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Supporting Information

Crafting Molecular Tools for 15-Lipoxygenase-1 in a Single Step

*A. Louka, N. Spacho, D. Korovesis, K. Adamis, C. Papadopoulos, E.-E. Kalaitzaki, N. Tavernarakis, C. G. Neochoritis, N. Eleftheriadis**

Crafting molecular tools in a single step

Anastasia Louka,[a] Ntaniela Spacho,[a] Dimitris Korovesis,[b] Konstantinos Adamis,[a] Christos Papadopoulos,[a] Eirini-Eleni Kalaitzaki,[a] Nektarios Tavernarakis,[b,c] Constantinos G. Neochoritis,[a] Nikolaos Eleftheriadis*[a]

[a] Dr. A. Louka, Ms. N. Spacho, Mr. K. Adamis, Mr. C. Papadopoulos, Ms. E.E. Kalaitzaki, Prof. C. G. Neochoritis, Prof. N. Eleftheriadis*
Department of Chemistry
University of Crete
Voutes, 70013 Heraklion, Greece
E-mail: n.eleftheriadis@uoc.gr

[b] Dr. D. Korovesis, Prof. N. Tavernarakis
Institute of Molecular Biology and Biotechnology
Foundation for Research and Technology-Hellas
Nikolaou Plastira 100, 70013, Heraklion, Greece

[c] Prof. N. Tavernarakis
Division of Basic Sciences, School of Medicine
University of Crete
Voutes, 70013 Heraklion, Greece

Supporting Information

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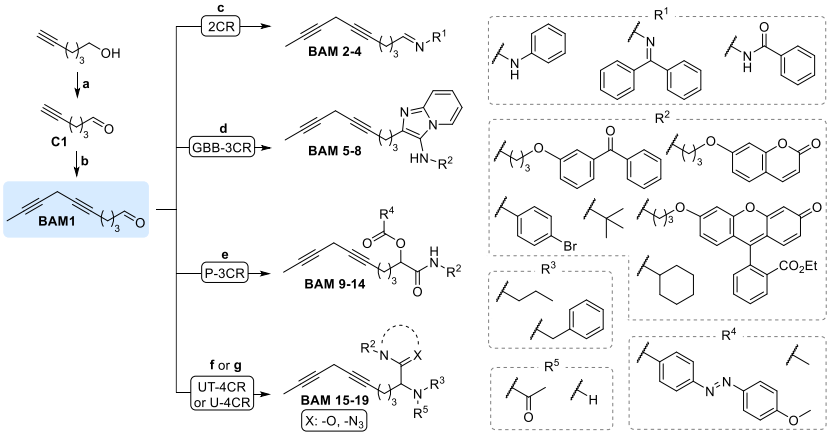
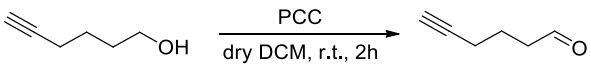
1. Experimental materials and methods

All the reagents and solvents were purchased from Sigma-Aldrich, AK Scientific, Fluorochem, Abcr GmbH, Acros, TCI, Alfa Aesar and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) carried out on Millipore precoated silica gel plates with a layer thickness of 0.20 mm, using UV light as a visualizing agent. When necessary, TLC plates were visualized with aqueous $KMnO_4$. Nuclear magnetic resonance spectra were recorded on Bruker Avance 500 spectrometers {^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz)}. Chemical shifts for ^{1}H NMR were reported as δ values and coupling constants were in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = double of doublets, tt = triplet of triplets, m = multiplet. Chemical shifts for ^{13}C NMR were reported in ppm relative to the solvent peak. High resolution mass spectra were recorded using an LTQ-Orbitrap-XL (Thermo) at a resolution of 60000@m/z400.

2. Synthetic procedures and analytical data



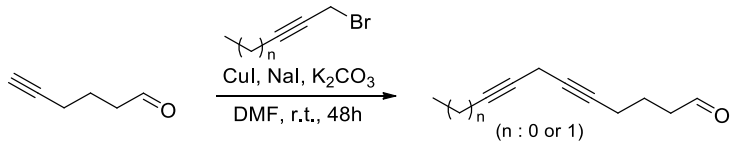
General procedure for the oxidation of 5-hexyn-1-ol to hex-5-ynal (C1)



A 25ml single-necked flame-dried round bottom flask with a magnetic stirrer under an argon atmosphere was equipped with 5-hexyn-1-ol (4.08 mmol, 400 mg) in dry DCM (10 ml), and then PCC (5.71 mmol, 1230 mg) was added at 0 ℃. The solution was stirred for 2 hours at room temperature. Then the reaction mixture was diluted with Et_2O and cooled to -20 ˚C for 1 hour. The mixture was filtered through a celite pad with Et_2O, and the filtrate was concentrated under reduced pressure. The aldehyde was used in the next step without any further purification.

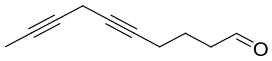
349 mg, 89% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 9.81 (s, 1H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.27 (dt, J_1 = 7.0 Hz, J_2 = 2.6 Hz, 2H), 1.98 (t, *J* = 2.5 Hz, 1H), 1.86 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 201.6, 83.2, 69.3, 42.5, 20.8, 17.8.

General procedure for the synthesis of Deca-5,8-diynal (BAM1) and Undeca-5,8-diynal (BAM1')



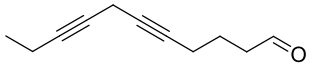
A mixture of CuI (0.5 equiv), NaI (1.0 equiv), K_2CO_3 (1.0 equiv), hex-5-ynal **C1** (1.0 equiv) and the corresponding bromide (1.0 equiv) were suspended in DMF. The suspension was stirred for 48 hours at room temperature. The mixture was diluted with EtOAc and filtered through a pad of celite. The organic layer was extracted with saturated aqueous NH_4Cl. Subsequently, the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The reaction mixture was purified through column chromatography (P.E:EtOAc = 55:1, v/v).

Deca-5,8-diynal (BAM1)



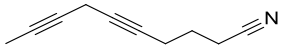
479 mg, 89% yield, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): 9.80 (t, *J* = 1.5 Hz, 1H), 3.07 – 3.01 (m, 2H), 2.57 (dt, J_1 = 7.0 Hz, J_2 = 1.5 Hz, 2H), 2.26 – 2.23 (m, 2H), 1.82 (t, *J* = 14 Hz, 2H), 1.79 (t, *J* = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 202.0, 79.1, 76.1, 75.7, 73.3, 42.8, 21.1, 18.1, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{10}H_{12}O$+H, calculated 149.0961; found 149.0960.

Undeca-5,8-diynal (BAM1')



124 mg, 92% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 9.80 (t, *J* = 1.5 Hz, 1H), 3.12 – 3.10 (m, 2H), 2.58 (dt, J_1 = 7.0 Hz, J_2 = 1.0 Hz, 2H), 2.25 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2H), 2.17 (qt, J_1 = 7.5 Hz, J_2 = 2.5 Hz, 2H), 1.83 (quint, *J* = 7.0 Hz, 2H), 1.12 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 202.0, 82.0, 79.0, 75.9, 73.5, 42.7, 21.1, 18.2, 13.8, 12.4, 9.6; HRMS (ESI) m/z : $[M+H]^+$: $C_{11}H_{14}O$+H, calculated 163.1117; found 163.1114.

General procedure for the synthesis of Deca-5,8-diynenitrile (C7)

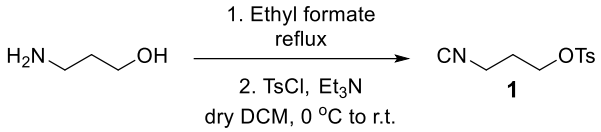


A mixture of CuI (0.54 mmol, 102 mg), NaI (1.07 mmol, 161 mg), K_2CO_3 (1.07 mmol, 148 mg), hexynenitrile (1.07 mmol, 100 mg) and 1-bromo-2-butyne (1.07 mmol, 143 mg) were suspended in DMF (4.0 mL). The suspension was stirred for 48 hours at room temperature. The mixture was diluted with EtOAc and filtered through a pad of celite

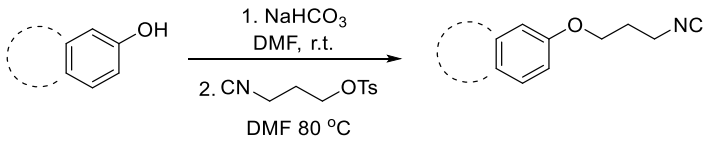
and the organic layer was washed with saturated aqueous NH_4Cl. Subsequently, the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure.

130 mg, 84% yield, yellow oil. ^1H NMR (500 MHz, $CDCl_3$): 3.11 – 3.08 (m, 2H), 2.48 (t, *J* = 7.0 Hz, 2H), 2.35 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2H), 1.85 (t, *J* = 14.0 Hz, 2H), 1.79 (t, *J* = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 119.3, 77.5, 76.2, 73.1, 70.3, 24.6, 17.9, 16.1, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{10}H_{11}N$+H, calculated 146.0964; found 146.0960.

General procedure for the synthesis of isocyanides 2-4 [61–63]

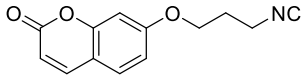


3-amino-propan-1-ol (20.0 mmol, 1 equiv) and ethyl formate (13.0 mL) were heated under reflux for 6 hours in an oil bath. Then, the volatile was removed under vacuo. The corresponding formamide (7.5 mmol, 1 equiv) was solubilized in dry DCM (14.0 mL) under nitrogen and after adding triethylamine (45.0 mmol, 6 equiv), the reaction mixture was cooled at 0 °C. TsCl (22.5 mmol, 3 equiv) was added and the reaction was stirred at room temperature for 5 hours. The mixture was quenched with a saturated aqueous Na_2CO_3 solution and stirred at 0°C for 30 minutes. Water was added and the aqueous phase was extracted with DCM (x3). The combined organic layers were dried over sodium sulfate and evaporated. The crude product was purified by flash filtration in silica using EtOAc as eluent solvent.



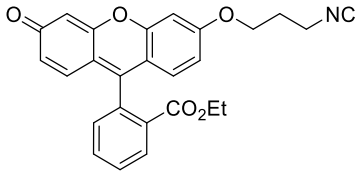
To a solution of hydroxyl compound (1 equiv) in anhydrous DMF was added $NaHCO_3$ (2 equiv) and the mixture was allowed to stir at room temperature for 15 minutes. 3-isocyanopropyl 4-methylbenzenesulfonate (1.2 equiv) dissolved in anhydrous DMF was added dropwise and the reaction mixture was allowed to stir overnight at 80˚C. After completion of the reaction, the solution was cooled to room temperature and the mixture was diluted with water and extracted with EtOAc. The organic phases were dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The residue was purified by silica gel chromatography (DCM:MeOH = 20:1, v/v) to afford the desired compounds.

7-(3-isocyanopropoxy)-2H-chromen-2-one (2)



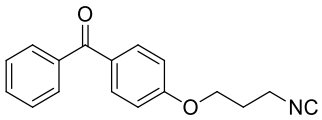
594 mg, 85% yield, off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.64 (d, *J* = 9.5 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 6.85 – 6.81 (m, 2H), 6.27 (d, *J* = 9.5 Hz, 1H), 4.18 (t, *J* = 5.5 Hz, 2H), 3.66 (tt, J_1 = 6.5 Hz, J_2 = 2.0 Hz, 2H), 2.23 – 2.17 (m, 2H).

Ethyl 2-(6-(3-isocyanopropoxy)-3-oxo-3H-xanthen-9-yl)benzoate (3)



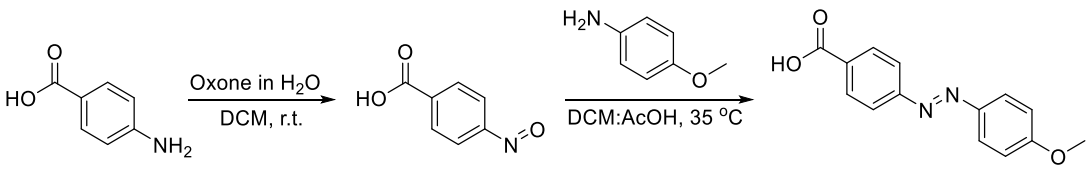
525 mg, 75% yield, bright orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.23 (dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.68 (dtd, J_1 = 30.0 Hz, J_2 = 7.5 Hz, J_3 = 1.5 Hz, 2H), 7.28 (dd, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 6.95 (d, *J* = 2.5 Hz, 1H), 6.88 (d, *J* = 21.5 Hz, 1H), 6.86 (d, *J* = 22.5 Hz, 1H), 6.72 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H), 6.50 (dd, J_1 = 9.5 Hz, J_2 = 2.0 Hz, 1H), 6.41 (d, *J* = 2.0 Hz, 1H), 4.20 (t, *J* = 5.5 Hz, 2H), 4.08 – 3.92 (m, 2H), 3.63 (t, *J* = 6.5 Hz, 2H), 2.19 – 2.17 (m, 2H), 0.94 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 185.6, 165.2, 162.6, 158.8, 157.2 (t, *J* = 5.0 Hz), 154.0, 150.0, 134.1, 132.5, 131.2, 130.6, 130.3, 130.3, 129.9, 129.6, 129.1, 117.8, 115.2, 113.2, 105.7, 101.0, 64.3, 61.2, 38.2 (t, $J_{C\text{-}N}$ = 6.5 Hz), 28.6, 13.6; HRMS (ESI) m/z : $[M+H]^+$: $C_{26}H_{21}NO_5$+H, calculated 428.1492; found 428.1490.

(4-(3-isocyanopropoxy)phenyl)(phenyl)methanone (4)



630 mg, 92% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 7.83 (d, *J* = 9.0 Hz, 2H), 7.75 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 2H), 7.57 (tt, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 4.20 (t, *J* = 5.5 Hz, 2H), 3.67 (t, *J* = 6.5 Hz, 2H), 2.22 – 2.17 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 195.5, 161.9, 157.0, 138.1, 132.6, 132.0, 129.7, 128.2, 114.0, 63.7, 38.4 (t, $J_{C\text{-}N}$ = 6.5 Hz), 28.9; HRMS (ESI) m/z : $[M+H]^+$: $C_{11}H_{14}O$+H, calculated 266.1175; found 266.1170.

General procedure for the synthesis of (*E*)-4-((4-methoxyphenyl)diazenyl)benzoic acid (5)[64]

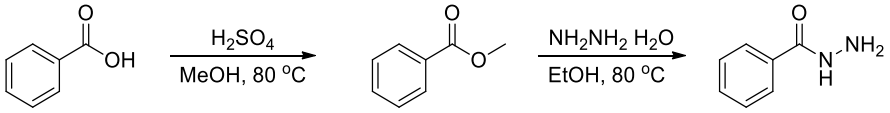


To a suspension of 4-aminobenzoic acid (1.0 g, 7.29 mmol) in DCM (11.0 mL) was added a solution of Oxone (8.97 g, 14.6 mmol) in water (45.0 mL). The resulting

suspension was stirred at room temperature for 1 hour, filtered, and dried to give 4-nitrosobenzoic acid. 1.05 g, 95% yield, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): 8.24 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 8.5 Hz, 2H).

A solution of 4-methoxyaniline (407 mg, 3.31 mmol) and nitroso (500 mg, 3.31 mmol) in DCM (20.0 mL) and acetic acid (20.0 mL) was stirred at 35 °C overnight. Then it was cooled to 0 °C and filtered to give azobenzene **5.** 456 mg, 54% yield, orange solid. ^{1}H NMR (500 MHz, DMSO-d_6): 8.10 (d, J = 8.5 Hz, 2H), 7.93 (d, J = 9.0 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 9.0 Hz, 2H), 3.87, (s, 3H).

General procedure for the synthesis of benzohydrazide (6)[65]

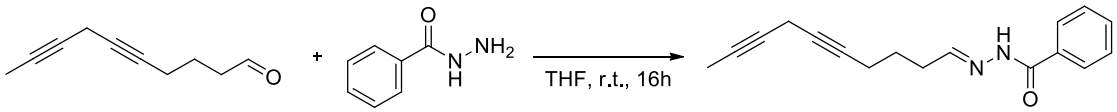


In a 25 ml round bottom flask charged with a magnetic stirrer a mixture of benzoic acid (1.0 g, 8.2 mmol) and methanol (8.0 mL) was refluxed at 80 °C for 2 hours in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled to room temperature and the residue was concentrated under reduced pressure. $NaHCO_3$ and Et_2O were used to extract the product. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to obtain the methyl benzoate which was used in the next step without any purification. 1.0 g, 89% yield, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.04 (d, J = 8.5 Hz, 2H), 7.57 – 7.54 (m, 1H), 7.44 (t, J = 7.5 Hz, 2H), 3.92 (s, 3H).

A mixture of methyl benzoate (1.0 g, 7.3 mmol) and hydrazine hydrate (0.86 mL, 8.8 mmol) was refluxed in ethanol (8 mL) at 80 °C for 2 hours with continuous stirring. The reaction was monitored by TLC. The mixture was concentrated on rotary evaporator. The solid so obtained was filtered, dried and recrystallized from ethanol to give benzohydrazide **6**. 0.89 g, 90 % yield, white solid.^{1}H NMR (500 MHz, DMSO-d_6): 9.73 (br s, 1H), 7.81 – 7.79 (m, 2H), 7.51 – 7.47 (m, 1H), 7.45 – 7.41 (m, 2H), 4.46 (br s, 2H).

General procedure for the synthesis of BAM2

***N*'-(deca-5,8-diyn-1-ylidene)benzohydrazide (BAM2)**

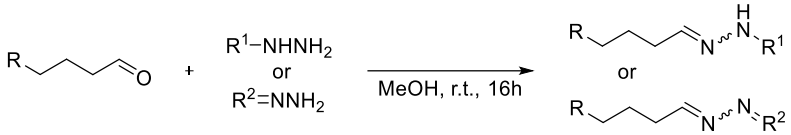


To a THF solution (0.4 mL) of benzohydrazide **6** (70 mg, 0.52 mmol), deca-5,8-diynal **BAM1** (90 mg, 0.61 mmol) was added and the mixture was stirred at room temperature for 16 hours. Upon completion of the reaction, THF was evaporated. The crude product was triturated with diethyl ether (25 mL), filtered and then dried under reduced pressure. The product was purified through column chromatography (P.E:EtOAc = 5:1 – 2:1, v/v)

116 mg, 84% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 9.38 (br, 1H), 7.80 (d, J = 7.5 Hz, 2H), 7.66 (br, 1H), 7.50 – 7.38 (m, 3H), 3.09 – 3.07 (m, 2H), 2.47 (br, 2H), 2.25 – 2.21

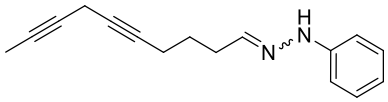
(m, 2H), 1.76 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 164.2, 152.2, 133.0, 131.8, 128.5, 127.3, 79.5, 76.0, 75.4, 73.5, 31.6, 25.6, 18.3, 9.6, 3.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{10}H_{11}N$+H, calculated 267.1492; found 267.1492.

General procedure for the synthesis of hydrazones BAM3-4 and C6



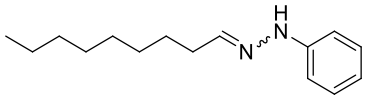
To a methanol solution (0.5 mL) of the corresponding hydrazine or hydrazone (0.50 mmol), the corresponding aldehyde (0.50 mmol) was added and the mixture was stirred at room temperature for 16 hours. Upon completion of the reaction, methanol was evaporated. The crude reaction mixture was treated with n-hexane (4.0 mL), the washes were collected and the solvent was concentrated under reduced pressure to give the product without any further purification.

1-(deca-5,8-diyn-1-ylidene)-2-phenylhydrazine (BAM3)



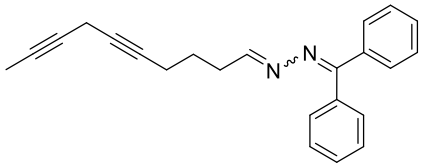
101 mg, 95 % yield, red oil, 3:1 *E*/*Z* mixture. ^{1}H NMR (500 MHz, $CDCl_3$): 7.27 – 7.21 (m, 4.0H), 7.09 (t, *J* = 5.0 Hz, 1H), 7.06 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 0.7H), 6.97 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 2H), 6.86 (tt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 0.35H), 6.81 (tt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 6.50 (t, *J* = 5.5 Hz, 0.35H), 3.15 – 3.12 (m, 0.7H), 3.12 – 3.09 (m, 2H), 2.42 – 2.38 (m, 2H), 2.37 – 2.33 (m, 0.7H), 2.31 – 2.25 (m, 2.7H), 1.80 – 1.78 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): 145.3, 145.1, 140.4, 139.6, 129.2, 129.2, 120.1, 119.5, 112.9, 112.5, 79.9, 79.2, 76.2, 76.0, 75.9, 75.1, 73.6, 73.3, 31.2, 26.0, 25,2, 24.9, 18.4, 18.3, 9.6, 9.6, 3.5, 3.4; HRMS (ESI) m/z : $[M+Na]^+$: $C_{16}H_{18}N_2$+Na, calculated 261.1366; found 261.1362.

1-nonylidene-2-phenylhydrazine (C6)



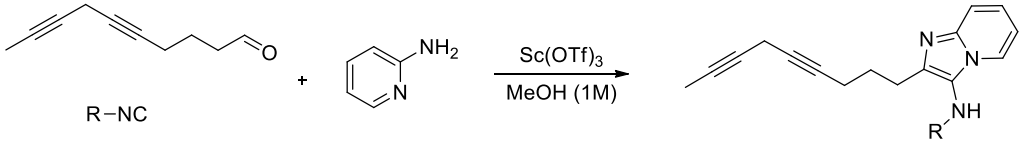
111 mg, 96 % yield, red oil, 3:1 *E*/*Z* mixture. ^{1}H NMR (500 MHz, $CDCl_3$): 7.27 – 7.21 (m, 2.8H), 7.07 (t, *J* = 5.5 Hz, 1H), 7.04 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 0.8H), 6.98 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 2H), 6.85 (tt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 0.35H), 6.81 (tt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 6.51 (t, *J* = 5.0 Hz, 0.35H), 2.32 – 2.28 (m, 2H), 2.22 – 2.18 (m, 0.8H), 1.63 – 1.57 (m, 0.8H), 1.56 – 1.50 (m, 2H), 1.31 – 1.28 (m, 13.5H), 0.90 – 0.87 (m, 4.05H); ^{13}C NMR (125 MHz, $CDCl_3$): 145.4, 145.2, 141.7, 141.3, 129.2, 129.2, 120.1, 119.5, 112.9, 112.5, 32.1, 31.8, 31.8, 29.4, 29.4, 29.3, 29.2, 29.2, 27.1, 26.2, 25.9, 22.6, 14.1; HRMS (ESI) m/z : $[M+H]^+$: $C_{15}H_{24}N_2$+H, calculated 233.2012; found 233.2009.

1-(deca-5,8-diyn-1-ylidene)-2-(diphenylmethylene)hydrazine (BAM4)



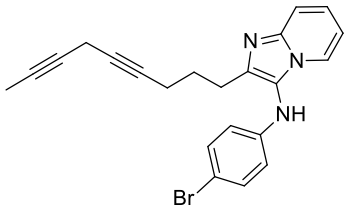
137 mg, 94 % yield, red oil, 3:1 *E*/*Z* mixture. ^{1}H NMR (500 MHz, $CDCl_3$): 7.83 – 7.80 (m, 1.2H), 7.67 – 7.62 (m, 2.3H), 7.55 – 7.46 (m, 2.0H), 7.42 – 7.40 (m, 7.8H), 7.31 – 7.27 (m, 1.8H), 7.24 – 7.21 (m, 1.9H), 7.11 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 0.2H), 7.03 (t, J = 5.0 Hz, 0.2H), 3.10 – 3.08 (m, 2.0H), 3.06 – 3.05 (m, 0.6H), 2.53 – 2.49 (m, 0.6H), 2.43 – 2.39 (m, 2.0H), 2.25 – 2.22 (m, 0.6H), 2.16 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2.0H), 1.79 (t, J = 2.5 Hz, 3.0H), 1.77 (t, J = 2.5 Hz, 0.9H), 1.74 – 1.67 (m, 2.6H) ; ^{13}C NMR (125 MHz, $CDCl_3$): 164.5, 161.3, 159.7, 156.3, 138.2, 135.3, 132.4, 130.0, 129.7, 129.4, 129.4, 128.8, 128.7, 128.7, 128.6, 128.5, 128.2, 128.1, 128.1, 127.8, 126.4, 126.4, 79.6, 79.4, 76.0, 75.9, 75.4, 75.2, 73.5, 73.4, 31.9, 31.6, 25.3, 25.2, 18.6, 18.2, 9.6, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{23}H_{22}N_2$+H, calculated 327.1856; found 327.1852.

General procedure for the synthesis of GBB – 3CR products



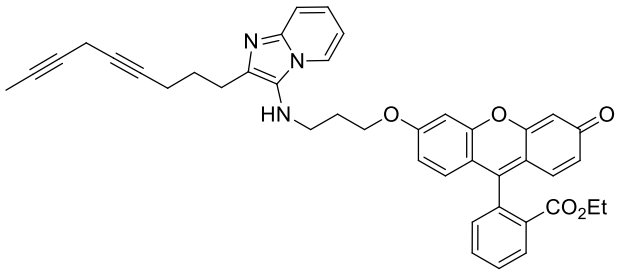
In a 4 ml reaction vial with a magnetic stirrer deca-5,8-diynal **BAM1** (0.50 mmol, 74 mg) and aminopyridine (0.50 mmol, 47 mg) were dissolved in MeOH (0.50 ml). After 10 minutes $Sc(OTf)_3$ (20 mol%) and the corresponding isocyanide (0.50 mmol) were added and the reaction was stirred overnight at room temperaure or 45 ˚C. The reaction was monitored by TLC. After the completion of the reaction the solvent was concentrated under reduced pressure and the product was purified through column chromatography (P.E:EtOAc = 5:1 – 0:1, v/v).

***N*-(4-bromophenyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM5)**



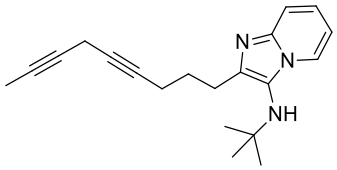
98 mg, 48% yield, brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.83 (d, J = 6.5 Hz, 1H), 7.63 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 8.5 Hz, 2H), 7.26 (t, J = 8.0 Hz, 1H), 6.83 (t, J = 6.5 Hz, 1H), 6.43 (d, J = 8.5 Hz, 2H), 5.97 (s, 1H), 3.04 – 3.02 (m, 2H), 2.82 (t, J = 7.5 Hz, 2H), 2.15 - 2.12 (tt, J_1 = 6.5 Hz, J_2 = 2.5 Hz, 2H), 1.96 – 1.91 (m, 2H), 1.78 (t, J = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 144.3, 141.9, 140.3, 132.4, 125.6, 122.7, 119.1, 116.7, 115.0, 112.6, 111.6, 79.8, 76.2, 75.3, 73.7, 27.3, 25.1, 17.9, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{22}H_{20}BrN_3$+H, calculated 406.0913; found 406.0911.

Ethyl 2-(6-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (BAM6)



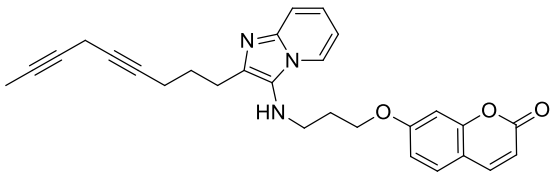
208 mg, 64% yield, bright orange – red solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.46 (d, *J* = 7.0 Hz, 1H), 8.26 (d, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 9.0 Hz, 1H), 7.74 – 7.64 (m, 4H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 2.5 Hz, 1H), 6.92 (dd, J_1 = 16.5 Hz, J_2 = 9.5 Hz, 2H), 6.80 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H), 6.60 (dd, J_1 = 9.5 Hz, J_2 = 2.0 Hz, 1H), 6.50 (d, *J* = 2.0 Hz, 2H), 4.34 (t, *J* = 6.0 Hz, 2H), 4.07 – 4.00 (m, 2H), 3.35 (br s, 2H), 3.04 – 3.00 (m, 4H), 2.23 – 2.18 (m, 4H), 2.00 – 1.94 (m, 2H), 1.74 (t, *J* = 2.5 Hz, 3H), 1.01 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 185.1, 165.2, 164.0, 159.4, 154.7, 136.5, 133.9, 132.7, 131.7, 131.2, 130.9, 130.5, 130.3, 129.9, 129.4, 129.0, 128.3, 124.5, 121.7, 119.2, 116.4, 115.1, 114.3, 112.5, 105.1, 100.9, 78.9, 76.0, 76.0, 73.3, 66.4, 61.4, 45.1, 29.8, 27.2, 23.0, 18.0, 15.2, 13.6, 9.5, 3.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{41}H_{37}N_3O_5$+H, calculated 652.2806; found 652.2802.

***N*-(tert-butyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM7)**



68 mg, 44% yield, brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.30 (d, *J* = 7.0 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 6.95 (t, *J* = 6.5 Hz, 1H), 3.11 – 3.10 (m, 2H), 3.06 (br, 1H), 2.99 (t, *J* = 7.5 Hz, 2H), 2.17 - 2.14 (m, 2H), 2.08 – 2.03 (m, 2H), 1.78 (t, *J* = 2.5 Hz, 3H), 1.22 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 139.9, 136.2, 127.1, 124.5, 123.8, 115.1, 113.1, 79.8, 76.1, 75.5, 73.4, 55.7, 30.2, 27.3, 24.7, 17.8, 9.7, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{20}H_{25}N_3$+H, calculated 308.2121; found 308.2118.

7-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-2*H*-chromen-2-one (BAM8)



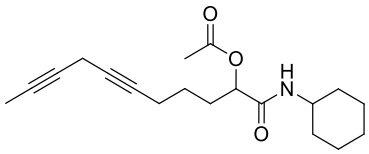
152 mg, 67% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 8.43 (d, *J* = 6.5 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.70 (t, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 9.5 Hz, 1H), 7.39 (d, *J* = 8.5 Hz,

1H), 7.28 (d, *J* = 7.0 Hz, 1H), 6.84 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H), 6.80 (d, *J* = 2.0 Hz, 1H), 6.22 (d, *J* = 9.5 Hz, 1H), 4.24 (t, *J* = 5.5 Hz, 2H), 4.00 (t, *J* = 6.5 Hz, 1H), 3.36 – 3.32 (m, 2H), 3.04 – 3.00 (m, 4H), 2.22 – 2.16 (m, 4H), 2.00 – 1.93 (m, 2H), 1.75 (t, *J* = 2.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 161.8, 161.2, 155.6, 143.6, 136.4, 132.0, 129.0, 128.2, 128.0, 124.2, 116.5, 112.9, 112.6, 112.6, 112.4, 101.3, 78.7, 76.0, 76.0, 73.2, 65.7, 45.1, 29.7, 26.9, 22.7, 17.8, 9.4, 3.3; HRMS (ESI) m/z : $[M+H]^+$: $C_{28}H_{27}N_3O_3$+H, calculated 454.2119; found 454.2117.

General procedure for the synthesis of Passerini products

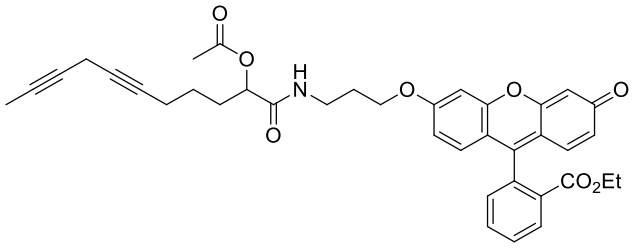
In a 4 ml reaction vial with a magnetic stirrer deca-5,8-diynal **BAM1** (0.50 mmol, 74 mg) or nonanal (0.50 mmol, 71 mg), isocyanide (0.50 mmol) and acetic acid (0.50 mmol, 29 µL) were dissolved in DCM (0.50 ml). The reaction was stirred overnight at room temperaure. The reaction was monitored by TLC. After the completion of the reaction the solvent was concentrated under reduced pressure and the product was purified through column chromatography (P.E:EtOAc = 5:1, v/v).

1-(cyclohexylamino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM9)



106 mg, 67% yield, pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): 5.84 (d, *J* = 8.0 Hz, 1H), 5.13 (dd, J_1 = 7.0 Hz, J_2 = 5.0 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.09 – 3.07 (m, 2H), 2.19 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2H), 2.15 (s, 3H), 1.95 – 1.87 (m, 4H), 1.79 (t, *J* = 2.5 Hz, 3H), 1.72 – 1.68 (m, 3H), 1.64 – 1.60 (m, 1H), 1.57 – 1.51 (m, 2H), 1.41 – 1.33 (m, 2H), 1.19 – 1.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 169.6, 168.5, 79.5, 76.0, 75.1, 73.7, 73.4, 48.0, 33.0, 33.0, 31.1, 25.5, 24.7, 23.9, 21.0, 18.5, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{19}H_{27}NO_3$+H, calculated 318.2064; found 318.2068.

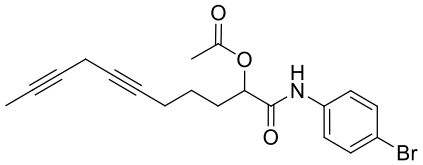
Ethyl 2-(6-(3-(2-acetoxyundeca-6,9-diynamido)propoxy)-3-oxo-3*H*-xanthen-9-yl) benzoate (BAM10)



260 mg, 82% yield, bright orange – red solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.25 (d, *J* = 8.0 Hz, 1H), 7.75 – 7.66 (m, 2H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.00 (s, 1H), 6.92 (dd, J_1 = 16.5 Hz,

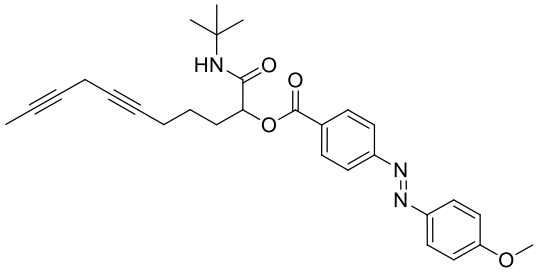
J_2 = 9.5 Hz, 2H), 6.78 (d, J = 9.0 Hz, 1H), 6.64, (d, J = 9.5 Hz, 2H), 6.57 (br s, 1H), 5.13 (dd, J_1 = 7.5 Hz, J_2 = 5.0 Hz, 1H), 4.15 (t, J = 6.0 Hz, 2H), 4.07 – 3.97 (m, 2H), 3.54 – 3.45 (m, 2H), 3.06 – 3.04 (m, 2H), 2.17 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2H), 2.13 (s, 3H), 2.10 – 2.05 (m, 2H), 2.00 – 1.86 (m, 2H), 1.76 (t, J = 2.5 Hz, 3H), 1.58 – 1.52 (m, 2H), 0.98 (t, J = 7.0 Hz, 3H) ; ^{13}C NMR (125 MHz, $CDCl_3$): 169.9, 169.8, 165.2, 163.8, 159.0, 154.6, 134.0, 132.6, 131.2, 130.6, 130.5, 130.3, 129.8, 129.2, 117.6, 115.2, 114.2, 105.4, 100.9, 79.4, 76.0, 75.2, 73.7, 73.4, 66.9, 61.4, 36.4, 31.0, 28.9, 24.1, 21.0, 18.4, 13.6, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{38}H_{37}NO_8$+H, calculated 636.2591; found 636.2591.

1-((4-bromophenyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM11)



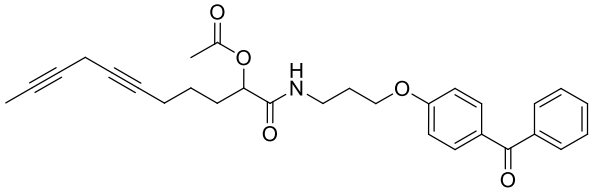
142 mg, 73% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 7.78 (s, br, 1H), 7.44 (s, 4H), 5.28 (dd, J_1 = 7.5 Hz, J_2 = 5.0 Hz, 1H), 3.10 – 3.07 (m, 2H), 2.25 – 2.20 (m, 2H), 2.21 (s, 3H), 2.02 – 1.96 (m, 2H), 1.77 (t, J = 7.0 Hz, 3H), 1.63 – 1.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 169.8, 167.7, 136.0, 132.0, 121.6, 117.5, 79.3, 76.1, 75.4, 73.9, 73.4, 30.9, 23.9, 20.9, 18.4, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{19}H_{20}BrNO_3$+H, calculated 390.0699; found 390.0692.

(*E*)-1-(tert-butylamino)-1-oxoundeca-6,9-diyn-2-yl 4-((4-methoxyphenyl)diazenyl) benzoate (BAM12)



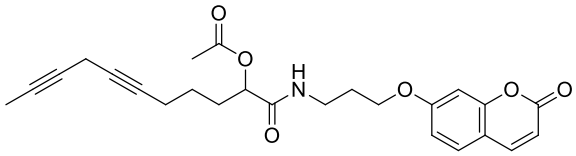
197 mg, 81% yield, orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.19 (d, J = 8.5 Hz, 2H), 7.97 (d, J = 9.0 Hz, 2H), 7.94 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 9.0 Hz, 2H), 5.90 (br s, 1H), 5.33 (dd, J_1 = 7.0 Hz, J_2 = 5.0 Hz, 1H), 3.91 (s, 3H), 3.08 – 3.07 (m, 2H), 2.26 – 2.23 (m, 2H), 2.13 – 2.04 (m, 2H), 1.77 (t, J = 2.5 Hz, 3H), 1.69 – 1.63 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 168.5, 164.9, 162.8, 155.8, 147.0, 130.7, 130.3, 125.3, 122.6, 114.4, 79.5, 76.0, 75.2, 74.5, 73.4, 55.6, 51.4, 31.1, 28.7, 24.1, 18.6, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{29}H_{33}N_3O_4$+H, calculated 488.2544; found 488.2541.

1-((3-(4-benzoylphenoxy)propyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM13)



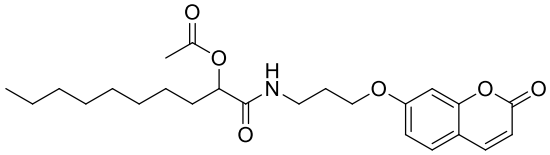
196 mg, 83% yield, pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.82 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 2H), 7.56 (tt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 6.95 (d, *J* = 8.5 Hz, 2H), 6.41 (br s, 1H), 5.15 (dd, J_1 = 7.0 Hz, J_2 = 5.0 Hz, 1H), 4.11 (t, *J* = 5.5 Hz, 2H), 3.53 – 3.47 (m, 2H), 3.07 – 3.05 (m, 2H), 2.21 – 2.17 (m, 2H), 2.11 (s, 3H), 2.08 – 2.03 (m, 2H), 1.99 – 1.84 (m, 2H), 1.77 (t, *J* = 2.5 Hz, 3H), 1.57 – 1.51 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 195.5, 169.8, 169.7, 162.2, 138.1, 132.6, 131.9, 130.4, 129.7, 128.2, 114.0, 79.4, 76.0, 75.2, 73.7, 73.4, 66.3, 36.9, 31.0, 28.8, 24.0, 20.9, 18.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{29}H_{31}NO_5$+H, calculated 474.2275; found 474.2269.

1-oxo-1-((3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)undeca-6,9-diyn-2-yl acetate (BAM14)



172 mg, 79% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 7.62 (d, *J* = 9.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.80 (s, 1H), 6.37 (br s, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.14 (dd, J_1 = 7.0 Hz, J_2 = 5.0 Hz, 1H), 4.08 (t, *J* = 6.0 Hz, 2H), 3.55 – 3.45 (m, 2H), 3.07 – 3.05 (m, 2H), 2.18 (tt, J_1 = 9.5 Hz, J_2 = 2.5 Hz, 2H), 2.13 (s, 3H), 2.08 – 2.03 (m, 2H), 2.00 – 1.86 (m, 2H), 1.77 (t, *J* = 2.5 Hz, 3H), 1.57 – 1.51 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): 169.8, 169.7, 161.8, 161.0, 155.7, 143.3, 128.8, 113.1, 112.7, 112.6, 101.5, 79.3, 75.9, 75.2, 73.7, 73.4, 66.5, 36.6, 31.0, 28.8, 24.0, 20.9, 18.4, 9.5, 3.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{25}H_{27}NO_6$+H, calculated 438.1911; found 438.1911.

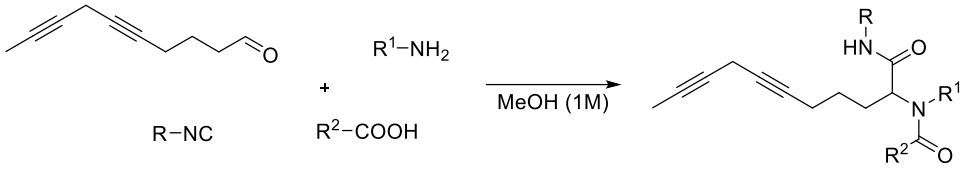
1-oxo-1-((3-((2-oxo-2H-chromen-7-yl)oxy)propyl)amino)decan-2-yl acetate (C3)



172 mg, 79% yield, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.62 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.79 (s, 1H), 6.38 (br s, 1H), 6.23 (d, *J* = 9.5 Hz, 1H), 5.12 (dd, J_1 = 7.0 Hz, J_2 = 5.5 Hz, 1H), 4.07 (t, *J* = 6.0 Hz, 2H), 3.56 – 3.44 (m, 2H), 2.12 (s, 3H), 2.08 – 2.03 (m, 2H), 1.86 – 1.75 (m, 2H), 1.31 – 1.22 (m, 2H), 0.85 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 170.1, 169.7, 161.8, 161.1, 155.8, 143.3, 128.8, 113.2, 112.7, 112.6, 101.5, 74.2, 66.5, 36.7, 31.8, 31.7, 29.3, 29.2, 29.1, 28.8,

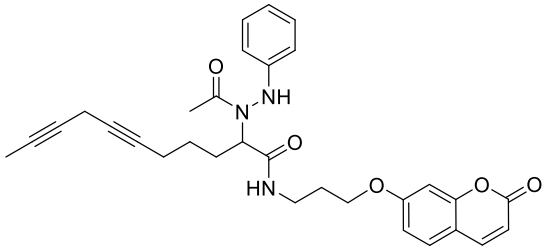
24.8, 22.6, 21.0, 14.0; HRMS (ESI) m/z : $[M+H]^+$: $C_{24}H_{33}NO_6$+H, calculated 432.2381; found 432.2377.

General procedure for the synthesis of U – 4CR products



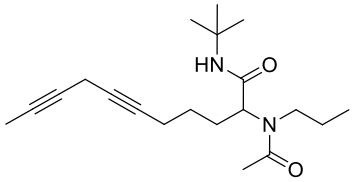
In a 4 ml reaction vial with a magnetic stirrer deca-5,8-diynal **BAM1** (0.50 mmol, 74 mg), amine (0.50 mmol), isocyanide (0.50 mmol) and acid (0.50 mmol) were dissolved in MeOH (0.50 ml). The reaction was stirred overnight at room temperature. The reaction was monitored by TLC. After the completion of the reaction the solvent was concentrated under reduced pressure and the product was purified through column chromatography (P.E:EtOAc = 5:1 – 3:1, v/v).

2-(1-acetyl-2-phenylhydrazineyl)-*N*-(3-((2-oxo-2H-chromen-7-yl)oxy)propyl)undeca-6,9-diynamide (BAM15)



111 mg, 42% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 7.61 (d, *J* = 9.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.21 (t, *J* = 7.5 Hz, 2H), 6.88 – 6.84 (m, 2H), 6.80 (s, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.23 (d, *J* = 9.5 Hz, 1H), 5.05 – 4.99 (m, 1H), 4.09 – 4.04 (m, 2H), 3.52 – 3.44 (m, 2H), 3.04 – 2.99 (m, 2H), 2.16 (s, 3H), 2.10 – 2.00 (m, 4H), 1.76 (t, *J* = 2.5 Hz, 3H), 1.66 – 1.62 (m, 1H), 1.50 – 1.36 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 176.8, 172.0, 171.0, 161.8, 161.1, 155.7, 143.3, 129.4, 129.3, 128.7, 113.0, 112.8, 112.5, 112.1, 101.3, 79.3, 75.9, 75.2, 73.3, 66.2, 66.1, 58.7, 36.4, 28.7, 25.3, 21.3, 18.2, 9.5, 3.4; HRMS (ESI) m/z : $[M+Na]^+$: $C_{31}H_{33}N_3O_5$+Na, calculated 550.2312; found 550.2307.

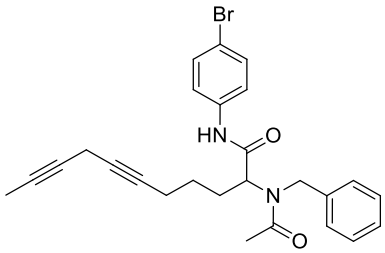
***N*-(*tert*-butyl)-2-(*N*-propylacetamido)undeca-6,9-diynamide (BAM16)**



63 mg, 38% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 6.46 (s, br, 1H), 4.69 (t, *J* = 7.5 Hz, 1H), 3.24 - 3.13 (m, 2H), 3.07 – 3.04 (m, 2H), 2.23 – 2.17 (m, 2H), 2.13 (s, 3H), 1.98 – 1.90 (m, 1H), 1.76 (t, *J* = 2.5 Hz, 3H), 1.66 – 1.59 (m, 1H), 1.52 – 1.34 (m, 4H), 1.27 (s, 9H), 0.86 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 172.2, 170.4, 79.7, 75.9, 74.9,

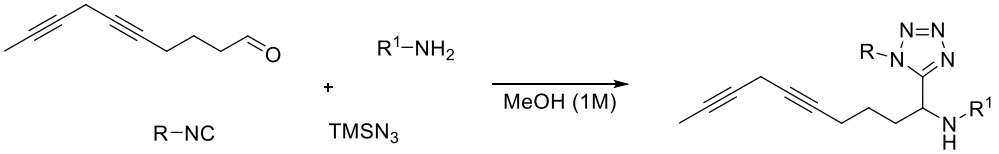
73.4, 57.4, 50.9, 47.5, 28.5, 27.1, 25.3, 23.0, 21.8, 18.6, 11.3, 9.6, 3.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{20}H_{32}N_2O_2$+H, calculated 333.2537; found 333.2539.

2-(*N*-benzylacetamido)-*N*-(4-bromophenyl)undeca-6,9-diynamide (BAM17)



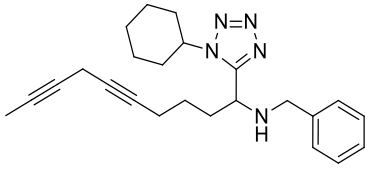
110 mg, 46% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 8.82 (s, br, 1H), 7.39 (d, *J* = 9.0 Hz, 2H), 7.33 (d, *J* = 9.0 Hz, 2H), 7.30 – 7.24 (m, 3H), 7.16 (d, *J* = 7.0 Hz, 2H), 5.00 (dd, J_1 = 8.0 Hz, J_2 = 7.0 Hz, 1H), 4.66 (d, *J* = 17.5 Hz, 1H), 4.59 (d, *J* = 17.5 Hz, 1H), 3.06-3.04 (m, 2H), 2.18 – 2.07 (m, 3H), 2.16 (s, 3H), 1.82 – 1.74 (m, 1H), 1.77 (t, *J* = 2.5 Hz, 3H), 1.57 – 1.48 (m, 1H), 1.48 – 1.39 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): 173.8, 168.6, 136.9, 136.6, 131.8, 128.9, 127.6, 126.1, 121.4, 116.6, 79.4, 76.0, 75.3, 73.4, 58.6, 49.5, 27.0, 25.4, 22.4, 18.5, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{26}H_{27}BrN_2O_2$+H, calculated 479.1329; found 479.1321.

General procedure for the synthesis of UT - 4CR products



In a 4 ml reaction vial with a magnetic stirrer deca-5,8-diynal **BAM1** (0.50 mmol, 74 mg), the amine (0.50 mmol), the isocyanide (0.50 mmol) and $TMSN_3$ (0.50 mmol, 58 mg) were dissolved in MeOH (0.50 ml). The reaction was stirred overnight at 45 ℃. The reaction was monitored by TLC. After the completion of the reaction the solvent was concentrated under reduced pressure and the product was purified through column chromatography (P.E:EtOAc = 5:1 – 2:1, v/v).

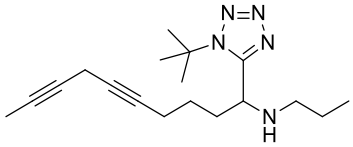
***N*-benzyl-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)deca-5,8-diyn-1-amine (BAM18)**



123 mg, 63% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 7.31 (d, *J* = 7.0 Hz, 2H), 7.27 – 7.25 (m, 3H), 4.34 (tt, J_1 = 11.5 Hz, J_2 = 3.5 Hz, 1H), 4.09 (t, *J* = 7.0 Hz, 1H), 3.73 (d, *J* = 13.0 Hz, 1H), 3.56 (d, *J* = 13.0 Hz, 1H), 3.06-3.04 (m, 2H), 2.19 (tt, J_1 = 7.0 Hz, J_2 = 2.5 Hz, 2H), 2.04 – 1.87 (m, 10H), 1.76 (t, *J* = 2.5 Hz, 1H), 1.62 – 1.55 (m, 1H), 1.48 – 1.41 (m, 1H), 1.36 – 1.31 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 155.2, 129.0, 128.5, 128.1,

127.4, 79.3, 76.0, 75.4, 73.3, 57.7, 51.7, 51.2, 33.6, 33.2, 33.0, 25.3, 24.8, 18.3, 9.6, 3.5; HRMS (ESI) m/z : $[M+H]^+$: $C_{24}H_{31}N_5$+H, calculated 390.2650; found 390.2649.

1-(1-(tert-butyl)-1*H*-tetrazol-5-yl)-*N*-propyldeca-5,8-diyn-1-amine (BAM19)



114 mg, 72% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 4.20 (t, *J* = 7.0 Hz, 1H), 3.05 - 3.03 (m, 2H), 2.43 – 2.33 (m, 2H), 2.22 – 2.18 (m, 2H), 1.95 – 1.90 (m, 2H), 1.76 – 1.74 (m, *5*H), 1.75 (s, 9H), 1.62 – 1.53 (m, 1H), 1.51 – 1.38 (m, 2H), 0.86 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 157.7, 79.5, 75.9, 75.2, 73.4, 61.1, 54.0, 49.4, 34.7, 30.2, 25.1, 23.2, 18.3, 11.6, 9.6, 3.4; HRMS (ESI) m/z : $[M+H]^+$: $C_{18}H_{29}N_5$+H, calculated 316.2496; found 316.2492.

3. Expression and purification of 15-LOX-1

Newly transformed BL21 (DE3) *E. coli* cells with the recombinant pET26b plasmid carrying the A-LOX-1 gene were obtained from an agar plate and were used to inoculate 10 ml of LB_{kan} medium. Both the agar plate and the LB medium were supplemented with 50 µg/ml kanamycin. After overnight growth at 30 ˚C, the preculture was used to inoculate 1 L of LB_{kan}. This culture was incubated at 37 ˚C until reaching an OD_{600} value of 0.6-0.8 (approximately 4 h). The expression of h-15-LOX-1-His6 protein was induced with 0.2 mM IPTG and the culture was incubated overnight at 18 ˚C before harvesting by centrifugation (6000 g, 20 minutes, 4 ˚C). The culture underwent stirring throughout the entire process. The pellet was resuspended in buffer A (50 mM HEPES, 300 mM NaCl, 5 mM imidazole, pH 7.5) to a final volume of 10 ml. For the lysis of the cells a sonicator was used (Branson Sonifier 250, G. Heinemann Ultraschallund Labortechnik) for 16 minutes with 40 – second sonication and 60 – second interval in between (duty cycle 50%, output 3). Prior to the sonification, 2.5 mM PMSF, 50 µg/ml DNase, 1 mM $MgCl_2$ and 5 mM DTT were added. Unbroken cells and debris were separated from the soluble proteins by centrifugation (15000 g, 30 minutes, 4 ˚C). The supernatant was incubated with a pre-equilibrated NEBExpress® Ni Resin column for 1 hour at 4 ˚C. The unbound proteins not bearing a His-tag were removed by gravity flow. The column was washed with 10 column volumes of each buffer: buffer A and subsequently buffer B (50 mM HEPES, 50 mM NaCl, 5 mM imidazole, pH 7.5). Retained proteins were eluted with 10 column volumes of buffer B supplemented with 200 mM imidazole (elution buffer). Fractions from all the steps were analysed using SDS-PAGE and the most purified ones were pooled and concentrated using Amicon® centrifugal filters with a 30 kDa cut-off membrane when necessary. A dialysis cellulose membrane (avg. flat width 43 mm) was used for

removing imidazole and for exchanging the elution buffer with buffer C (50 mM HEPES, 50 mM NaCl, 50% glycerol, pH 7.5) overnight at 4 ℃. The purified protein was aliquoted and stored at -20 ℃.

4. Enzyme inhibition studies

Each measurement was performed in triplicate and all data were processed with Microsoft Excel Professional Plus 2016 and GraphPad Prism 8 software.

Activity assay

The h-15-LOX-1 was expressed in BL21 (DE3) *E. coli* cells and the cell lysate was used for the activity assay with no further purification. The conversion of linoleic acid to 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13(S)-HpODE) was observed through UV absorbance at 234 nm over time with a ThermoFisher Varioskan Plate Reader and a Greiner Bio-One F-Bottom 96-well plate. The measurement took place for 20 minutes with an interval time of 20 seconds. Only the linear part was used for the determination of the enzymatic activity, typically extending over the first 1-5 minutes depending on the enzyme concentration. After that, the conversion rate slows down due to the consumption of the substrate. The activity assay was used for the determination of the optimum concentration of the cell lysate (x200 times dilution in assay buffer: 50 mM HEPES, 50 mM NaCl, pH 7,5). Linoleic acid (Sigma Aldrich, L1376) was diluted in ethanol.

Screening UV assay

For the evaluation of the inhibitory potency of the compounds the same experimental approach based on the absorption of 13(S)-HpODE at 234 nm was used. All the compounds were dissolved in DMSO at a final concentration of 2 mM. Then, they were diluted with assay buffer and tested at 50 μM. Each compound was mixed with the diluted cell lysate and after a 10-minute incubation at room temperature, the linoleic acid was added at a final concentration of 25 μM. All the values were normalized by setting as 100% the absence of the inhibitor. Compounds in test samples with enzyme activity less than 50% were considered as hits.

	Volume (μl)		
Components	Positive Control	Test Sample	Blank
Assay Buffer	135	135	145
DMSO	5	-	5
Cell lysate	50	50	50
Inhibitor 2 mM	-	5	-
Linoleic acid	10	10	-

Determination of the half maximal inhibitor concentration (IC_{50})

The half maximal inhibitor concentration (IC_{50}) was also determined by a comparable method using the absorption of 13(S)-HpODE at 234 nm. All the compounds were firstly dissolved in DMSO and then diluted with assay buffer at a final concentration of 200 µM. The desired concentrations, ranging from 0,78 to 100 µM, were achieved by serial dilution. Each concentration was mixed with the diluted cell lysate and after a 10 or 20-minute incubation at room temperature, the linoleic acid was added at the same final concentration as above. The 100% was set by the absence of the inhibitor, while the 0% was set by the absence of the substrate. DMSO, diluted in assay buffer, was added to both the positive control and the blank sample at the same concentration as the test sample.

	Volume (µl)		
Components	Positive Control	Test Sample	Blank
Assay Buffer	90	40	100
DMSO 5%	50	-	50
Cell lysate	50	50	50
Inhibitor 200 µM	-	100	-
Linoleic acid	10	10	-

Michaelis – Menten kinetics

The absorption of 13(S)-HpODE at 234 nm was employed once again for the kinetics study. Four different concentrations of the linoleic acid were tested this time: 5 µM, 10 µM, 20 µM and 40 µM. The enzyme activity was determined in the absence or presence of specified concentrations of the inhibitors based on their IC_{50} value. As previously, 100 µl of the inhibitor solution were mixed with 40 µl of assay buffer and 50 µl of the diluted cell lysate. In absence of inhibitor, the corresponding volume is replaced with assay buffer. After a 10-minute incubation at room temperature, 10 µl of linoleic acid ranging from 800 µM to 100 µM. The reaction velocity V was plotted against the substate concentration [S] in a Michaelis – Menten plot for the determination of the K_m and V_{max} in the presence of the inhibitor. The reciprocal of the substrate concentration 1/[S] was plotted against the reciprocal of the reaction velocity 1/V in a Lineweaver Burk plot.

5. IC_{50} graphs of BAM compounds

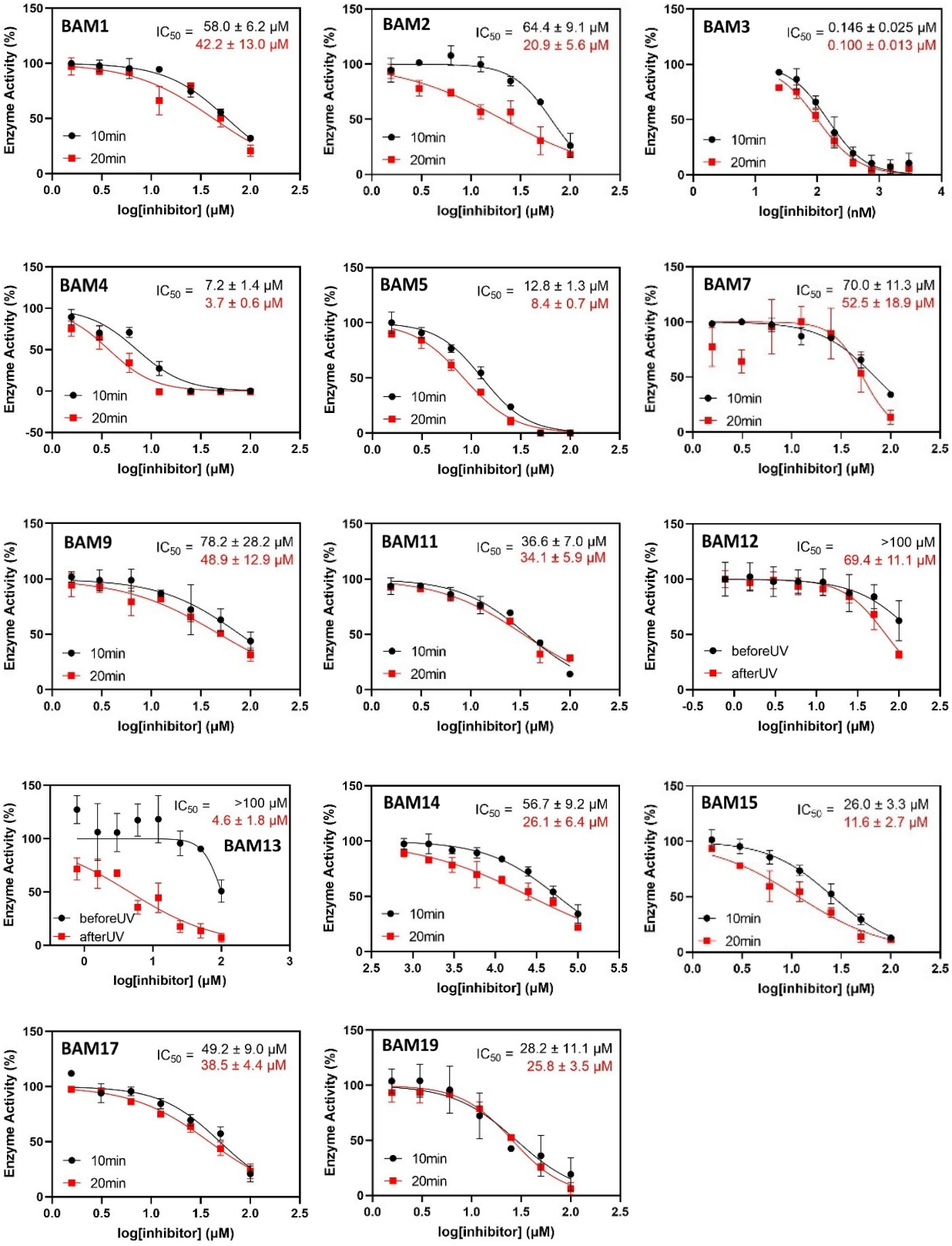


Fig. S1. IC_{50} graphs of BAM compounds.

6. Screening (at 50 uM) and IC_{50} graphs of control compounds

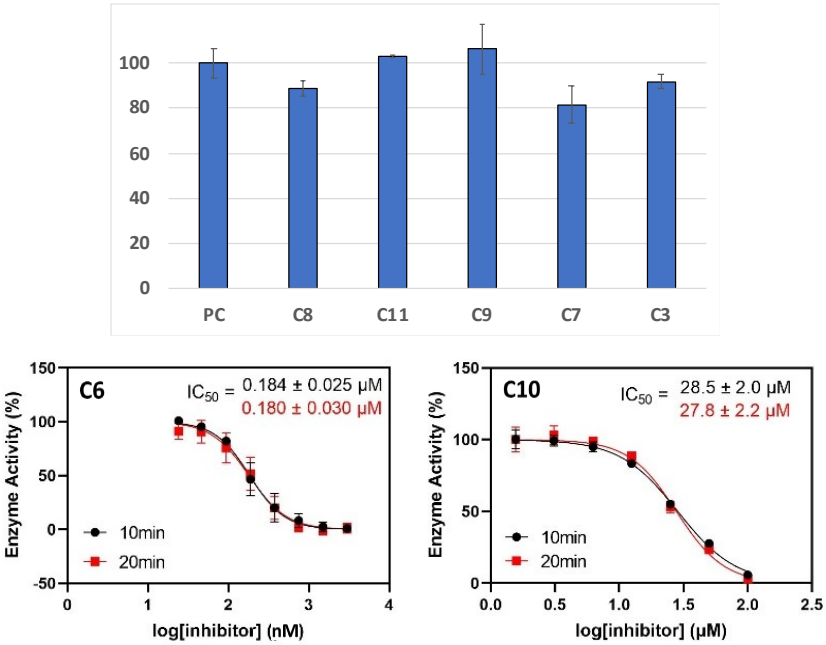
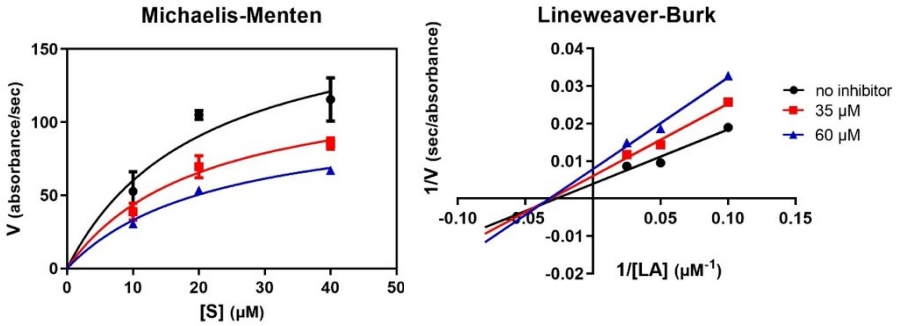


Fig. S2. Inhibitory screening against 15-LOX-1 at 50 µM and IC_{50} graphs of control compounds.



BAM1(µM)	K_m^{app} (µM)	V_{max}^{app} (absorbance/s)
0	20,5	183,0
35	20,5	132,7
60	22,7	107,8

These are the best fit values calculated by Prism 8.

Fig. S3. The kinetic analysis of **BAM1**. A Michaelis-Menten graph (left) and a Lineweaver-Burk graph (right). Two different concentrations of the inhibitor, one higher than the IC_{50} value (blue) and one lower (red) are compared with the absence of the inhibitor (black)

7. MS experiments

Pre-incubated with **BAM3** purified 15-LOX-1 was loaded in a 12.5% polyacrylamide gel and ran an SDS-PAGE. Fixation of the gel was performed with solution compatible with mass spectrometry: 30% methanol, 10% acetic acid for 1 hour and washed with distilled water for 15 minutes for 4 times. Staining was performed with “blue silver” Coomasie colloidal blue stain (0.12% Coomassie Blue G-250, 10% ammonium sulfate, 10% phosphoric acid, 20% methanol) overnight. Gel was then washed with dH_2O for 3 times. Protein bands were excised from polyacrylamide gel and cut in to small pieces. Gel pieces were covered with 100 µL 50% ACN/dH_2O and shaken for 10 minutes, the solution was then removed and gel pieces were covered with 100 µL 50 mM Ammonium bicarbonate and shaken for 10 minutes. For complete destaining of the gel pieces, previous steps were repeated for six times. For the reduction and alkylation of the cysteine residues, gel pieces were covered with 100 µL DTT and shaken for 45 minutes at 56°C followed by 45 minutes shaking at room temperature with 100 µL 55 mM iodoacetamide. Protein was digested in 50 µL of diluted Trypsin solution and incubated overnight at 37°C. The next day, the supernatant was collected. For the extraction from the gel matrix of generated peptides, gel pieces were shaken for 20 minutes first in 50 µL 50% ACN and finally in 50 µL 0.1% TFA/50% ACN. Peptide solutions were centrifuged by Speed Vac until dry powder remained and then analyzed by means of liquid chromatography combined with mass spectrometry analysis (nanoLC-MS/MS).[66] The nanoLC-MS/MS analysis was performed on an EASY-nLC system (Proxeon, software version 2.7.6 #1) coupled with an LTQ-Orbitrap XL ETD (Thermo Scientific, Bremen, Germany) through a nanoES ion source (Proxeon). Data were acquired with Xcalibur software (LTQ Tune 2.5.5 sp1, Thermo Scientific). The MS raw data were loaded in Proteome Discoverer 1.1.0.263 (Thermo Scientific) and run using Mascot 2.3.01 (Matrix Science, London, UK) and Sequest (Thermo Scientific) search algorithms.[67]

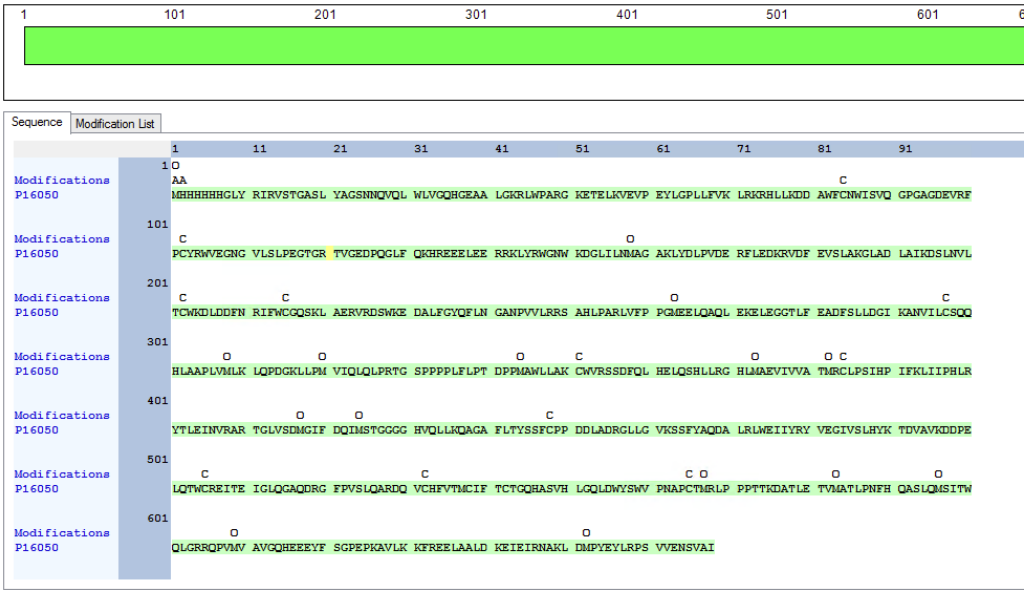


Fig. S4. The high level of sequence coverage (100%) of human 15-LOX-1

No Modification	High	[R].YTLEINVR.[A]			1	1	P16050	P16050 [401-408]	1007,55202	High	High	2,06	39	R.YTLEINVR.A	[401-408]						
	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		High	Sequest HT (A22)	Ambiguous	[R].YTLEINVR.[A]		1	P16050	0	2	0,5874	0,0000	1	1	504,27933	1007,55139	1007,55202	-0,63	-0,00031	1,044	42,3290
Y395	Medium	[R].YTLEINVRAR.[T]	1xELE_BAM3 [Y1]		1	1	P16050	P16050 [401-410]	1470,82159	n/a	Medium	1,26		R.YTLEINVRAR	[401-410]						
(Y401)	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		Medium	Sequest HT (A22)	Ambiguous	[R].yTLEINVRAR.[T]	Y1(BAM3)	1	P16050	1	2	0,1825	0,0000	1	1	735,90840	1470,80952	1470,82159	-8,21	-0,00604	149,556	38,2790
T396	Medium	[R].YTLEINVRAR.[T]	1xELE_BAM3 [T2]		1	1	P16050	P16050 [401-410]	1470,82159	n/a	Medium	1,16		R.YTLEINVRAR	[401-410]						
(T402)	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		Medium	Sequest HT (A22)	Ambiguous	[R].YtLEINVRAR.[T]	T2(BAM3)	1	P16050	1	2	0,3103	0,0000	1	1	735,90840	1470,80952	1470,82159	-8,21	-0,00604	149,556	38,2790
L397	Medium	[R].YTLEINVRAR.[T]	1xELE_BAM3 [L3]		1	1	P16050	P16050 [401-410]	1470,82159	n/a	Medium	1,09		R.YTLEINVRAR	[401-410]						
(L403)	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		Medium	Sequest HT (A22)	Ambiguous	[R].YTlEINVRAR.[T]	L3(BAM3)	1	P16050	1	2	0,0917	0,0000	1	1	735,90840	1470,80952	1470,82159	-8,21	-0,00604	149,556	38,2790
E398	Low	[R].YTLEINVRAR.[T]	1xELE_BAM3 [E4]		1	1	P16050	P16050 [401-410]	1470,82159	n/a	Low	0,74		R.YTLEINVRAR	[401-410]						
(E404)	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		Low	Sequest HT (A22)	Ambiguous	[R].YTLeINVRAR.[T]	E4(BAM3)	1	P16050	1	2	0,0000	0,0000	1	1	735,90840	1470,80952	1470,82159	-8,21	-0,00604	149,556	38,2790
I399	Low	[R].YTLEINVRAR.[T]	1xELE_BAM3 [I5]		1	1	P16050	P16050 [401-410]	1470,82159	n/a	Low	0,78		R.YTLEINVRAR	[401-410]						
(I405)	Checked	Confidence	Identifying Node	PSM Ambiguity	Annotated Sequence	Modification	# Proteins	Master Protein Acce	# Missed Cleavages	Charge	DeltaScore	DeltaCn	Rank	Search Engine	m/z [Da]	MH+ [Da]	Theo. MH+ [Da]	DeltaM [ppm	Deltam/z [Da	Ion Inject Tin	RT [min]
		Low	Sequest HT (A22)	Ambiguous	[R].YTLEiNVRAR.[T]	I5(BAM3)	1	P16050	1	2	0,0128	0,0000	1	1	735,90840	1470,80952	1470,82159	-8,21	-0,00604	149,556	38,2790

Fig. S5. Identified modifications of peptide sequence [YTLEINVRAR] with amino acids Y395, T396, L397 and E398 displaying a mass increase of +230 Da, corresponding to an adduct of **BAM3**.

8. Imaging-based experiments and microscopy

Methods

Cell culture

Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. RAW 264.7 murine macrophages were grown in high-glucose DMEM medium (Sigma) supplemented with 10% FBS (VWR), 100 U/mL penicillin/streptomycin (Sigma-Aldrich) and 2 mM GlutaMax (Fisher Scientific). The DMSO concentration for all small-molecule cell experiments was kept at 0.1%.

Imaging-based cell permeability assay

RAW 264.7 cells were seeded on uncoated 35 mm no. 1.5 glass-bottomed dishes (Ibidi) with 1.0 x 10^5 cells per sample. Cells were grown for one day. The medium was changed to complete medium without phenol-red. Live cells were imaged before and after replacing the medium with **BAM10** (5 µM) dissolved in complete medium without phenol-red (*in situ* labelling). Confocal images of equatorial regions of the cells were collected over a period of 24 minutes. Wherever indicated, cells were washed with PBS (thrice) before addition of fresh complete medium without phenol-red.

Cell-based competition assay

RAW 264.7 cells were seeded on uncoated 35 mm no. 1.5 glass-bottomed dishes (Ibidi) with 1.0 x 10^5 cells per sample. Cells were grown for one day. The medium was replaced with medium containing DMSO or competitor (**BAM3** or ThioLox) at the indicated concentration and incubated for 1 hour in the incubator. Cells were then washed with PBS and subsequently treated either with **BAM10** (5 µM) with DMSO or competitor dissolved in complete medium without phenol-red. After 16 minutes, live cells were imaged.

Cell-based irreversibility assay

RAW 264.7 cells were seeded on uncoated 35 mm no. 1.5 glass-bottomed dishes (Ibidi) with 1.0 x 10^5 cells per sample. Cells were grown for one day. The medium was replaced with medium containing **BAM10** (5 µM) and incubated for the indicated time. The labelling medium was then replaced by complete medium without phenol-red containing DMSO or competitor (**BAM3** or ThioLox) at the indicated concentration and incubated for 1 hour. Finally, live cells were imaged.

Caenorhabditis elegans

Nematodes were maintained and manipulated following established procedures.[68] The N2 wild-type Bristol isolate strain (CGC) was used for this study. Nematodes were maintained on nematode growth media (NGM). The OP50 *E.coli* (KRT) strain was used as a food source. For RNAi experiments, NGM plates were seeded with HT115 (DE3) bacteria (KRT), carrying the desired RNAi plasmid construct. For imaging, animals were

anesthetized on a 20 mM levamisole drop on the coverslips. For all small-molecule experiments, plates were allowed to absorb the small molecule and dry before placing the animals on the plates. The DMSO concentration was kept at 0.1%.

RNAi treatment

Specific gene downregulation was achieved using the RNA interference technique (RNAi). Animals were fed bacteria expressing double-stranded (ds) RNA with the gene of interest. RNAi of the selected gene was achieved using the Ahringer library. For synchronized animals, egg laying was performed on RNAi plates containing 2 mM IPTG with either the pL4440 vector (empty vector - PL) as a control or the test RNAi constructs. They were allowed for 2 days to grow (L4 stage) and then transferred to new RNAi plates treated with small molecules or DMSO.

Growth and imaging of BAM10-treated *C. elegans*

L4 larvae were transferred on OP50-seeded NGM plates containing DMSO, paraquat (8 mM, Sigma-Aldrich) or **BAM10** (5 µM) and were grown for 16 hours (day one of adulthood - D1). Full plates images were obtained through a camera placed on the eyepiece of the stereoscope. Up to 25 animals per condition were transferred on coverslips and then imaged on either epifluorescence (used for calculating animal size and total fluorescence) or a confocal Airyscan microscope (for high-resolution imaging). For competition experiments, animals were grown on co-treated (**BAM10** and competitor) plates.

Microscopy

Epifluorescence microscopy was performed using Zeiss AxioImager Z2 epifluorescence microscope. A 5x lens objective (Air).

Super-resolution imaging was performed using a Zeiss LSM 900 with the Airyscan 2 Super-Resolution module. The pinhole for all experiments was set at 1 Airy unit. Images were acquired at 2048×2048 line resolution using a LD LCI PlanApochromat 40x/1.2 Multi immersion (water, silicon oil, glycerine) objective at room temperature. High-resolution representative images were acquired using a 1.4 NA Zeiss 63× oil objective with z-sections at 150% of the optimal lateral resolution through the thickness of the samples; maximum intensity projections are presented as representative images. Samples were excited with 488nm laser line. Laser power, gain settings, magnification, zoom, pixel size, were held constant across all samples. Images were AiryScan processed using the Zen Blue software. The brightness and contrast range for selected images shown in the figures were kept the same between conditions that were compared. Images were analysed in ImageJ/Fiji.[69] Specific analyses are described below.

Imaging analysis

Imaging analysis of all data was done using ImageJ/Fiji.[69] Plots were generated using Microsoft Excel or GraphPad Prism 5.0. Intensity distribution histograms were generated by extracting fluorescence intensity values of pixels contributing to cell labelling to create a frequency distribution for each condition. Images had been previously manually thresholded to extract values only from cells. Intensity plot profile values were extracted using an ImageJ built-in plugin (Plot profile) using a line across the cell. The mean intensity of manually thresholded images and the corresponding area were extracting using an ImageJ built-in plugin (Measure) to generate box-plots of the competition experiments.

9. Other Supplementary Figures

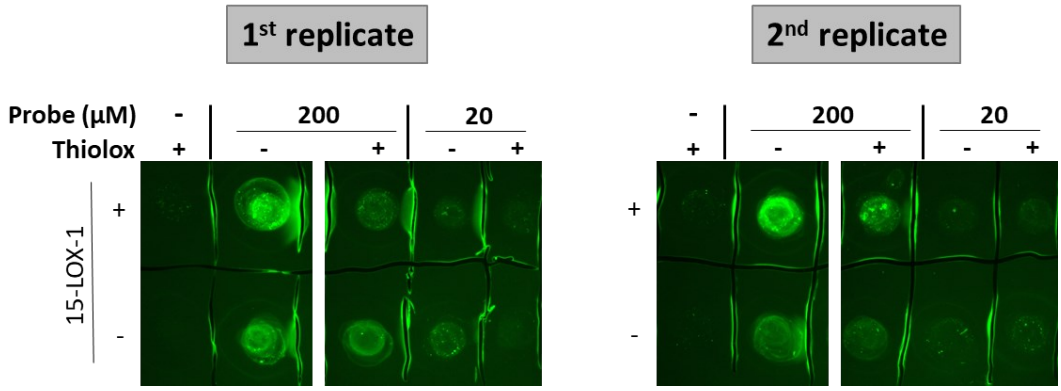


Fig. S6. Evaluate of **BAM10** covalent binding on purified 15-LOX-1. Dot blots of **BAM10**-treated samples. Fluorescence imaging of 15-LOX-1 after preincubation with DMSO or ThioLox (200 µM) followed by labeling with **BAM10** (200/20 µM) for 30min and acetone precipitation protocol. Control experiments without the 15-LOX-1 we also performed. The entire volume of the resuspended protein sample was spotted in a membrane and images were recorded on a fluorescence stereoscope. Dot blots of the two replicates are shown.

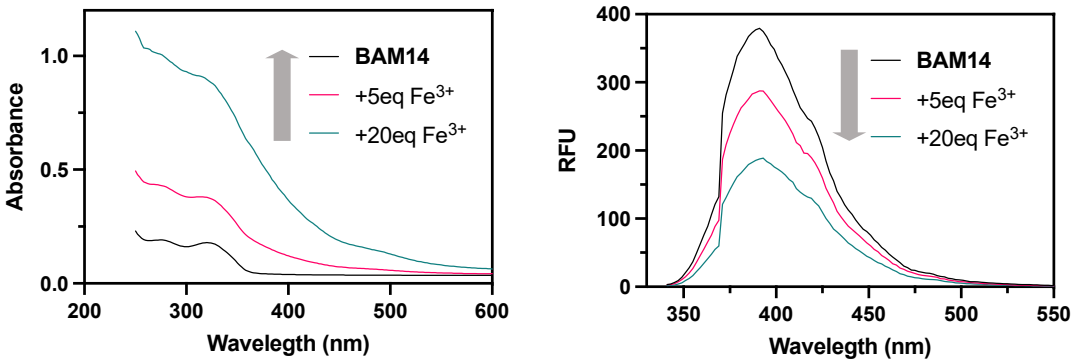


Fig. S7. The absorbance and the fluorescence spectra of **BAM14** in the presence of an accumulated amount of iron.

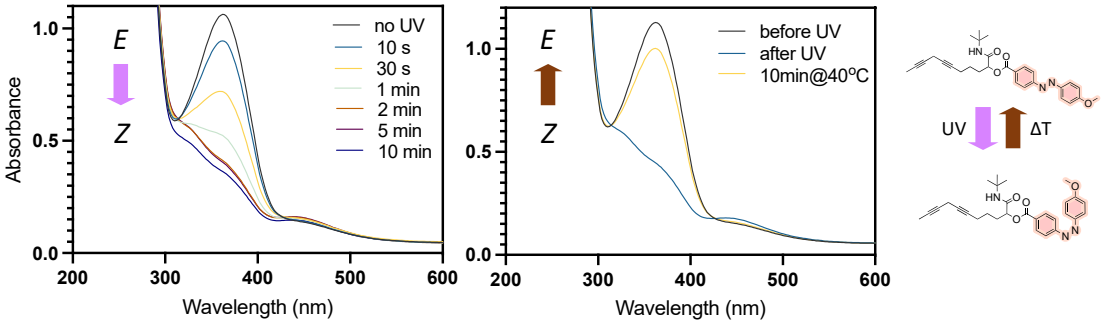


Fig. S8. Absorption spectra of BAM12 show the conversion from the E to Z conformation upon increasing time exposure on UV light, and returned to the E conformation after heat treatment.

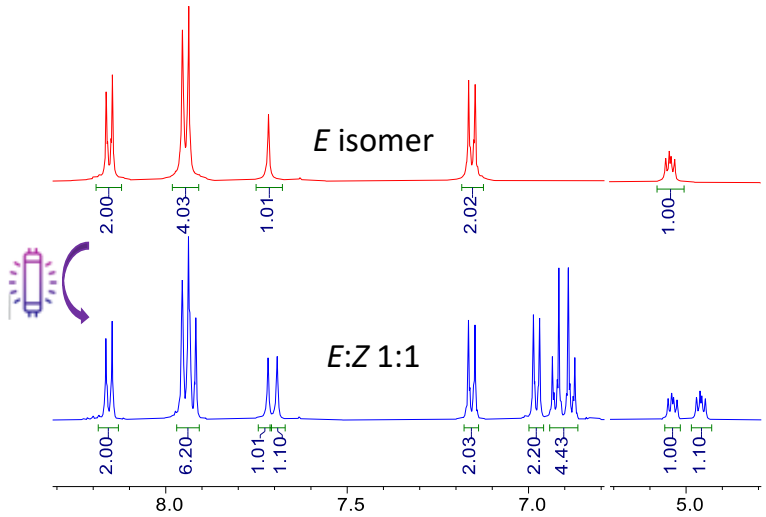


Fig. S9. ^{1}H NMR spectra of BAM12 as E isomer and in ratio of 1:1 E:Z isomers after UV irradiation

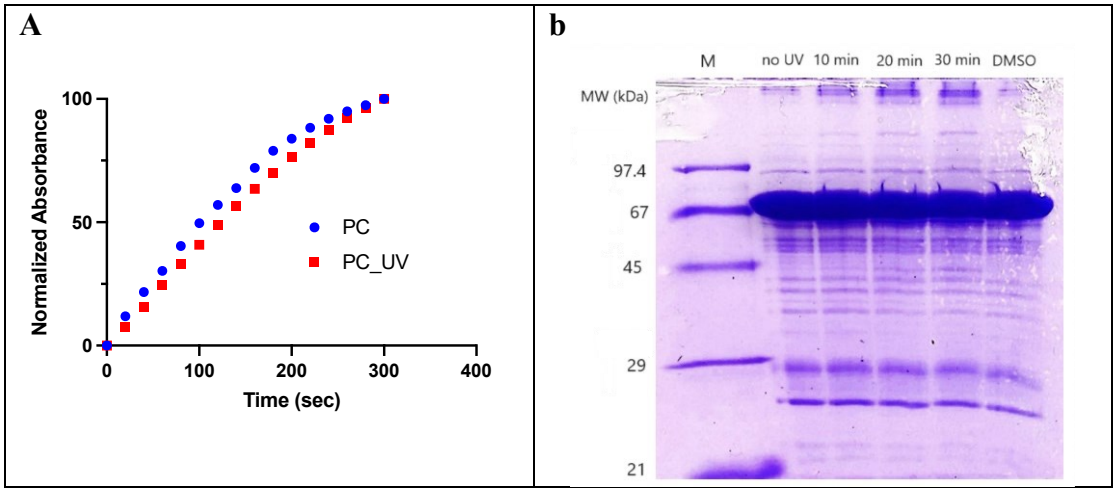


Fig. S10. **a**, Enzyme activity of human 15-LOX-1 (PC), without and upon UV irradiation for 10 min. Normalized absorbance (234nm) has calculated over time for better comparison. **b**, SDS-page reveals increasing crosslinked protein species, after incubation of **BAM13** with cell lysate (with overexpressed 15-LOX-1) and application of UV irradiation for 0, 10, 20 and 30 min.

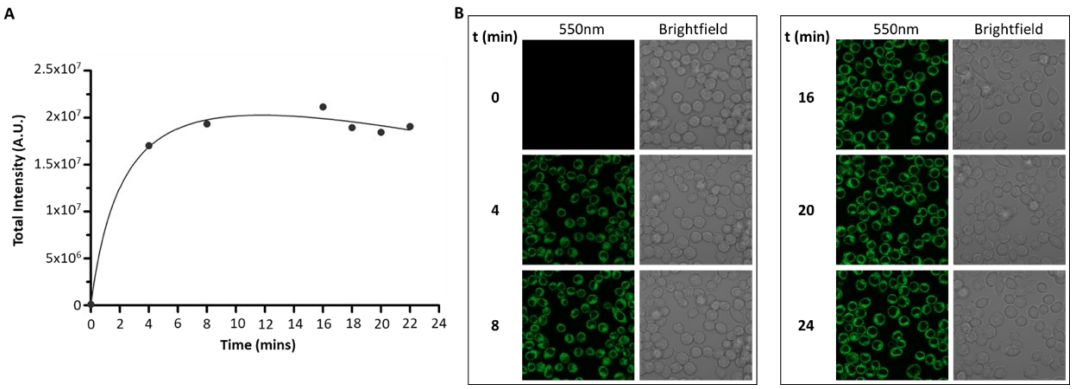


Fig. S11. Evaluate of **BAM10** cell permeability in live RAW264.7 cells. (A) Plot of the quantified cell intensity of images in (B). Quantification indicates that **BAM10** is taken up rapidly by the cells and reaches saturation within 16 mins. (B) Example images of the imaging-based assay used to evaluate cell permeability of **BAM10** probe in cells. Images were recorded prior and after the *in situ* addition of **BAM10** in different time points, as indicated. Fluorescence images are shown in the left and brightfield images on the left.

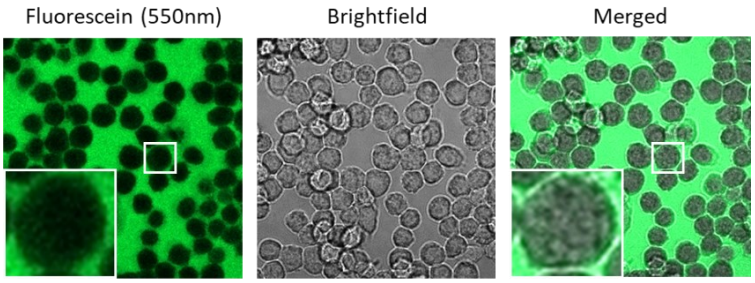


Fig. S12. Free fluorescein used as a control in the imaging-based cell permeability assay. Images of RAW 264.7 live cells treated with free fluorescein. The compound was unable to cross plasma membrane in the time course of our assay.

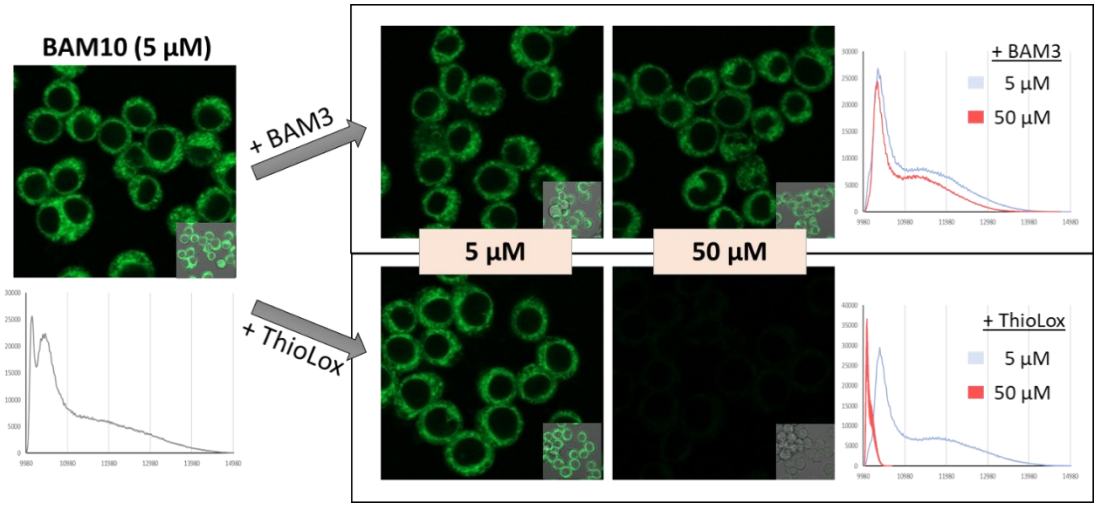


Fig. S13. **BAM10** intensity distribution in the cell-based competition assay. Examples of images of live cells pretreated with either DMSO (left image) or competitor (right box) and subsequently labelled with **BAM10**. **BAM3** (top row) and ThioLox (bottom row) were used as competitor in either equimolar (middle) or 10-times (right) concentration. Histograms show how the intensity distribution of **BAM10** changes when cells had been pre-treated with either competitor. The scale on both x and y axis of the histograms was kept the same in all conditions. The insets in the images show the merged channels. Experiments were performed in triplicates.

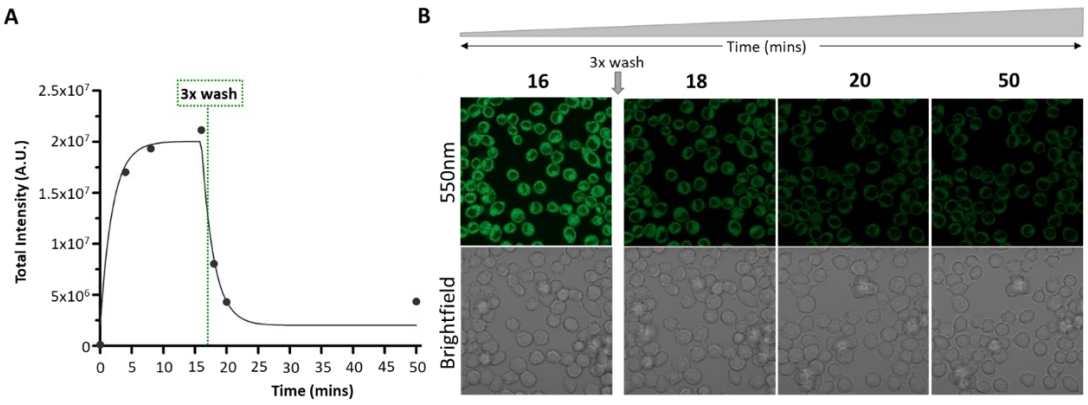


Fig. S14. Assessment of **BAM10** retention in RAW 264.7 cells. (A) Plot showing the total **BAM10** intensity in cells following washes. Four minutes after washes, the **BAM10** intensity is roughly decreased by a factor of 4, but remains constant for 30 minutes, indicative of covalent binding to protein target. (B) Example images of the imaging-based assay used to evaluate cell permeability of **BAM10** probe in cells. Images were recorded prior and after the *in situ* addition of **BAM10** in different time points, as indicated. Fluorescence images are shown on the top row and brightfield on the bottom row.

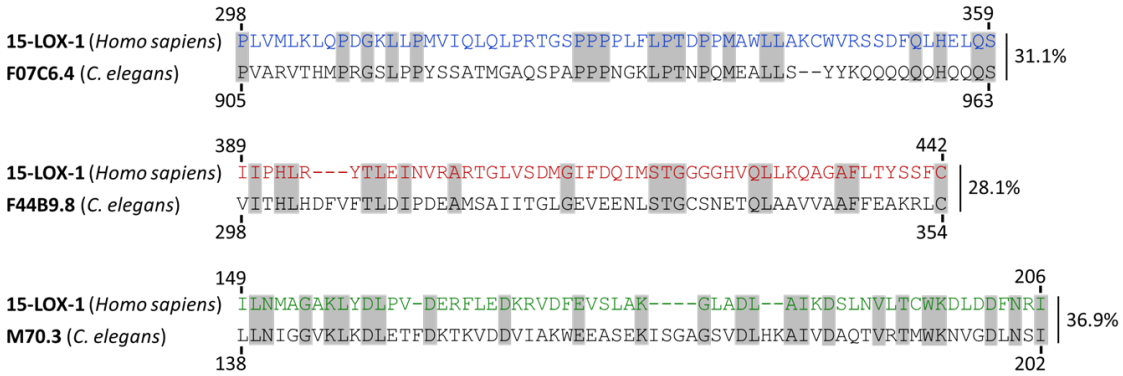


Fig. S15. Sequence similarity of the human 15-LOX-1 and *C. elegans* gene hits identified by BLAST. Protein sequence alignment homo sapiens 15-LOX-1 and the identified *C. elegans* gene (F07C6.4, F44B9.8 and M70.3). The percentage of sequence identity for each gene is shown on the right. M70.3 exhibits the highest similarity with 15-LOX-1. The coloured 15-LOX-1 sequences correspond to the coloured structure in Fig. 5e.

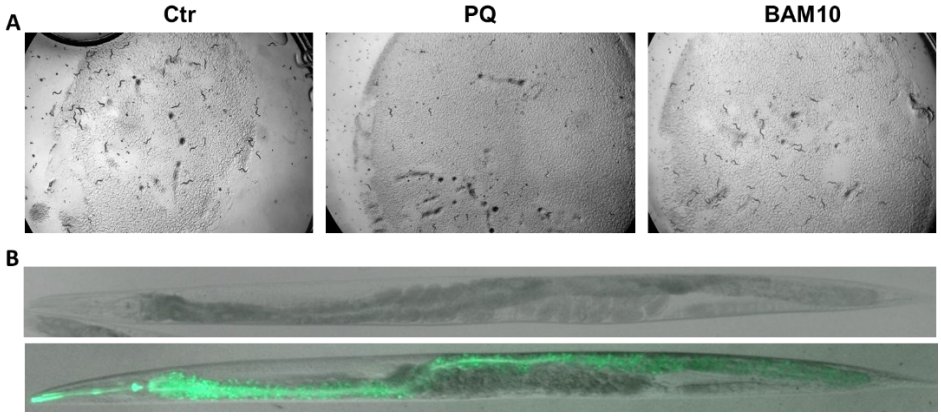
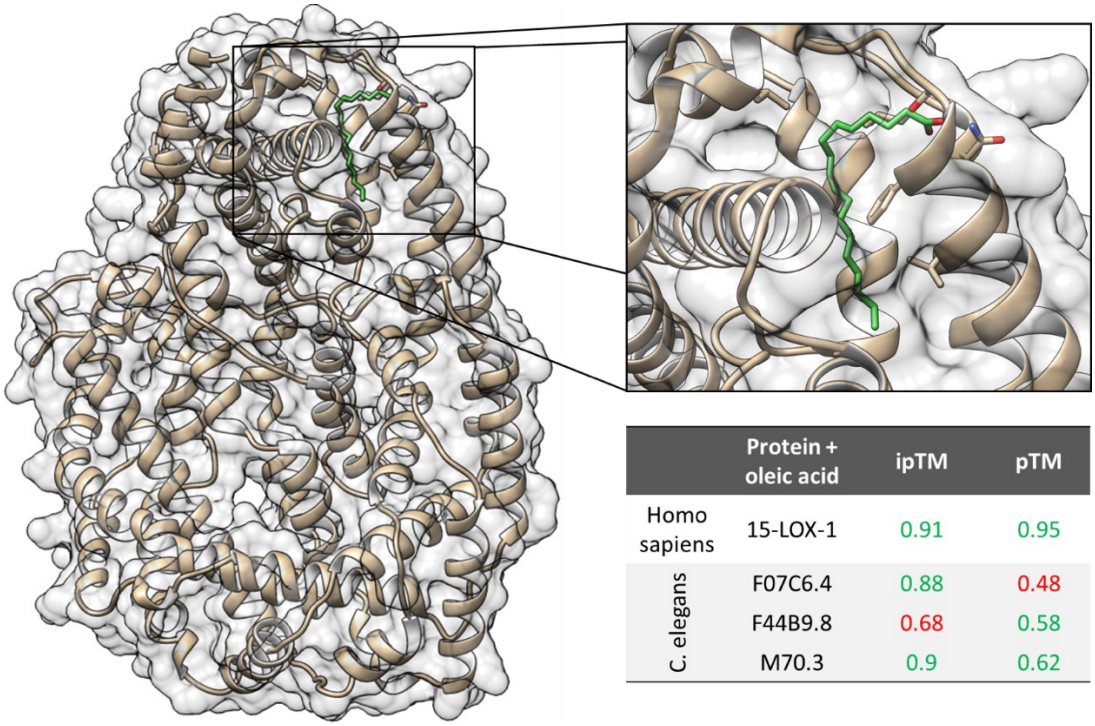


Fig. S16. Evaluation of the effect of **BAM10** in *C. elegans* development and its uptake. (A) Stereoscope images of *C. elegans* on NGM plates treated with DMSO (Ctr), paraquat (PQ) or **BAM10**. L4 larvae (N2 strain) were transferred onto pre-treated plates and cultured for 16 h, where they reached early adulthood (D1 animals). *C. elegans* grown on **BAM10** reached similar sizes and showed normal egg development in their gonads (see (B)), comparable to control samples. Additionally, unlike the negative control (PQ-treated plates), **BAM10** does not appear to repel animals from the food source. (B) Example epifluorescence fluorescence images of whole worms. 16-hour incubation of animals with **BAM10** leads to significantly higher fluorescence intensity on the pharynx and the intestine compared to the normal autofluorescence that *C. elegans* exhibit.

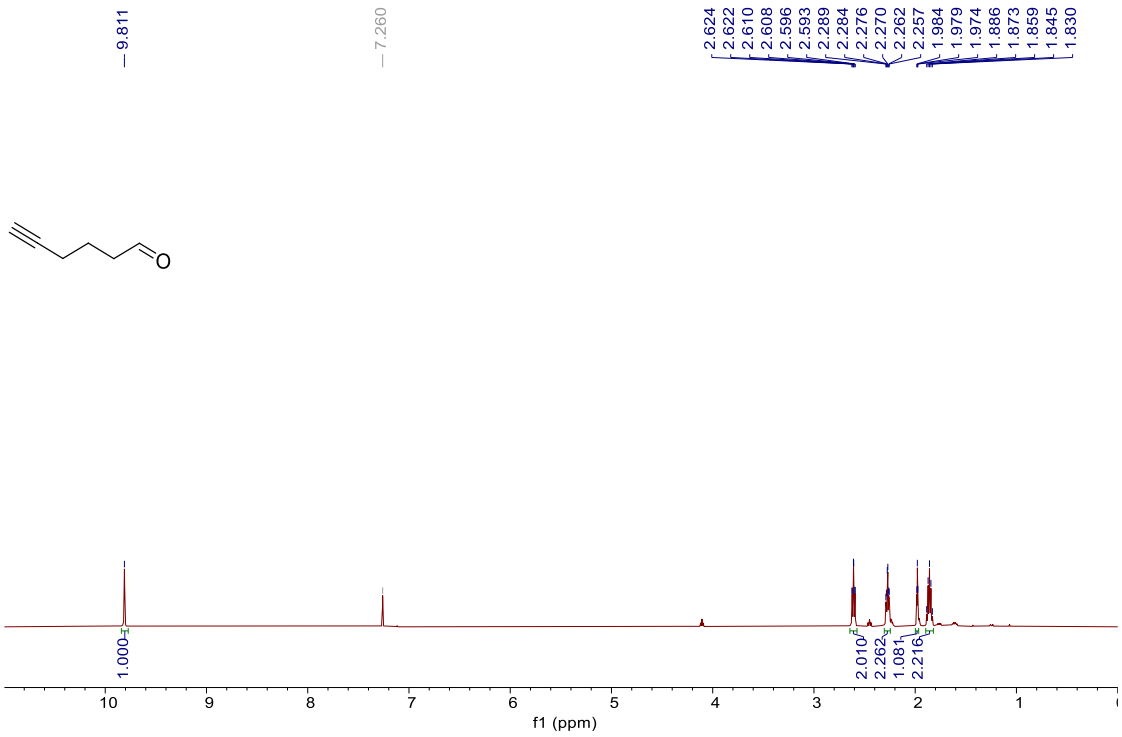


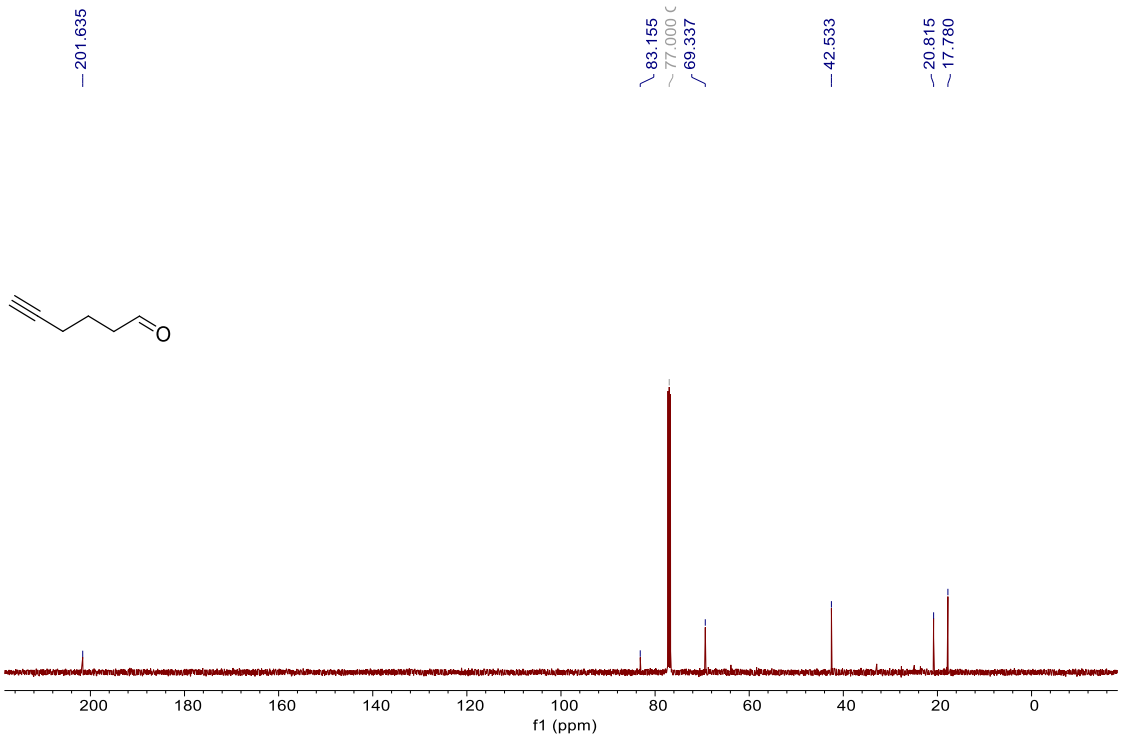
	Protein + oleic acid	ipTM	pTM
Homo sapiens	15-LOX-1	0.91	0.95
C. elegans	F07C6.4	0.88	0.48
	F44B9.8	0.68	0.58
	M70.3	0.9	0.62

Fig. S17. **Structure and accuracy of the predicted complex of M70.3 with oleic acid**. AlphaFold3 server was used to predict the potential interaction of the only available unsaturated lipid molecule with the *C. elegans* genes identified by BLAST. The overall predicted structure of M70.3 with oleic acid is shown on the left. Protein is shown on transparent gray surface with tin ribbon. Oleic acid is shown in green stick. Zoom-in bound-M70.3 is show on the right. The table show the predicted template modeling (pTM) and the interface predicted template modeling (ipTM) scores for complexes of oleic acid with 15-LOX-1, F07C6.4, F44B9.8 and M70.3. pTM scores above 0.5 and ipTM scores above 0.8 indicate high confidence in the overall structure and interface accuracy of the predicted models, respectively. 15-LOX-1 exhibits the highest values, as expected. From the three predicted complexes, only M70.3 gives values that meet both criteria for a high-confidence overall structure.

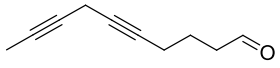
10. Exemplary copies of NMR spectra of novel compounds

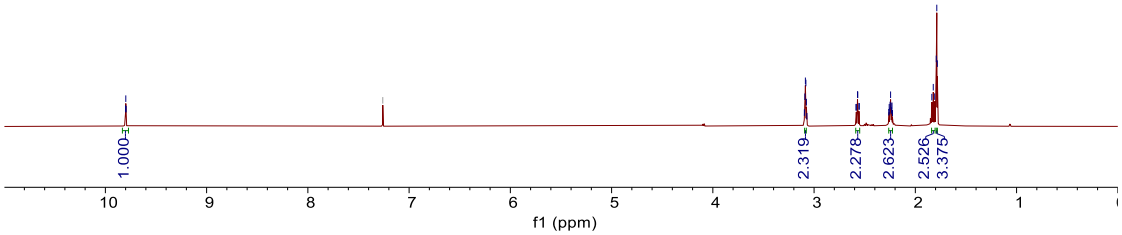
Hex-5-ynal (C1)

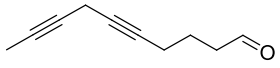


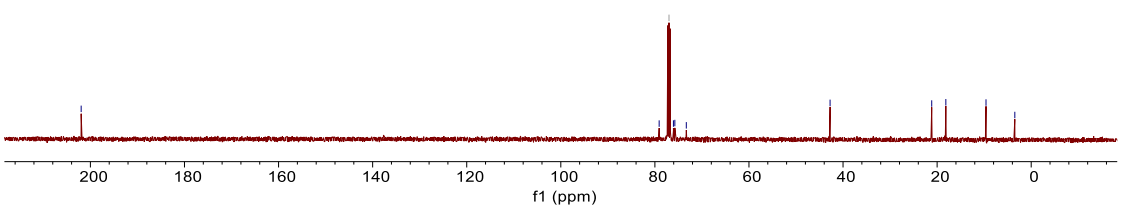


Deca-5,8-diynal (BAM1)

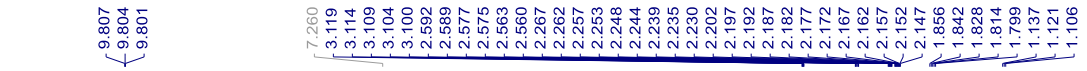


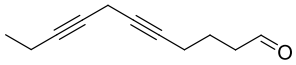


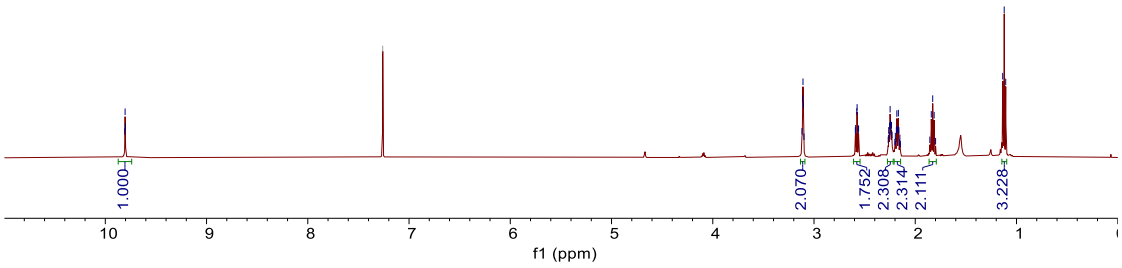


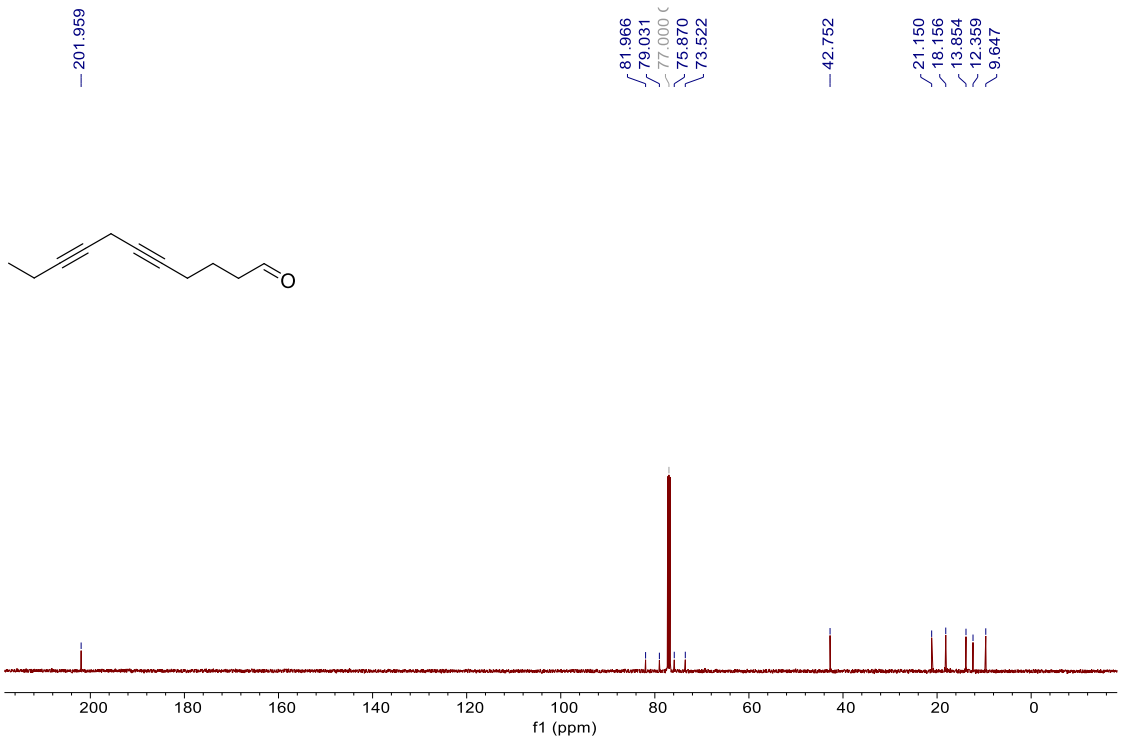


Undeca-5,8-diynal (BAM1')

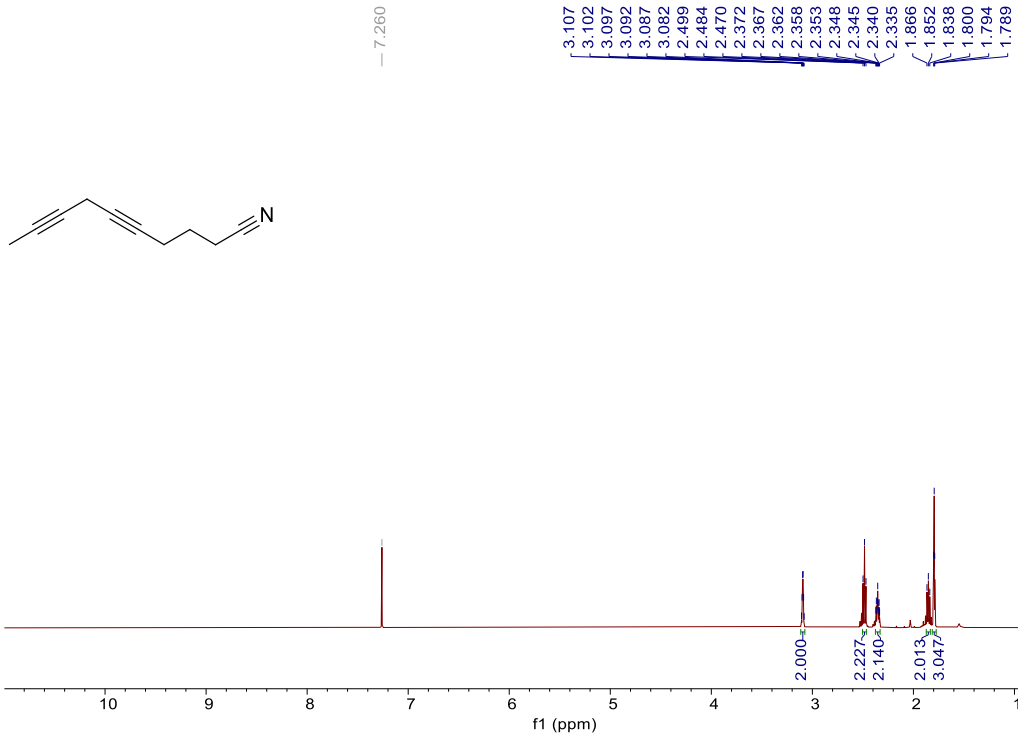


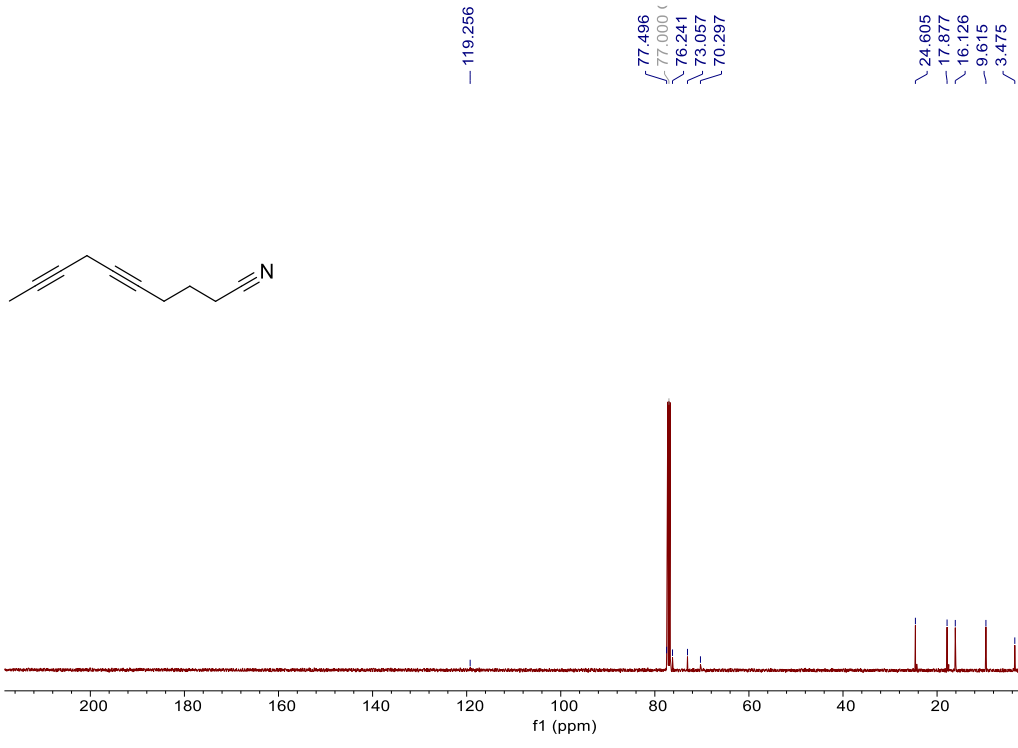




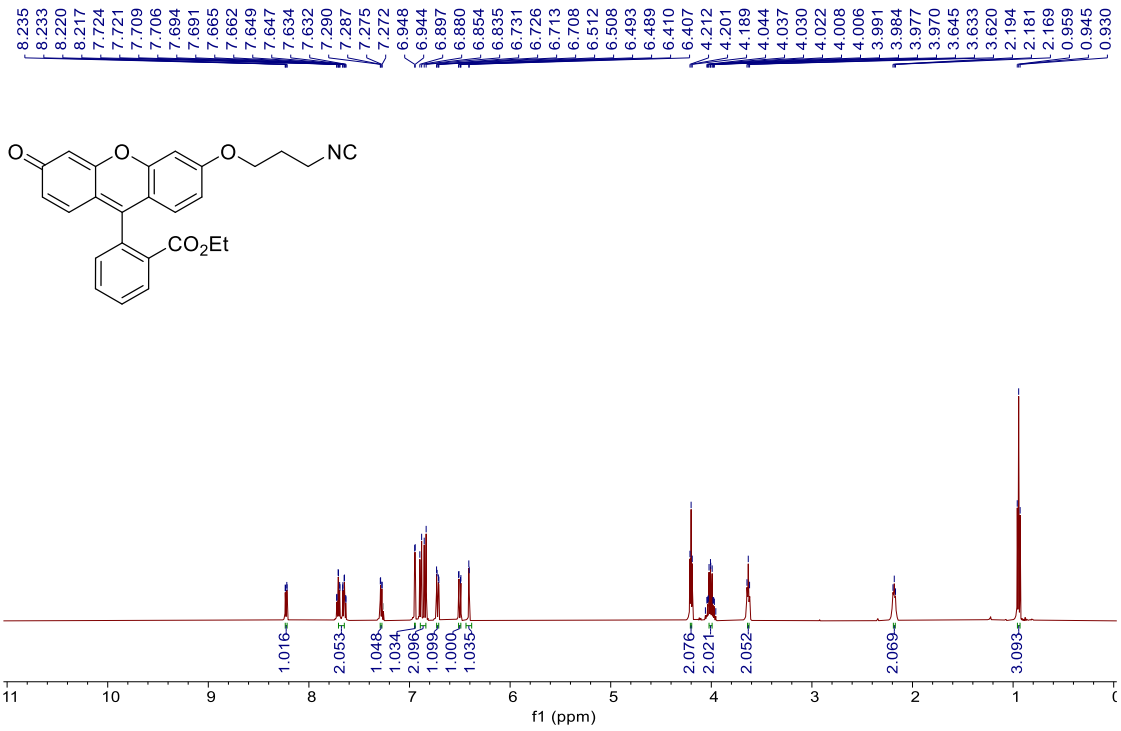


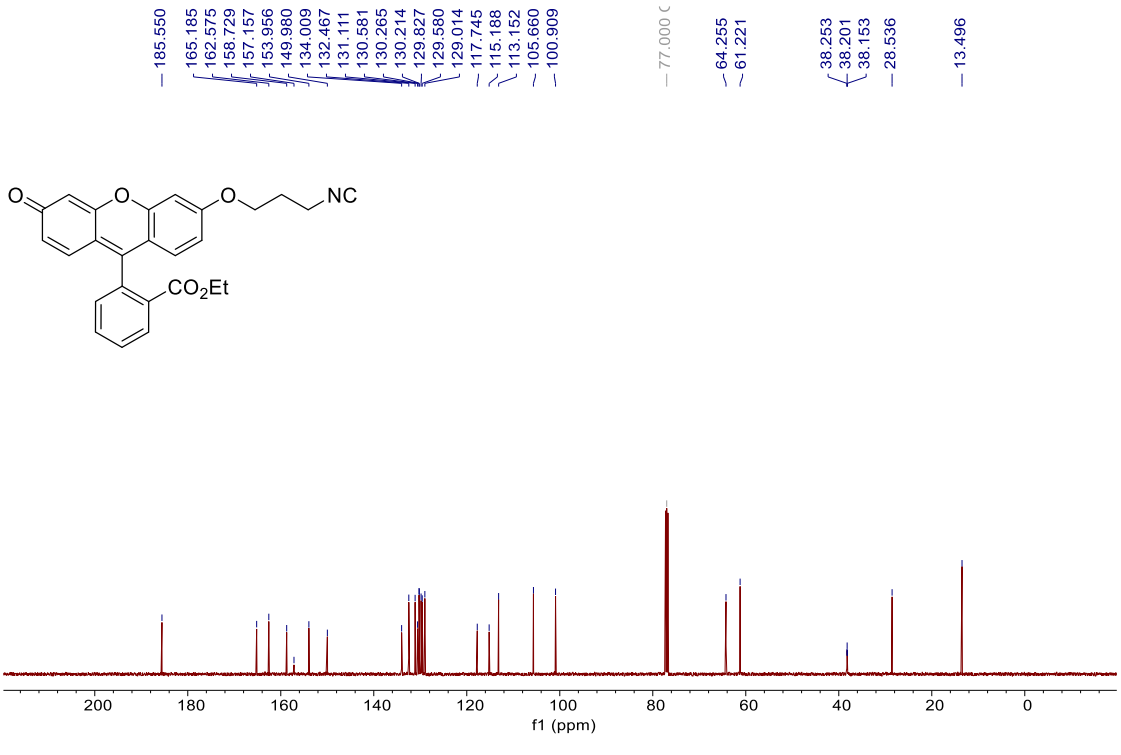
Deca-5,8-diynenitrile (C7)



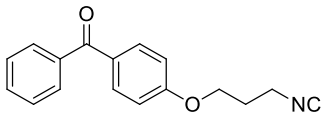


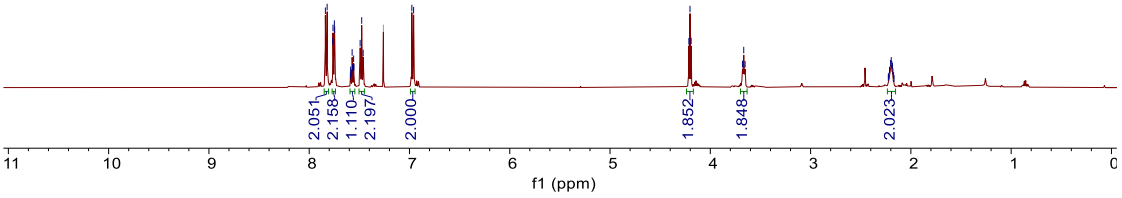
Ethyl 2-(6-(3-isocyanopropoxy)-3-oxo-3H-xanthen-9-yl)benzoate (3)

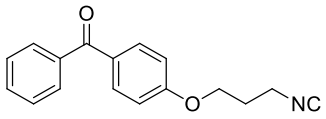


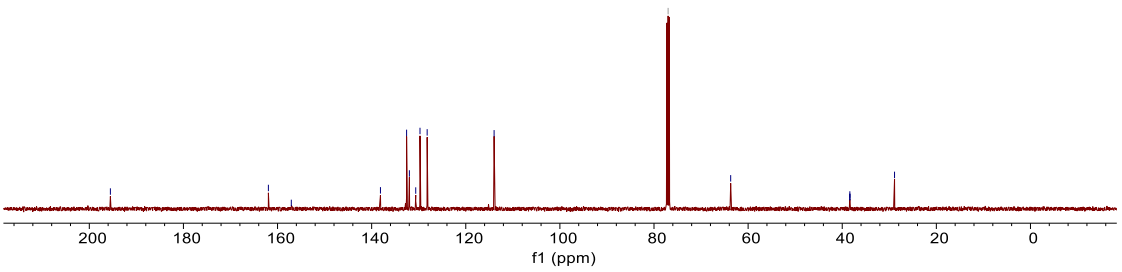


(4-(3-isocyanopropoxy)phenyl)(phenyl)methanone (4)

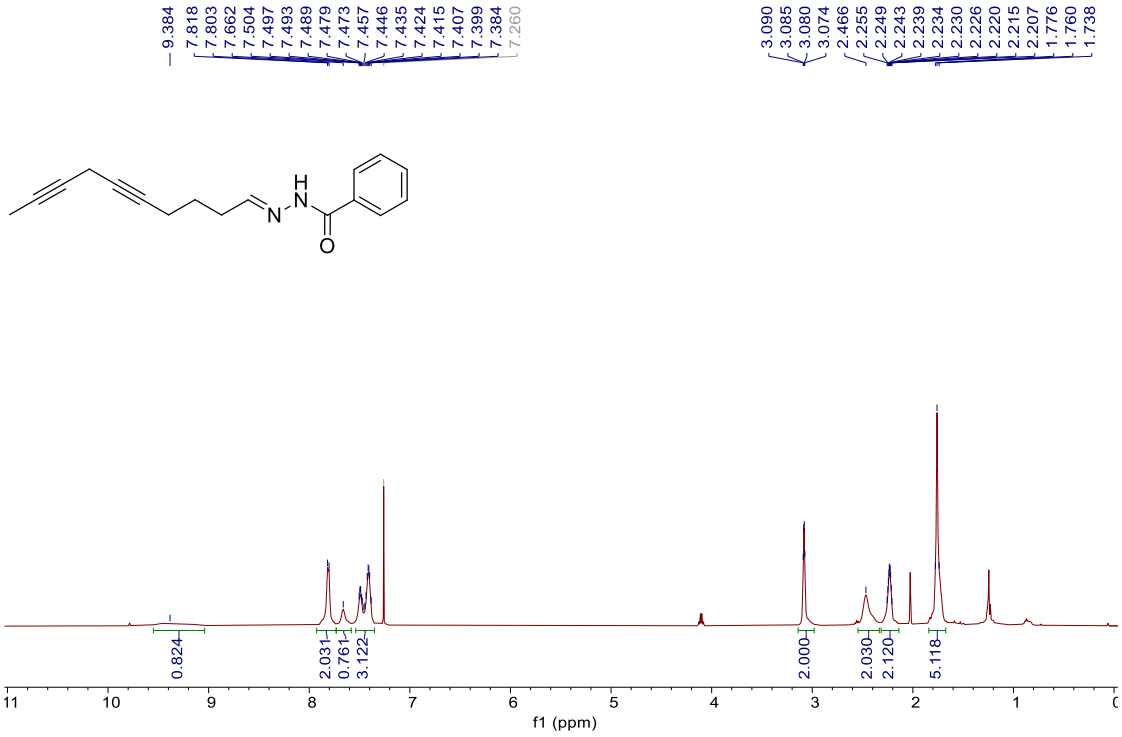


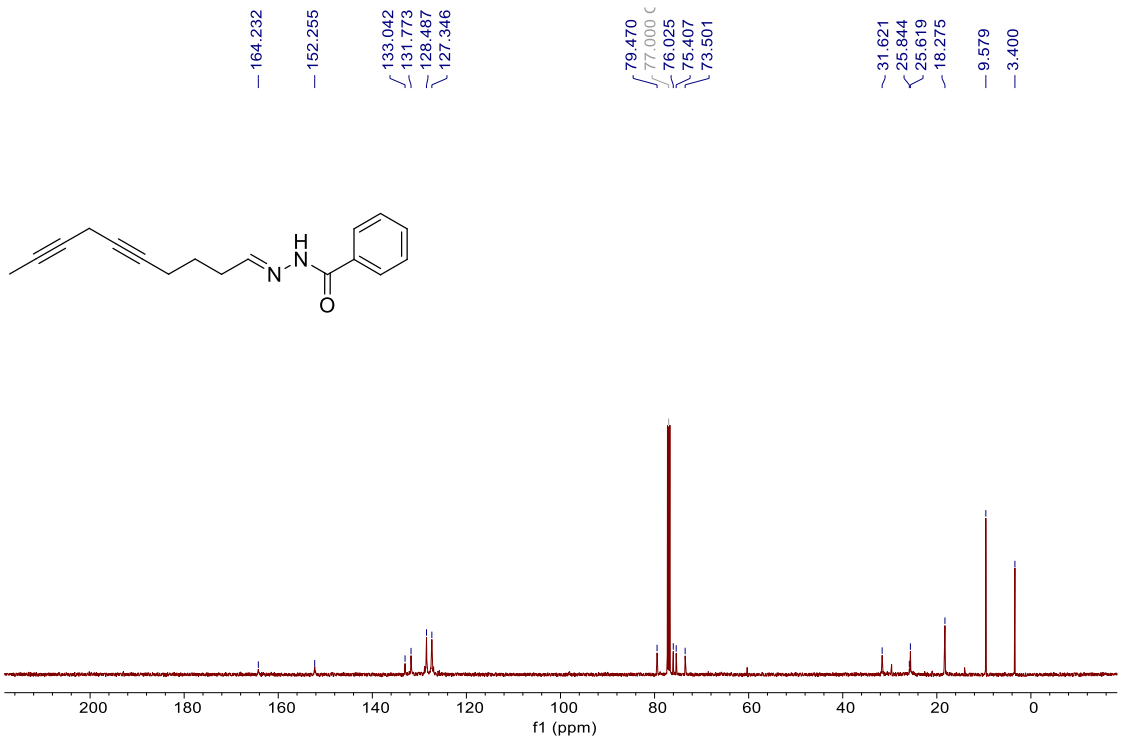




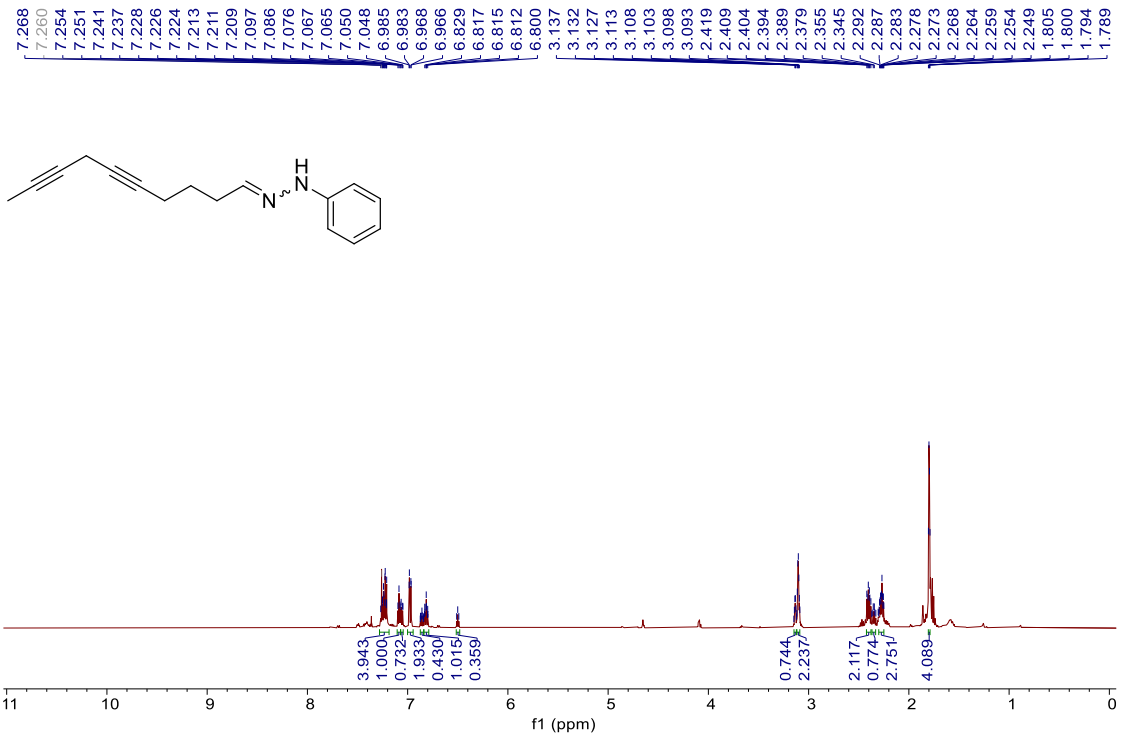


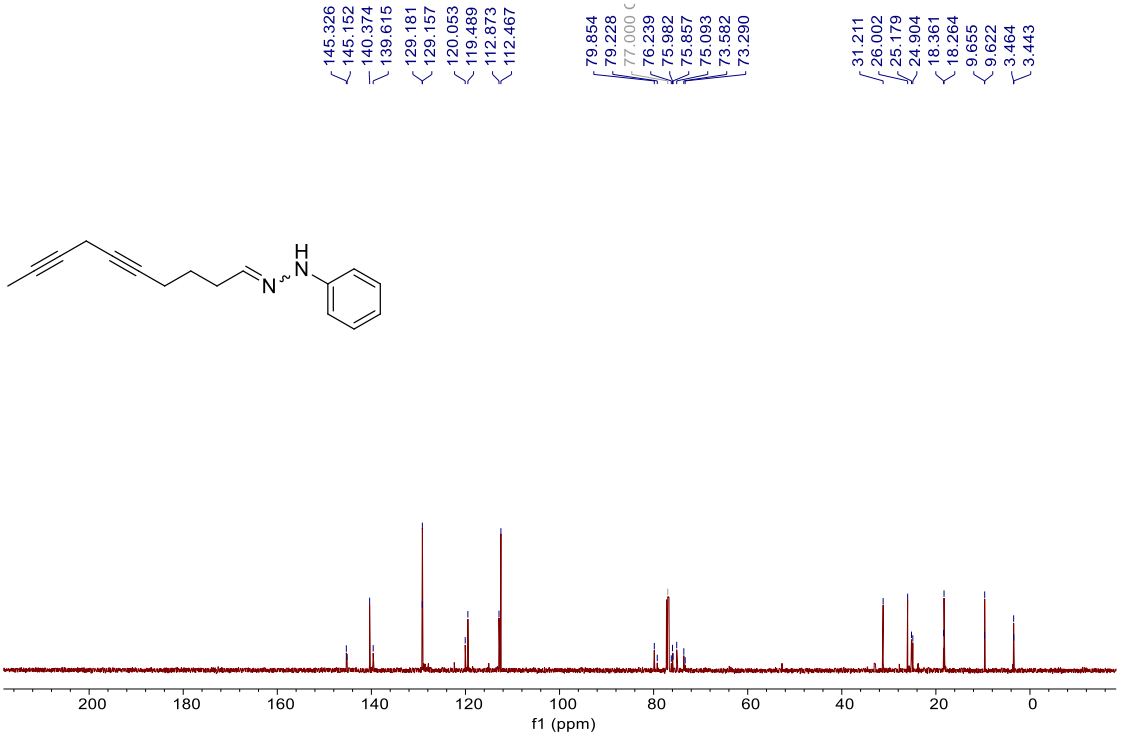
N′-(deca-5,8-diyn-1-ylidene)benzohydrazide (BAM2)



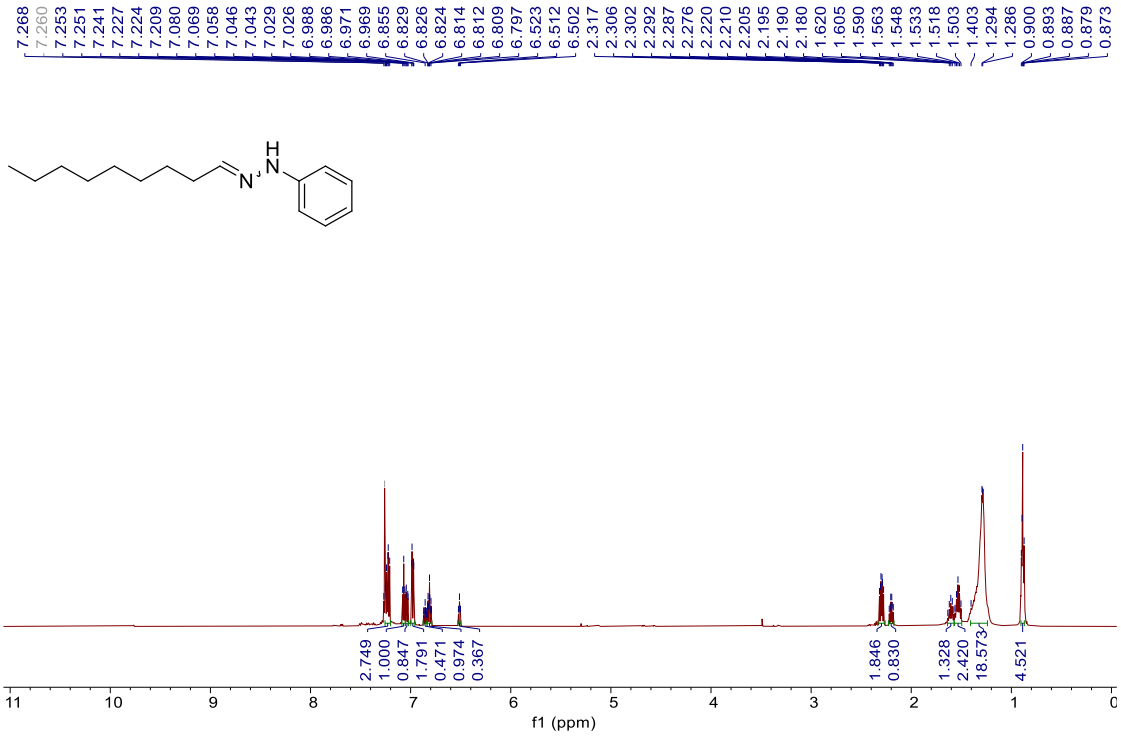


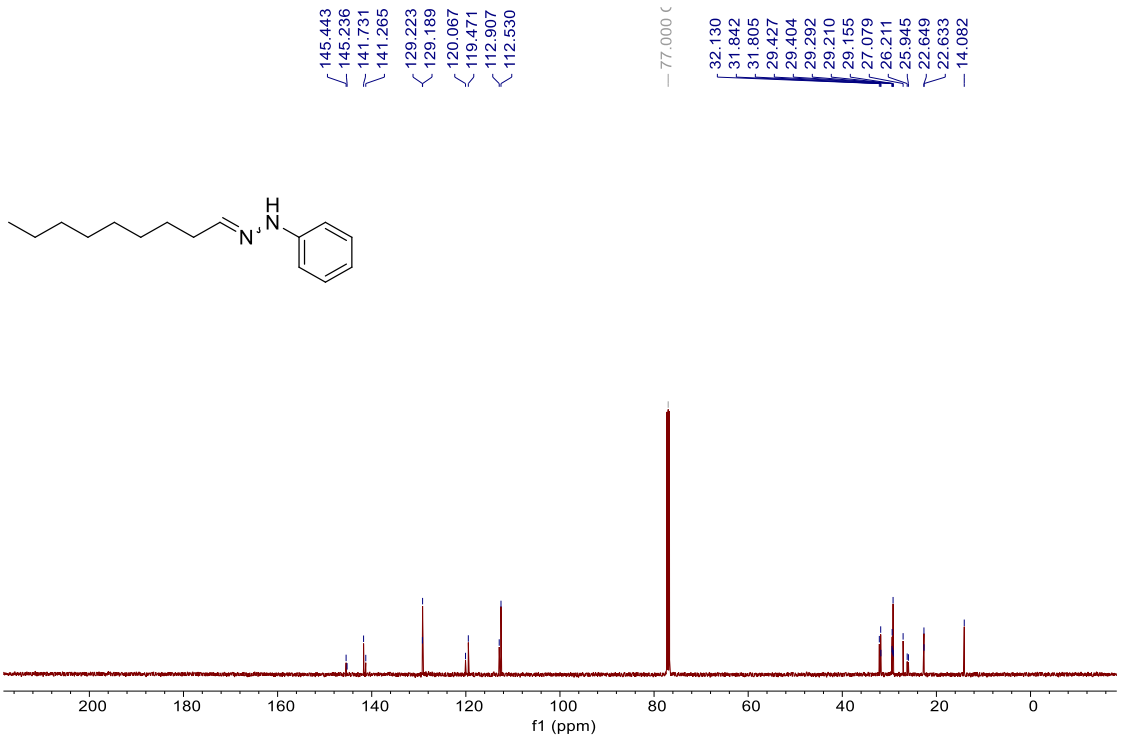
1-(deca-5,8-diyn-1-ylidene)-2-phenylhydrazine (BAM3)



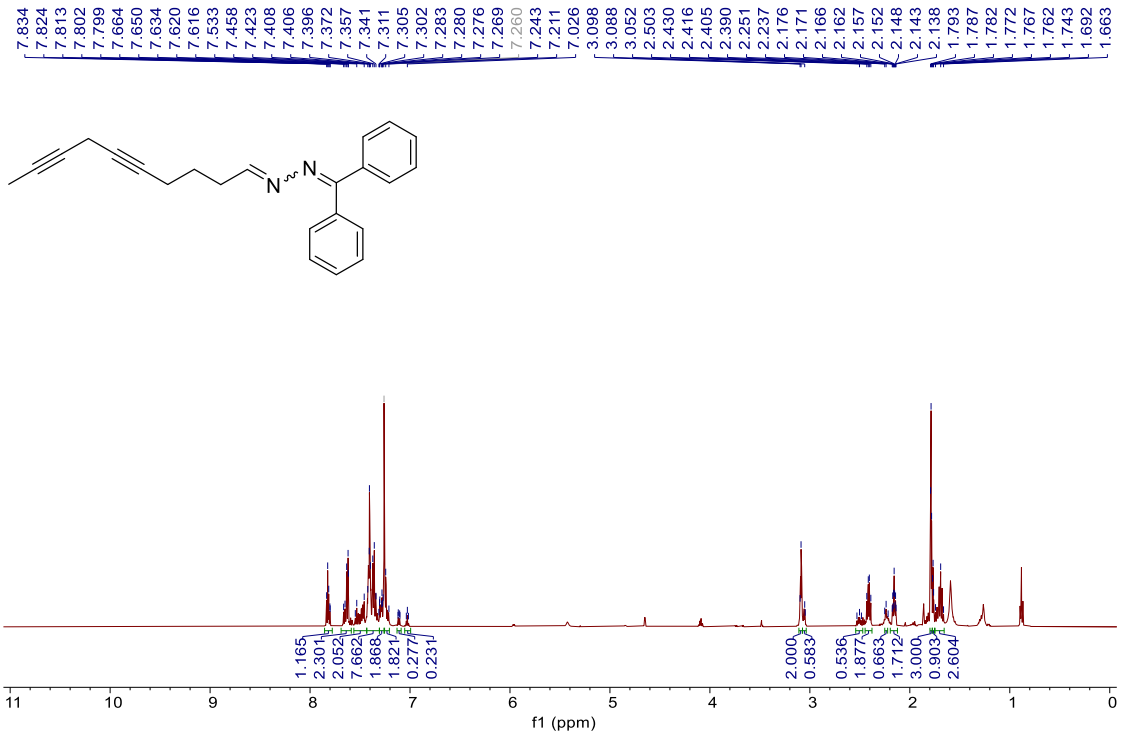


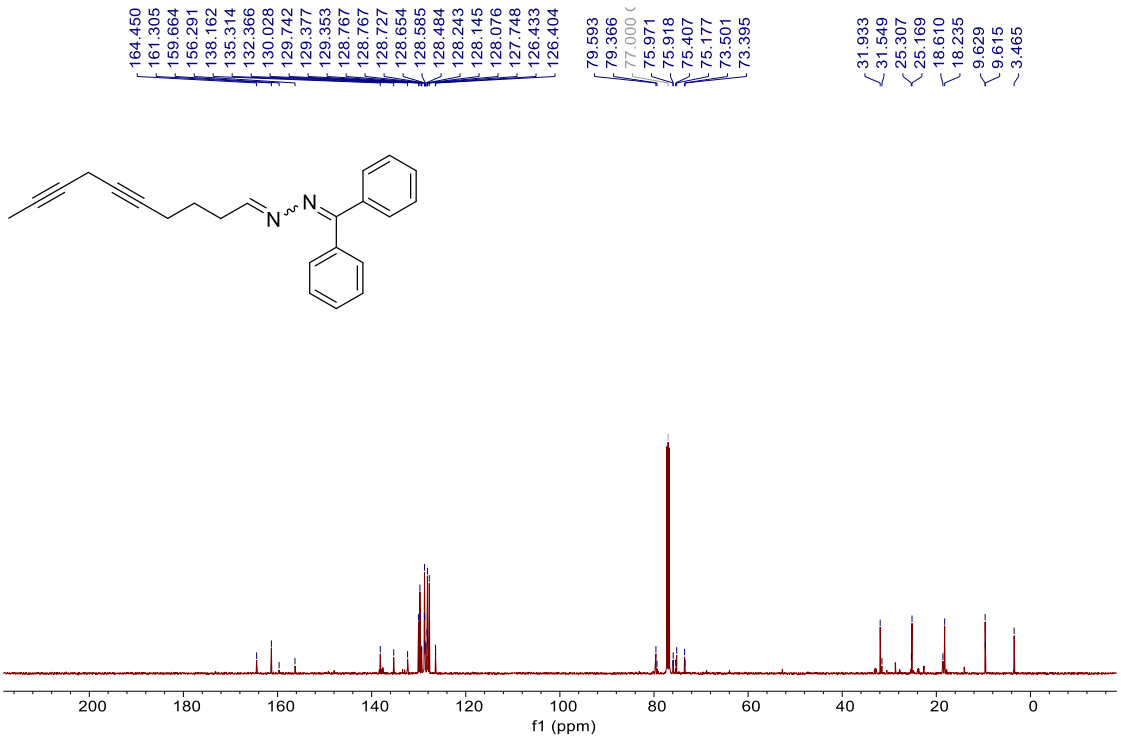
1-nonylidene-2-phenylhydrazine (C6)



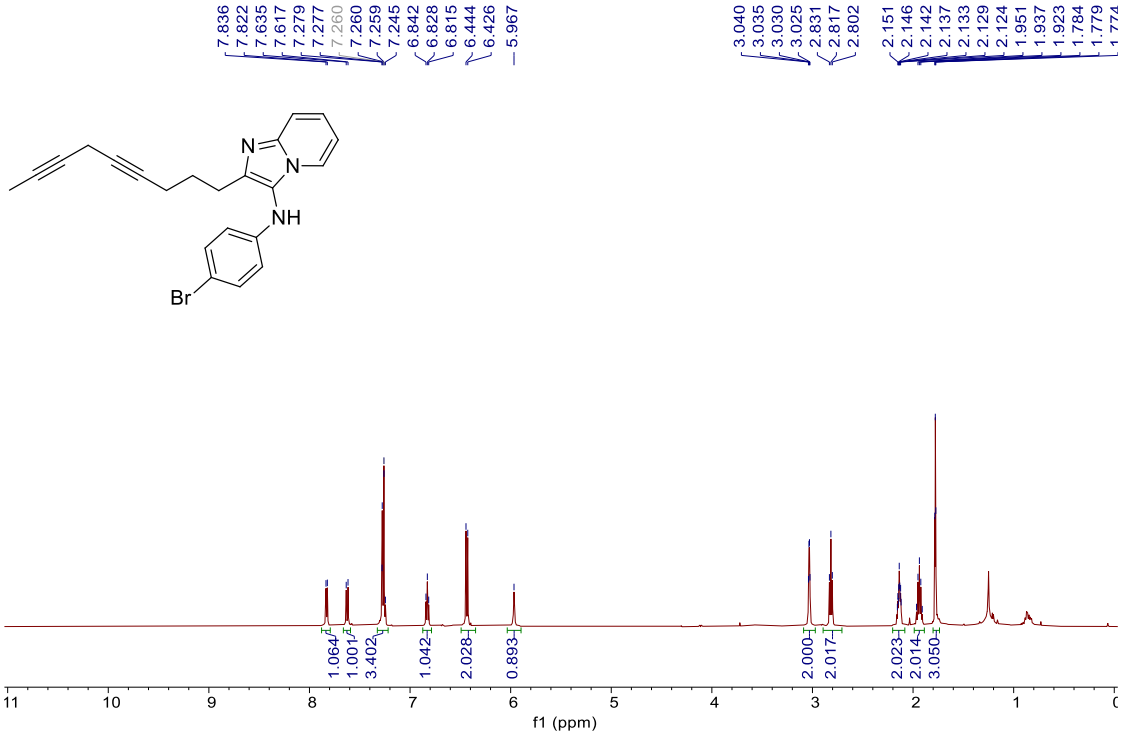


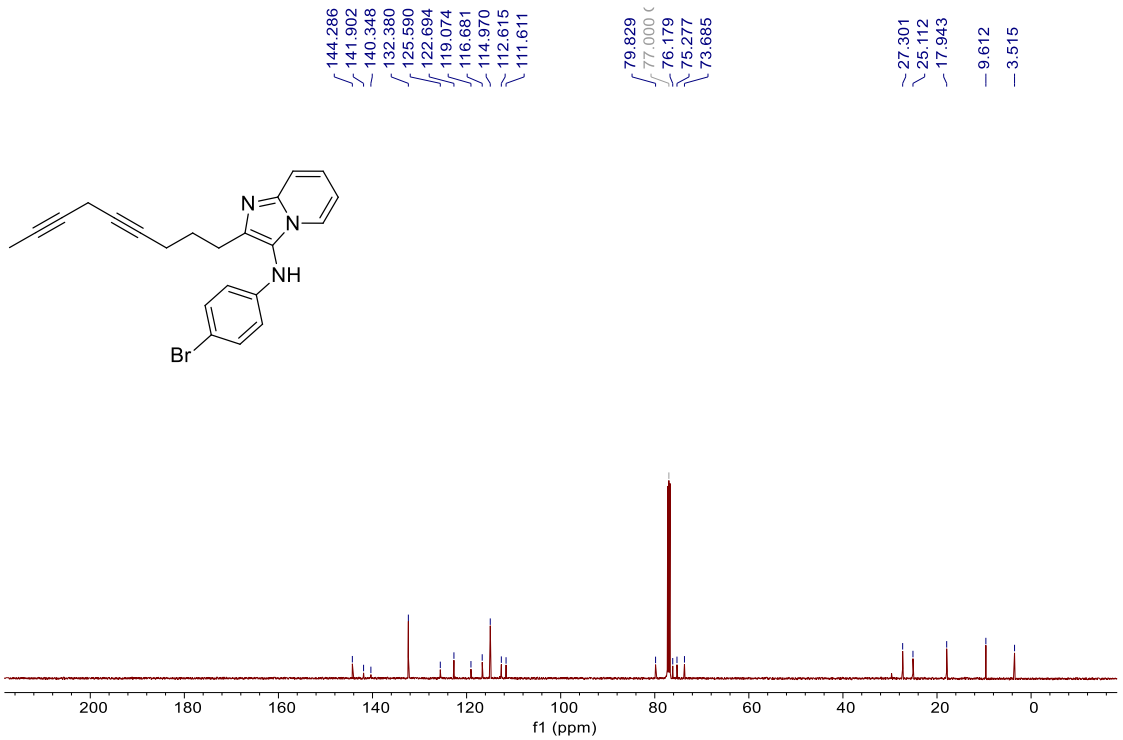
1-(deca-5,8-diyn-1-ylidene)-2-(diphenylmethylene)hydrazine (BAM4)



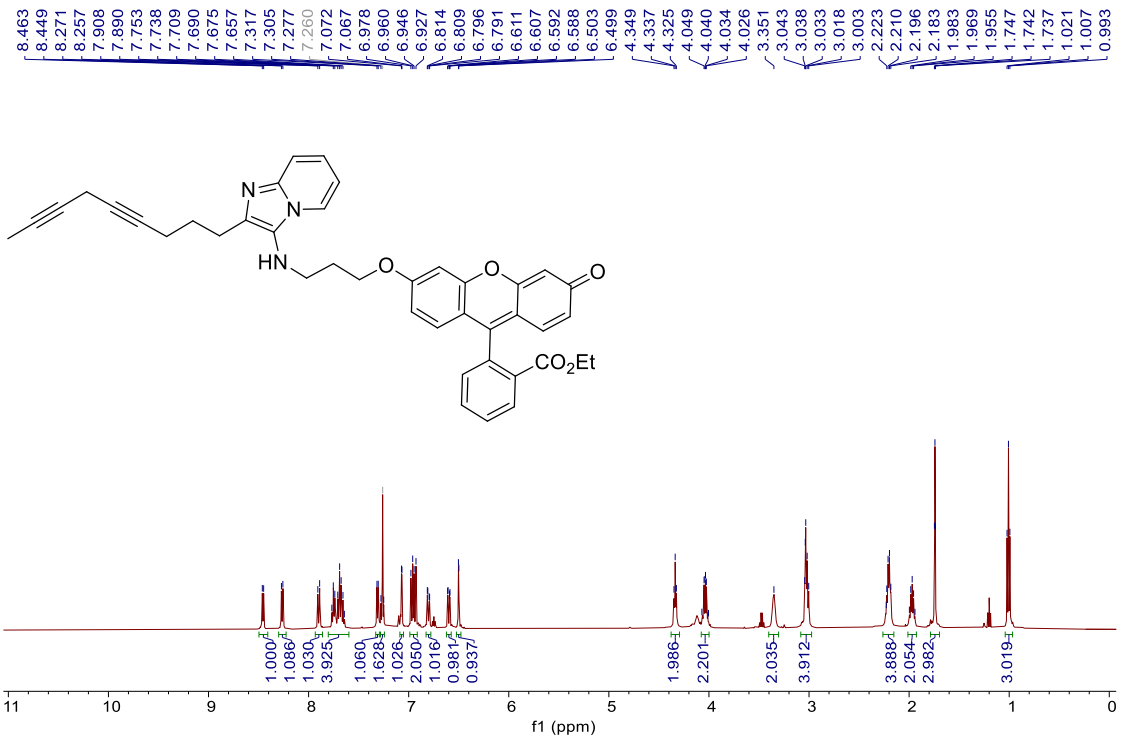


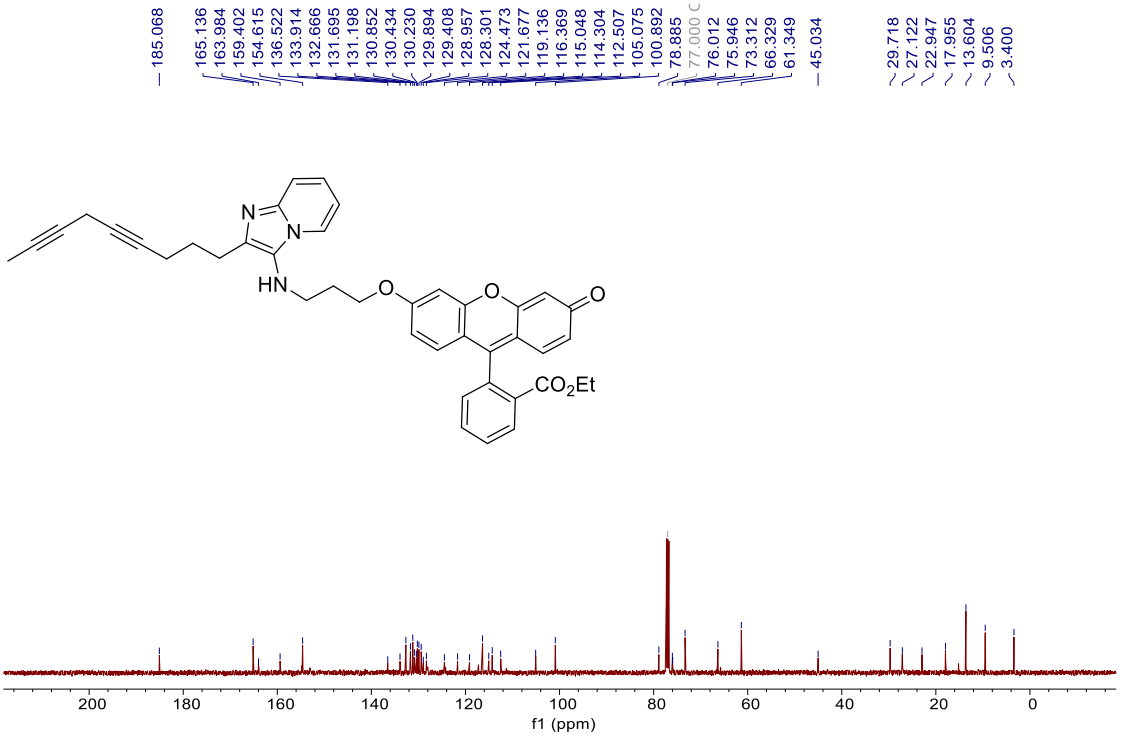
N-(4-bromophenyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM5)



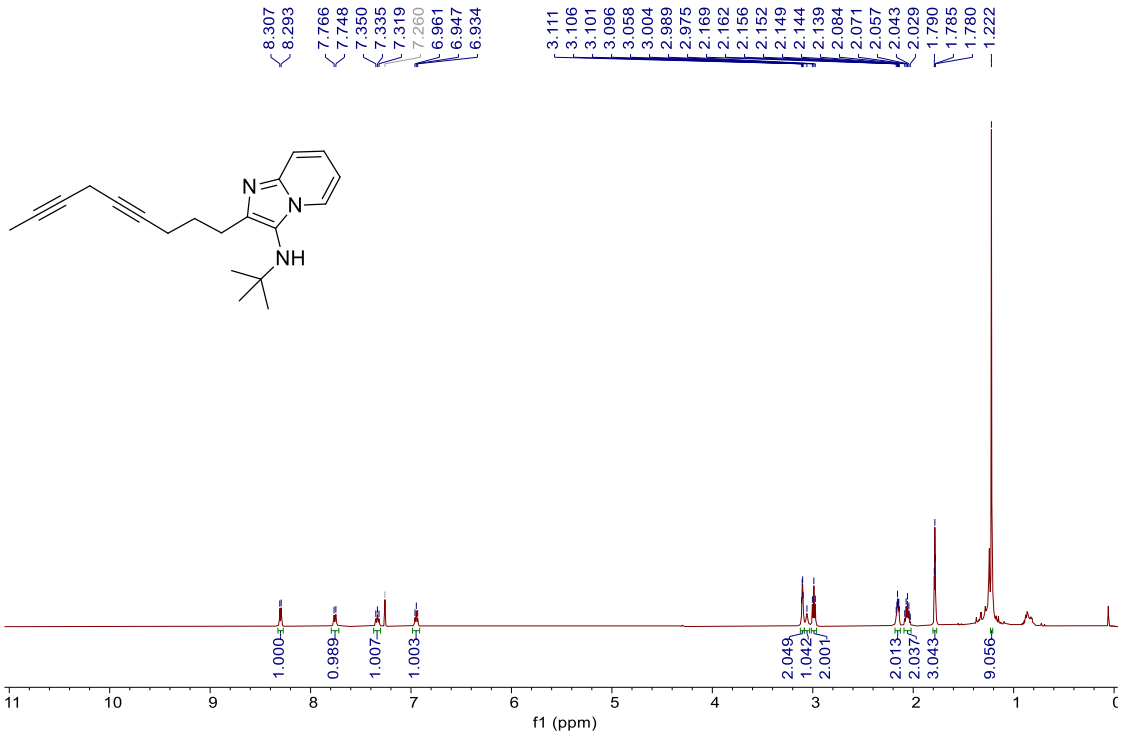


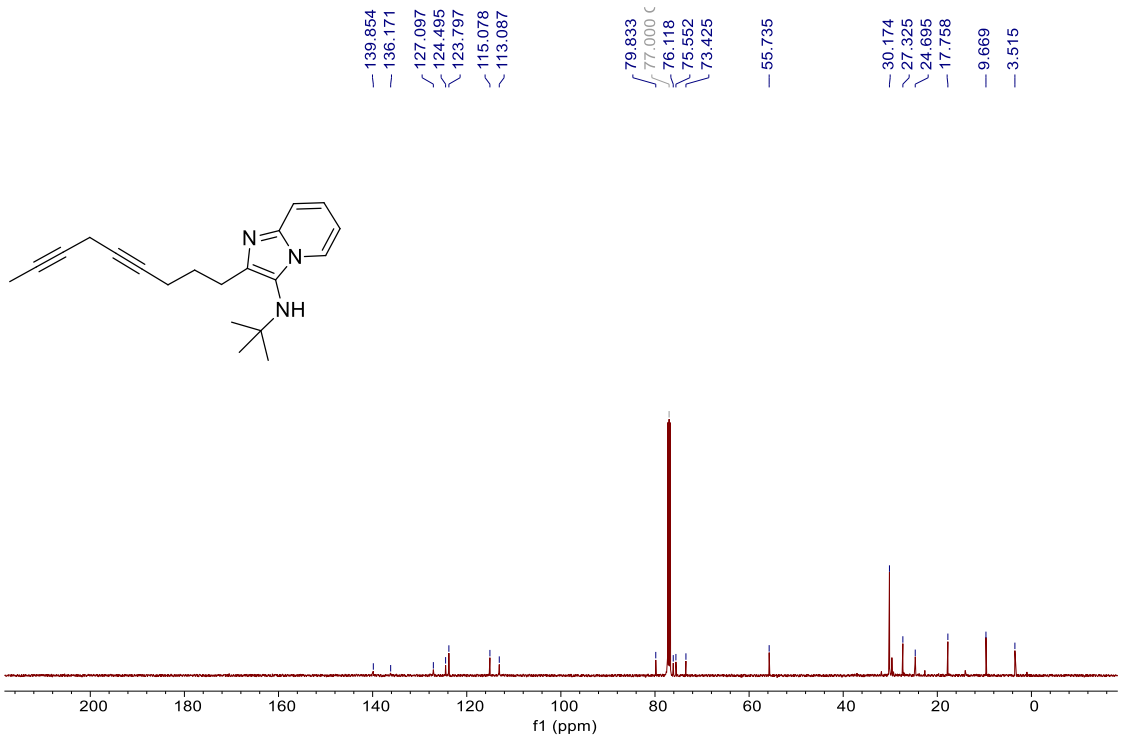
Ethyl 2-(6-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3H-xanthen-9-yl)benzoate (BAM6)



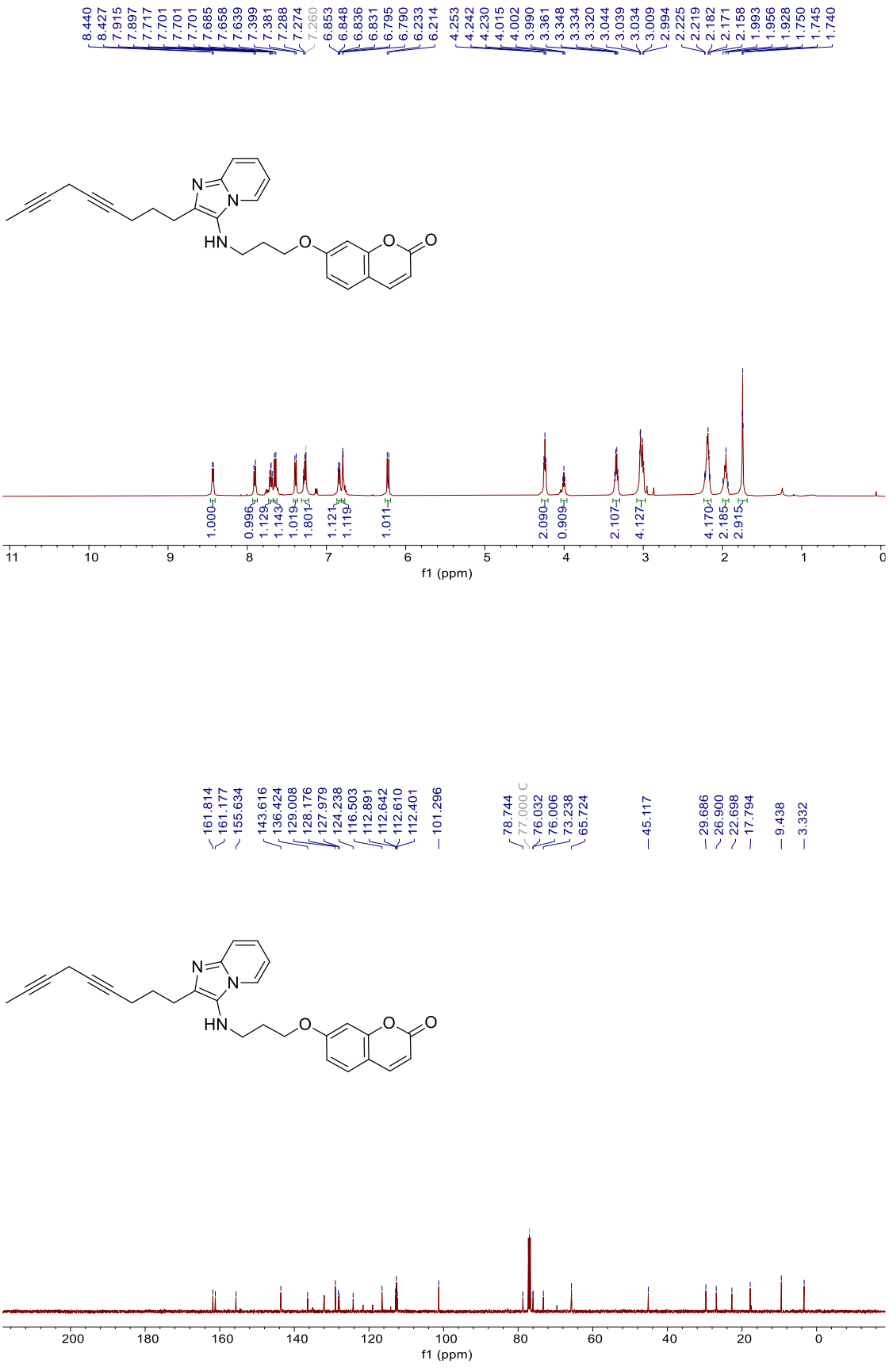


_N_-(tert-butyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM7)

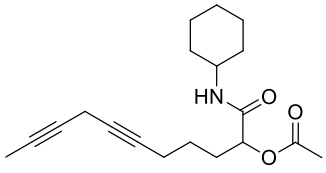


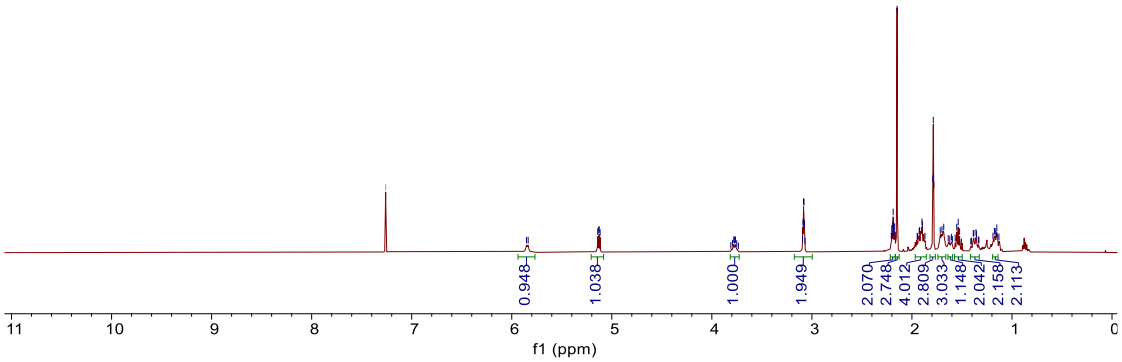


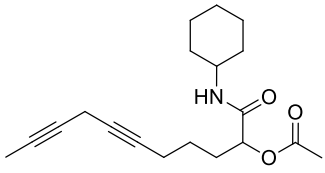
7-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-2*H*-chromen-2-one (BAM8)

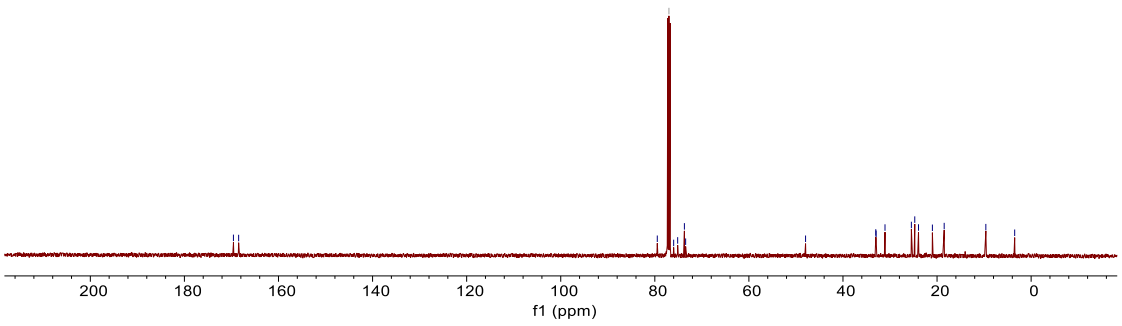


1-(cyclohexylamino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM9)

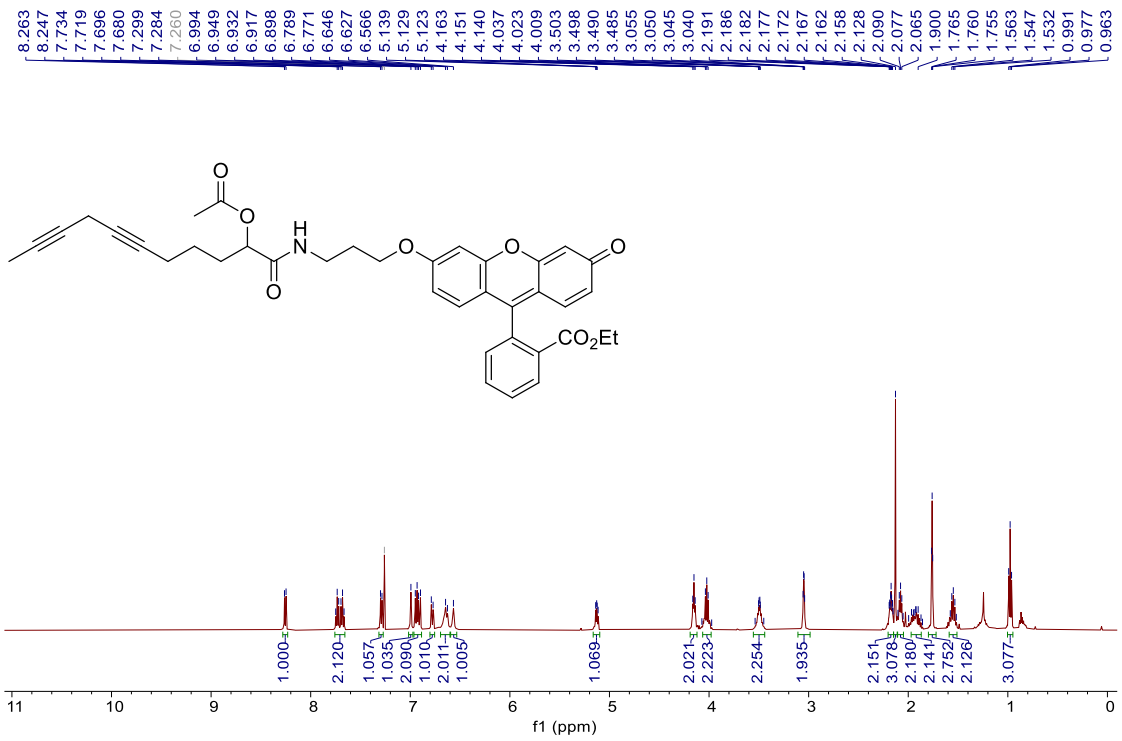


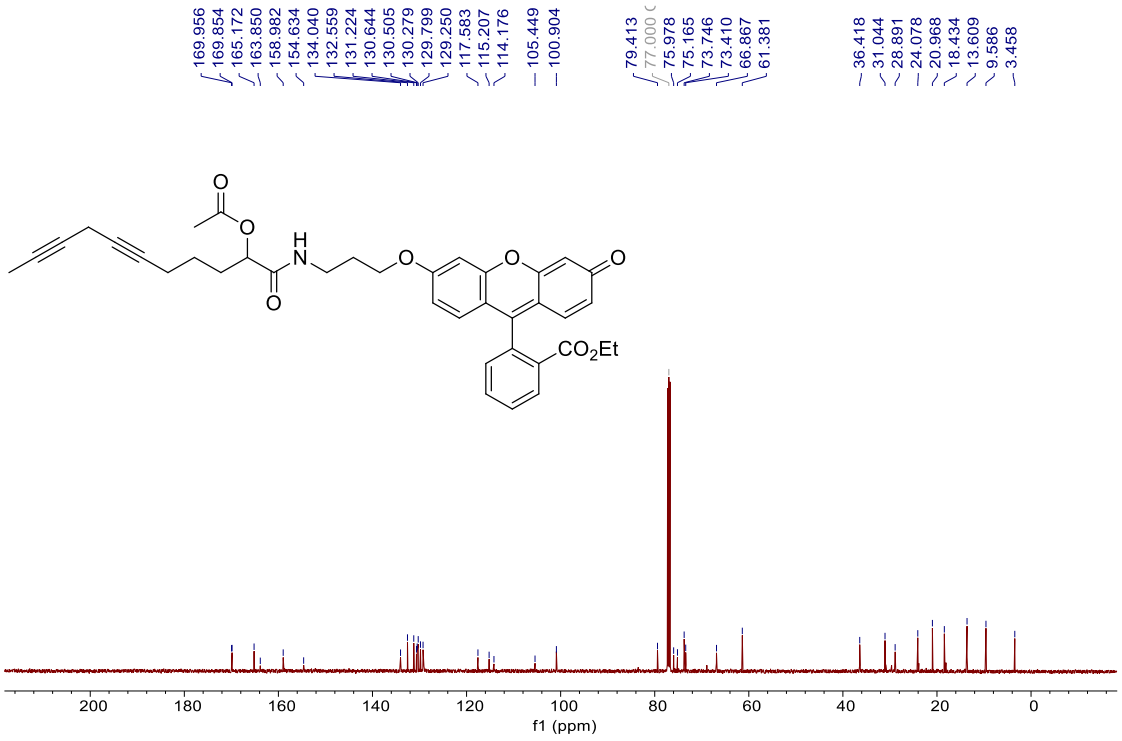




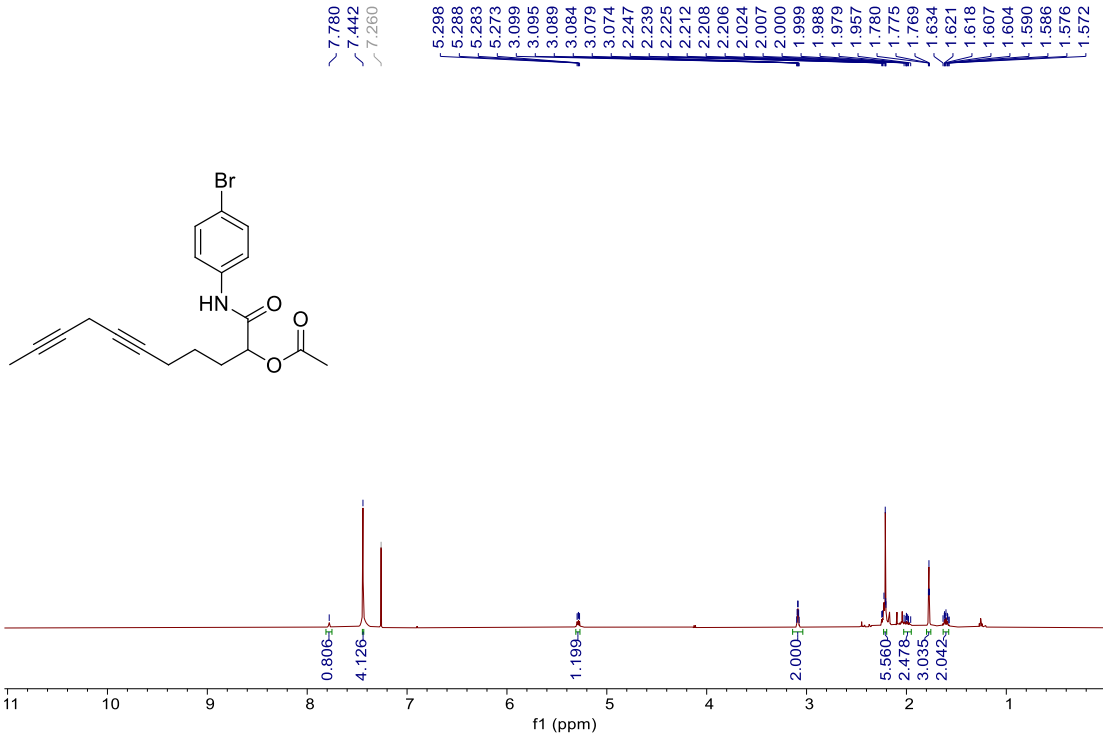


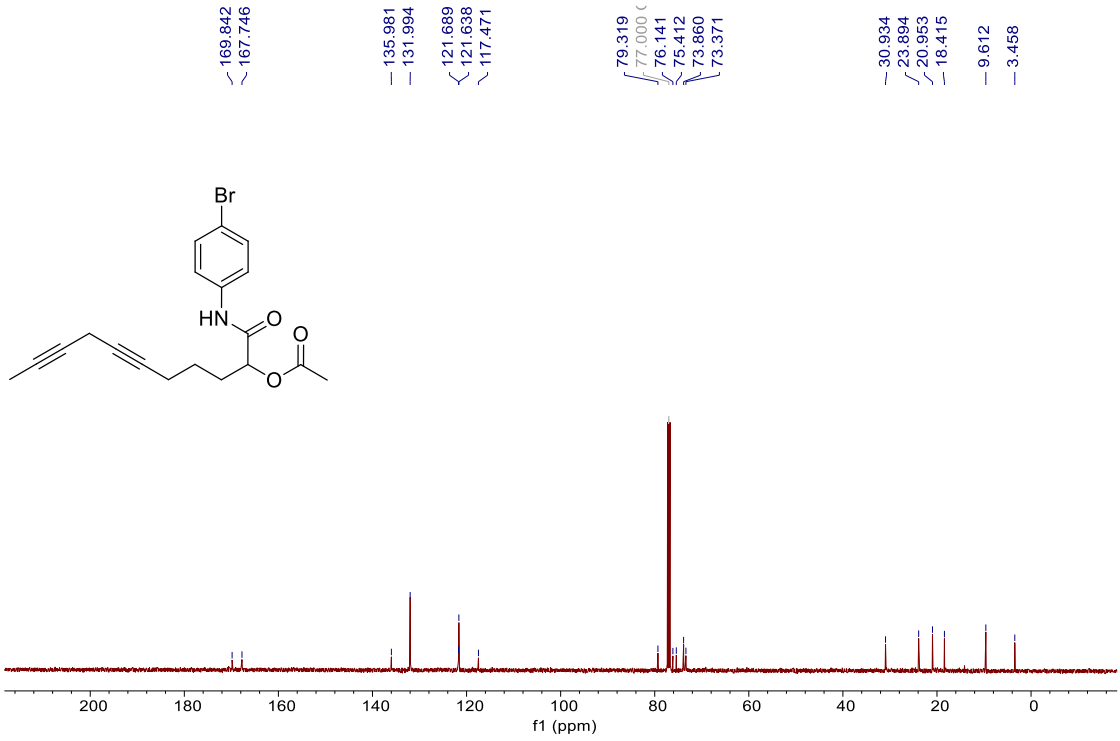
Ethyl 2-(6-(3-(2-acetoxyundeca-6,9-diynamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (BAM10)



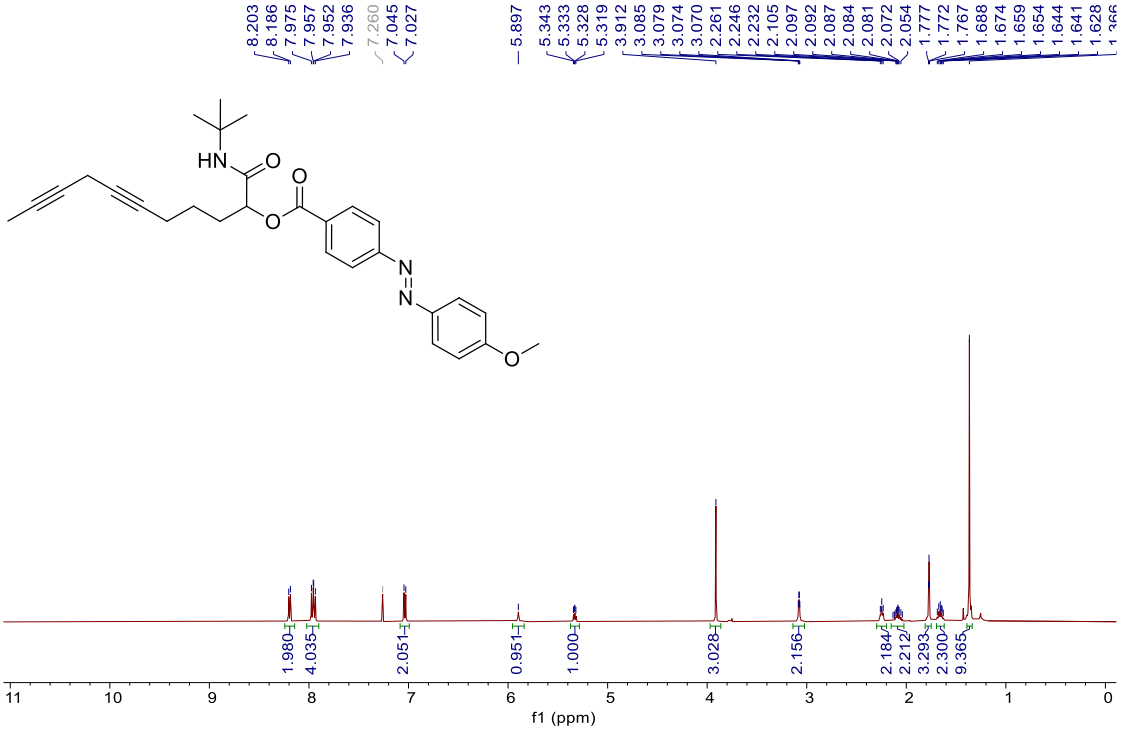


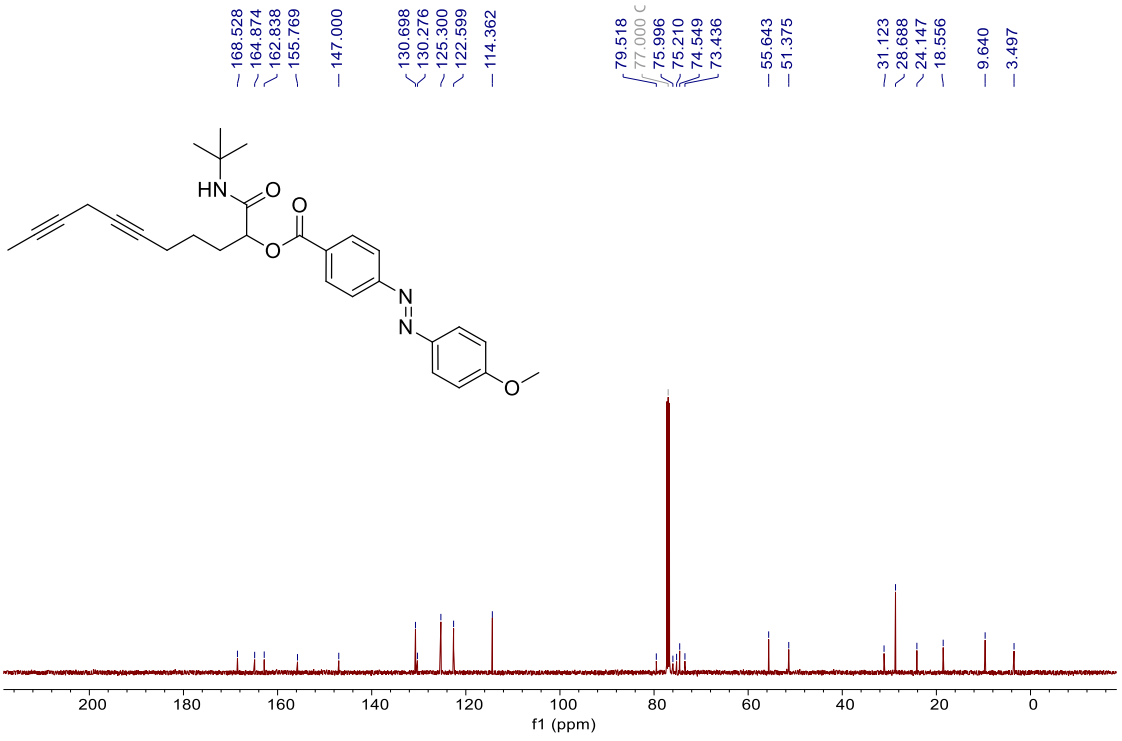
1-((4-bromophenyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM11)



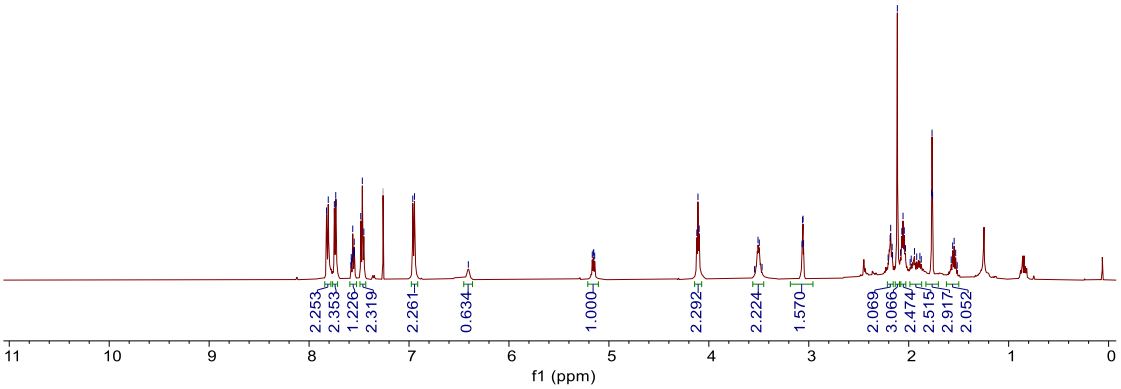


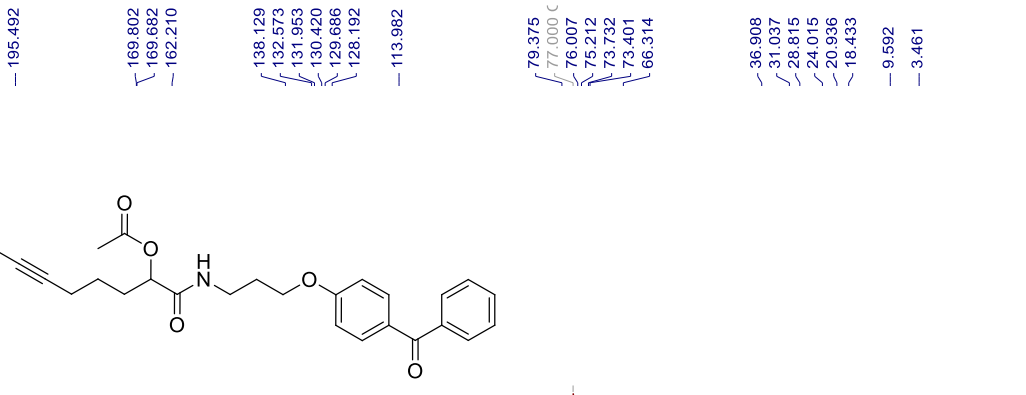
(E)-1-(tert-butylamino)-1-oxoundeca-6,9-diyn-2-yl4-((4-methoxyphenyl)diazenyl) benzoate (BAM12)

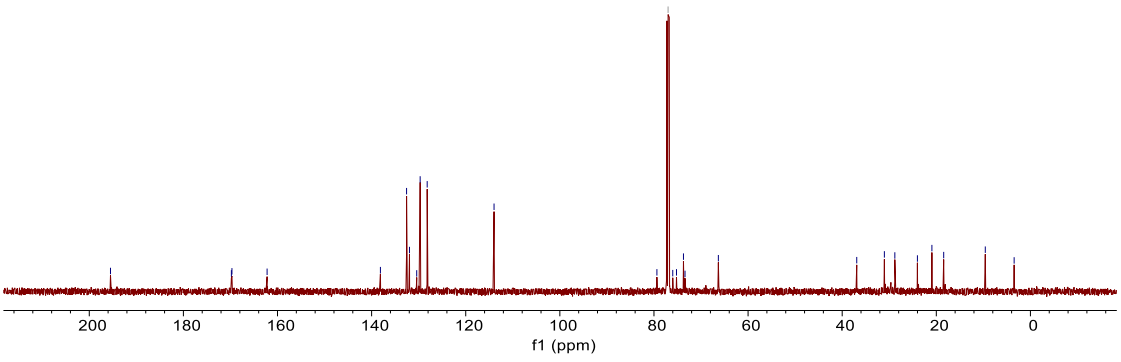




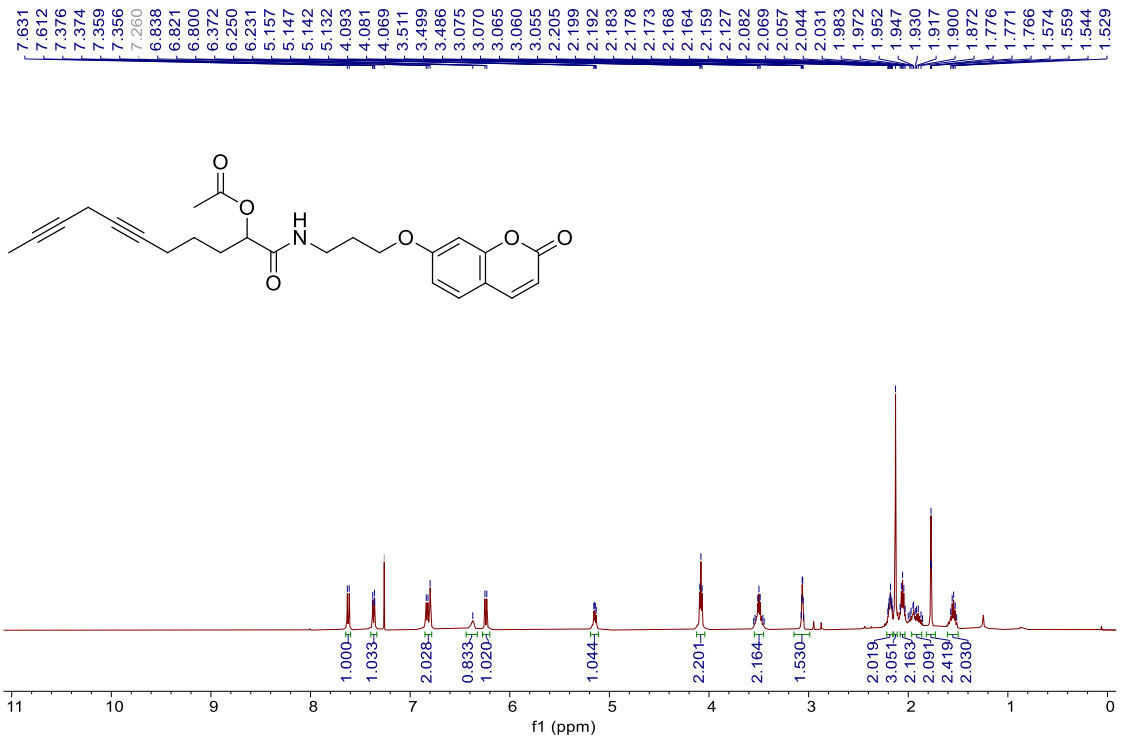
1-((3-(4-benzoylphenoxy)propyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM13)

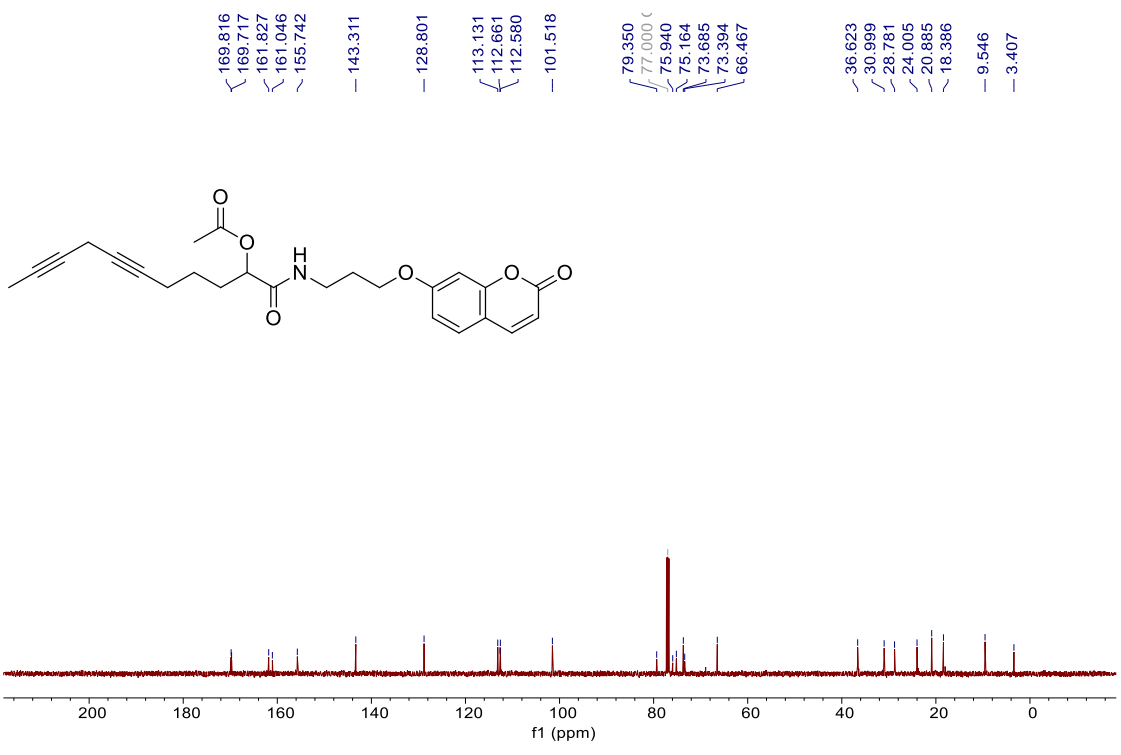




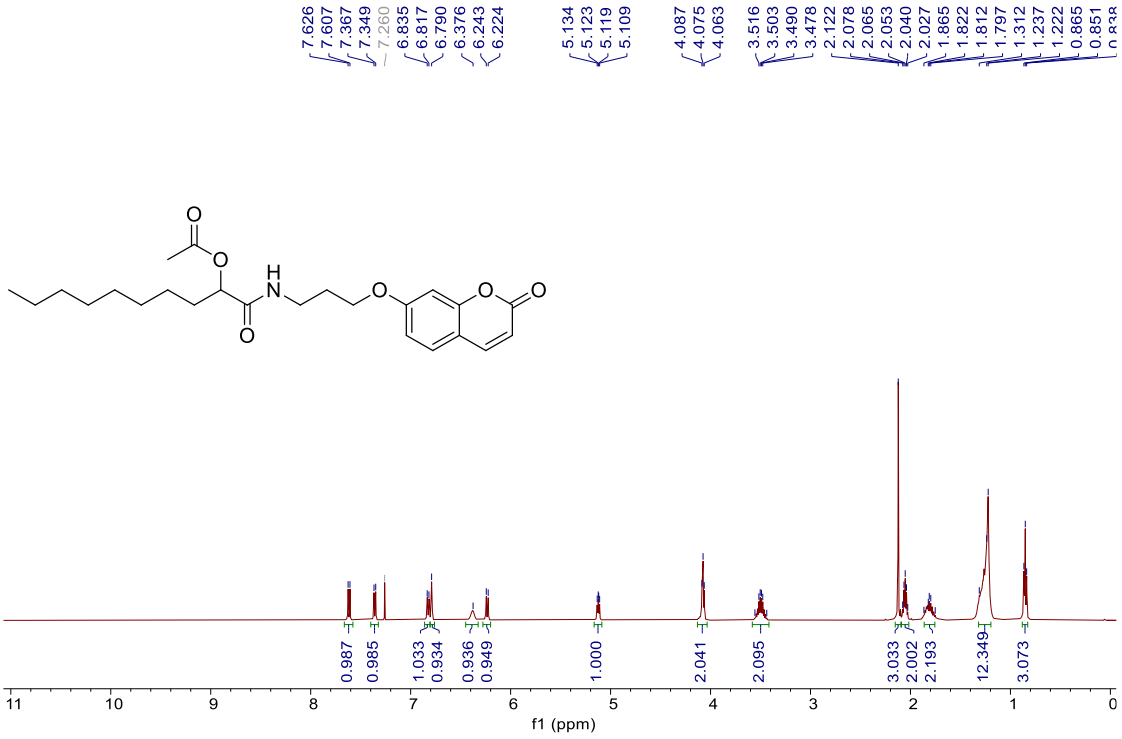


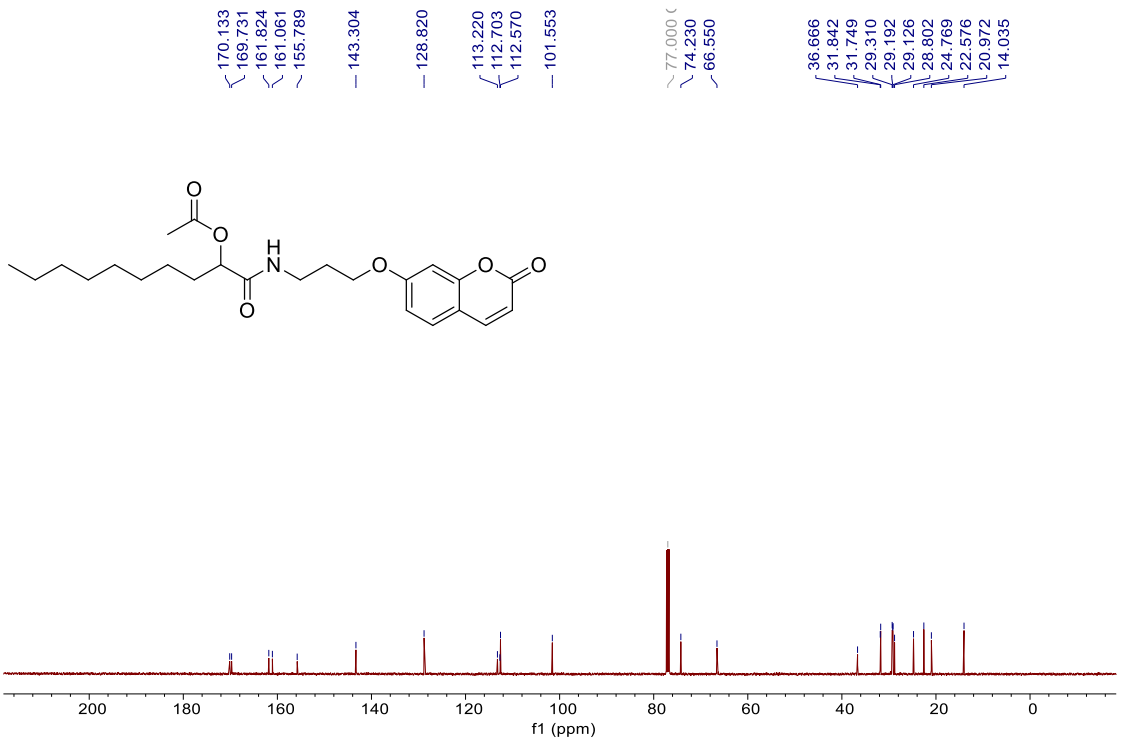
1-oxo-1-((3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)undeca-6,9-diyn-2-yl acetate (BAM14)



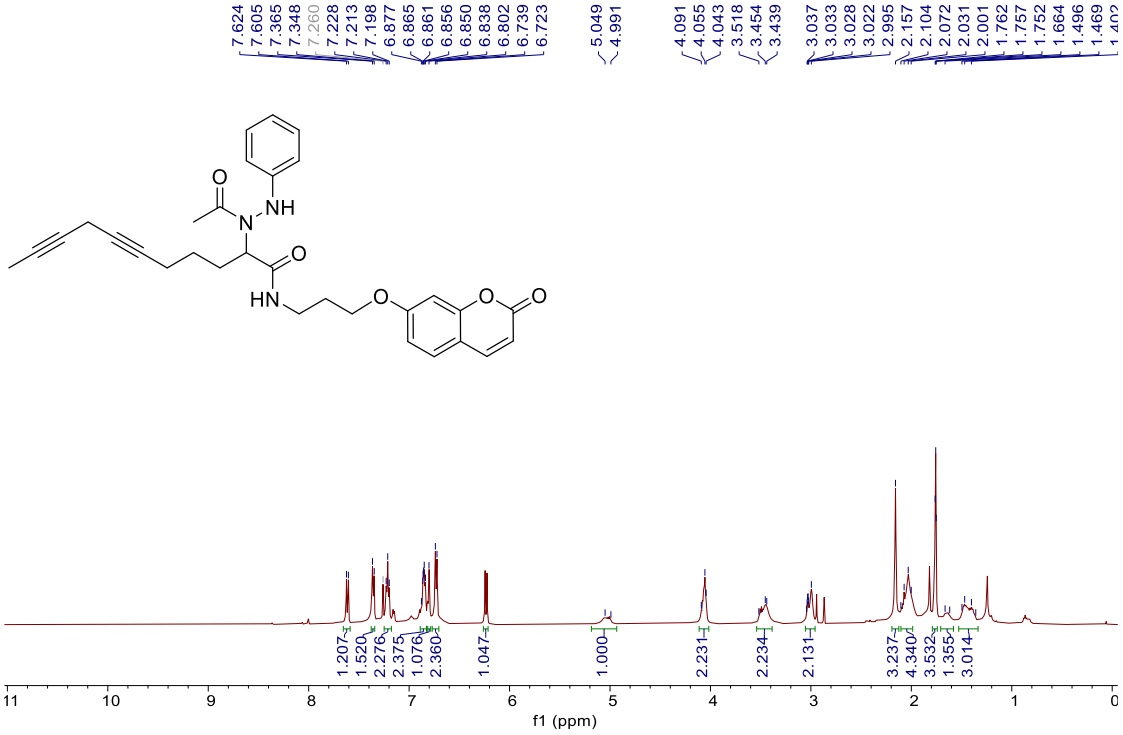


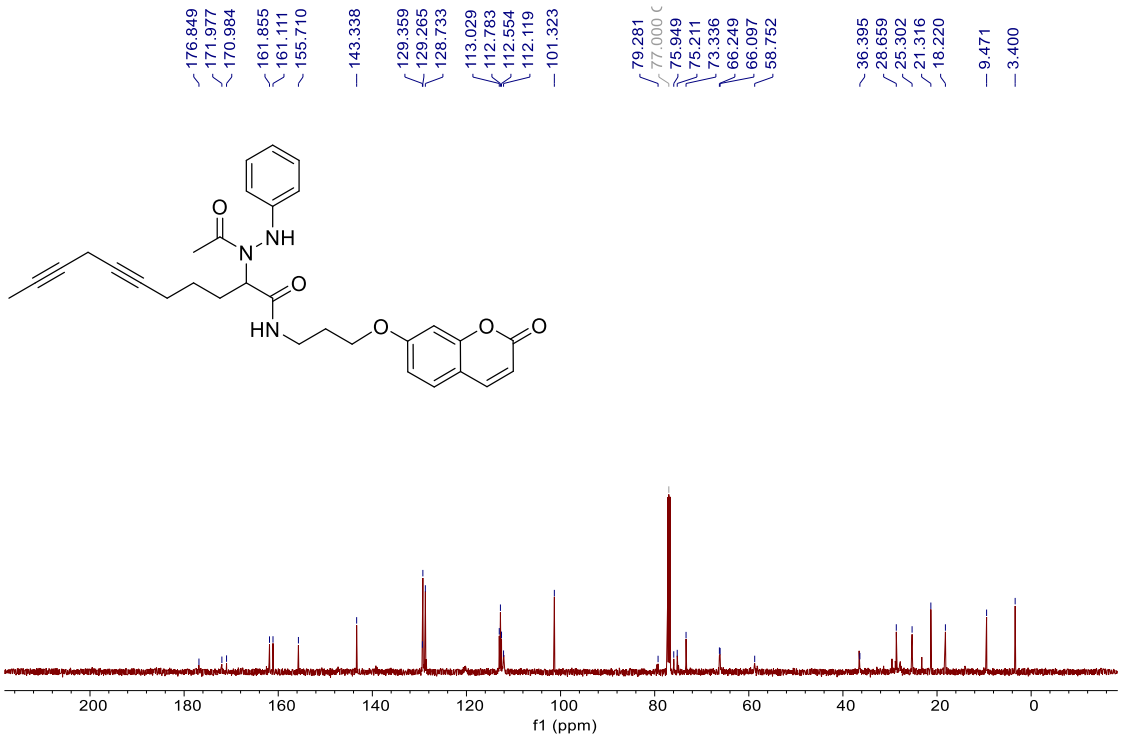
1-oxo-1-((3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)decan-2-yl acetate (C3)



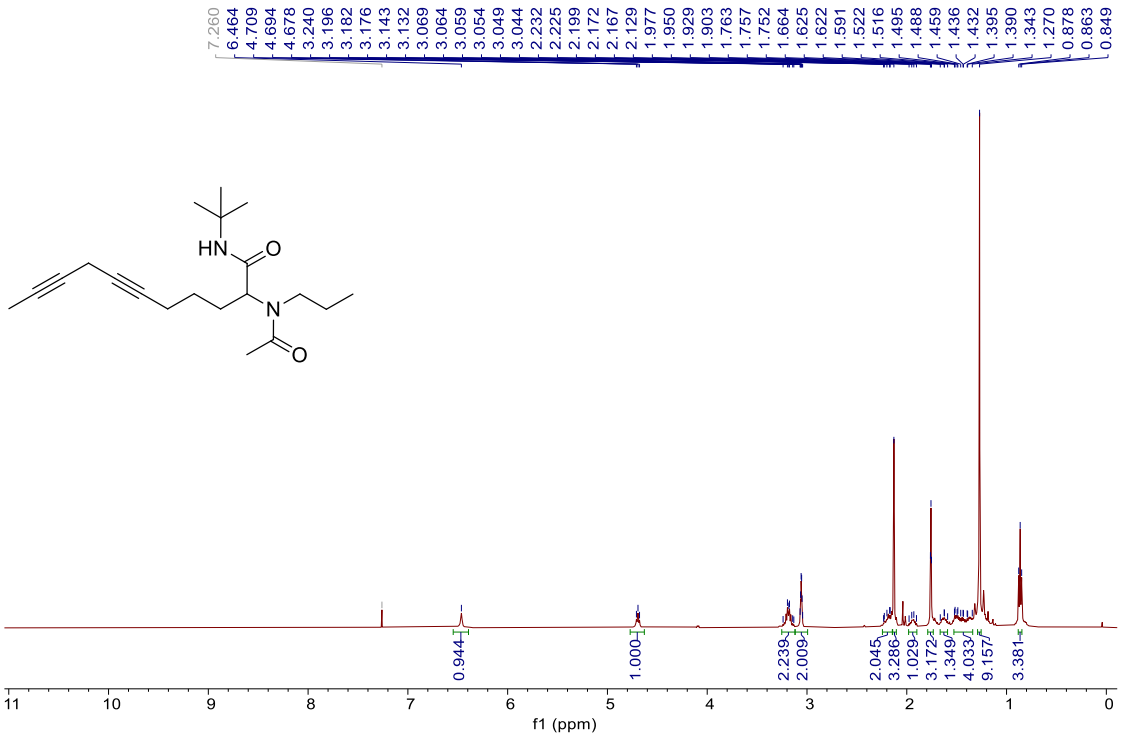


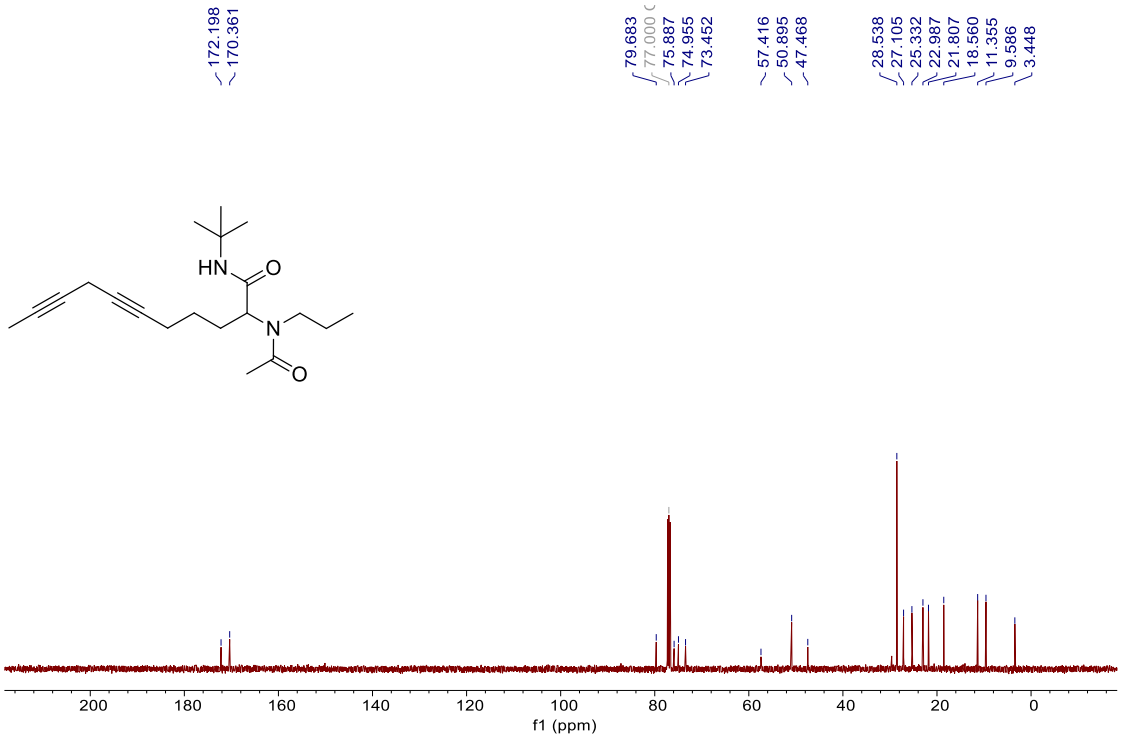
2-(1-acetyl-2-phenylhydrazineyl)-*N*-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)undeca-6,9-diynamide (BAM15)



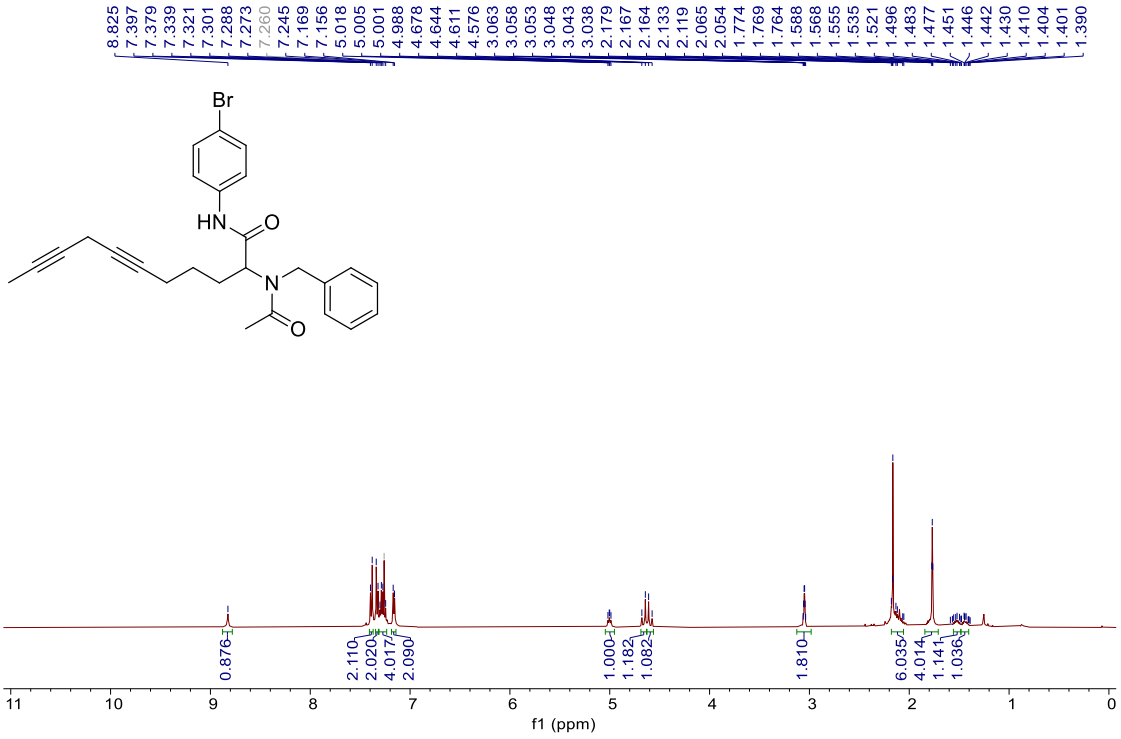


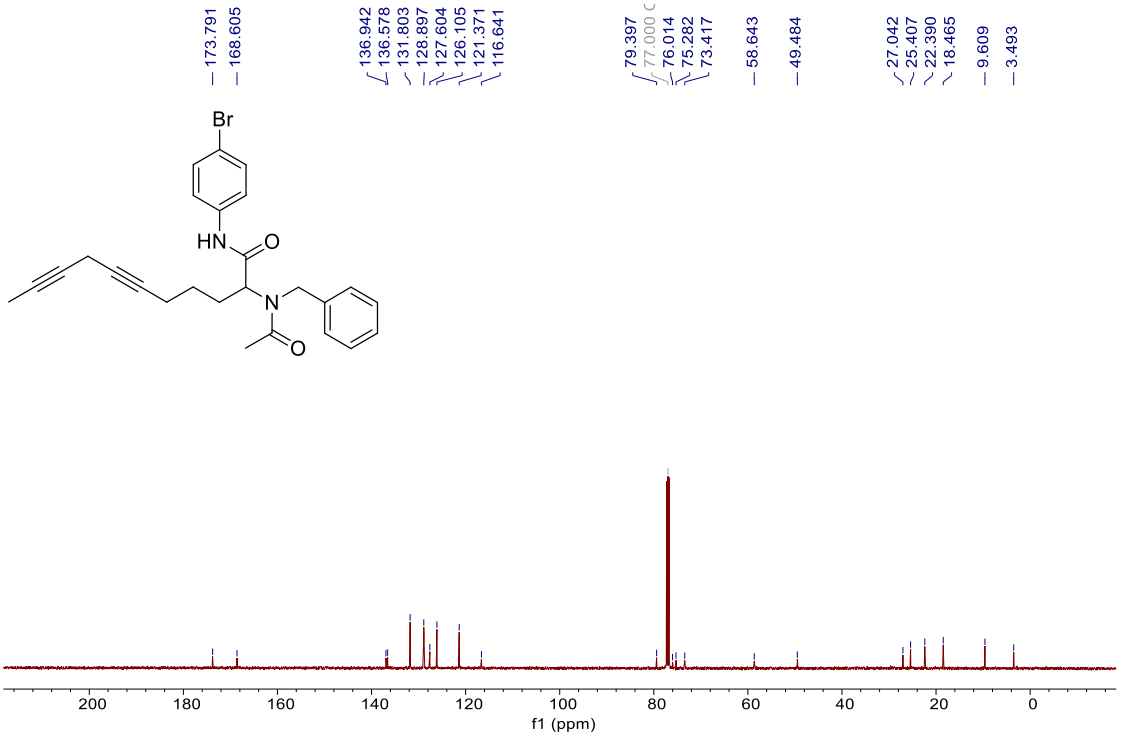
_N_-(_tert_-butyl)-2-(_N_-propylacetamido)undeca-6,9-diynamide (BAM16)



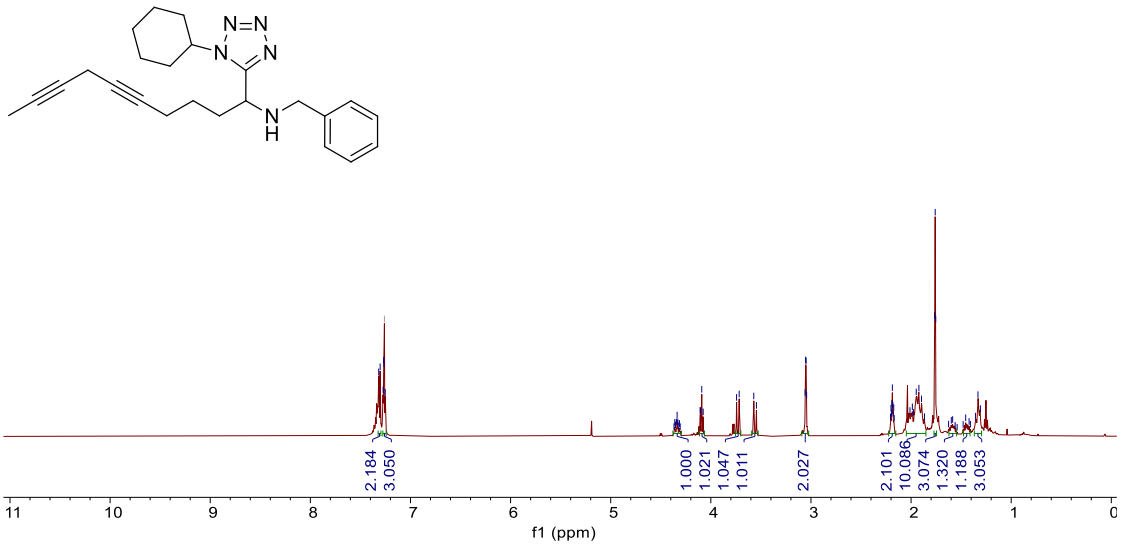


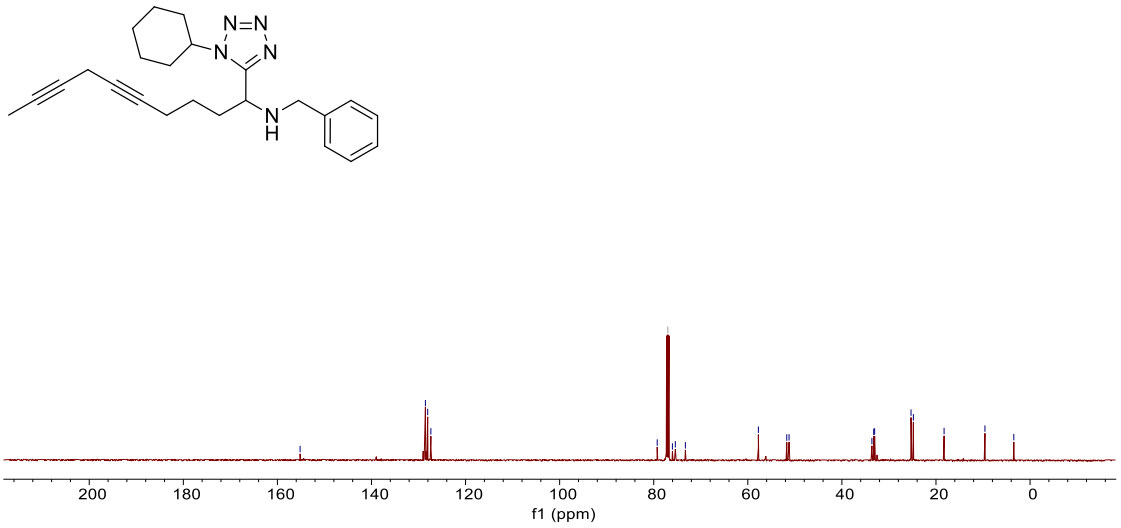
2-(*N*-benzylacetamido)-*N*-(4-bromophenyl)undeca-6,9-diynamide (BAM17)



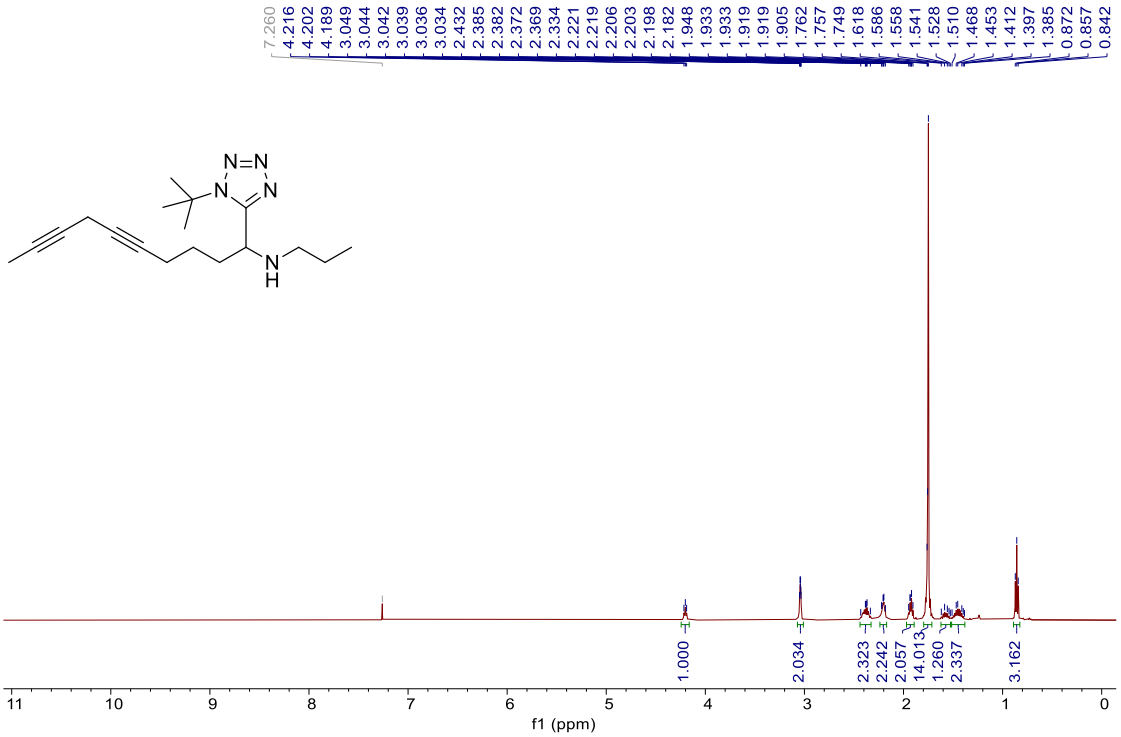


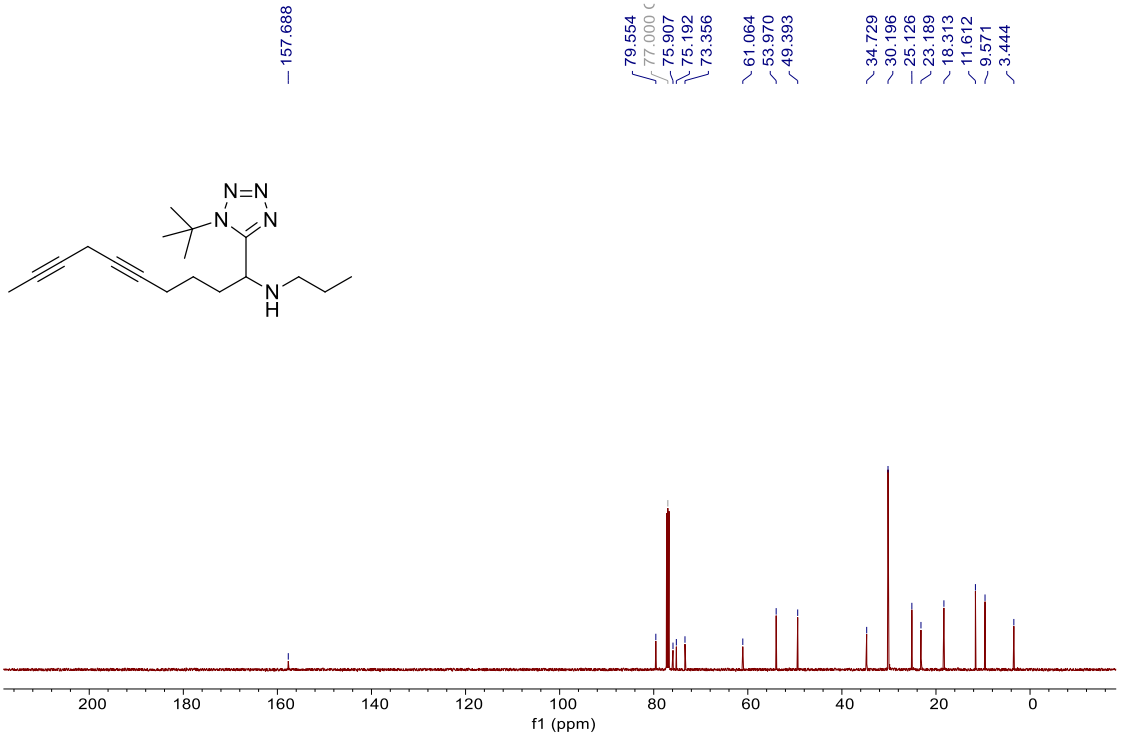
N-benzyl-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)deca-5,8-diyn-1-amine (BAM18)



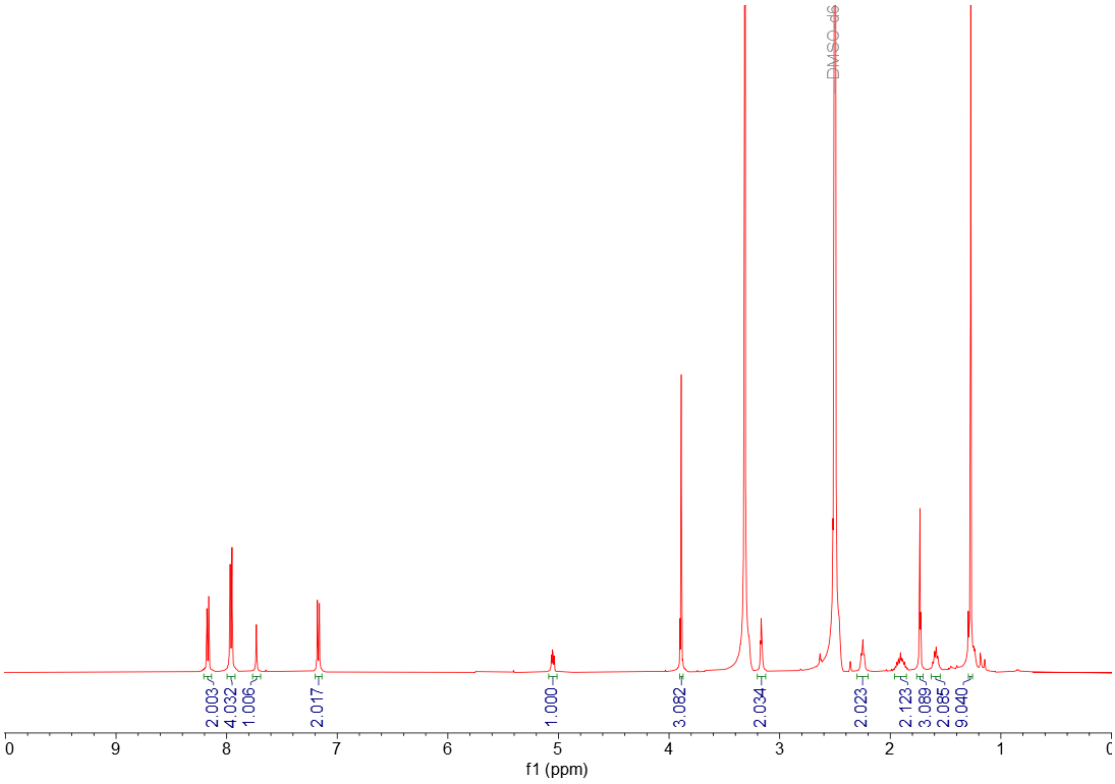


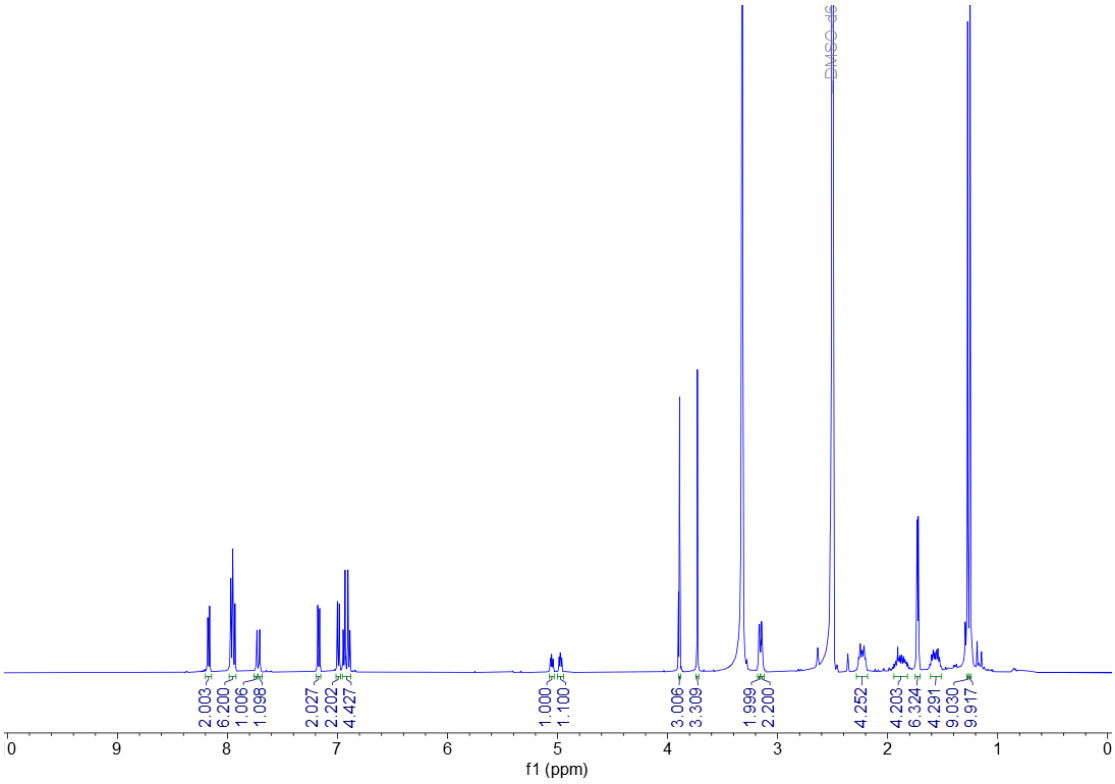
1-(1-(tert-butyl)-1*H*-tetrazol-5-yl)-*N*-propyldeca-5,8-diyn-1-amine (BAM19)





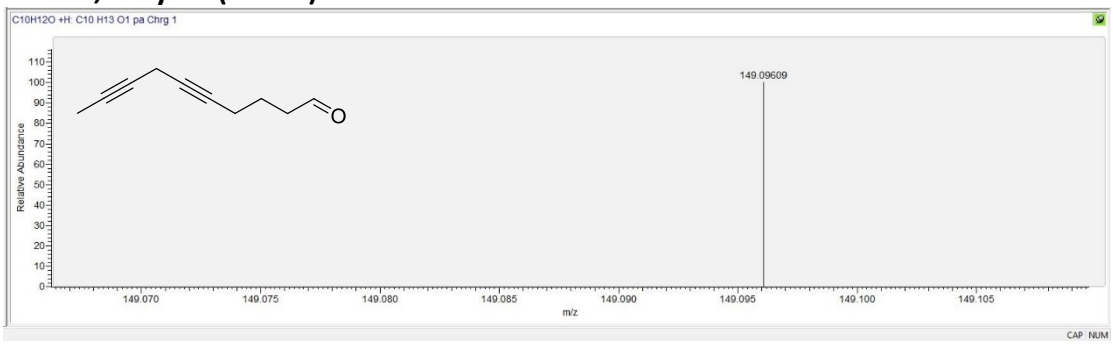
1.1 ^{1}H NMR spectra of BAM12 before and after UV irradiation in DMSO-d_6



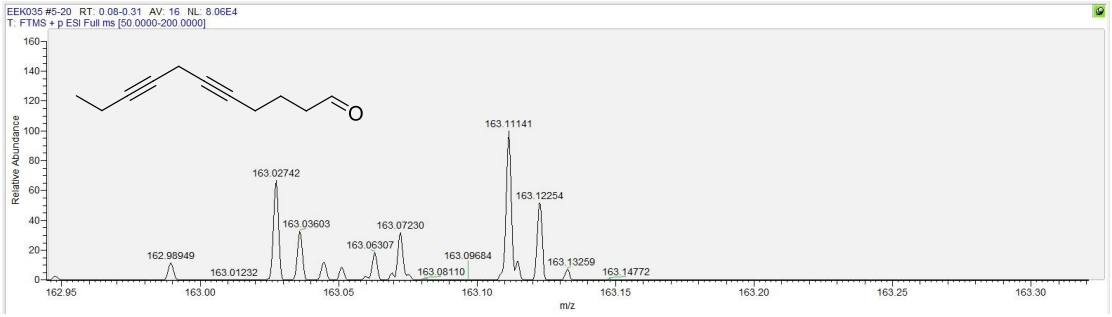


11. HRMS spectra

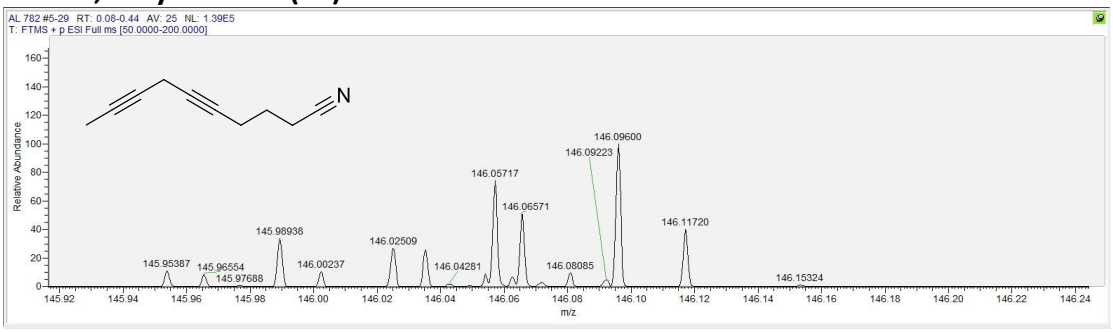
Deca-5,8-diynal (BAM1)



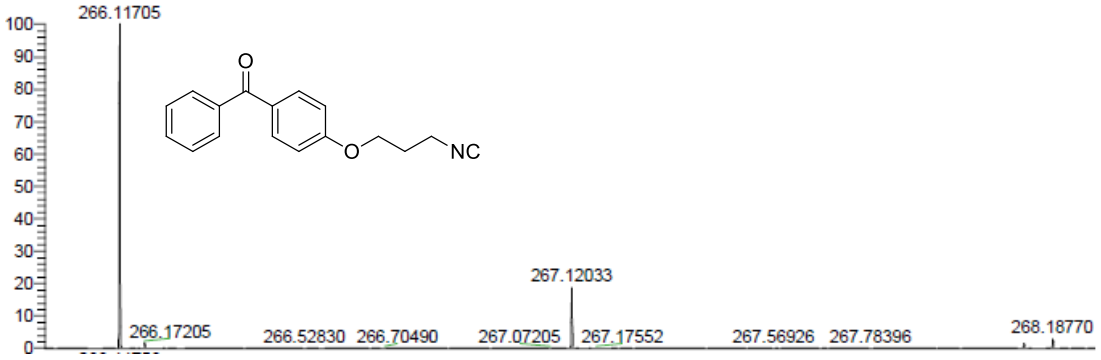
Undeca-5,8-diynal (BAM1')



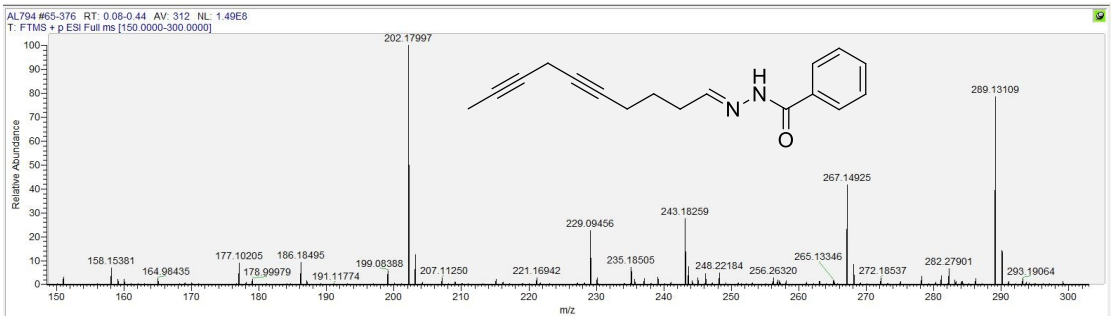
Deca-5,8-diynenitrile (C7)



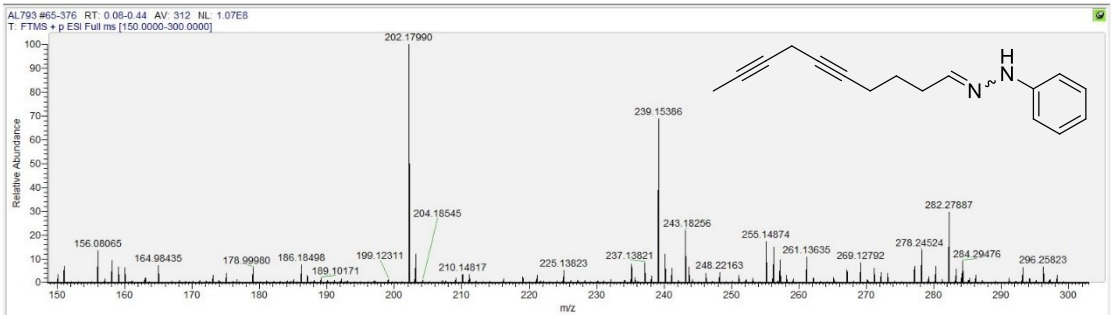
(4-(3-isocyanopropoxy)phenyl)(phenyl)methanone (4)



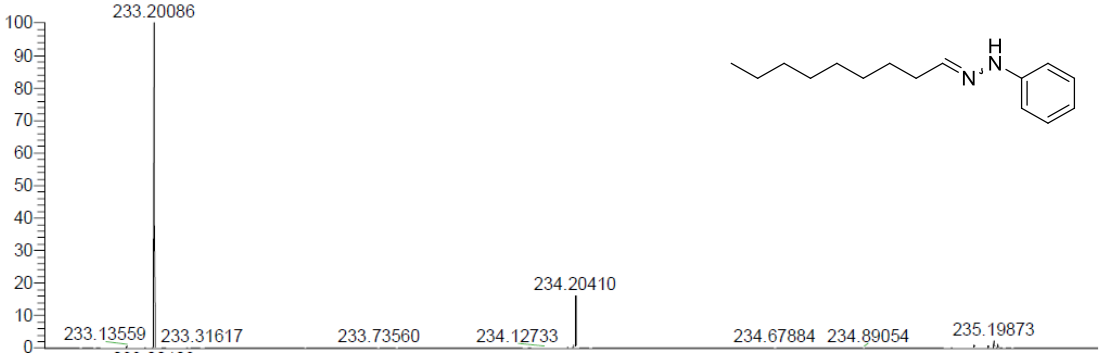
N'-(deca-5,8-diyn-1-ylidene)benzohydrazide (BAM2)



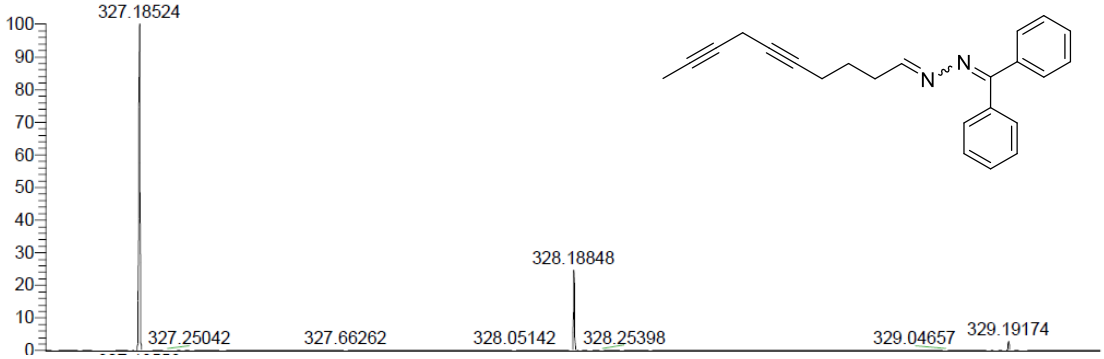
1-(deca-5,8-diyn-1-ylidene)-2-phenylhydrazine (BAM3)



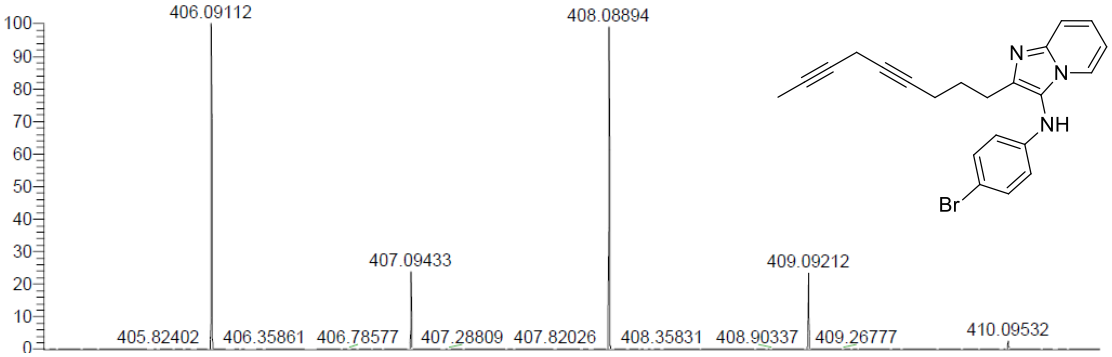
1-nonylidene-2-phenylhydrazine (C6)



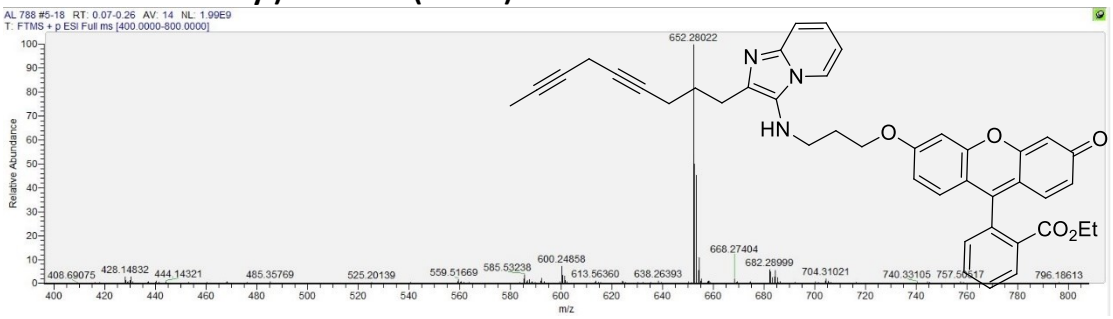
1-(deca-5,8-diyn-1-ylidene)-2-(diphenylmethylene)hydrazine (BAM4)



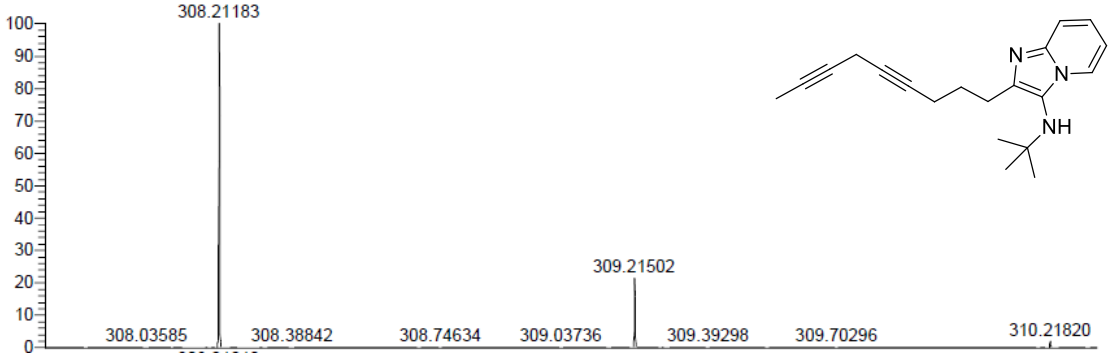
_N_-(4-bromophenyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM5)



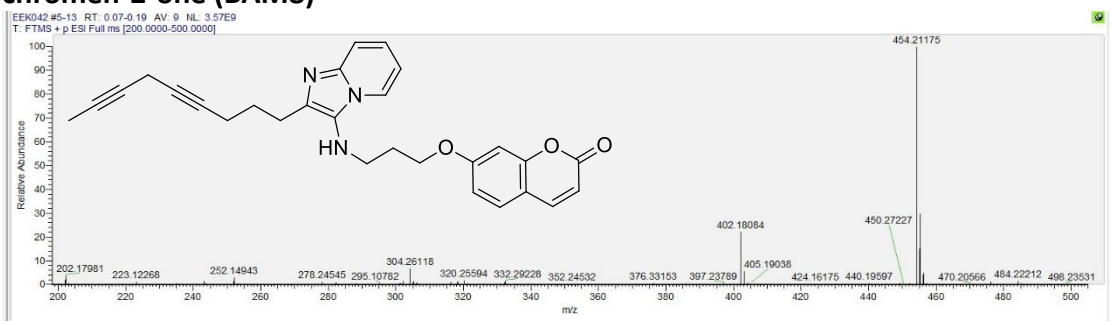
Ethyl 2-(6-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (BAM6)



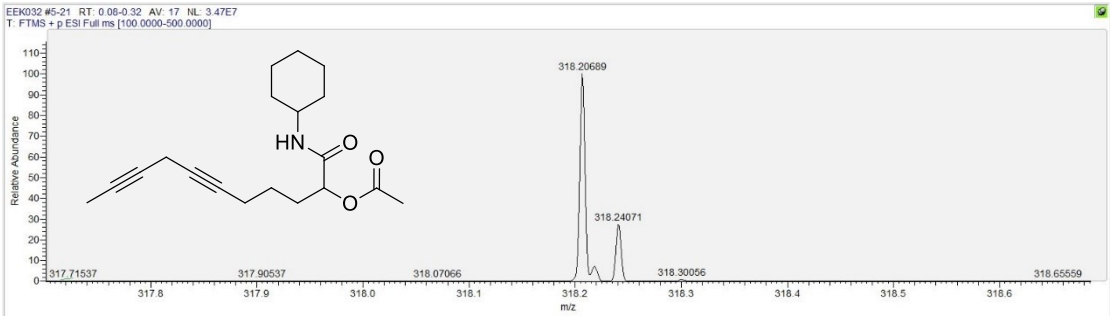
_N_-(tert-butyl)-2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-amine (BAM7)



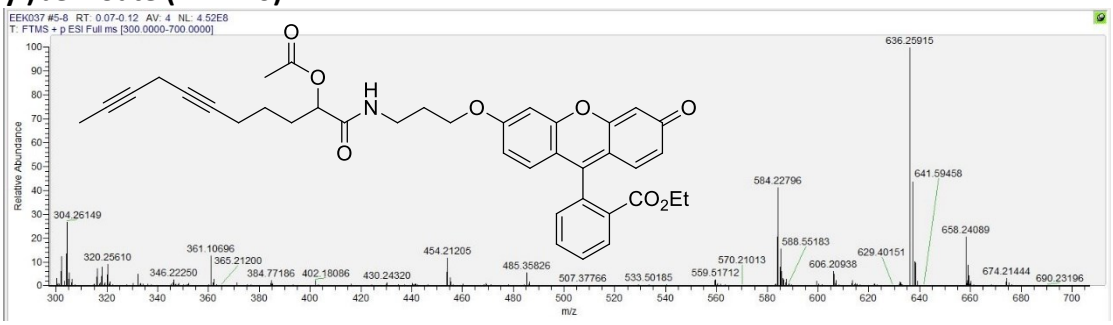
7-(3-((2-(nona-4,7-diyn-1-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-2*H*-chromen-2-one (BAM8)



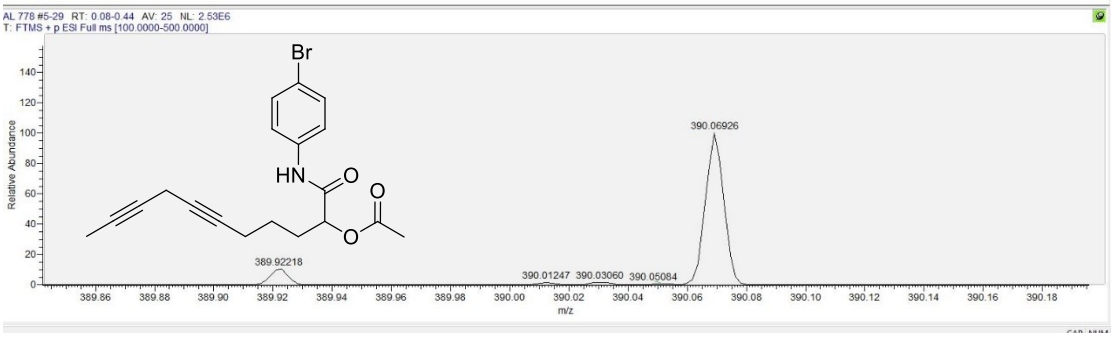
1-(cyclohexylamino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM9)



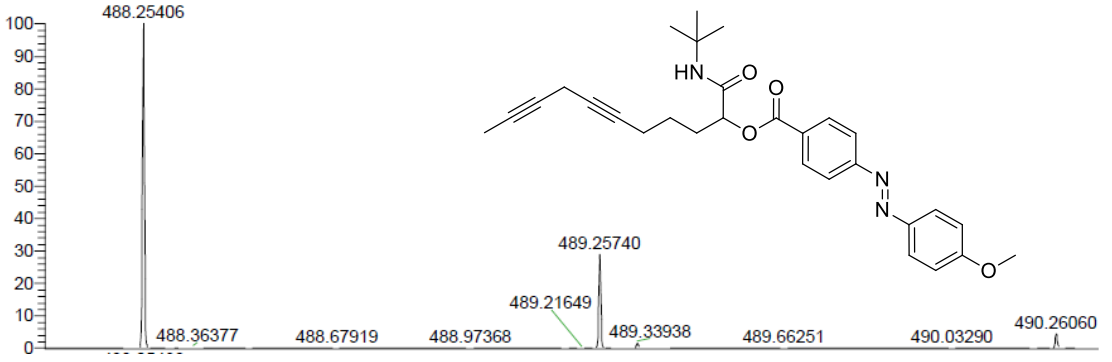
Ethyl 2-(6-(3-(2-acetoxyundeca-6,9-diynamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (BAM10)



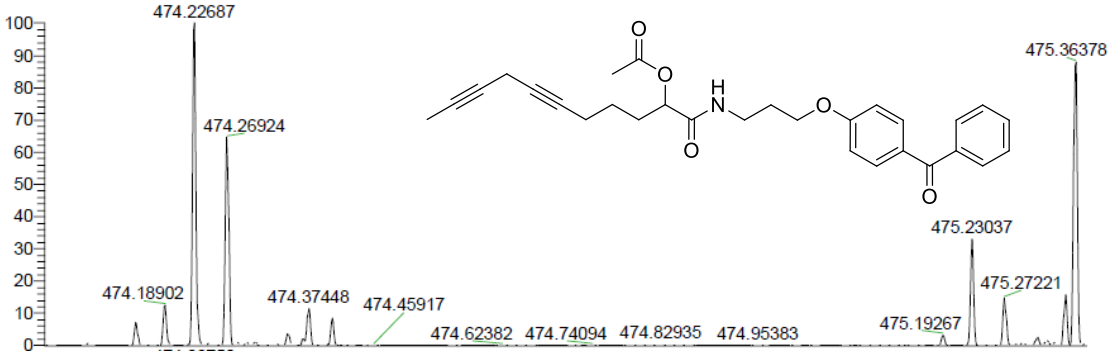
1-((4-bromophenyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM11)



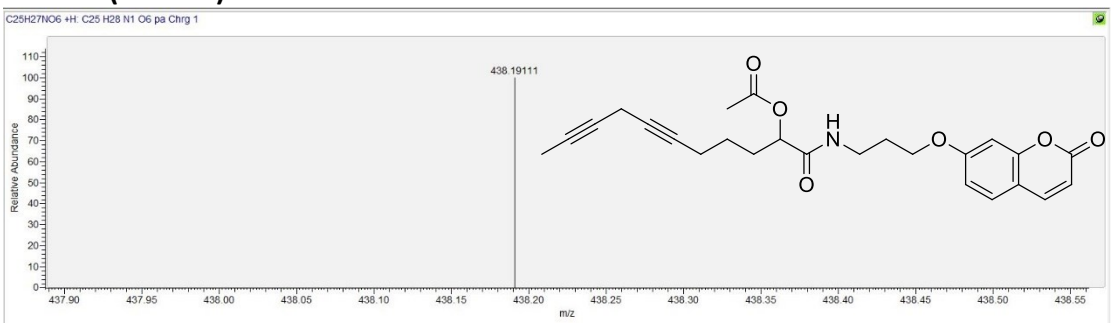
(*E*)-1-(tert-butylamino)-1-oxoundeca-6,9-diyn-2-yl4-((4-methoxyphenyl)diazenyl) benzoate (BAM12)



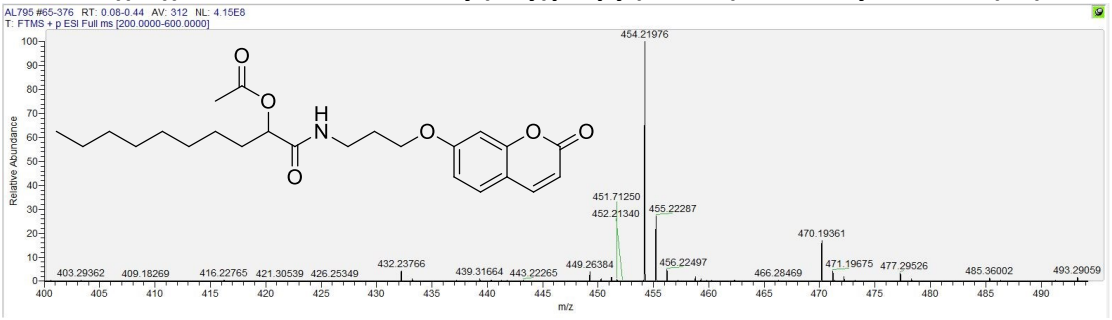
1-((3-(4-benzoylphenoxy)propyl)amino)-1-oxoundeca-6,9-diyn-2-yl acetate (BAM13)



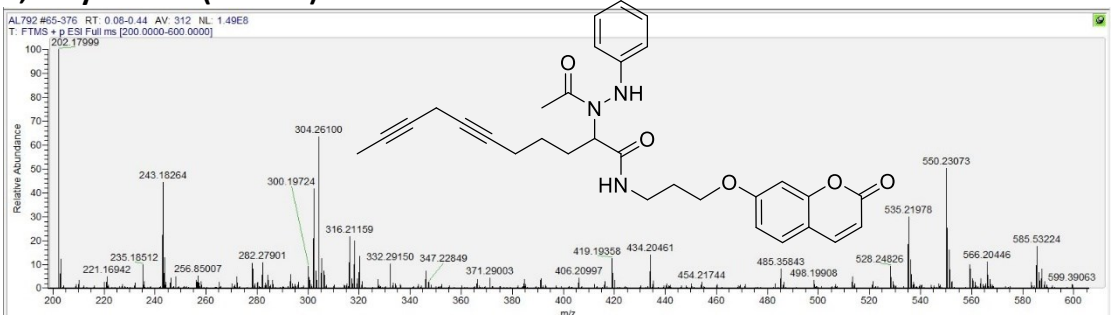
1-oxo-1-((3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)undeca-6,9-diyn-2-yl acetate (BAM14)



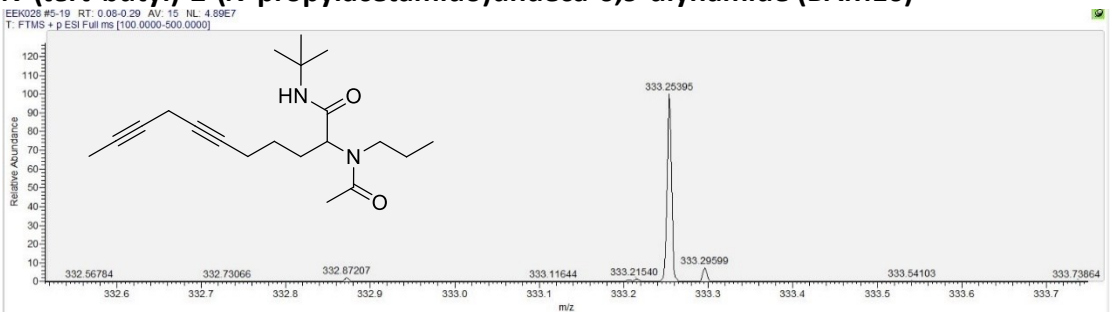
1-oxo-1-((3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)amino)decan-2-yl acetate (C3)



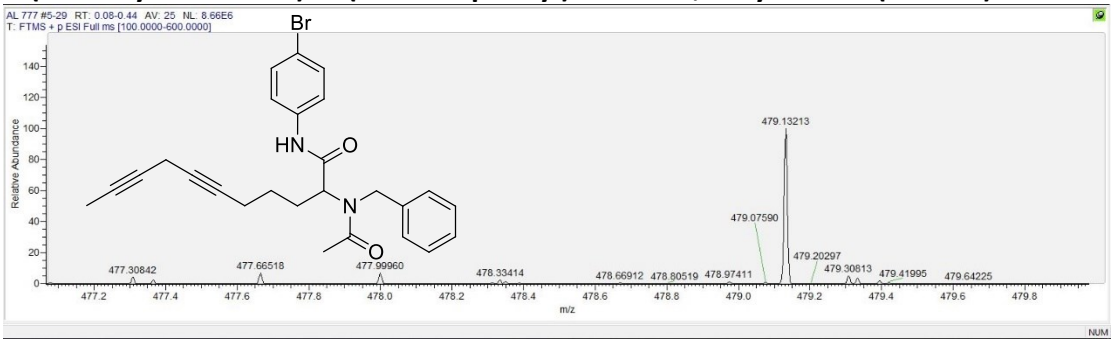
2-(1-acetyl-2-phenylhydrazineyl)-*N*-(3-((2-oxo-2*H*-chromen-7-yl)oxy)propyl)undeca-6,9-diynamide (BAM15)



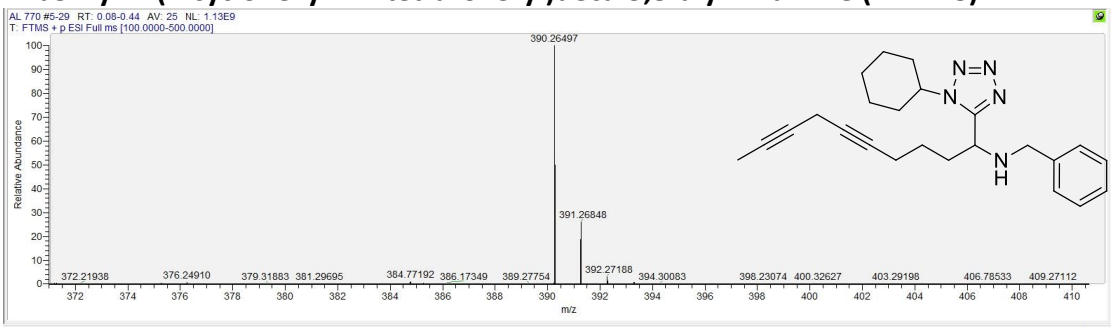
***N*-(*tert*-butyl)-2-(*N*-propylacetamido)undeca-6,9-diynamide (BAM16)**



2-(*N*-benzylacetamido)-*N*-(4-bromophenyl)undeca-6,9-diynamide (BAM17)



***N*-benzyl-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)deca-5,8-diyn-1-amine (BAM18)**



1-(1-(tert-butyl)-1*H*-tetrazol-5-yl)-*N*-propyldeca-5,8-diyn-1-amine (BAM19)

12. References and Notes

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