+$.

HRMS (ESI) calculated for $C_{43}H_{65}N_{13}O_7$ [M+2H]2+, 437.755947 found 437.755273; calculated for $C_{43}H_{64}N_{13}O_7$ [M+H]+, 874.504618 found 874.503436; calculated for $C_{43}H_{63}N_{13}NaO_7$, [M+Na]+ 896.486562, found 896.486214.

- **tert-butyl (15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)carbamate (13)**



To a solution of 2,5-dioxopyrrolidin-1-yl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (300 mg, 0.879 mmol) in DMF (3 ml) was added *tert*-butyl (3-(3-aminopropoxy)propyl)carbamate **11** (282 mg, 0.265 mmol). After overnight stirring at room temperature under argon, the solvent was evaporated and the residue was solubilized in DCM. The solution was then washed with water, brine and dried over $MgSO_4$. The crude was then purified by silica gel chromatography (DCM:MeOH 90:10) to afford the product as a transparent oil (145 mg, 0.0852 mmol, yield 30%).

^{1}H NMR (400 MHz, MeOD) δ 5.12 (dd, *J* = 7.7, 4.8 Hz, 1H), 4.94 (dd, *J* = 7.8, 4.4 Hz, 1H), 4.23 (ddd, *J* = 8.7, 5.0, 2.1 Hz, 8H), 4.14 (dd, *J* = 11.5, 6.0 Hz, 4H), 3.93 (dd, *J* = 3.1, 1.5 Hz, 1H), 3.88 (t, *J* = 6.8 Hz, 2H), 3.83 (dd, *J* = 9.2, 5.0 Hz, 1H), 3.74 (t, *J* = 6.8 Hz, 2H), 3.56 (dd,

J = 12.7, 4.9 Hz, 1H), 2.83 (t, *J* = 7.3 Hz, 2H), 2.36 (dtd, *J* = 19.7, 13.0, 6.6 Hz, 6H), 2.25 (ddd, *J* = 20.5, 10.6, 4.1 Hz, 3H), 2.05 (s, 11H).

^{13}C NMR (101 MHz, MeOD) δ 172.98, 163.10, 155.46, 76.90, 68.57, 68.27, 66.96, 66.90, 60.40, 58.65, 54.05, 38.11, 35.75, 34.86, 33.90, 27.95, 27.45, 26.85, 26.55, 25.87, 23.94, 23.35.

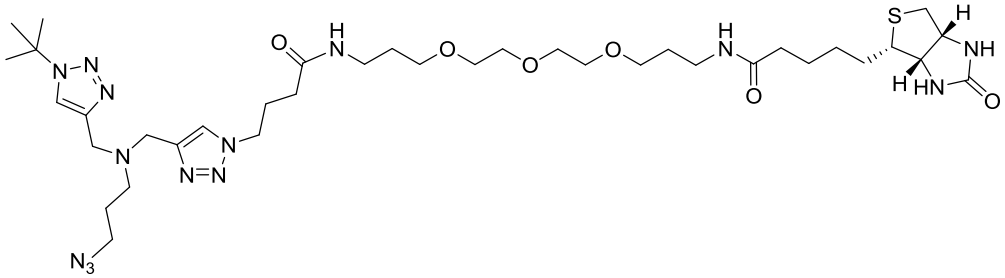
IR (cm^{-1}) 3298, 2927, 2864, 1698, 1641, 1527, 1462, 1365, 1211, 1166, 1075, 651.

LC-MS (ESI+), *m/z:* 547.5 $[M+H]^+$.

HRMS (ESI) calculated for $C_{25}H_{47}N_4O_7S$ [M+H]+, 547.315997 found 547.316126; calculated for $C_{25}H_{46}N_4NaO_7S$, [M+Na]+ 569.297942, found 569.297809.

Data in agreement with literature.[6]

- **N-(18-(4-(((3-azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-15-oxo-4,7,10-trioxa-14-azaoctadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide ($CA_{2\text{-}6}$)**



To a solution of tert-butyl (15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)carbamate (**13)** (145 mg, 0.265 mmol) in DCM (2 ml) was added TFA (2 ml, 26.6 mmol). The reaction mixture was stirred for 2h at room temperature under argon. After completion, the mixture was evaporated and directly used for coupling. In a second flask a suspension of N-hydrosuccinimide (18 mg, 0.158 mmol) in dry THF (2 ml) and 5-(4-(((3-Azidopropyl)((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)pentanoic acid **$CA_{2\text{-}2}$** (57 mg, 0.131 mmol), was added EDC (30 mg, 0.158 mmol) at 0 °C under argon. After 3 h of stirring, the reaction was warmed up to room temperature and stirred for 24 h. After completion, the solvent was evaporated. The residue was solubilized in DMF (3 ml) and to this solution was added successively triethylamine (89 µl, 0.66 mmol) and (66 mg, 0.132 mmol) under argon. The reaction was stirred overnight at room temperature. The solvent was then evaporated and the residue purified via HPLC (with gradient H_2O:acetronitrile from 95:5 to 70:30, flux 21.2

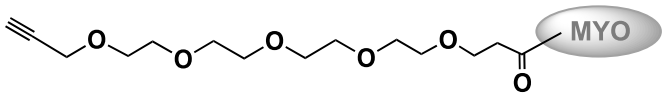
ml/min, column Phenomenex Synergi Fusion-RP 150 x 21.2 mm, 5 µm) to afford the product as a transparent oil (22 mg, 0.026 mmol, yield 20%).

^{1}H NMR (400 MHz, MeOD) δ 8.07 (s, 1H), 8.00 (s, 1H), 4.51 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.46 (d, *J* = 6.2 Hz, 2H), 4.32 (dd, *J* = 7.8, 4.5 Hz, 1H), 3.77 (d, *J* = 5.4 Hz, 4H), 3.62 (ddd, *J* = 8.8, 6.3, 3.4 Hz, 8H), 3.53 (t, *J* = 6.1 Hz, 4H), 3.37 (t, *J* = 6.6 Hz, 2H), 3.27 (t, *J* = 6.8 Hz, 4H), 3.22 (dd, *J* = 9.0, 4.8 Hz, 1H), 2.94 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.72 (d, *J* = 12.7 Hz, 1H), 2.54 (t, *J* = 6.6 Hz, 2H), 2.22 (dd, *J* = 9.8, 5.2 Hz, 6H), 1.86 – 1.72 (m, 7H), 1.70 (s, 9H), 1.68 – 1.57 (m, 4H), 1.46 (dd, *J* = 15.2, 7.5 Hz, 2H).

IR (cm^{-1}) 3385, 2987, 2703, 2101, 1670, 1464, 1399, 1196, 1126, 828, 798, 718.

LC-MS (ESI+), *m/z:* 833.8 $[M+H]^+$.

III - MYO-ALK preparation and characterisation



a) Preparation

11 mL of PBNa buffer 100 mM pH 7,4 containing 22 mg (1,29 μmoles, 2mg/mL) of Myoglobin from equine heart -MYO- were treated for 1h30 at room temperature with 3,5 equivalents of Alkynepeg$_4$-*N*-hydroxysuccinimidyl–ALK-peg$_4$NHS-1,8 mg (4,5 μmoles) being previously dissolved in 400 μL of DMF. Incubation was followed by an overnight dialysis against PBK 25 mM pH 8 at 4°C to remove excess of chemicals. Aliquots of 11,75 nmoles of Myoglobin were freeze-dried and kept at -20°C.

b) Characterization

ESI-MS

LC-ESI-MS analysis of the obtained MYO-ALK solution indicated that a mixture of alkyne modified myoglobins was obtained and none unmodified MYO remains. The ESI-MS spectra for MYO-ALK sample differs drastically from the one of the single MYO starting material (top panel vs lower panel, Figure S1).

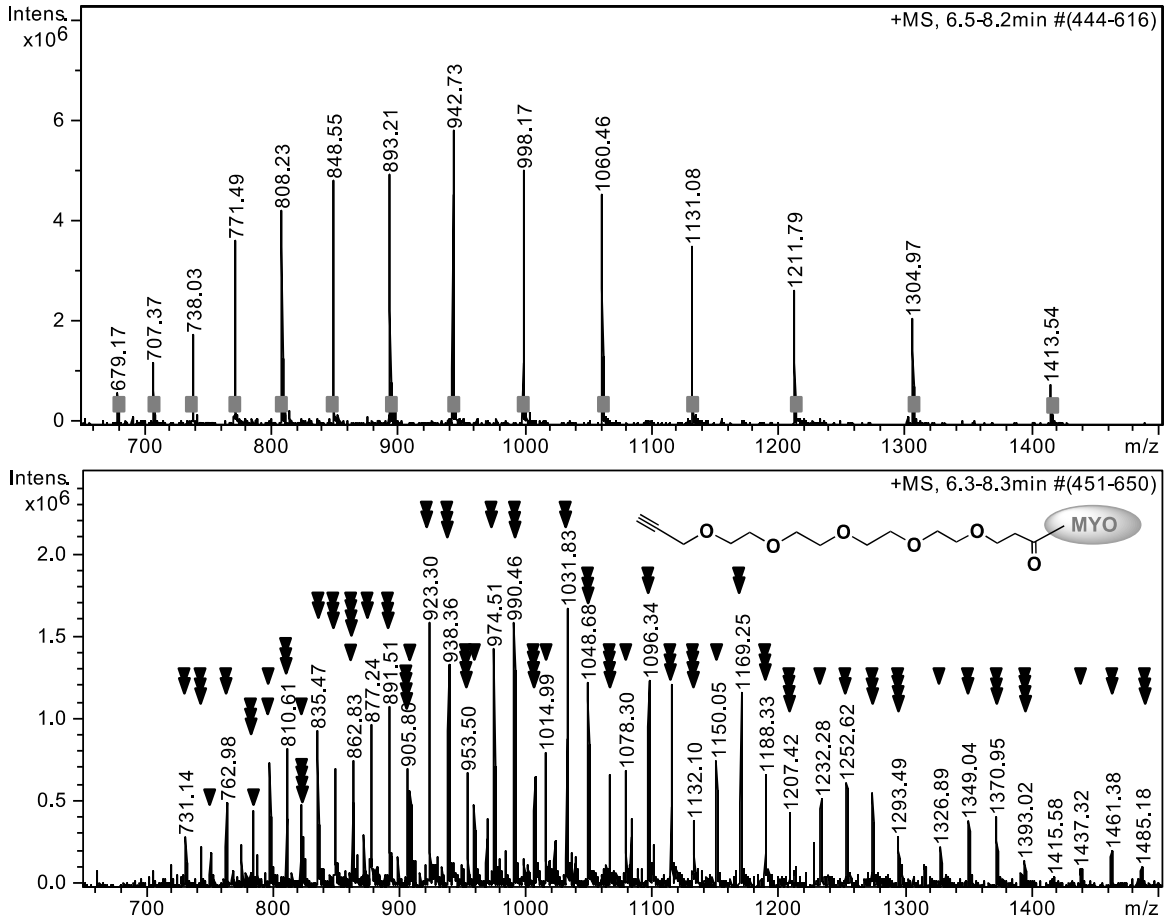


Figure S1 : ESI-MS spectra obtained for MYO starting material (upper panel) and for MYO-ALK sample **(lower panel)**. Grey square refers to the set of multicharged states of unmodified Myoglobin (MYO, top panel), black triangle to the set relative to Myoglobin bearing one alkyne function (MYO-1ALK, lower panel), black double triangles to the set relative to Myoglobin bearing two alkyne functions (MYO-2ALK, lower panel), black triple triangles to the set relative to Myoglobin bearing three alkyne functions (MYO-3ALK, lower panel) and black quartet triangles to the set relative to Myoglobin bearing four alkyne functions (MYO-4ALK, lower panel).

The ESI-MS spectra of MYO-ALK sample (lower panel, Figure S1) shows the presence of four major set of multicharged states (Table S2) corresponding to the alkyne modified myoglobins that bear respectively :

- one alkyne function (Myoglobin-1ALK = MYO-1ALK ; single black triangle),
- two alkyne functions (Myoglobin-2ALK = MYO-2ALK ; double black triangles),
- three alkyne functions (Myoglobin-3ALK = MYO-3ALK; triple black triangles),
- four alkyne functions (Myoglobin-4ALK = MYO-4ALK ; quartet black triangles).

	m/z			
charge z	MYO-1ALK	MYO-2ALK	MYO-3ALK	MYO-4ALK
25+	690.61	-	713.56	-
24+	719.32	731.14	743.10	-
23+	750.46	762.98	775.34	787.79
22+	784.50	797.62	810.61	823.74
21+	821.87	835.47	849.06	862.83
20+	862.83	877.24	891.51	905.80
19+	908.24	923.30	938.36	953.50
18+	958.64	974.51	990.46	1006.41
17+	1014.99	1031.83	1048.68	1065.47
16+	1078.30	1096.34	1114.17	1132.10
15+	1150.05	1169.25	1188.33	1207.42
14+	1232.28	1252.62	1273.15	1293.49
13+	1326.89	1349.04	1370.95	1393.02
12+	1437.32	1461.38	1485.18	1509.10

Table S2 : m/z for each charge state of all type of MYO-ALK adduct

The corresponding deconvoluted spectra (Figure S3) indicate the respective measured molecular weight:

- MYO-1ALK : 17237,08 +/-0,65 Da (expected for $C_{783}H_{1234}N_{210}O_{224}S_2$: 17237.62 Da),
- MYO-2ALK : 17524,05 Da +/- 0,61 Da (expected for $C_{797}H_{1256}N_{210}O_{230}S_2$: 17523.94 Da),
- MYO-3ALK : 17810,11 Da +/- 0,42 Da (expected for $C_{811}H_{1278}N_{210}O_{236}S_2$: 17810.26 Da),
- MYO-4ALK : 18096,90 +/- 0,72 Da (expected for $C_{825}H_{1300}N_{210}O_{242}S_2$: 18096,58 Da).

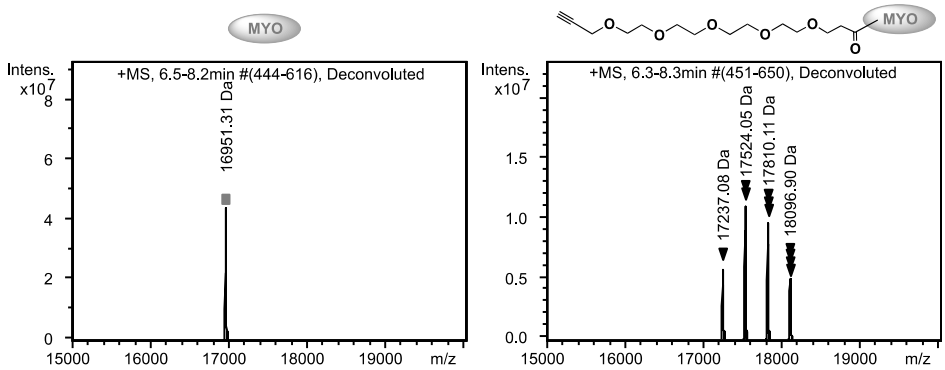


Figure S3: Comparison of ESI-MS deconvoluted spectra of MYO starting material (left panel) and from MYO-ALK **(right panel)**. Grey square refers to unmodified Myoglobin (MYO, left panel), black triangle to Myoglobin bearing one alkyne function (MYO-1ALK, right panel), black double

triangles to Myoglobin bearing two alkyne functions (MYO-2ALK, right panel), black triple triangles to Myoglobin bearing three alkyne functions (MYO-3ALK, right panel) and black quartet triangles to Myoglobin bearing four alkyne functions (MYO-4ALK, right panel).

As alkyne chemical modification on Myoglobin may affect poorly the nebullisation ability of the protein species in ESI experiment, ratio of each species could be extrapolated from integration of peaks areas in ESI-MS spectra. From these percentages (Figure S4) an average of alkyne functions per myoglobin unit could be estimated and was found to be around 2,8 alkyne function per myoglobin.

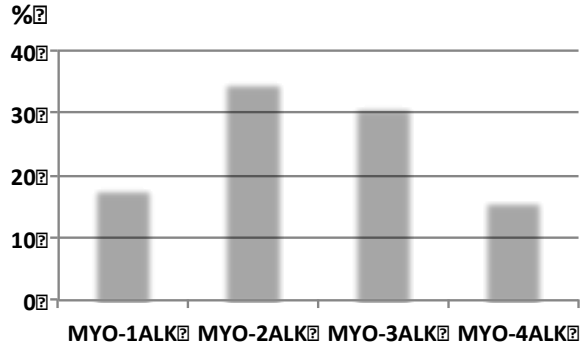


Figure S4 : Estimated relative abundances of different alkyne myoglobins in MYO-ALK sample

MALDI-MS

The presence of the four protein species in MYO-ALK sample **were** confirmed using MALDI-MS (lower panel, Figure S5)

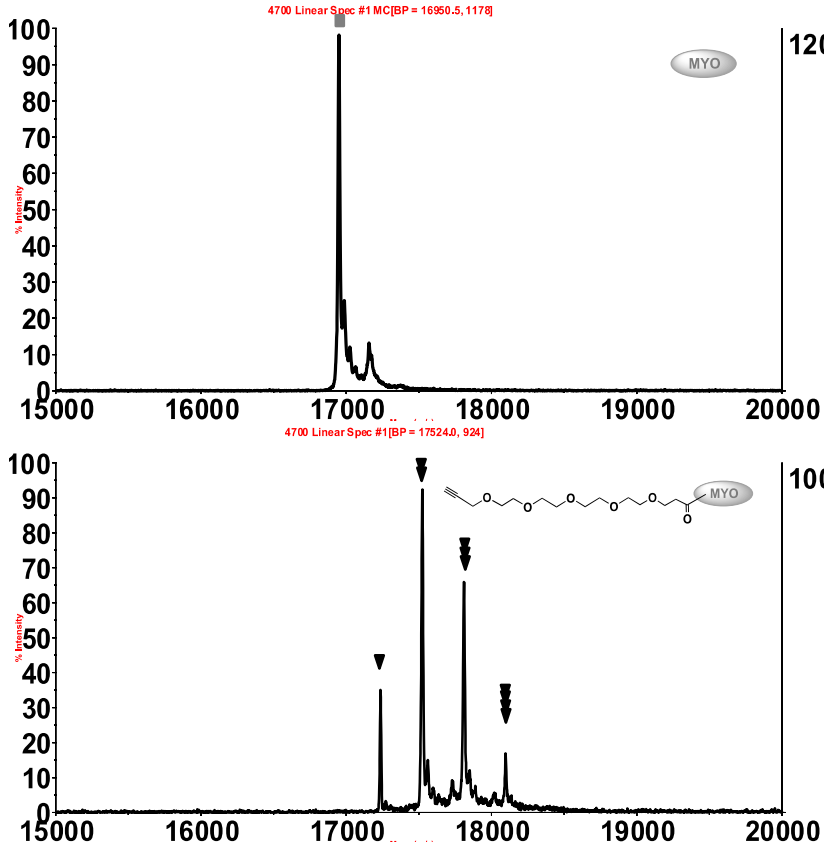
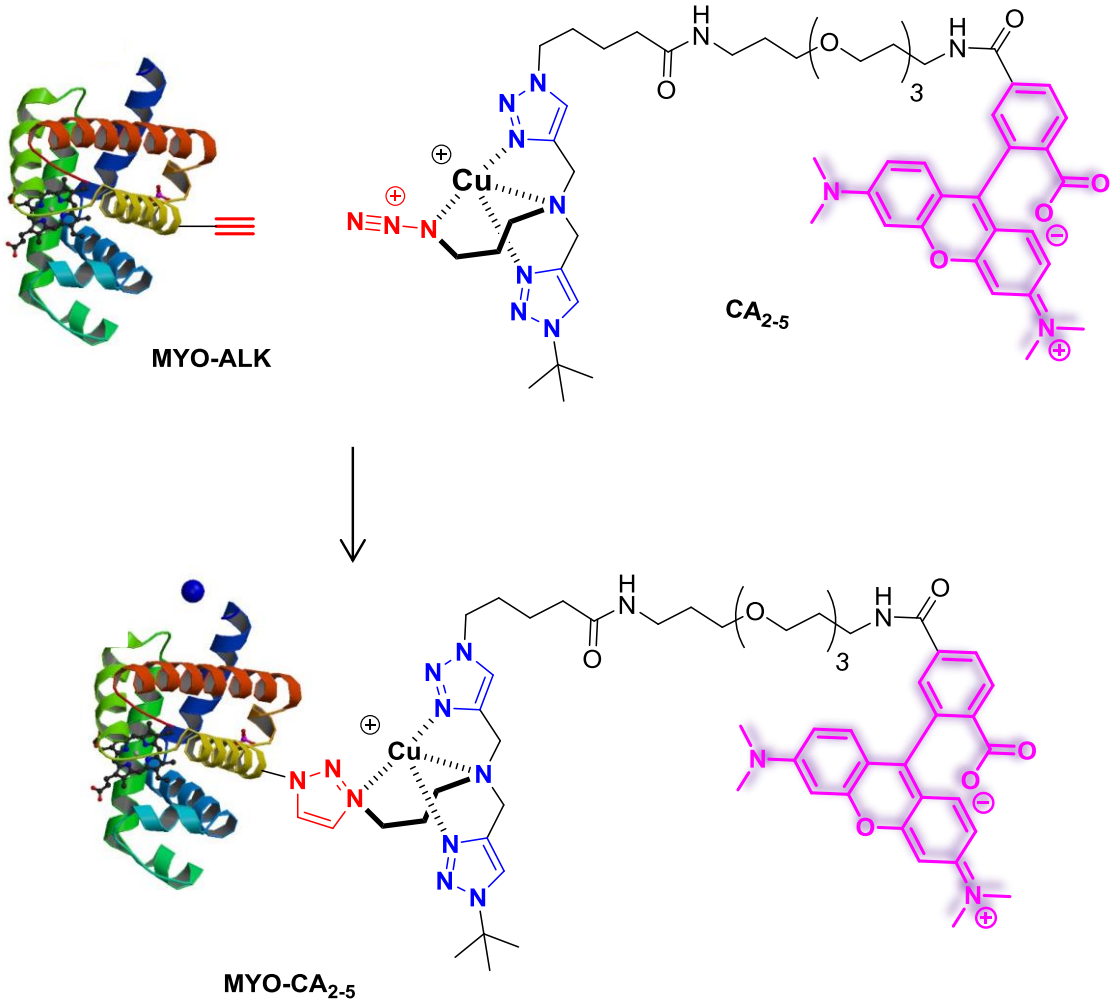


Figure **S5**: Comparison of MALDI-MS spectra of MYO starting material **(upper panel)** and from MYO-ALK **(lower panel)**. Grey square refers to unmodified Myoglobin (MYO, top panel), black triangle to Myoglobin bearing one alkyne function (MYO-1ALK, lower panel), black double triangles to Myoglobin bearing two alkyne functions (MYO-2ALK, lower panel), black triple triangles to Myoglobin bearing three alkyne functions (MYO-3ALK, lower panel) and black quartet triangles to Myoglobin bearing four alkyne functions (MYO-4ALK, lower panel).

IV – MYO-ALK labeling experiments

General



Labeling experiments on MYO-ALK sample use pre-mix solutions of **CA_{2-5}** or **A_1**/THPTA with $CuSO_4$, AscNa. After SDS-PAGE (4-20% gradient polyacrylamide gel after five minutes 95°C boiling), gel obtained were scanned using Molecular Imager® VersaDoc™ MP 4000 Molecular Digital Imaging System from Biorad (light source : green LED ; fluorescence collection 605 BP) before a silver staining step using standard protocol.

*Pre-mix of **CA_{2-5}** solution*

Equal volumes (x µL) of a DMF solution of **CA_{2-5}** at 625 µM and aqueous solution of $CuS0_4$ at 1,2 mM were first mixed and incubated at RT for 30 minutes before the addition of volume of x µL of AscNa freshly prepared in water at 6 mM. The resulting solution containing **CA_{2-5}**, $CuSO_4$, AscNa at respective concentrations of 208 µM, 400 µM and 2 mM was incubated for further 30 minutes at RT before addition on MYO-ALK.

*Pre-mix **A_1** solution*

The same protocol was followed to prepare *Pre-mix* $\mathbf{A_1}$ *solution* except that THPTA was mixed together A_1 and $CuS0_4$ before the addition of AscNa solution. The solution prepared contained thus A_1, $CuSO_4$, THPTA and AscNa at respective concentrations of 208 μM, 400 μM, 208 μM and 2 mM before addition on MYO-ALK.

Figure 1b) MYO-ALK labeling by $CA_{2\text{-}5}$ and specificity

2,7 μL of *pre-mix* $\boldsymbol{CA_{2\text{-}5}}$ *solution* were added to 26,6 μL buffered solutions (PBK buffer 50 mM pH 8) of MYO and MYO-ALK at the same 5,5 μM to lead to reaction mixtures containing 5 μM of MYO or MYO-ALK and $\mathbf{CA_{2\text{-}5}}$/$CuSO_4$/AscNa at respective concentrations 19 μM/38 μM/190 μM.

After 1h, reaction were quenched by the addition of Laemmli buffer 4X for immediate SDS-PAGE analysis. Volumes corresponding to ~500 ng of protein were loaded in gel wells.

Figure 1c) Comparison in labeling potencies of $CA_{2\text{-}5}$ and A_1 towards 1, 3 and 5 μM concentrations of MYO-ALK

8 μL of *pre-mix* $CA_{2\text{-}5}$ *solution* or *pre-mix* A_1 *solution* were added to 80 μL buffered solutions (PBK buffer 50 mM pH 8) of MYO-ALK at 1,1 μM, 3,3 μM or 5,5 μM. Resulting mixtures contain 1 μM, 3 μM or 5 μM of MYO-ALK and :

- $\mathbf{CA_{2\text{-}5}}$ /$CuSO_4$/AscNa at respective concentrations 19 μM/38 μM/190 μM

or

- A_1/$CuSO_4$/THPTA/AscNa at respective concentrations 19 μM/38 μM/19 μM/190 μM

After 1h, an equal volume (24 μL) of each reaction mixtures was collected and quenched with 8 μL of Laemmli buffer 4X for subsequent SDS-PAGE analysis. 16 μL of these samples (corresponding respectively to ~200 ng, ~600 ng and ~1 μg depending on the initial protein concentration 1 μM, 3 μM or 5 μM) were loaded in gel wells.

Figure 2) Labeling of 3 μM of MYO-ALK by $CA_{2\text{-}5}$ over time

14 μL *pre-mix* $CA_{2\text{-}5}$ *solution* was added to 140 μL buffered solution (PBK buffer 50 mM pH 8) of MYO-ALK at 3,3 μM concentration to lead to a solution containing MYO-ALK at 3 μM and $CA_{2\text{-}5}$/$CuSO_4$/AscNa were respectively at 19 μM/38 μM/ 190 μM.

After 1, 3, 5, 10, 20, 30, 60 minutes incubation at RT, an equal volume (16 µL) of the reaction mixture was collected over time and quenched with 6 µL of Laemmli buffer 4X for subsequent SDS-PAGE analysis. 16 µL of each these samples (corresponding respectively to ~600 ng, were loaded in gel wells.

V - REFERENCES

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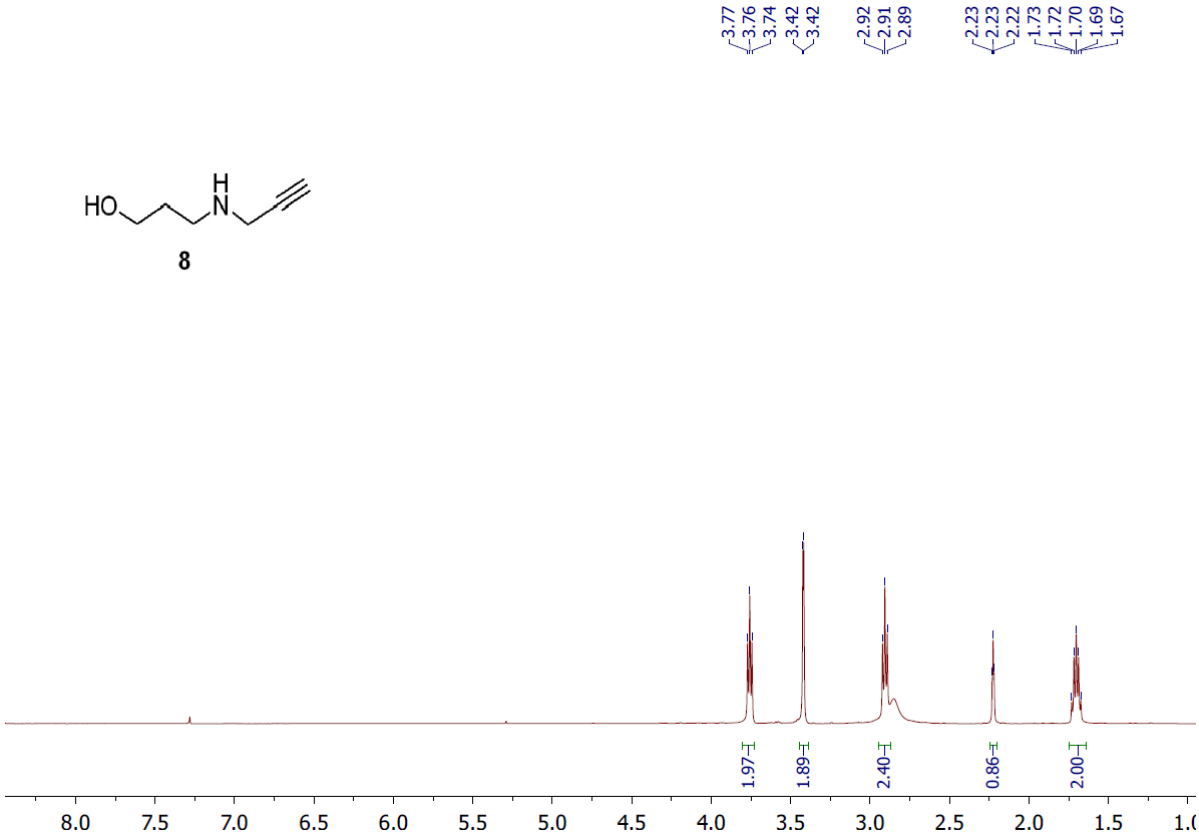
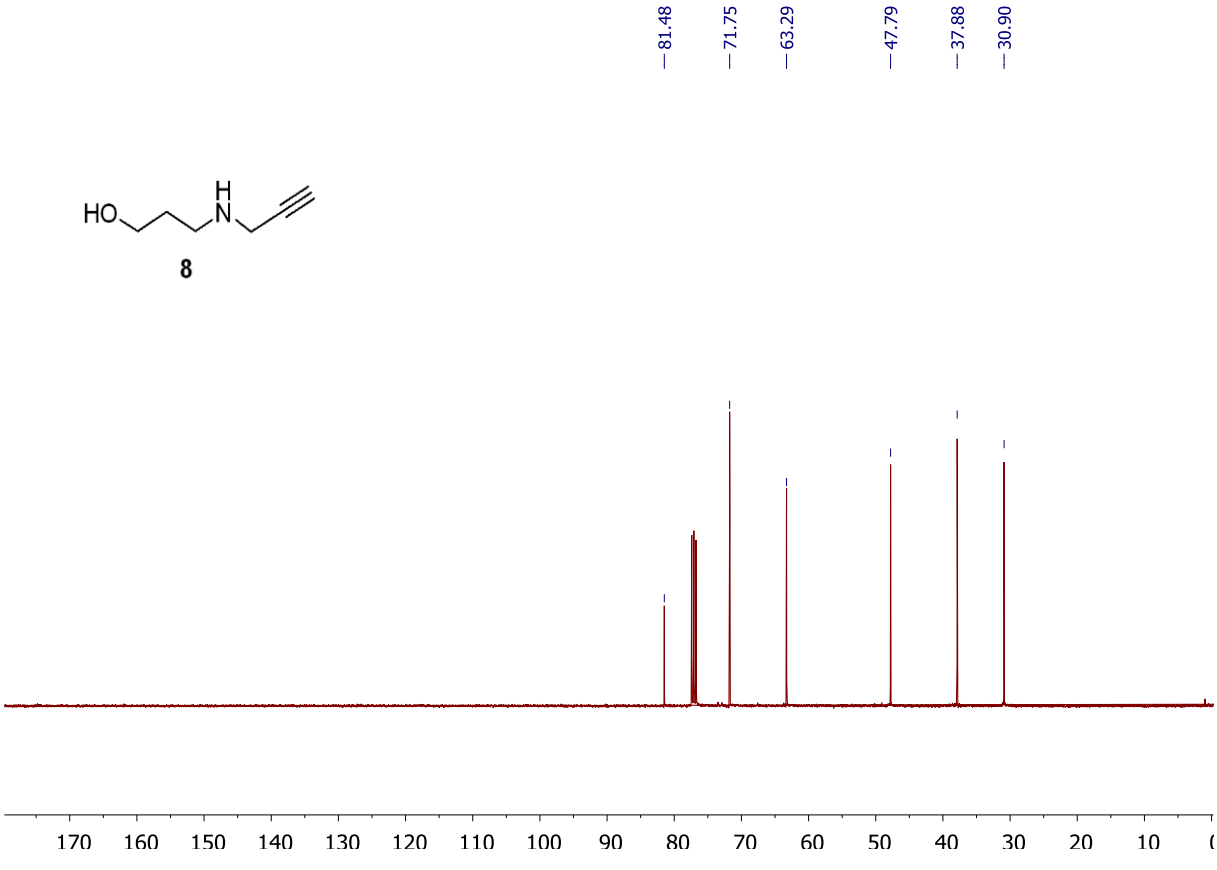
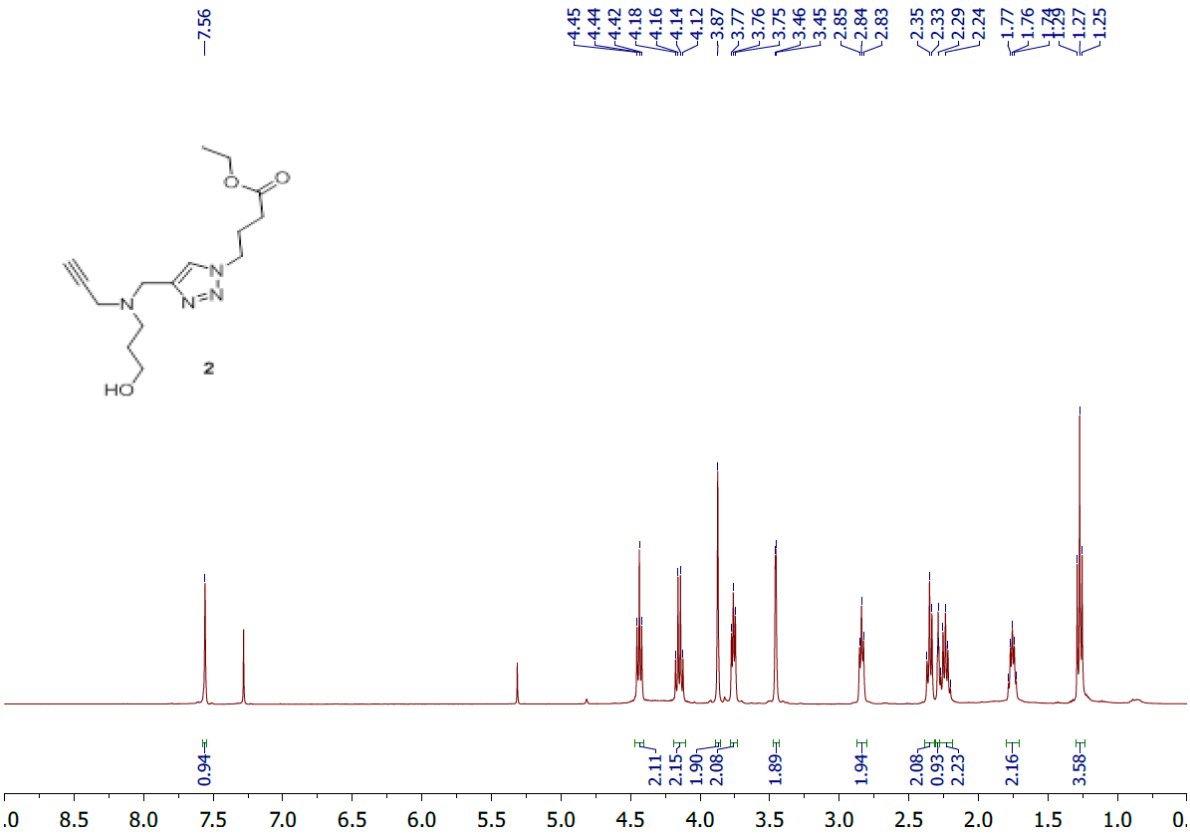
VI - NMR SPECTRA



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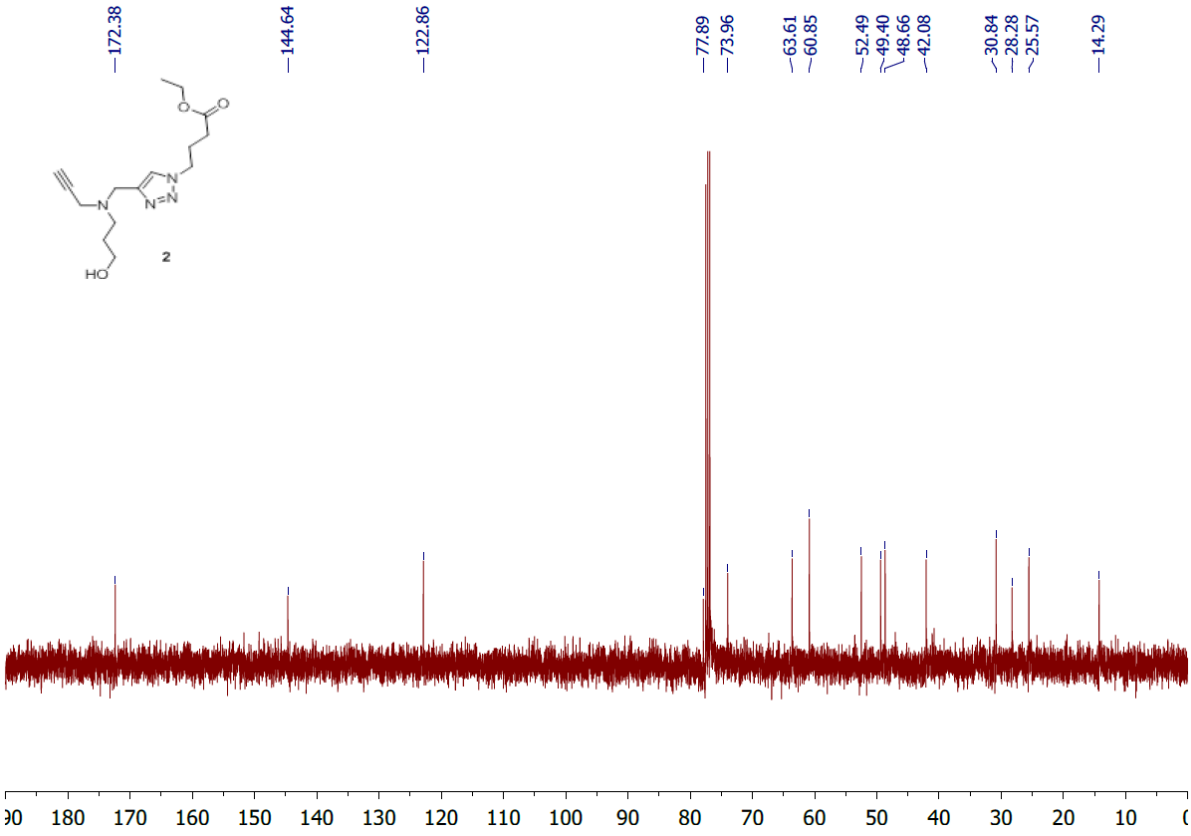
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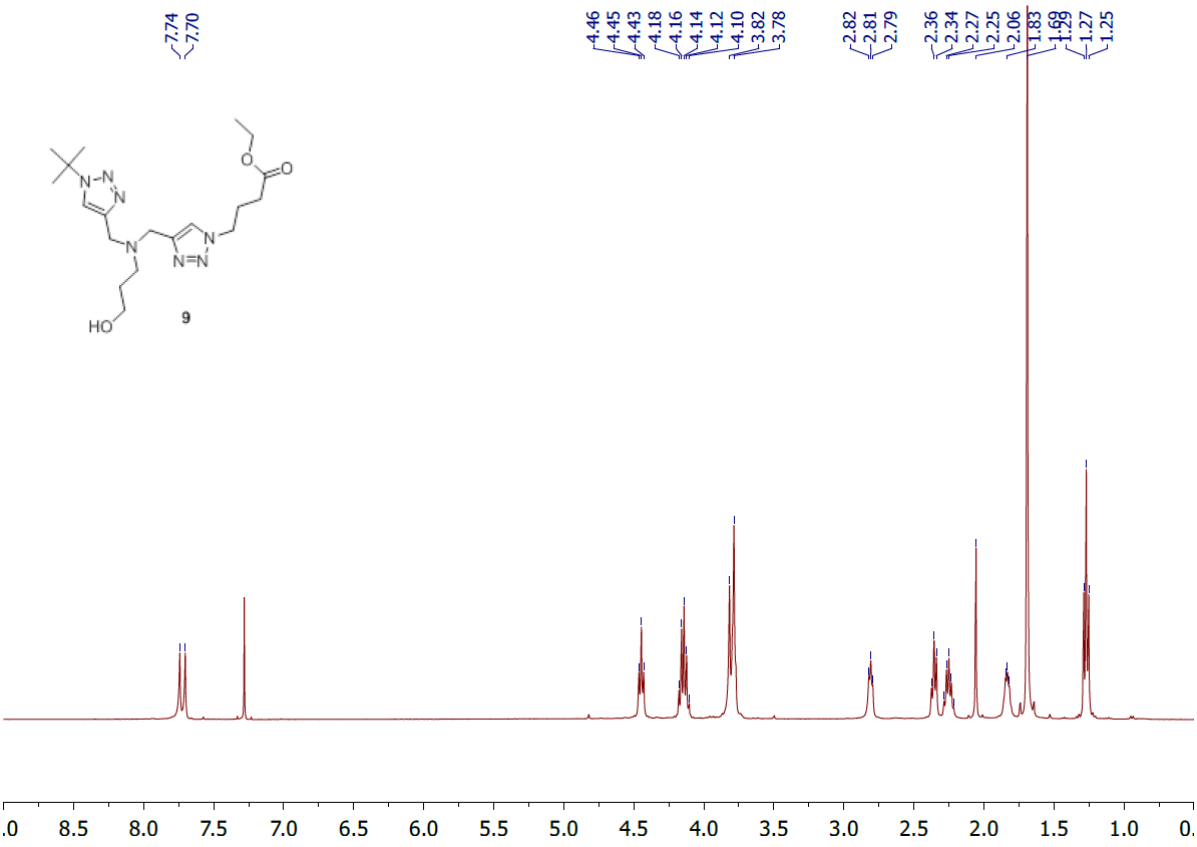
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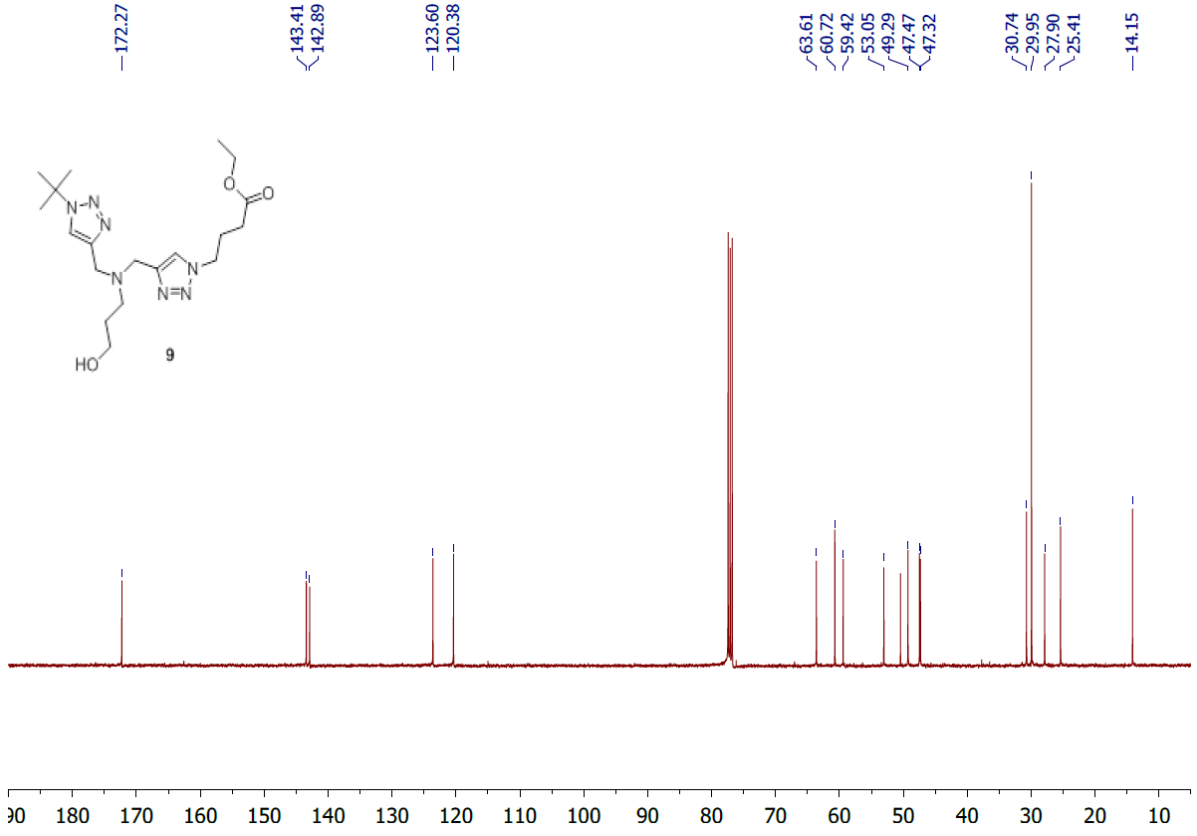
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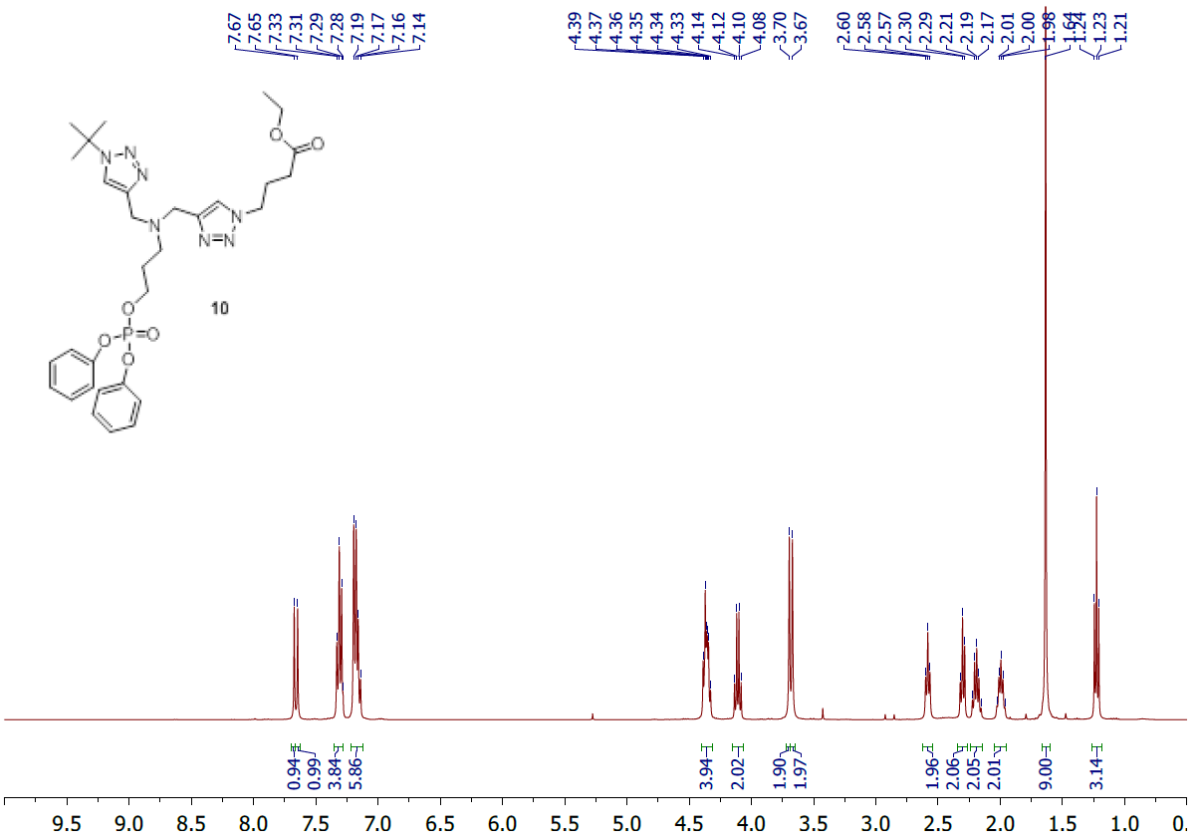
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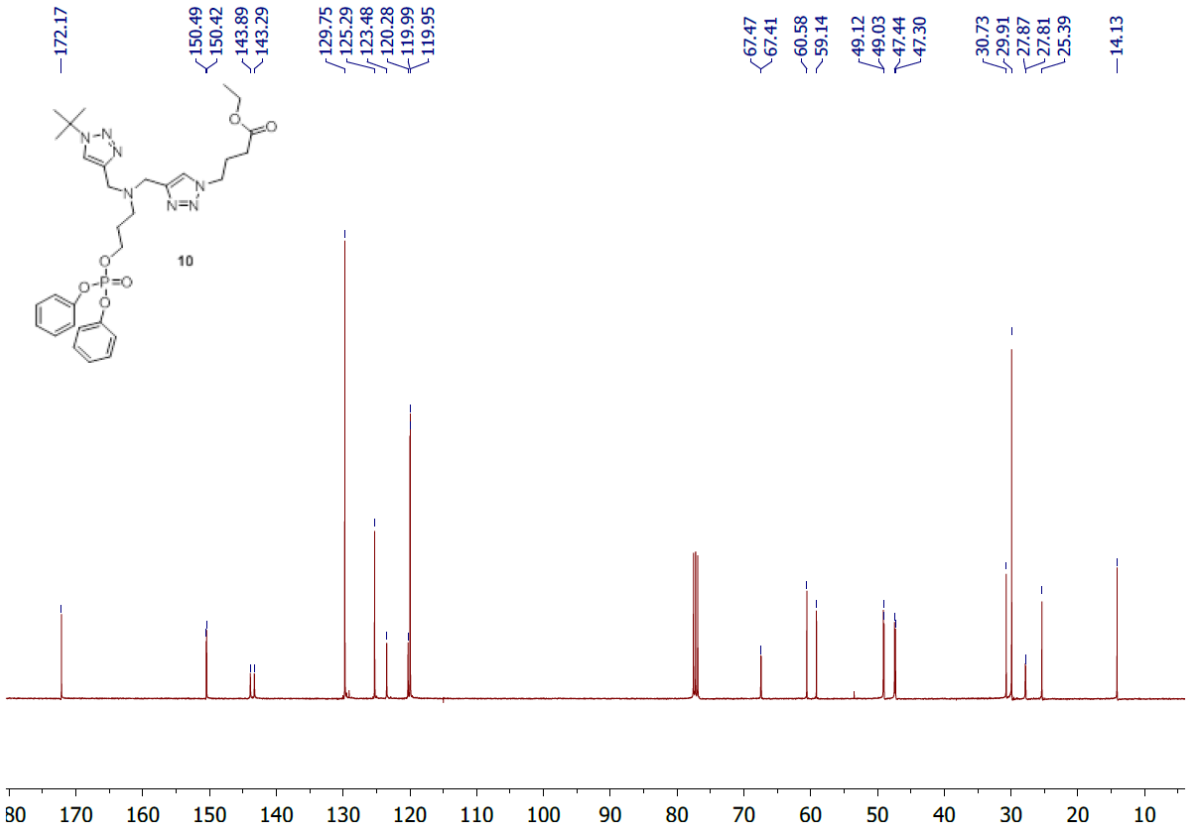
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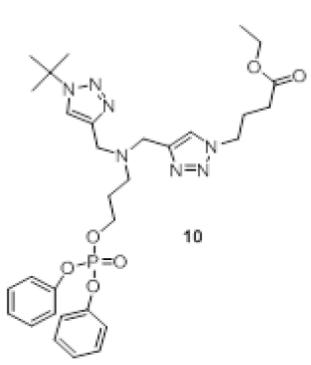

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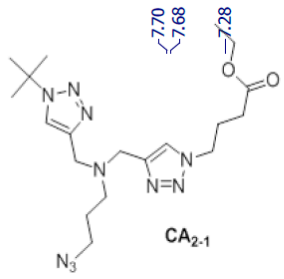

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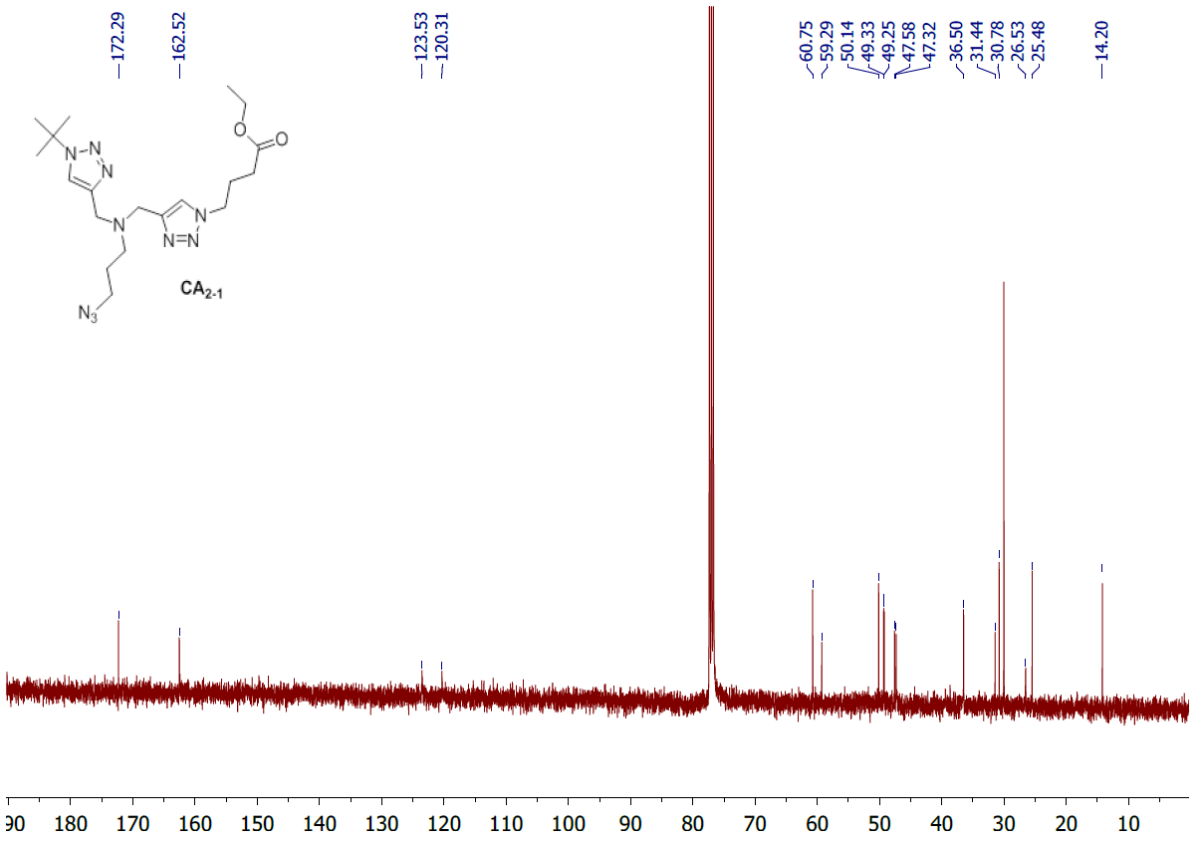
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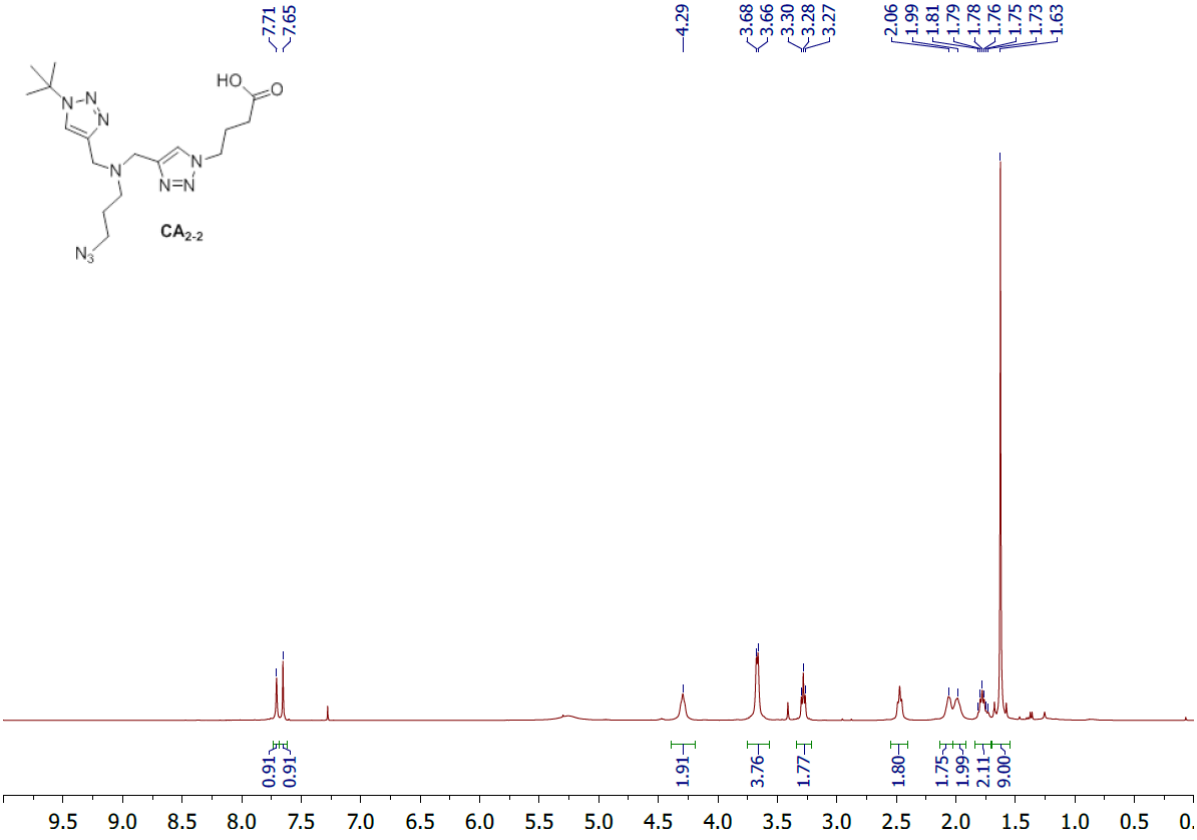
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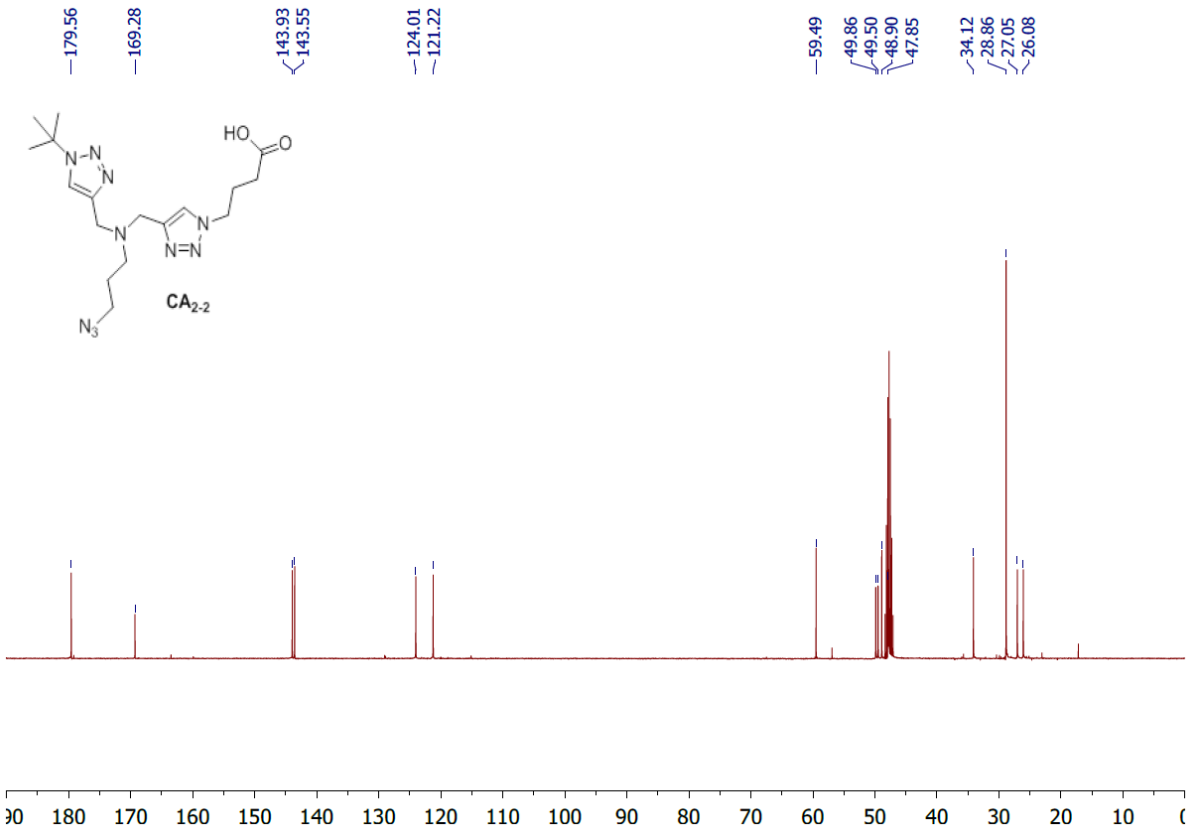
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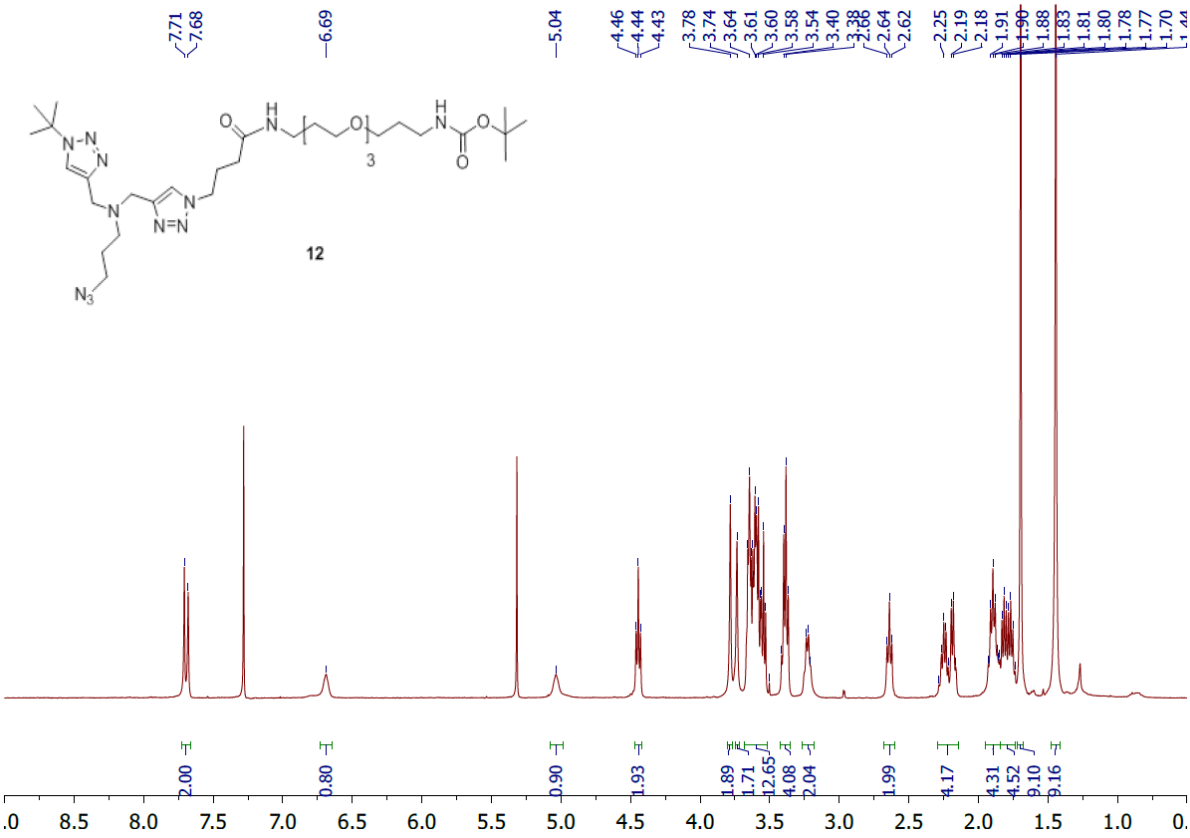
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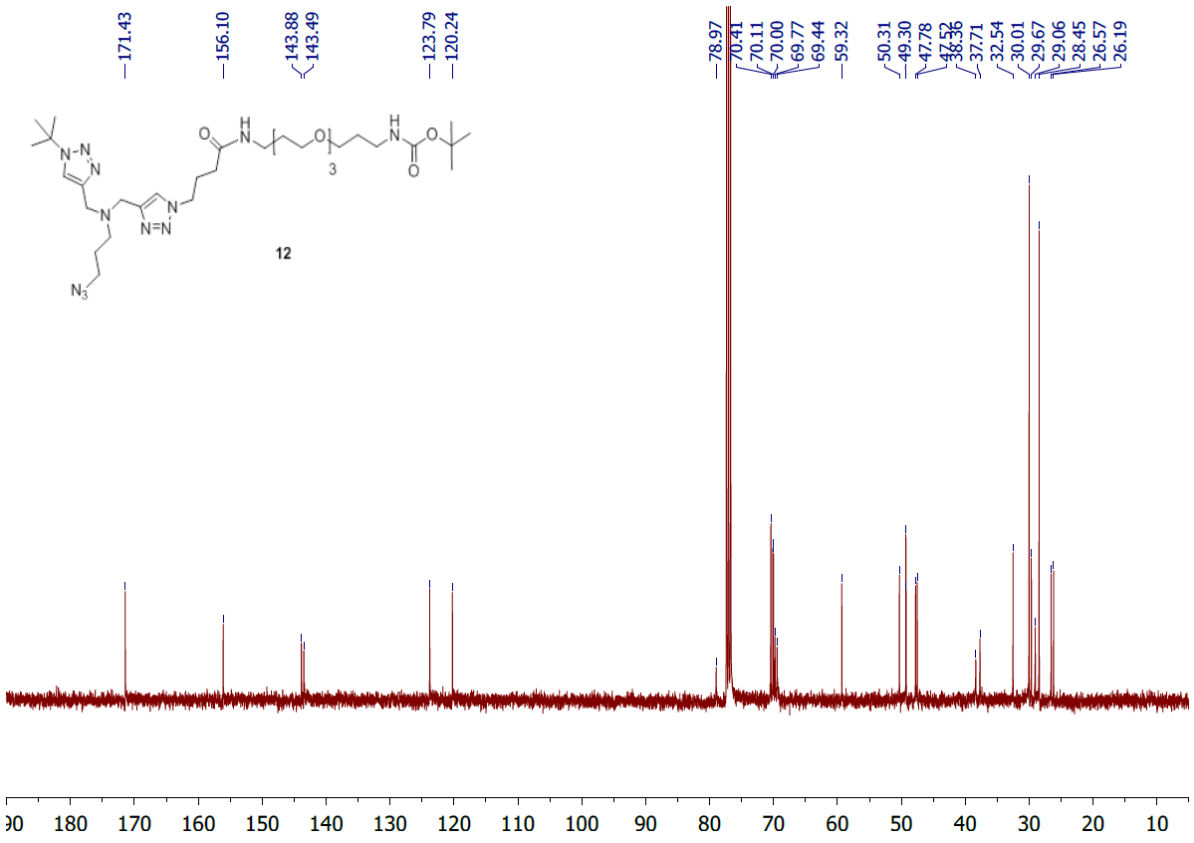
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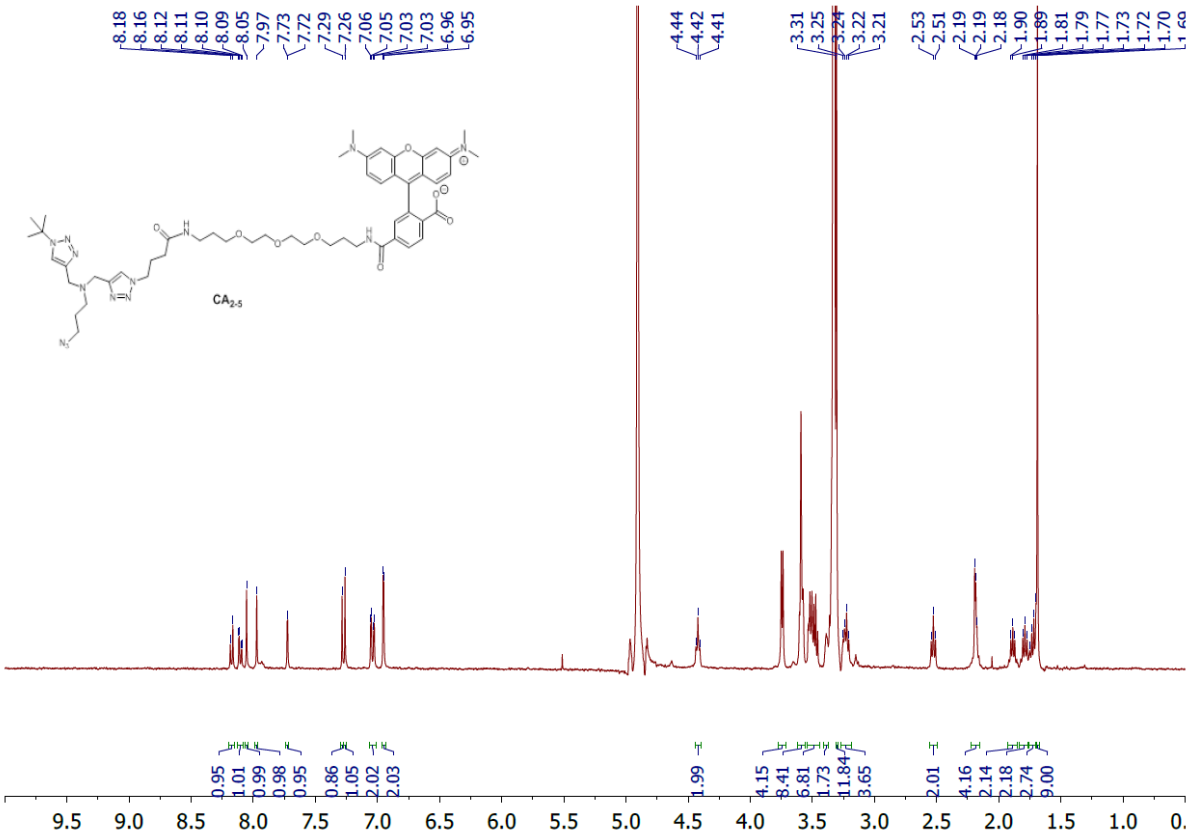
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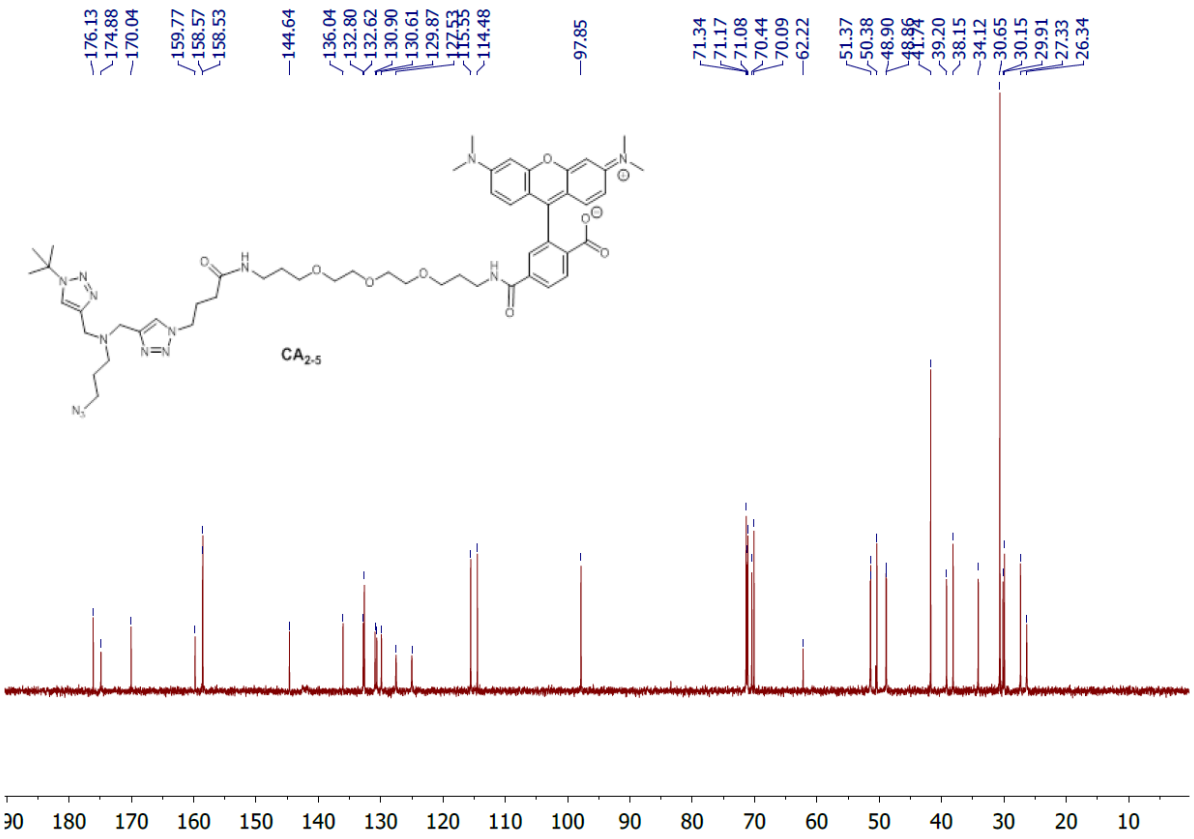
CA2-5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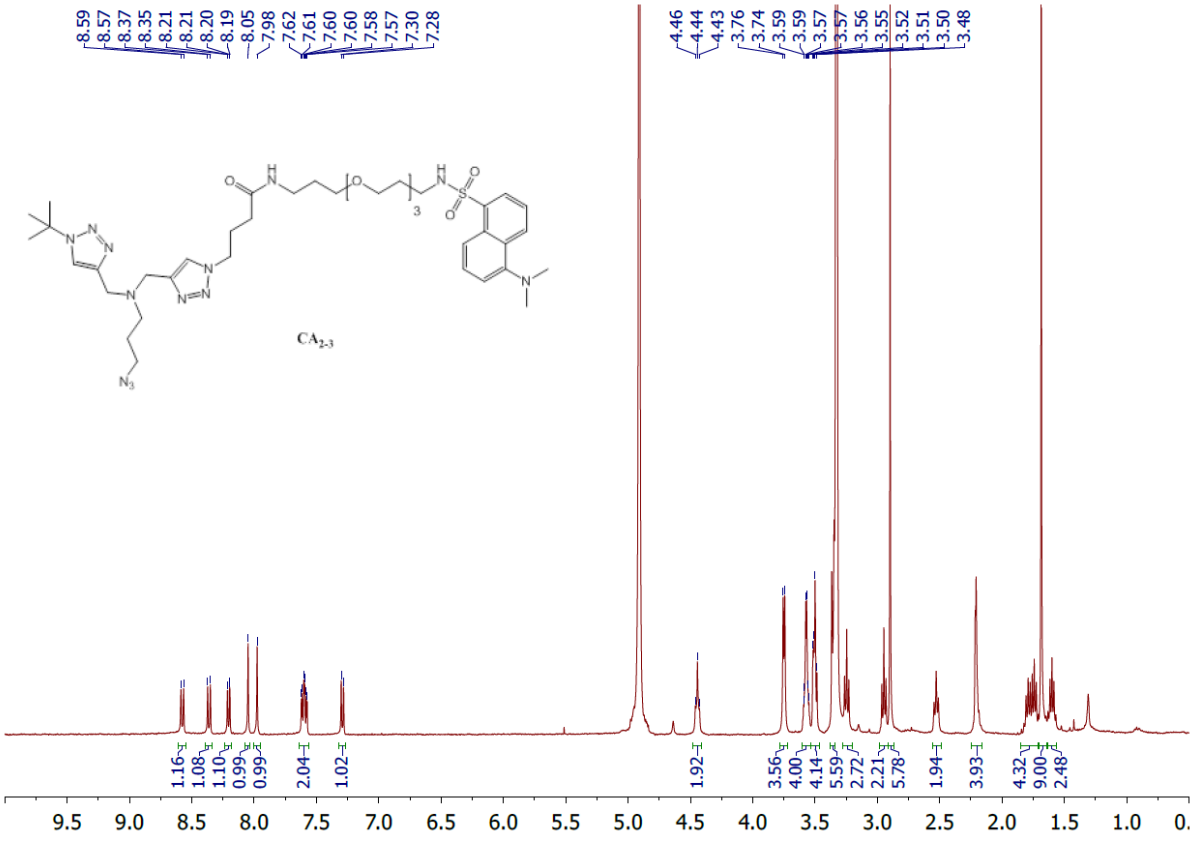
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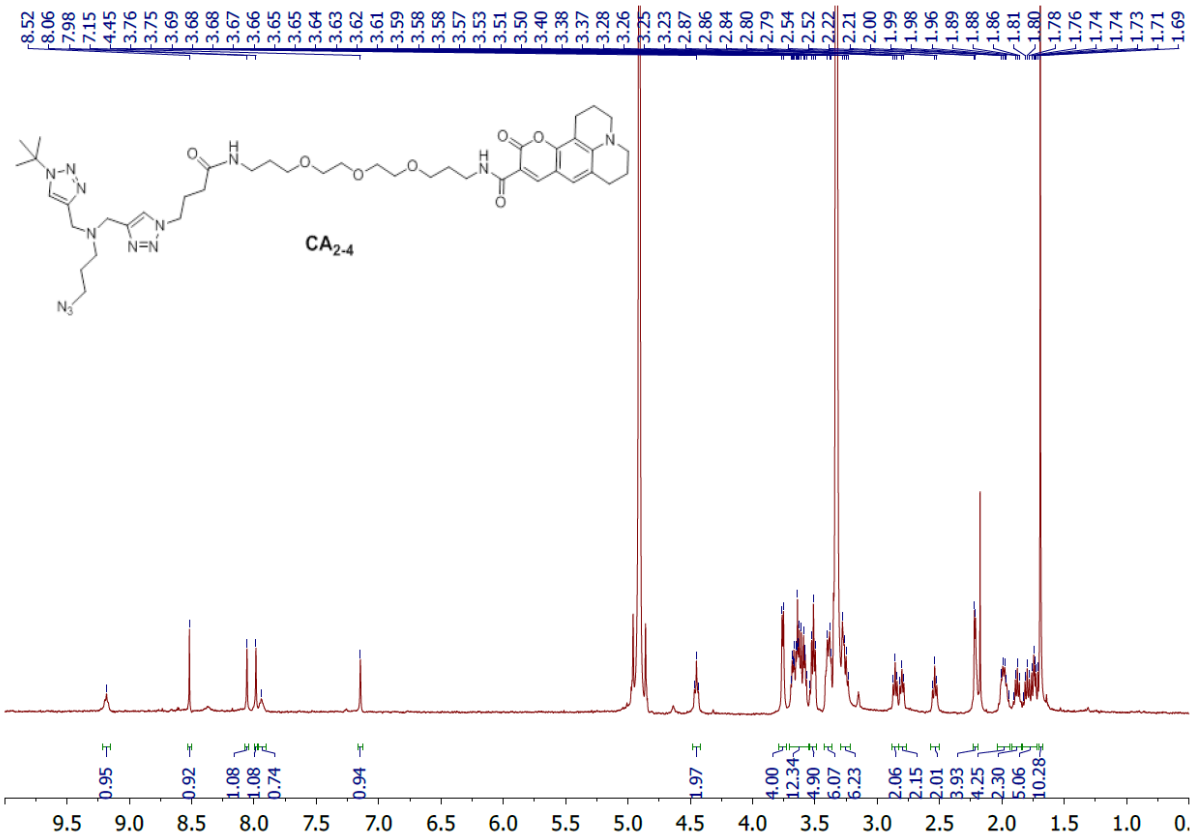
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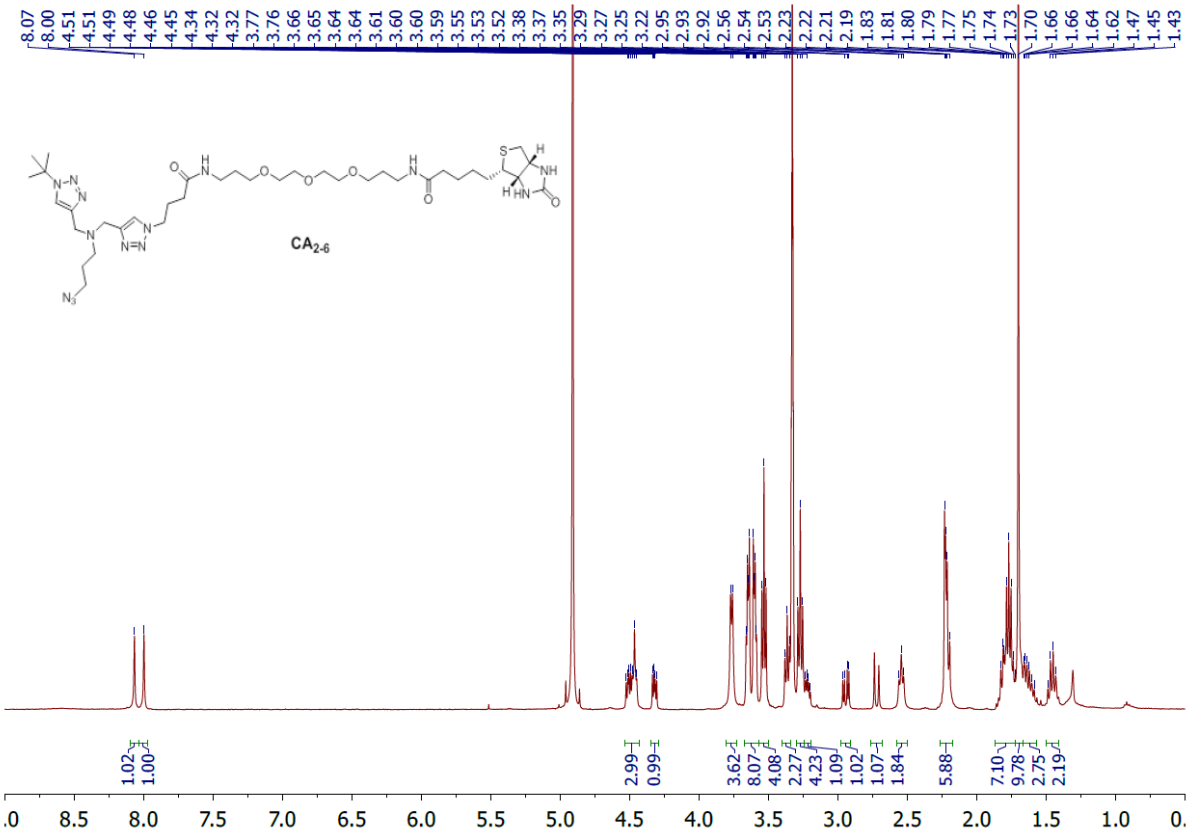
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