The Evolution of Fluorescein into a Potential Theranostic Tool

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Supporting Information

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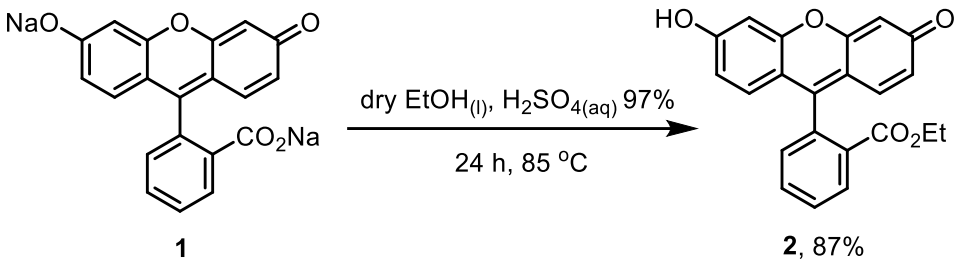
1. Experimental methods and materials

All reagents and solvents were purchased from Sigma-Aldrich, Thermo Fisher Scientific, AK Scientific, Fluorochem, Abcr GmbH, Acros Organics and used without further purification. Thin layer chromatography (TLC) was performed on Millipore silica gel coated plates (thickness 0.20 mm, particle size 25 µm). Nuclear magnetic resonance spectra were recorded on Bruker Avance 500 spectrometers {^{1}H NMR (500 MHz), ^{13}C NMR (126 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 = single, br s = broad single, d = double, t = triple, q = quad, br q = broad quad, quin = quintet, dd = double of double, tt = triple of triple, dtd = double of triple of triple, m = multiple. Chemical shifts for ^{13}C NMR were reported in ppm relative to the solvent peak. High resolution mass spectra were recorded using LTQ-Orbitrap-XL (Thermo) at a resolution of 60000@m/z400. The gas chromatograph (GC-MS) used is a Shimadzu Nexis GC-2030 type, which is equipped with a MEGA-5 HT capillary column (30m × 0.25mm diameter × 0.25µm thickness) and is coupled to a 24 Shimadzu GCMS-mass detector. QP2020NX. Finally, the Varioscan Plate reader spectrometer from Thermo Fisher Scientific was used to obtain the absorption and emission spectra. Purification of the final compounds was performed by flash column chromatography with SiO_2 support material (silica gel 60, SDS, 230-400 mesh ASTM) and using of an automated chromatographic separation system Reveleris™ Flash Chromatography System from Grace. Control of the reactions was performed by thin layer chromatography (TLC), or by obtaining a ^{1}H NMR spectrum through the reaction (*in situ*). The drying of the organic extracts was carried out with Na_2SO_4 and the concentration of the mixtures in order to remove the solvents in a rotavaporator.

2. Synthetic Procedures and analytical data

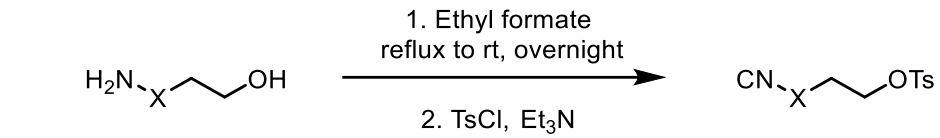
Procedure for synthesis of fluorescein ethyl ester



1 **2**, 87%

Ethanol (absolute, ≥99%), was passed with external pressure through a SiO_2 column in a round-bottom flask with activated molecular sieves 3 Å and left for three days under an argon atmosphere. Fluorescein disodium salt (**1**) (25 mmol, 1.0 equiv) and ethanol (200 mL) were added to a pre-dried round bottom flask. The reaction mixture was then cooled to 0 °C and concentrated H_2SO_4, 97% (5.0 mL) was added dropwise. The reaction was allowed at 85 °C under reflux and inert argon atmosphere for 24 h. Evaporation of the solvent under vacuum was followed and redissolution of the mixture in $CHCl_3$. H_2SO_4 neutralization and consequently quenching of the reaction mixture took place by gradual addition of saturated aqueous Na_2CO_3 solution until two phases are formed and stirring for 40 min, at 0 °C. Attention when adding the saturated aqueous Na_2CO_3 solution, fast addition causes rapid release of caustic foam and overflow of the flask. The two phases were then transferred to a separatory funnel and the organic phase was extracted with water (x4). The aqueous phase was then extracted with $CHCl_3$ (x3). The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum, giving the desired product (**2**) in 87% yield.

General procedures for the synthesis of isocyanides

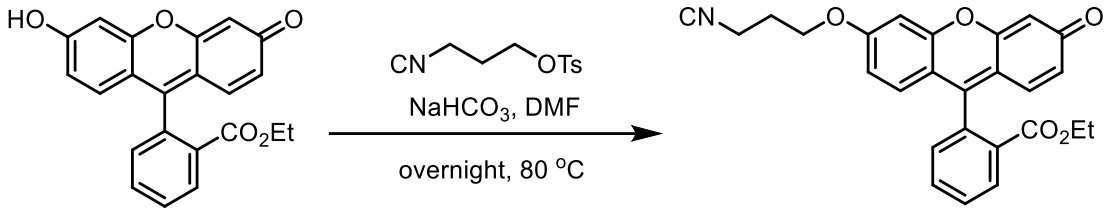


X = -CH_2-, -$(CH_2)_4$-, -CH_2CH_2O-

60-72%

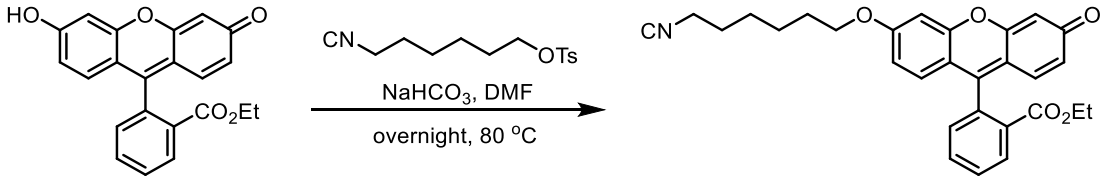
The amino alcohol (20.0 mmol, 1.0 equiv) and the ethyl formate (13.0 mL) were refluxed for 8 h in an oil bath to form the corresponding formamide and then left overnight under stirring at room temperature. Then, the volatiles were removed under vacuum. The corresponding formamide (7.5 mmol, 1.0 equiv) was dissolved in dry DCM (14.0 mL) under argon and after the addition of triethylamine (45.0 mmol, 6.0 equiv), the reaction mixture was cooled to 0 °C. Then, TsCl (22.5 mmol, 3.0 equiv) was added and the reaction was stirred at room temperature for 5-7 h. The mixture was then quenched with saturated aqueous Na_2CO_3 solution until two phases were formed under vigorous stirring at 0 °C for 40 min. The two phases were then transferred to a separatory funnel and the organic phase was extracted with water (x3). The aqueous phase was then extracted with DCM (x3). The combined organic layers were dried

over Na_2SO_4 and evaporated in vacuum. The crude product was purified by filtration through silica gel under reduce pressure, using EtOAc (300 mL) as eluent solvent. Activated charcoal was added to the resulting EtOAc supernatant. The mixture was stirred for 5 min and again filtered through celite in a vacuum filter to remove activated carbon. Removal of the solvent in a vacuum rotavaporator gave the desired isocynides in 60-72% yield, with 89% purity (measured by GC-MS).



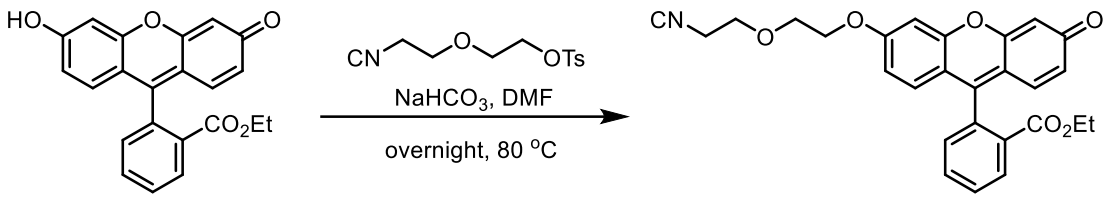
2 **3a**, 75%

The fluorescein ethyl ester (**2**) (15 mmol, 1.0 equiv) was added to a sealed tube and dissolved in DMF (60 mL). Then, 3-isocyanopropyl-4-methylbenzenesulfonate (18 mmol, 1.2 equiv) and $NaHCO_3$ (30 mmol, 2.0 equiv) were added. The reaction mixture was left at 80 °C, under stirring, overnight. After the end of the reaction, the mixture was allowed to reach room temperature, quantitatively transferred to a separatory funnel and extracted with EtOAc/H_2O (x5), in order to remove DMF. The aqueous phase was then extracted with EtOAc (x1). The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. The reaction mixture was purified by liquid column chromatography with eluent solvents PE/EtOAc to give the desired product (**3a**) in 75% yield.



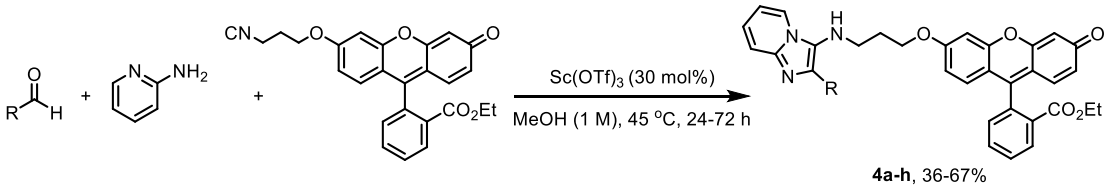
2 **3b**, 72%

The fluorescein ethyl ester (**2**) (1 mmol, 1.0 equiv) was added to a sealed tube and dissolved in DMF (4 mL). Then, 6-isocyanohexyl-4-methylbenzenesulfonate (1.2 mmol, 1.2 equiv) and $NaHCO_3$ (2 mmol, 2.0 equiv) were added. The reaction mixture was left at 80 °C, under stirring, overnight. After the end of the reaction, the mixture was allowed to reach room temperature, quantitatively transferred to a separatory funnel and extracted with EtOAc/H_2O (x5), in order to remove DMF. The aqueous phase was then extracted with EtOAc (x1). The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. The reaction mixture was purified by liquid column chromatography with eluent solvents PE/EtOAc to give the desired product (**3b**) in 72% yield.



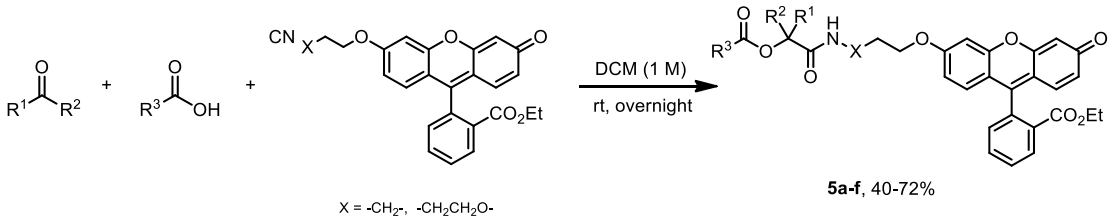
The fluorescein ethyl ester (**2**) (3 mmol, 1.0 equiv) was added to a sealed tube and dissolved in DMF (12 mL). Then, 2-(2-isocyanoethoxy)ethyl-4-methylbenzenesulfonate (3.6 mmol, 1.2 equiv) and $NaHCO_3$ (6 mmol, 2.0 equiv) were added. The reaction mixture was left at 80 °C, under stirring, overnight. After the end of the reaction, the mixture was allowed to reach room temperature, quantitatively transferred to a separatory funnel and extracted with EtOAc/H_2O (x5), in order to remove DMF. The aqueous phase was then extracted with EtOAc (x1). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The reaction mixture was purified by liquid column chromatography with eluent solvents PE/EtOAc, giving the desired product (**3c**) in 79% yield.

General method for the GBB-3CR



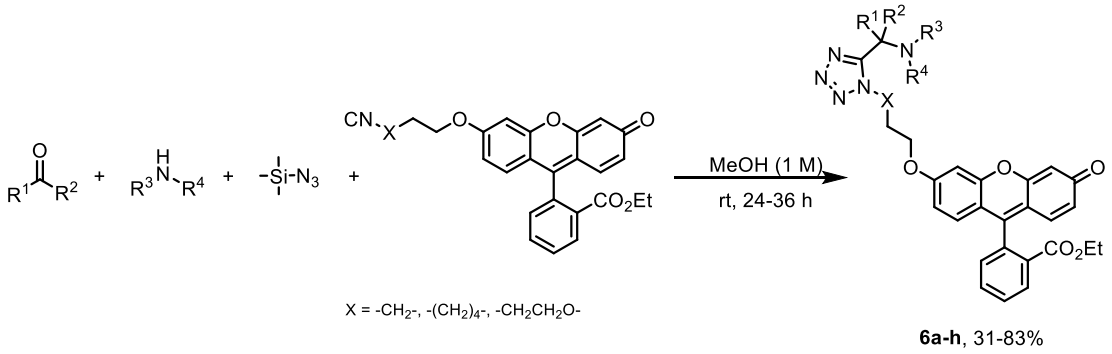
To a stirred solution of aldehyde (0.7 mmol, 1.0 equiv) in MeOH (0.7 mL), 2-aminopyridine (0.7 mmol, 1.0 equiv) and scandium triflate (0.2 mmol, 0.3 equiv) were added, the mixture was left for 20 min under stirring at 45 °C. Addition of the isocyanide **3a** (0.7 mmol, 1.0 equiv) was followed. The final reaction mixture was left under stirring for 24-72 h at 45°C. The solvent was removed under vacuum. Chromatographic separation with PE/EtOAc/DCM/MeOH as eluent solvents, gave the desired products (**4a-f**, **4h**) in 36-67% yield. In the case of product **4g**, the solution of stirred isophthalaldehyde (1.4 mmol, 2.0 equiv) in MeOH (1.4 mL) was cooled to 0 °C in an ice bath and 2-aminopyridine (0.7 mmol, 1.0 equiv) also dissolved in methanol (1.4 mL) was added slowly dropwise, under vigorous stirring, over 30 min. The solution was left in the ice bath, to reach from 0 °C to room temperature, under stirring for 24 h, in order to fully form the intermediate single imine, instead of the double one. The solvent was then evaporated, the solid redissolved in MeOH (0.7 mL), the scandium triflate catalyst (0.2 mmol, 0.3 equiv) and isocyanide 3a were added. The mixture was left to react for a total of 48 h, at a temperature of 45°C. Chromatographic separation with PE/EtOAc/DCM/MeOH as eluent solvents followed, giving the desired product **4g** in 41% yield.

General method for the P-3CR



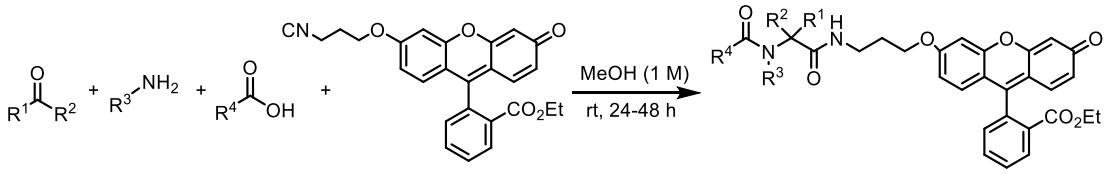
To a stirred solution of the aldehyde or ketone (0.7 mmol, 1.0 equiv) in DCM (0.7 mL), the carboxylic acid (0.7 mmol, 1.0 equiv) was added and the mixture was left to stir for 5 min at room temperature. This was followed by addition of the corresponding isocyanide (0.7 mmol, 1.0 equiv). The final reaction mixture was left under stirring overnight at room temperature. The solvent was removed under vacuum. Chromatographic separation with PE/EtOAc/DCM/MeOH as eluent solvents followed, giving the desired products (**5a-f**) in 40-72% yield. Product **5a** should be noted that was made on a 0.1 mmol scale, namely to a stirred solution of benzaldehyde (0.1 mmol, 1.0 equiv) in DCM (0.1 mL), (1S,2S)-2-phenylcyclopropane-1-carboxylic acid (0.1 mmol, 1.0 equiv) was added and the mixture was allowed to stir for 5 min at room temperature. This was followed by addition of isocyanide 3a (0.1 mmol, 1.0 equiv). The final reaction mixture was left to stir overnight at room temperature. The solvent was removed under vacuum. Chromatographic separation with PE/EtOAc/DCM/MeOH as eluent solvents gave the desired product **5a** in 40% yield, as a 50:50 mixture of diastereomers. Compound **5e** was accessed by hydrolysis of **5f**.[1]

General method for the UT-4CR



To a stirred solution of aldehyde (0.5-1.0 mmol, 1.0 equiv) in MeOH (0.5-1.0 mL), the amine (0.5-1 mmol, 1.0 equiv) was added and the mixture was allowed to stir for 20 min at room temperature. This was followed by addition of the corresponding isocyanide (0.5-1 mmol, 1.0 equiv) and trimethylsilyl azide (0.5-1 mmol, 1.0 equiv). The final reaction mixture was left under stirring for 24-36 h at room temperature. The solvent was removed under vacuum. Chromatographic separation followed with eluent solvents PE/EtOAc for the products **6a-c** and **6e-h**, while in product **6d** PE/EtOAc/DCM/MeOH, giving the desired products (**6a, 6c-h**) in 36-67% yield. In the case of **6b** in MeOH solvent, the reaction stuck at the imine intermediate, which was insoluble in it. For this reason, 3 drops of $HCCl_3$ were added and the temperature was raised to 45 °C.

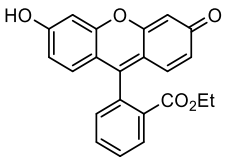
General method for the U-4CR



7a-d, 38-49%

To a stirred solution of aldehyde (0.3-0.7 mmol, 1.0 equiv) in MeOH (0.3-0.7 mL), the amine (0.3-0.5 mmol, 1.0 equiv) was added and the mixture was allowed to stir for 20 min at room temperature. This is followed by addition of the corresponding carboxylic acid (0.3-0.5 mmol, 1.0 equiv) and the isocyanide **3a** (0.3-0.5 mmol, 1.0 equiv). The final reaction mixture was left under stirring for 24-48 h at room temperature. The solvent was removed under vacuum. Chromatographic separation with PE/EtOAc/DCM/MeOH as eluent solvents followed, giving the desired products (**7a-d**) in 38-49% yield.

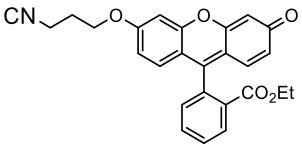
Ethyl 2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoate (2)



2, 87%

7838 mg, 87% yield, red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.71 (dtd, *J* = 16.4, 7.4, 1.0 Hz, 2H), 7.32 (d, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 9.2 Hz, 2H), 6.91 (s, 2H), 6.81 (d, *J* = 9.0 Hz, 2H), 4.00 (q, *J* = 7.1 Hz, 2H), 0.92 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 175.6, 165.3, 157. 9, 156.0, 134.2, 132.5, 131.3, 130.72, 130.6, 130.3, 129.9, 122.2, 115.0, 103.8, 61.5, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{22}H_{16}O_5$+H, calculated 361.1076; found 361.1068.

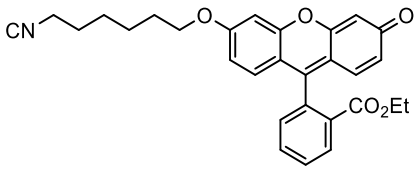
Ethyl 2-(6-(3-isocyanopropoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3a)



3a, 75%

4809 mg, 75% yield, orange powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.68 (dtd, *J* = 29.9, 7.6, 1.3 Hz, 2H), 7.28 (dd, *J* = 7.5, 1.1 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 21.7 Hz, 1H), 6.86 (d, *J* = 22.5 Hz, 1H), 6.72 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.50 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.41 (d, *J* = 1.9 Hz, 1H), 4.20 (t, *J* = 5.7 Hz, 2H), 4.08 – 3.92 (m, 2H), 3.63 (t, *J* = 6.3 Hz, 2H), 2.18 (tt, *J* = 5.9 Hz, 2H), 0.94 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 165.2, 162.6, 158.8, 157.2 (t, *J* = 4.8 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* = 6.3 Hz), 28.6, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{26}H_{21}NO_5$+H, calculated 428.1492; found 428.1490.

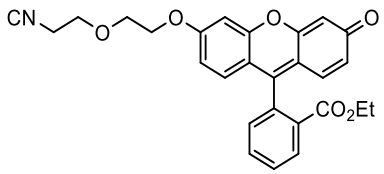
Ethyl 2-(6-((6-isocyanohexyl)oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3b)



3b, 72%

338 mg, 72% yield, red-brown powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.8 Hz, 1H), 7.69 (dtd, *J* = 29.2, 7.5, 1.2 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.88 (d, *J* = 13.8 Hz, 1H), 6.86 (d, *J* = 14.6 Hz, 1H), 6.72 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.53 (dd, *J* = 9.7, 1.5 Hz, 1H), 6.44 (d, *J* = 1.5 Hz, 1H), 4.07 (t, *J* = 6.3 Hz, 2H), 4.05 – 3.95 (m, 2H), 3.45 – 3.38 (m, 2H), 1.92 – 1.81 (m, 2H), 1.76 – 1.68 (m, 2H), 1.62 – 1.49 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 165.3, 163.5, 158.9, 155.9 (t, *J* = 5.6 Hz), 154.2, 150.2, 134.2, 132.5, 131.2, 130.7, 1304, 130.2, 129.8, 129.6, 129.0, 117.6, 114.8, 113.6, 105.7, 100.7, 68.5, 61.3, 41.4 (t, *J* = 6.4 Hz), 28.9, 28.7, 26.0, 25.2, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{29}H_{27}NO_5$+H, calculated 470.1962; found 470.1959.

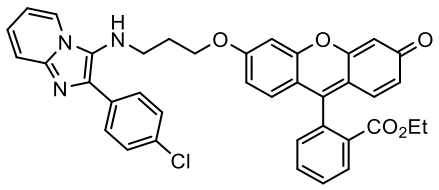
Ethyl 2-(6-(2-(2-isocyanoethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3c)



3c, 79%

1084 mg, 79% yield, yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.66 (dtd, *J* = 30.5, 7.6, 1.3 Hz, 2H), 7.26 (dd, *J* = 7.5, 1.1 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.85 (d, *J* = 18.5 Hz, 1H), 6.84 (d, *J* = 19.3 Hz, 1H), 6.74 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.47 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.38 (d, *J* = 1.9 Hz, 1H), 4.29 – 4.17 (m, 2H), 4.07 – 3.93 (m, 2H), 3.93 – 3.86 (m, 2H), 3.75 – 3.70 (m, 2H), 3.63 – 3.53 (m, 2H), 0.91 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.5, 165.2, 162.9, 158.8, 157.4 (t, *J* = 4.8 Hz), 153.9, 150.2, 134.0, 132.5, 131.1, 130.5, 130.2, 130.2, 129.7, 129.6, 128.9, 117.6, 115.0, 113.5, 105.5, 100.9, 69.4, 68.7, 67.9, 61.2, 41.6 (t, *J* = 6.9 Hz), 13.5. HRMS (ESI) m/z: $[M+H]^+$: $C_{27}H_{23}NO_6$+H, calculated 458.1604; found 458.1598.

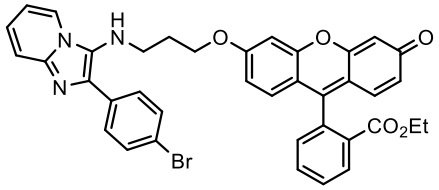
Ethyl 2-(6-(3-((2-(4-chlorophenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4a)



4a, 38%

171 mg, 38% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.04 (dt, *J* = 6.9, 1.1 Hz, 1H), 7.96 – 7.89 (m, 2H), 7.70 (dtd, *J* = 30.4, 7.6, 1.4 Hz, 2H), 7.53 (dt, *J* = 8.9, 0.9 Hz, 1H), 7.38 – 7.28 (m, 3H), 7.14 (ddd, *J* = 9.1, 6.7, 1.3 Hz, 1H), 6.91 (s, 1H), 6.89 (d, *J* = 19.2 Hz, 1H), 6.87 (d, *J* = 17.7 Hz, 1H), 6.77 (td, *J* = 6.8, 1.0 Hz, 1H), 6.67 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.53 (dd, *J* = 9.7, 2.0 Hz, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 4.19 (t, *J* = 5.9 Hz, 2H), 4.10 – 3.95 (m, 2H), 3.36 (t, *J* = 5.8 Hz, 1H), 3.27 (dt, *J* = 6.5 Hz, 2H), 2.08 (tt, *J* = 6.3 Hz, 2H), 0.97 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.8, 165.4, 163.1, 158.9, 154.2, 150.3, 141.8, 135.4, 134.2, 133.2, 132.8, 132.6, 131.3, 130.8, 130.4, 130.4, 130.0, 129.7, 129.1, 128.8, 128.4, 125.5, 124.4, 122.3, 117.8, 117.7, 115.2, 113.5, 112.1, 105.8, 100.8, 66.5, 61.4, 44.9, 29.7, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{38}H_{30}ClN_3O_5$+H, calculated 644.1947; found 644.1944.

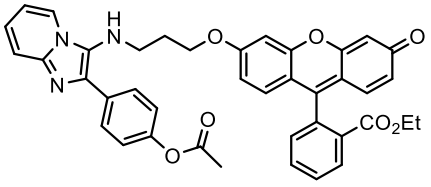
Ethyl 2-(6-(3-((2-(4-bromophenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4b)



4b, 40%

193 mg, 40% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.06 (dt, *J* = 6.8, 1.1 Hz, 1H), 7.90 – 7.84 (m, 2H), 7.71 (dtd, *J* = 30.3, 7.6, 1.4 Hz, 2H), 7.56 (dt, *J* = 9.1, 1.9 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.33 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.17 (ddd, *J* = 9.0, 6.7, 1.2 Hz, 1H), 6.95 – 6.86 (m, 2H), 6.81 (td, *J* = 6.8, 1.0 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.68 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.56 (dd, *J* = 9.7, 2.0 Hz, 1H), 6.48 (d, *J* = 1.9 Hz, 1H), 4.20 (t, *J* = 5.9 Hz, 2H), 4.09 – 3.98 (m, 2H), 3.33 – 3.24 (m, 2H), 2.09 (tt, *J* = 6.2 Hz, 2H), 0.98 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 165.2, 163.3, 159.1, 154.3, 151.1, 141.6, 135.0, 134.1, 133.0, 132.6, 131.6, 131.2, 130.5, 130.4, 130.3, 130.0, 129.7, 129.6, 129.1, 128.7, 125.8, 124.6, 122.4, 121.4, 117.6, 117.3, 113.7, 112.1, 105.6, 100.8, 66.4, 61.3, 44.8, 29.6, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{38}H_{30}BrN_3O_5$+H, calculated 688.1442; found 688.1443.

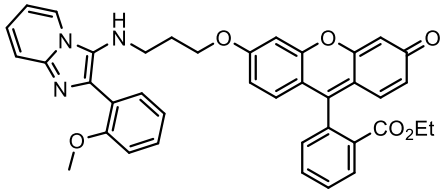
Ethyl 2-(6-(3-((2-(4-acetoxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4c)



4c, 48 %

224 mg, 48% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 6.8 Hz, 1H), 7.99 (d, *J* = 8.6 Hz, 2H), 7.70 (dtd, *J* = 29.8, 7.5, 1.1 Hz, 2H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.90 (d, *J* = 14.0 Hz, 1H), 6.88 (d, *J* = 14.8 Hz, 1H), 6.79 (t, *J* = 6.7 Hz, 1H), 6.71 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.55 (dd, *J* = 9.6, 1.9 Hz, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 4.20 (t, *J* = 5.8 Hz, 2H), 4.07 – 3.99 (m, 2H), 3.29 (t, *J* = 6.4 Hz, 2H), 2.29 (s, 3H), 2.12 – 2.06 (m, 2H), 0.97 (t, *J* = 7.1 Hz, 3H).^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.67, 169.41, 165.28, 163.22, 158.99, 154.25, 150.62, 150.04, 141.45, 135.23, 134.18, 132.56, 131.66, 131.20, 130.69, 130.39, 129.78, 129.68, 129.09, 128.13, 125.56, 124.41, 122.37, 121.75, 117.6, 117.4, 116.0, 115.0, 113.6, 112.1, 105.7, 100.8, 66.4, 61.3, 44.8, 29.7, 21.1, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{40}H_{33}N_3O_7$+H, calculated 668.2391; found 668.2387.

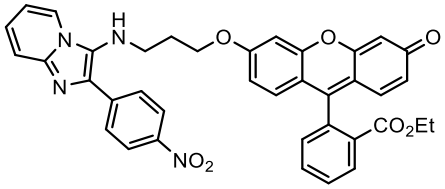
Ethyl 2-(6-(3-((2-(2-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4d)



4d, 67%

300 mg, 67% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 7.8, 1.1 Hz, 1H), 8.19 (d, *J* = 6.9 Hz, 1H), 7.79 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.75 – 7.61 (m, 3H), 7.33 (ddd, *J* = 8.3, 7.4, 1.7 Hz, 1H), 7.29 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.23 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.06 (td, *J* = 7.5, 0.7 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.90 – 6.82 (m, 3H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.62 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.53 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 4.07 – 3.92 (m, 4H), 3.84 (s, 3H), 3.08 (t, *J* = 6.4 Hz, 2H), 1.88 (tt, *J* = 6.3 Hz, 2H), 0.96 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 165.3, 163.3, 159.0, 156.1, 154.2, 150.7, 140.3, 134.2, 132.6, 131.5, 131.2, 130.7, 130.4, 130.4, 129.9, 129.8, 129.7, 129.0, 127.8, 125.6, 122.8, 121.7, 121.3, 117.6, 116.2, 115.0, 113.6, 112.9, 112.4, 111.9, 105.6, 100.7, 65.9, 61.3, 56.4, 44.1, 29.4, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{39}H_{33}N_3O_6$+H, calculated 640.2448; found 640.2442.

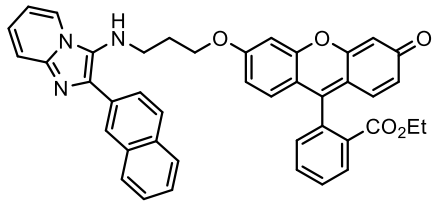
Ethyl 2-(6-(3-((2-(4-nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4e)



4e, 36%

165 mg, 36% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 – 8.19 (m, 3H), 8.16 – 8.13 (m, 2H), 8.10 – 8.02 (m, 1H), 7.70 (dtd, *J* = 36.3, 7.6, 1.2 Hz, 2H), 7.55 (d, *J* = 9.1 Hz, 1H), 7.34 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.18 (ddd, *J* = 8.9, 6.7, 1.0 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.89 (d, *J* = 22.3 Hz, 1H), 6.87 (d, *J* = 23.1 Hz, 1H), 6.81 (td, *J* = 6.8, 0.8 Hz, 1H), 6.68 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.51 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 4.29 – 4.18 (m, 1H), 4.06 – 3.98 (m, 2H), 3.34 – 3.29 (m, 2H), 2.14 (tt, *J* = 6.2 Hz, 2H), 0.97 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 165.3, 163.0, 159.0, 154.2, 150.5, 146.5, 142.1, 140.9, 134.2, 133.9, 132.6, 131.2, 130.7, 130.5, 130.4, 129.8, 129.7, 129.2, 127.4, 127.3, 125.1, 123.9, 122.5, 118.0, 117.8, 115.2, 113.6, 112.5, 105.7, 100.8, 66.5, 61.4, 45.1, 29.8, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{38}H_{30}CN_4O_7$+H, calculated 655.2193; found 655.2186.

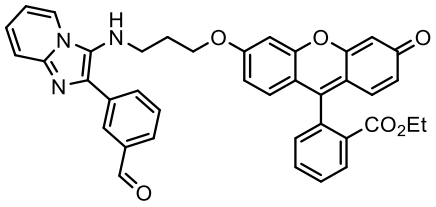
Ethyl 2-(6-(3-((2-(naphthalen-2-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4f)



4f, 62%

286 mg, 62% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.24 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.10 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.06 (ddd, *J* = 6.8, 1.5, 1.1 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.69 (dtd, *J* = 28.5, 7.6, 1.4 Hz, 2H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.44 – 7.36 (m, 3H), 7.28 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.14 (ddd, *J* = 8.9, 6.7, 1.2 Hz, 1H), 6.85 (d, *J* = 18.3 Hz, 1H), 6.83 (d, *J* = 17.6 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.77 (td, *J* = 6.7, 0.9 Hz, 1H), 6.60 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.54 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.46 (d, *J* = 1.9 Hz, 1H), 4.11 (t, *J* = 5.9 Hz, 2H), 4.04 – 3.92 (m, 2H), 3.29 (t, *J* = 6.3 Hz, 2H), 2.03 (tt, *J* = 6.1 Hz, 2H), 0.94 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 165.3, 163.2, 159.0, 154.2, 150.7, 141.7, 136.2, 134.2, 133.6, 132.7, 132.5, 131.5, 131.2, 130.7, 130.4, 130.4, 130.0, 129.7, 129.7, 129.0, 128.2, 127.6, 126.2, 126.1, 126.0, 125.9, 125., 124.3, 122.4, 117.6, 117.4, 115.0, 113.6, 112.0, 105.6, 100.7, 66.4, 61.3, 44.8, 29.6, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{42}H_{34}ClN_3O_5$+H, calculated 660.2498; found 660.2481.

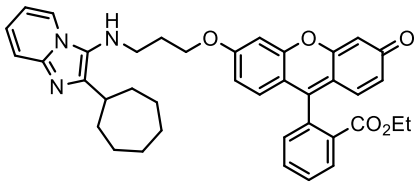
Ethyl 2-(6-(3-((2-(3-formylphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4g)



4g, 41%

183 mg, 41% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.55 (dd, *J* = 2.1, 1.5 Hz, 1H), 8.33 (ddd, *J* = 7.7, 1.8, 1.2 Hz, 1H), 8.25 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.11 (d, *J* = 6.8 Hz, 1H), 7.80 (ddd, *J* = 7.6, 1.7, 1.2 Hz, 1H), 7.70 (dtd, *J* = 31.0, 7.6, 1.3 Hz, 2H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.31 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.21 (ddd, *J* = 9.0, 6.7, 1.1 Hz, 1H), 6.92 – 6.80 (m, 4H), 6.68 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.55 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.46 (d, *J* = 1.9 Hz, 1H), 4.23 (t, *J* = 5.9 Hz, 2H), 4.10 – 3.90 (m, 2H), 3.33 (t, *J* = 6.6 Hz, 2H), 2.13 (tt, *J* = 6.3 Hz, 2H), 0.98 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 192.3, 185.8, 165.4, 163.1, 159.0, 154.2, 150.3, 141.7, 136.8, 134.3, 133.0, 132.6, 131.3, 130.8, 130.4, 130.4, 130.0, 129.7, 129.6, 129.5, 129.1, 128.8, 128.0, 127.9, 126.1, 125.1, 122.5, 117.8, 117.6, 115.2, 113.5, 112.5, 105.8, 100.9, 66.6, 61.4, 45.1, 29.8, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{39}H_{31}N_3O_6$+H, calculated 638.2286; found 638.2297.

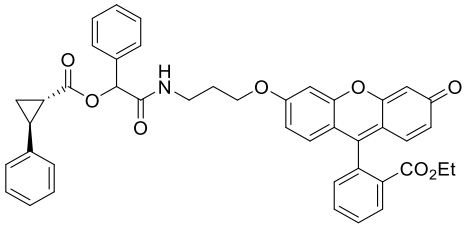
Ethyl 2-(6-(3-((2-cycloheptylimidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4h)



4h, 43%

190 mg, 43% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.8, 1.1 Hz, 1H), 8.07 (d, *J* = 6.7 Hz, 1H), 7.76 – 7.62 (m, 3H), 7.30 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.22 – 7.16 (m, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.90 (d, *J* = 19.2 Hz, 1H), 6.88 (d, *J* = 20.0 Hz, 1H), 6.82 (dd, *J* = 6.6 Hz, 1H), 6.74 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.55 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 4.28 (t, *J* = 5.9 Hz, 2H), 4.15 (t, *J* = 6.1 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.58 – 3.45 (m, 1H), 3.24 (t, *J* = 6.7 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.17 – 2.05 (m, 2H), 2.01 – 1.93 (m, 2H), 1.91 – 1.78 (m, 3H), 1.69 – 1.59 (m, 3H), 1.57 – 1.49 (m, 3H), 0.98 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.8, 165.3, 163.2, 163.2, 161.5, 159.0, 154.3, 150.5, 150.4, 134.3, 132.6, 131.3, 130.8, 130.4, 130.4, 130.0, 129.7, 129.1, 123.7, 122.5, 117.8, 115.2, 113.7, 105.8, 105.7, 100.9, 100.8, 66.5, 61.4, 45.9, 38.2, 34.9, 30.9, 29.9, 28.4, 27.8, 27.3, 26.4, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{39}H_{39}N_3O_5$+H, calculated 630.2968; found 630.2962.

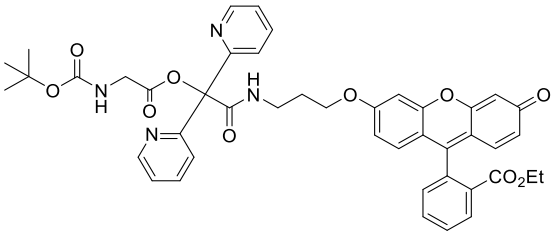
Ethyl 2-(3-oxo-6-(3-(2-phenyl-2-(((1S,2S)-2-phenylcyclopropane-1-carbonyl)oxy) acetamido)propoxy)-3*H*-xanthen-9-yl)benzoate (5a)



5a, 40%

190 mg, 40% yield, red solid. Mixture of diastereomers, ^{1}H NMR (500 MHz, $CDCl_3$), (50:50), δ 8.31 – 8.24 (m, 2H), 8.12 – 8.05 (m, 2H), 7.77 – 7.68 (m, 5H), 7.57 (d, *J* = 7.3 Hz, 2H), 7.48 – 7.27 (m, 14H), 7.25 – 7.21 (m, 1H), 7.08 – 7.03 (m, 2H), 6.98 – 6.90 (m, 5H), 6.77 – 6.67 (m, 7H), 6.29 (d, *J* = 2.5 Hz, 1H), 6.12 – 6.07 (m, 1H), 4.15 – 4.09 (m, 4H), 4.06 – 3.99 (m, 4H), 3.61 – 3.45 (m, 5H), 2.59 – 2.51 (m, 1H), 2.14 – 1.99 (m, 6H), 1.66 – 1.60 (m, 2H), 1.42 – 1.37 (m, 1H), 0.98 (td, *J* = 7.1, 2.6 Hz, 6H), 0.90 – 0.86 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.7, 165.2, 159.0, 135.4, 134.1, 133.7, 133.7, 132.6, 131.3, 130.7, 130.5, 130.3, 129.9, 129.2, 129.1, 129.0, 128.8, 128.8, 128.6, 128.6, 128.6, 128.5, 127.3, 127.2, 126.7, 126.7, 126.1, 126.1, 105.5, 100.9, 76.1, 66.9, 61.4, 36.7, 29.7, 27.0, 24.00, 17.6, 13.6. ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 172.0, 171.8, 168.8, 168.7, 168.7, 165.2, 165.2, 159.0, 154.6, 154.6, 139.5, 139.4, 135.6, 135.5, 134.1, 134.1, 133.7, 133.7, 132.6, 131.3, 130.7, 130.5, 130.4, 130.4, 129.9, 129.8, 129.2, 129.2, 129.2, 129.1, 129.1, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 127.3, 127.3, 126.8, 126.8, 126.2, 126.1, 117.7, 115.3, 114.3, 105.6, 100.9, 100.9, 76.1, 75.9, 75.9, 66.9, 66.8, 61.5, 36.8, 36.6, 29.7, 28.9, 28.9, 27.0, 27.0, 24.1, 24.1, 24.0, 17.8 17.8, 17.6, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{43}H_{37}N_1O_8$+H, calculated 696.2597; found 696.2589.

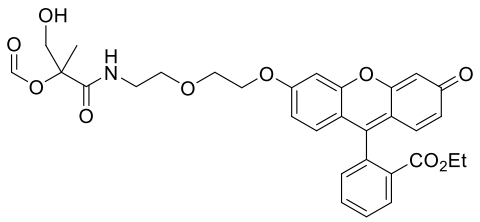
Ethyl 2-(6-((2,2-dimethyl-4,7,10-trioxo-9,9-di(pyridin-2-yl)-3,8-dioxa-5,11-diaza tetradecan-14-yl)oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5b)



5b, 42%

231 mg, 42% yield, red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.65 (d, *J* = 5.5 Hz, 1H), 10.64 (s, 1H), 8.43 (ddd, *J* = 4.9, 1.7, 1.1 Hz, 1H), 8.40 (d, *J* = 4.4 Hz, 1H), 8.24 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.74 – 7.64 (m, 6H), 7.29 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.17 (ddd, *J* = 7.0, 4.9, 1.2 Hz, 1H), 6.88 – 6.84 (m, 3H), 6.71 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.55 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 5.22 (t, *J* = 5.2 Hz, 1H), 4.18 (d, *J* = 5.9 Hz, 2H), 4.12 (t, *J* = 6.3 Hz, 2H), 4.06 – 3.99 (m, 2H), 3.63 – 3.57 (m, 2H), 2.10 (tt, *J* = 6.4 Hz, 2H), 1.43 (s, 9H), 0.96 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 169.3, 167.8, 165.3, 163.7, 159.0, 158.3, 156.0, 154.3, 150.8, 147.8, 147.8, 137.5, 134.2, 132.5, 131.2, 130.7, 130.4, 129.7, 129.6, 128.9, 122.9, 121.2, 117.4, 114.7, 114.1, 105.5, 100.6, 84.8, 80.1, 66.2, 61.3, 43.1, 36.2, 28.8, 28.3, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{44}H_{42}N_4O_{10}$+H, calculated 787.2979; found 787.2974.

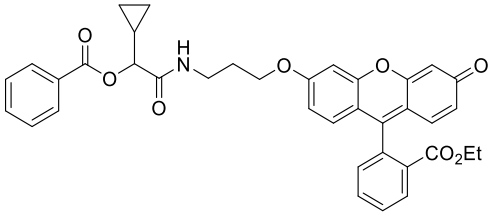
Ethyl 2-(6-(2-(2-(2-(formyloxy)-3-hydroxy-2-methylpropanamido)ethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5c)



5c, 56%

226 mg, 56% yield, orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.04 (d, *J* = 0.7 Hz, 1H), 7.70 (dtd, *J* = 28.8, 7.6, 1.2 Hz, 2H), 7.29 (dd, *J* = 7.6, 1.0 Hz, 2H), 7.01 (dd, *J* = 3.4, 2.8 Hz, 1H), 6.92 (dd, *J* = 9.0, 1.7 Hz, 1H), 6.88 (d, *J* = 9.7 Hz, 1H), 6.80 (ddd, *J* = 9.0, 2.3, 1.1 Hz, 1H), 6.56 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.49 (t, *J* = 1.7 Hz, 1H), 4.40 (d, *J* = 11.3 Hz, 1H), 4.27 – 4.22 (m, 3H), 4.06 – 3.99 (m, 2H), 3.89 – 3.84 (m, 2H), 3.63 (t, *J* = 5.2 Hz, 2H), 3.52 – 3.45 (m, 2H), 1.41 (d, *J* = 6.1 Hz, 3H), 0.98 (td, *J* = 7.1, 4.4 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.1, 173.5, 173.5, 165.3, 165.3, 163.7, 163.7, 161.0, 161.0, 159.1, 154.4, 151.6, 151.6, 134.2, 134.13, 132.6, 132.6, 131.2, 130.6, 130.6, 130.5, 130.5, 130.4, 129.8, 129., 129.4, 129.2, 117.6, 115.2, 115.1, 114.3, 114.2, 105.5, 101.0, 100.9, 77.3, 77.0, 76.8, 74.6, 74.5, 70.0, 70.0, 69.2, 69.2, 69.1, 69.1, 68.2, 61.5, 61.5, 39.0, 23.1, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{31}H_{31}NO_{10}$+H, calculated 578.2026; found 578.2021.

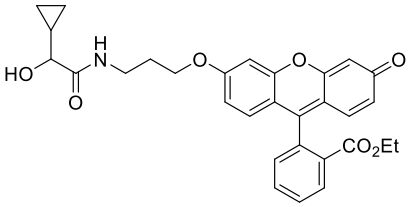
Ethyl 2-(6-(3-(2-(benzoyloxy)-2-cyclopropylacetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5d)



5d, 61%

265 mg, 61% yield, orange-red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.8 Hz, 1H), 8.04 (dd, *J* = 7.7, 2.1 Hz, 2H), 7.69 (dt, *J* = 26.3, 7.5 Hz, 2H), 7.51 (dd, *J* = 10.6, 4.1 Hz, 1H), 7.39 (td, *J* = 7.7, 3.5 Hz, 2H), 7.28 – 7.23 (m, 1H), 6.89 (d, *J* = 2.2 Hz, 1H), 6.88 – 6.80 (m, 3H), 6.67 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.55 (d, *J* = 9.6 Hz, 1H), 6.46 (s, 1H), 4.80 (dd, *J* = 8.3, 2.9 Hz, 1H), 4.17 – 4.08 (m, 2H), 4.04 – 3.94 (m, 2H), 3.56 – 3.47 (m, 2H), 2.06 (tt, *J* = 6.2 Hz, 2H), 1.45 – 1.35 (m, 1H), 0.94 (td, *J* = 7.1, 2.6 Hz, 3H), 0.74 – 0.65 (m, 1H), 0.65 – 0.58 (m, 2H), 0.55 – 0.47 (m, 1H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.1, 169.7, 165.7, 165.2, 163.6, 158.9, 154.3, 151.3, 134.1, 133.4, 133.4, 132.5, 131.2, 130.6, 130.4, 130.3, 129.7, 129.7, 129.4, 129.3, 129.0, 128.4, 117.4, 114.9, 113.9, 113.8, 105.4, 100.8, 77.9, 66.8, 66.8, 61.3, 36.3, 28.8, 13.6, 13.1, 3.1, 2.9. HRMS (ESI) m/z: $[M+Na]^+$: $C_{37}H_{34}NO_8$+Na, calculated 642.2104; found 642.2098.

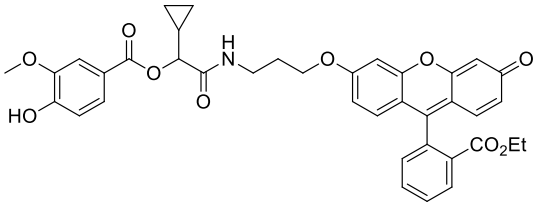
Ethyl 2-(6-(3-(2-cyclopropyl-2-hydroxyacetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5e)



5e, 52%

258 mg, 52% yield, red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.69 (dtd, *J* = 27.0, 7.5, 1.3 Hz, 2H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.07 (br q, *J* = 5.3 Hz, 1H), 6.97 – 6.93 (m, 1H), 6.90 (d, *J* = 15.1 Hz, 1H), 6.88 (d, *J* = 15.9 Hz, 1H), 6.75 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.52 (dd, *J* = 9.6, 1.7 Hz, 1H), 6.44 (dd, *J* = 2.0 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 4.06 – 3.96 (m, 2H), 3.63 (d, *J* = 7.4 Hz, 1H), 3.57 – 3.44 (m, 2H), 2.08 (tt, *J* = 6.2 Hz, 2H), 1.16 – 1.09 (m, 1H), 0.96 (t, *J* = 7.1 Hz, 3H), 0.62 – 0.52 (m, 2H), 0.51 – 0.45 (m, 1H), 0.45 – 0.37 (m, 1H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 174.0, 165.2, 163.5, 159.1, 154.3, 151.3, 134.1, 132.6, 131.2, 130.6, 130.5, 130.3, 129.7, 129.4, 129.1, 117.4, 115.1, 113.9, 113.9, 105.5, 100.9, 100.8, 74.1, 74.1, 66.9, 66.8, 61.4, 36.4, 36.3, 28.9, 28.9, 15.5, 13.6, 2.4, 1.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{30}H_{29}NO_7$+H, calculated 516.2022; found 516.2025.

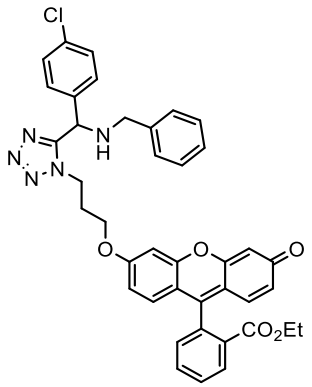
2-((3-((9-(2-(ethoxycarbonyl)phenyl)-3-oxo-3*H*-xanthen-6-yl)oxy)propyl)amino)-2-oxoethyl 4-hydroxy-3-methoxybenzoate (5f)



5f, 45%

280 mg, 45% yield, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 8.25 (d, *J*= 7.8 Hz, 1H), 7.74-7.71 (m, 1H), 7.68 (dd, J_1= 8.3Hz, J_2= 1.8 Hz, 2H), 7.58 (br s, 1H), 7.29 (d, *J*= 7.8 Hz, 2H), 6.95-6.88 (m, 4H), 6.75 (dd, J_1= 8.9 Hz, J_2= 2.4Hz, 1H), 6.59 (dd, J_1 = 9.7 Hz, J_2 = 1.9 Hz, 1H), 6.51 (br s, 1H), 4.15 (t, *J*= 5.9 Hz, 2H), 4.05-3.99 (m, 2H), 3.93 (s, 3H), 3.66 (t, *J*= 5.6 Hz, 1H), 3.54-3.50 (m, 2H), 2.09 (t, *J*= 6.2 Hz, 2H), 1.72 (quint, *J* = 5.8 Hz, 1H), 1.15-1.09 (m, 1H), 0.96 (t, *J* = 7.1 Hz, 3H), 0.64-0.40 (m, 4H) ^{13}C NMR (125 MHz, $CDCl_3$): 185.7, 173.7, 170.1, 165.3, 163.4, 161.6, 159.1, 154.3, 150.6, 146.2, 134.1, 132.6, 131.2, 130.6, 130.5, 130.3, 129.7, 129.6, 129.1, 124.9, 121.7, 117.5, 115.0, 114.1, 113.9, 112.1, 105.6, 100.9, 74.6, 66.8, 61.4, 56.1, 29.7, 28.9, 15.7, 13.6, 2.6, 1.9.

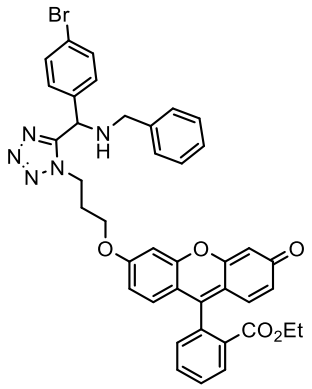
Ethyl 2-(6-(3-(5-((benzylamino)(4-chlorophenyl)methyl)-1*H*-tetrazol-1-yl) propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6a)



6a, 61%

427 mg, 61% yield, orange powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.8 Hz, 1H), 7.69 (dt, *J* = 28.4, 7.5 Hz, 2H), 7.37 – 7.14 (m, 11H), 6.91 – 6.79 (m, 2H), 6.76 (dd, *J* = 2.1 Hz, 1H), 6.55 (ddd, *J* = 8.9, 4.3, 2.5 Hz, 1H), 6.51 (dd, *J* = 9.7, 1.8 Hz, 1H), 6.42 (d, *J* = 1.8 Hz, 1H), 5.15 (s, 1H), 4.50 – 4.28 (m, 2H), 4.06 – 3.97 (m, 2H), 3.97 – 3.87 (m, 2H), 3.71 (dd, *J* = 13.2, 6.2 Hz, 1H), 3.66 (dd, *J* = 13.2, 7.7 Hz, 1H), 2.43 (br s, 1H), 2.22 (t, *J* = 6.3 Hz, 2H), 0.96 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 165.2, 162.3, 158.8, 155.2, 154.0, 150.0, 138.4, 135.9, 134.6, 134.2, 132.6, 131.2, 130.7, 130.4, 130.3, 130.0, 129.7, 129.3, 129.1, 128.8, 128.6, 128.6, 128.3, 128.2, 127.6, 117.9, 115.4, 113.0, 113.0, 105.8, 100.9, 64.8, 61.3, 55.4, 55.4, 51.2, 44.3, 28.6, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{40}H_{34}ClN_5O_5$+H, calculated 700.2321; found 700.2321.

Ethyl 2-(6-(3-(5-((benzylamino)(4-bromophenyl)methyl)-1*H*-tetrazol-1-yl) propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6b)

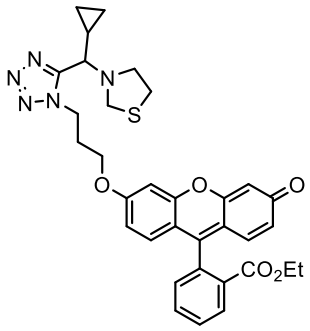


6b, 63%

235 mg, 63% yield, orange powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, *J* = 7.8 Hz, 1H), 7.67 (dt, *J* = 29.7, 7.5 Hz, 2H), 7.38 (dd, *J* = 8.1, 3.0 Hz, 2H), 7.30 – 7.14 (m, 8H), 6.85 (t, *J* = 9.6 Hz, 2H), 6.76 (dd, *J* = 2.7 Hz, 1H), 6.58 – 6.52 (m, 1H), 6.47 (d, *J* = 9.7 Hz, 1H), 6.40 (s, 1H), 5.13 (s, 1H), 4.47 – 4.30 (m, 2H), 4.04 – 3.95 (m, 2H), 3.94 – 3.84 (m, 2H), 3.68 (dd, *J* = 13.2, 5.8 Hz, 1H), 3.62 (dd, *J* = 13.2, 8.1 Hz, 1H), 2.49 (br s, 1H), 2.20 (tt, *J* = 6.0 Hz, 2H), 0.94 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.4, 165.0, 162.4, 158.7, 155.1, 153.9, 150.3, 138.3, 136.3, 133.9, 132.5, 132.0,

131.0, 130.4, 130.3, 130.2, 129.6, 129.1, 129.0, 128.4, 128.4, 128.4, 128.1, 128.1, 127.4, 122.5, 117.6, 115.1, 113.1, 105.5, 100.7, 77.3, 64.8, 61.2, 55.3, 55.3, 51.0, 44.2, 28.4, 13.5. HRMS (ESI) m/z: $[M+H]^+$: $C_{40}H_{34}BrN_5O_5$+H, calculated 744.1816; found 744.1820.

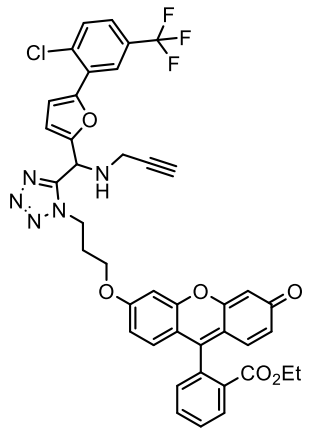
Ethyl 2-(6-(3-(5-(cyclopropyl(thiazolidin-3-yl)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6c)



6c, 52%

223 mg, 52% yield, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.4 Hz, 1H), 7.68 (dt, *J* = 28.5, 6.8 Hz, 2H), 7.28 (d, *J* = 6.8 Hz, 1H), 6.93 (d, *J* = 1.7 Hz, 1H), 6.87 (d, *J* = 16.6 Hz, 1H), 6.85 (d, *J* = 17.4 Hz, 1H), 6.72 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.51 (d, *J* = 9.7 Hz, 1H), 6.42 (s, 1H), 4.43 (d, *J* = 9.4 Hz, 1H), 4.14 (t, *J* = 5.7 Hz, 2H), 4.04 – 3.97 (m, 2H), 3.94 (d, *J* = 9.5 Hz, 1H), 3.54 – 3.46 (m, 2H), 3.27 (dd, *J* = 12.3, 6.7 Hz, 1H), 3.05 (dt, *J* = 12.1, 5.9 Hz, 1H), 2.91 – 2.84 (m, 1H), 2.84 – 2.67 (m, 1H), 2.19 (d, *J* = 9.3 Hz, 1H), 2.14 – 1.97 (m, 2H), 0.95 (t, *J* = 7.1 Hz, 3H), 0.87 – 0.75 (m, 1H), 0.69 – 0.60 (m, 1H), 0.58 – 0.52 (m, 1H), 0.52 – 0.40 (m, 2H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 173.0, 165.3, 163.2, 158.9, 154.2, 134.1, 132.5, 131.1, 130.7, 130.3, 129.8, 129.7, 129.0, 117.7, 115.0, 113.6, 113.6, 105.7, 100.7, 100.7, 69.6, 66.9, 61.3, 57.2, 55.3, 36.4, 29.6, 29.1, 14.2, 13.6, 6.1, 1.4. HRMS (ESI) m/z: $[M+H]^+$: $C_{33}H_{33}N_5O_5S$+H, calculated 612.2281; found 612.2273.

Ethyl 2-(6-(3-(5-((5-(2-chloro-5-(trifluoromethyl)phenyl)furan-2-yl)(prop-2-yn-1-ylamino)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6d)

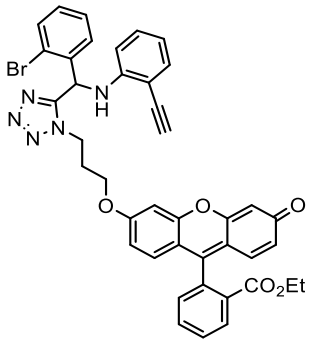


6d, 31%

242 mg, 31% yield, orange-red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.25 (td, *J* = 7.9, 1.1 Hz, 1H), 7.81 – 7.59 (m, 4H), 7.30 (d, *J* = 7.4 Hz, 2H), 6.98 – 6.88 (m, 4H), 6.85 (d, *J* = 1.9 Hz, 1H), 6.76 (dd, *J* = 9.3, 2.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 2.4 Hz, 1H),

6.58 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.49 (d, *J* = 1.9 Hz, 1H), 4.70 (t, *J* = 6.7 Hz, 2H), 4.13 (t, *J* = 5.6 Hz, 2H), 4.04 (ddd, *J* = 16.8, 9.0, 5.3 Hz, 2H), 3.99 (dd, *J* = 14.3, 7.2 Hz, 2H), 2.52 (tt, *J* = 6.2 Hz, 2H), 0.99 (t, *J* = 7.1 Hz, 1H), 0.91 (t, *J* = 7.1 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) δ 185.7, 165.3, 165.2, 162.8, 159.2, 157.6, 155.1, 154.3, 151.7, 142.9, 134.2, 134.0, 132.7, 132.5, 131.3, 131.2, 130.7, 130.6, 130.5, 130.3, 130.3, 129.9, 129.8, 129.6, 129.4, 122.0, 117.8, 115.5, 115.1, 113.6, 105.6, 103.8, 101.0, 65.0, 61.4, 61.4, 45.2, 29.8, 13.7, 13.5. HRMS (ESI) m/z: $[M+H]^+$: $C_{41}H_{31}ClF_3N_5O_6$+H, calculated 782.1988; found 782.1984.

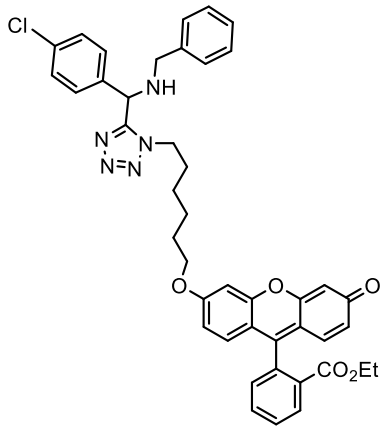
Ethyl 2-(6-(3-(5-((2-bromophenyl)((2-ethynylphenyl)amino)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6e)



6e, 67 %

506 mg, 67% yield, brown-red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.77 – 7.64 (m, 2H), 7.55 (dt, *J* = 8.8, 1.5 Hz, 2H), 7.36 – 7.27 (m, 2H), 7.22 – 7.16 (m, 1H), 7.10 – 7.01 (m, 1H), 6.88 – 6.75 (m, 3H), 6.67 – 6.52 (m, 3H), 6.49 – 6.40 (m, 2H), 6.34 – 6.28 (m, 1H), 5.68 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.79 – 4.67 (m, 2H), 4.11 – 3.96 (m, 4H), 3.43 (d, *J* = 4.7 Hz, 1H), 2.47 (tt, *J* = 6.0 Hz, 2H), 0.97 (td, *J* = 7.1, 1.3 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) δ 185.8, 185.78, 165.3, 162.3, 158.8, 154.9, 154.0, 153.9, 149.92, 149.9, 146.1, 135.9, 135.8, 134.2, 134.2, 133.1, 133.0, 133.0, 132.6, 131.29, 131.26, 130.8, 130.6, 130.5, 130.4, 130.3, 130.3, 130.1, 130.08, 129.7, 129.2, 129.04, 129.01, 129.0, 128.84, 128.82, 123.2, 118.7, 118.5, 118.0, 115.43, 115.37, 113.3, 112.7, 110.8, 110.7, 108.1, 108.0, 105.90, 105.88, 101.3, 100.9, 84.3, 84.2, 79.7, 79.6, 64.61, 64.57, 61.4, 51.31, 51.27, 44.68, 44.66, 29.1, 29.0, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{41}H_{32}BrN_5O_5$+H, calculated 754.1665; found 754.1661.

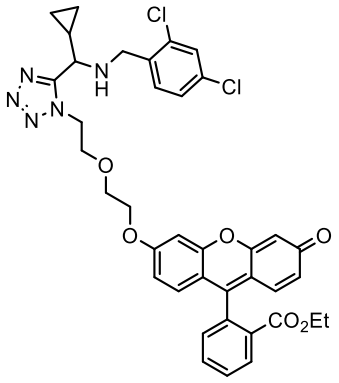
Ethyl 2-(6-((6-(5-((benzylamino)(4-chlorophenyl)methyl)-1*H*-tetrazol-1-yl)hexyl) oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6f)



6f, 53%

275 mg, 53% yield, orange powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.69 (dtd, *J* = 29.0, 7.5, 1.3 Hz, 2H), 7.41 – 7.22 (m, 10H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 14.1 Hz, 1H), 6.86 (d, *J* = 14.8 Hz, 1H), 6.70 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.53 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 5.12 (s, 1H), 4.21 – 4.06 (m, 2H), 4.06 – 3.92 (m, 4H), 3.77 (d, *J* = 13.3 Hz, 1H), 3.72 (d, *J* = 13.3 Hz, 1H), 2.49 (br s, 1H), 1.69 (td, *J* = 13.5, 6.7 Hz, 2H), 1.65 – 1.53 (m, 2H), 1.35 (dd, *J* = 14.9, 7.0 Hz, 2H), 1.22 – 1.12 (m, 2H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 165.3, 163.5, 158.9, 154.9, 154.2, 150.3, 138.5, 136.2, 134.6, 134.2, 132.5, 131.2, 130.7, 130.9, 130.3, 129.8, 129.6, 129.3, 129.0, 128.9, 128.6, 128.3, 127.6, 117.6, 114.9, 113.7, 105.7, 100.7, 68.4, 61.3, 55.3, 51.2, 47.3, 29.1, 28.6, 26.0, 25.3, 13.6. HRMS (ESI) m/z: [M+H]$^{+}$: $C_{43}H_{40}ClN_5O_5$+H, calculated 742.2791; found 742.2789.

Ethyl 2-(6-(2-(2-(5-(cyclopropyl((2,4-dichlorobenzyl)amino)methyl)-1*H*-tetrazol-1-yl)ethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6g)

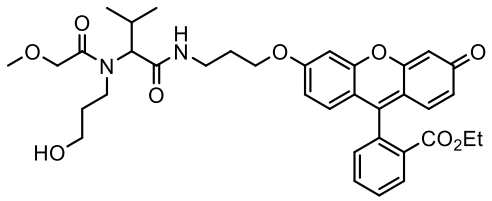


6g, 71%

237 mg, 71% yield, orange powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.7 Hz, 1H), 7.66 (dtd, *J* = 33.1, 7.6, 1.2 Hz, 2H), 7.31 – 7.19 (m, 2H), 7.14 (d, *J* = 8.2 Hz, 1H), 7.08 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.85 – 6.76 (m, 3H), 6.65 – 6.57 (m, 1H), 6.49 – 6.44 (m, 1H), 6.40 – 6.36 (m, 1H), 4.83 – 4.60 (m, 2H), 4.05 – 3.89 (m, 6H), 3.75 (s, 2H), 3.68 (dd, *J* = 14.0, 2.3 Hz, 1H), 3.57 (dd, *J* = 14.0, 1.0 Hz, 1H), 3.49 (dd, *J* = 9.1, 1.2 Hz, 1H), 2.17 (br s, 1H), 1.37 – 1.28 (m, 1H), 0.91 (td, *J* = 7.1, 2.2 Hz, 3H), 0.56 (s, 1H), 0.42 – 0.33 (m, 1H), 0.29 – 0.15 (m, 2H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.5,

165.1, 162.6, 158.7, 156.0, 153.9, 150.0, 135.1, 134.1, 134.0, 133.5, 132.5, 131.1, 130.9, 130.9, 130.5, 130.2, 130.2, 129.7, 129.6, 129.1, 128.9, 127.0, 117.6, 115.0, 113.2, 113.2, 105.8, 100.6, 100.6, 69.2, 69.1, 67.7, 61.2, 57.8, 48.3, 47.5, 15.0, 13.5, 4.8, 2.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{38}H_{35}Cl_2N_5O_6$+H, calculated 728.2043; found 728.2036.

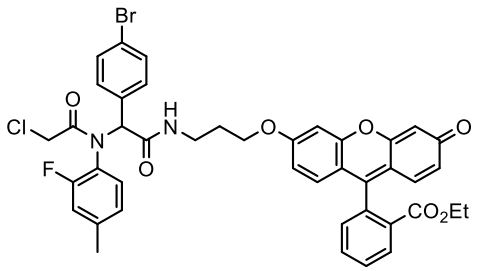
Ethyl 2-(6-(3-(2-(*N*-(3-hydroxypropyl)-2-methoxyacetamido)-3-methyl butanamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7a)



7a, 38%

123 mg, 38% yield, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 1.7 Hz, 1H), 7.83 – 7.57 (m, 2H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.01 – 6.84 (m, 3H), 6.80 – 6.68 (m, 1H), 6.54 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.45 (d, *J* = 1.8 Hz, 1H), 4.68 (s, 1H), 4.42 (d, *J* = 12.3 Hz, 1H), 4.24 – 3.96 (m, 7H), 3.94 (dd, *J* = 12.3, 1.9 Hz, 1H), 3.80 – 3.66 (m, 1H), 3.60 – 3.51 (m, 2H), 3.52 – 3.38 (m, 7H), 3.34 – 3.10 (m, 1H), 2.69 – 2.51 (m, 1H), 2.51 – 2.36 (m, 1H), 2.10 – 1.97 (m, 2H), 1.85 – 1.66 (m, 2H), 1.08 – 0.91 (m, 6H), 0.86 (d, *J* = 6.6 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.8, 171.6, 171.2, 169.8, 169.6, 165.4, 165.4, 163.3, 163.0, 159.0, 158.9, 154.3, 154.2, 150.3, 150.1, 134.3, 134.3, 132.6, 131.3, 130.8, 130.5, 130.3, 130.0, 130.0, 129.8, 129.7, 129.7, 129.1, 129.0, 117.9, 117.8, 115.2, 115.1, 113.9, 113.8, 113.5, 113.4, 105.9, 105.8, 102.3, 100.9, 100.8, 100.8, 100.7, 74.0, 71.4, 66.8, 66.5, 66.4, 61.4, 61.4, 59.4, 59.3, 59.3, 59.2, 38.9, 36.7, 36.4, 36.4, 31.9, 31.9, 31.3, 29.0, 28.9, 26.7, 26.3, 26.2, 20.0, 19.8, 19.1, 18.7, 13.7. HRMS (ESI) m/z: $[M+H]^+$: $C_{36}H_{42}N_2O_9$+H, calculated 647.2969; found 647.2968.

Ethyl 2-(6-(3-(2-(4-bromophenyl)-2-(2-chloro-*N*-(2-fluoro-4-methylphenyl) acetamido)acetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7b)

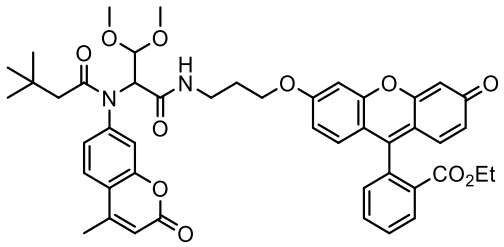


7b, 49%

279 mg, 49% yield, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.8 Hz, 1H), 7.76 – 7.58 (m, 3H), 7.27 (ddd, *J* = 7.5, 1.9, 1.3 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.00 (dd, *J* = 8.2, 1.3 Hz, 2H), 6.92 – 6.79 (m, 4H), 6.69 – 6.65 (m, 1H), 6.63 (dd, *J* = 8.9, 2.2 Hz, 2H), 6.49 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.40 (dd, *J* = 1.8, 1.0 Hz, 1H), 6.00 (d, *J* = 1.7 Hz, 1H), 4.11 (dt, *J* = 9.5, 6.1 Hz, 1H), 4.07 – 3.96 (m, 3H), 3.83 (s, 2H), 3.62 – 3.51 (m, 1H), 3.51 – 3.36 (m, 1H), 2.27 (s, 3H), 2.13 – 1.96 (m, 2H), 0.94 (td, *J* = 7.1, 0.9 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.6, 169.4, 167.1, 165.3, 163.3, 159.0,

154.2, 150.4, 142.5, 142.4 (d, *J* = 7.5 Hz), 134.3 (d, *J* = 2.9 Hz), 132.5, 132.3, 132.0, 131.8, 131.6, 131.4, 131.2, 130.9, 130.8, 130.4 (d, *J* = 10.6 Hz), 129.8, 129.6, 129.0, 125.6, 123.2, 122.7 (d, *J* = 12.5 Hz), 117.6, 116.6 (d, *J* = 20.0 Hz), 115.0, 113.6, 105.7, 101.0, 66.5, 64.6, 61.3, 42.4, 36.9, 28.8, 21.2, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{42}H_{35}BrClFN_2O_7$+H, calculated 813.1378; found 813.1375.

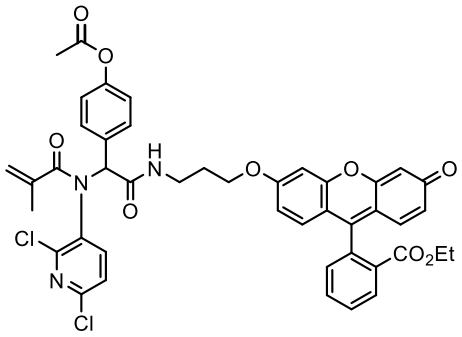
Ethyl 2-(6-(3-(2-(3,3-dimethyl-*N*-(4-methyl-2-oxo-2*H*-chromen-7-yl)butanamido)-3,3-dimethoxypropanamido)propoxy)-3*H*-xanthen-9-yl)benzoate (7c)



7c, 43%

104 mg, 43% yield, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 7.9 Hz, 1H), 8.09 (s, 1H), 7.69 (dtd, *J* = 28.3, 7.5, 1.2 Hz, 3H), 7.29 (d, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 2H), 6.90 – 6.85 (m, 3H), 6.74 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.54 (dd, *J* = 9.7, 1.7 Hz, 1H), 6.44 (d, *J* = 1.7 Hz, 1H), 6.37 (t, *J* = 5.8 Hz, 1H), 5.20 (d, *J* = 4.3 Hz, 1H), 4.62 (d, *J* = 4.3 Hz, 1H), 4.12 (t, *J* = 6.1 Hz, 2H), 4.07 – 3.98 (m, 2H), 3.50 (dt, *J* = 12.5, 4.9 Hz, 2H), 3.44 (s, 3H), 3.43 (s, 3H), 2.34 (d, *J* = 13.6 Hz, 1H), 2.31 (d, *J* = 13.6 Hz, 1H), 2.06 (tt, *J* = 6.3 Hz, 2H), 1.25 (s, 3H), 1.05 (s, 9H), 0.96 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 185.7, 170.9, 167.0, 165.3, 163.3, 158.9, 154.2, 150.4, 141.1, 134.2, 132.5, 131.2, 130.7, 130.4, 130.3, 129.9, 129.7, 129.5, 129.0, 128.7, 127.0, 123.5, 119.1, 118.2, 117.7, 115.0, 113.7, 105.7, 103.6, 100.8, 72.2, 66.5, 61.3, 56.3, 55.8, 47.6, 36.4, 31.0, 29.7, 29.5, 28.8, 17.9, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{46}H_{48}N_2O_{11}$+H, calculated 805.3336; found 805.3335.

Ethyl 2-(6-(3-(2-(4-acetoxyphenyl)-2-(*N*-(2,6-dichloropyridin-3-yl) methacrylamido)acetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7d)



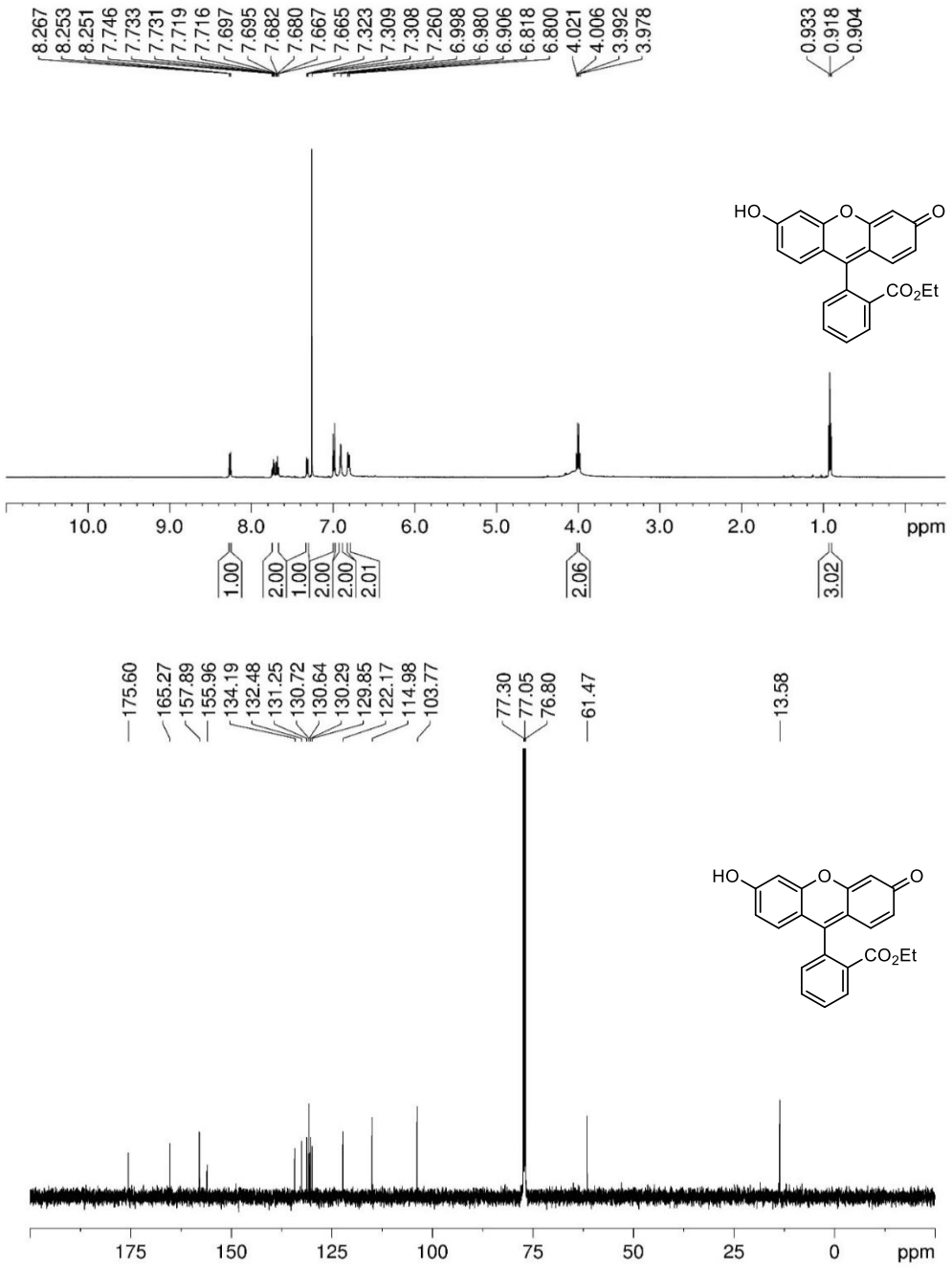
7d, 38%

94 mg, 38% yield, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 7.8 Hz, 1H), 7.69 (dtd, *J* = 26.5, 7.5, 1.0 Hz, 3H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 6.8 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 2H), 6.91 – 6.84 (m, 6H), 6.71 (d, *J* = 8.9 Hz, 1H), 6.54 (dd, *J* = 9.7, 1.8 Hz, 1H), 6.44 (s, 1H), 6.03 (s, 1H), 4.07 (t, *J* = 6.0 Hz, 2H), 4.03 – 3.98 (m, 2H), 3.59 – 3.39 (m, 2H), 2.26 (s, 3H), 2.15 (s, 3H), 2.05 (tt, *J* = 6.8 Hz, 2H), 0.97 (td,

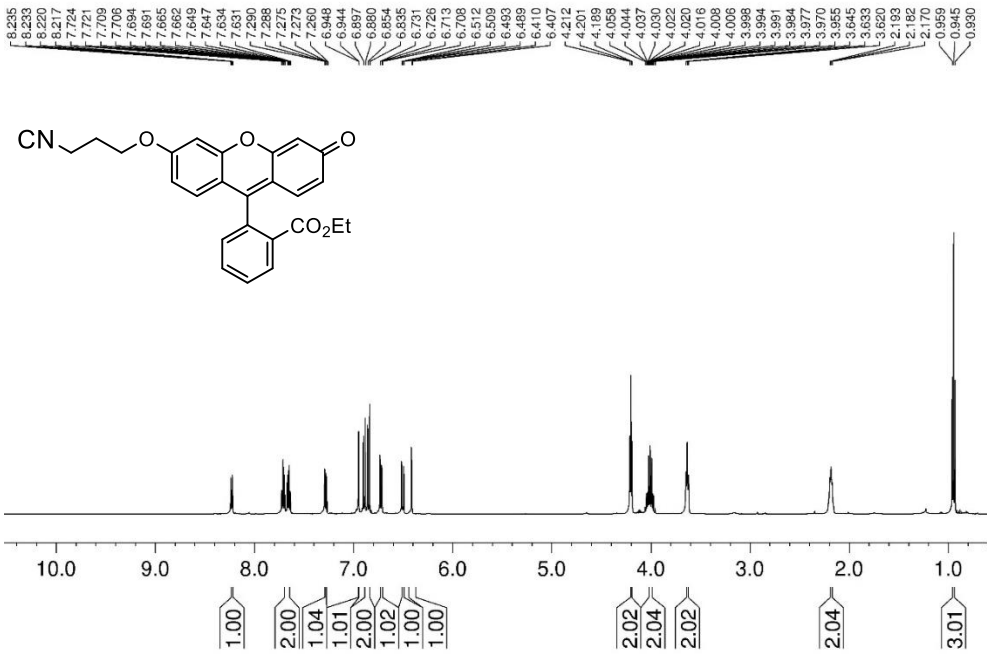
J = 7.1, 2.8 Hz, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) δ 185.5, 169.4, 169.2, 168.5, 165.3, 163.4, 159.0, 154.3, 151.1, 150.9, 150.8, 134.2, 133.0, 132.6, 131.2, 130.7, 130.4, 130.4, 129.7, 129.7, 129.1, 128.6, 122.0, 117.6, 115.1, 113.7, 113.7, 105.6, 100.9, 100.9, 77.3, 77.0, 76.8, 75.0, 66.6, 66.6, 61.4, 36.6, 36.6, 28.8, 21.1, 21.0, 13.6. HRMS (ESI) m/z: $[M+H]^+$: $C_{44}H_{37}Cl_2N_3O_9$+H, calculated 822.1985; found 822.1942.

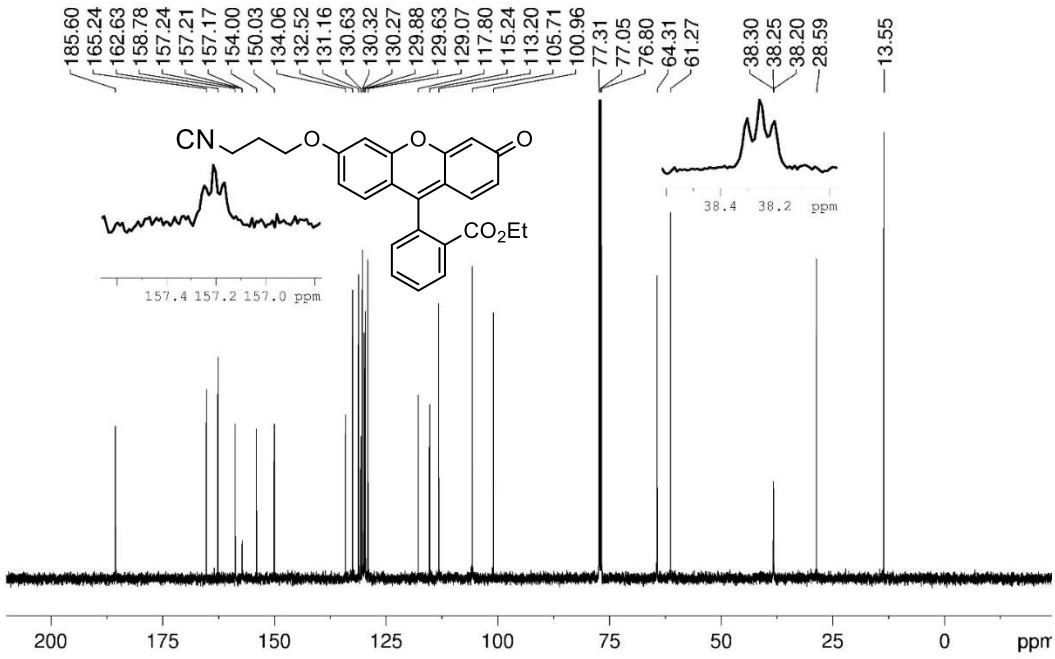
3. Exemplary copies of NMR spectra of novel compounds

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

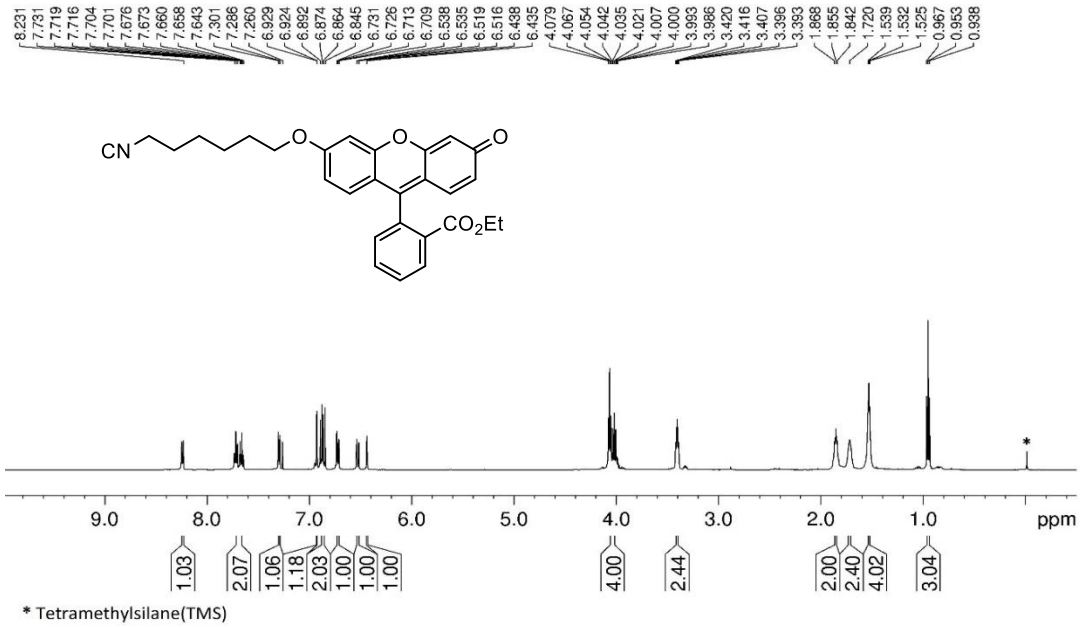


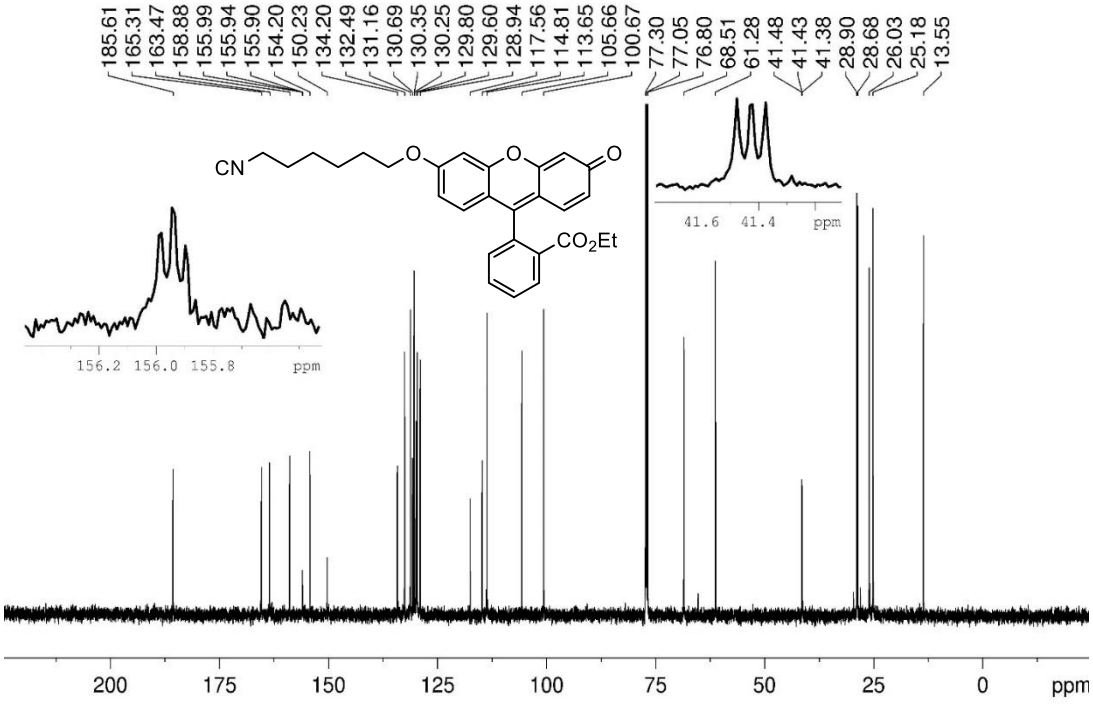
Ethyl 2-(6-(3-isocyanopropoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3a)



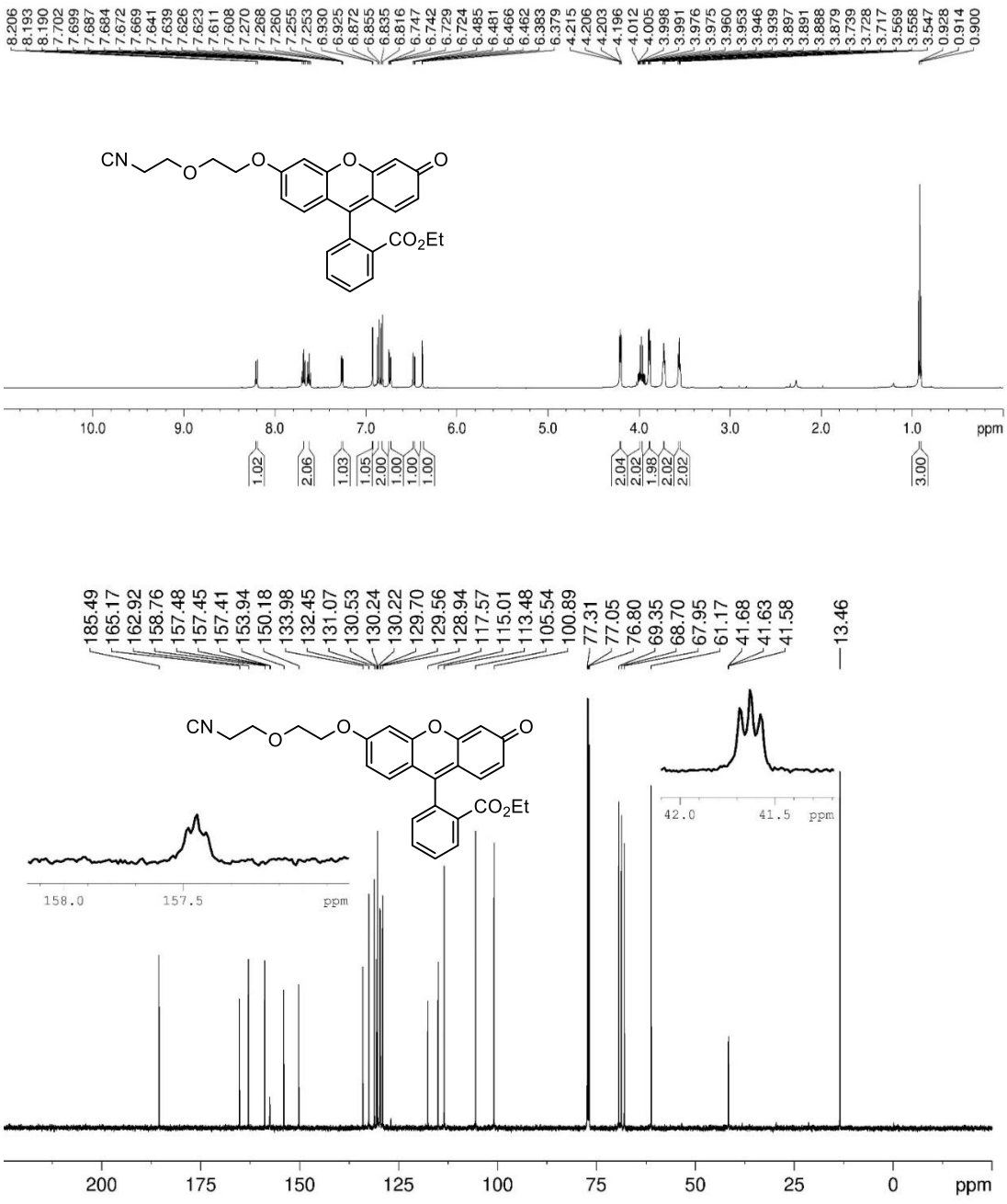


Ethyl 2-(6-((6-isocyanohexyl)oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3b)

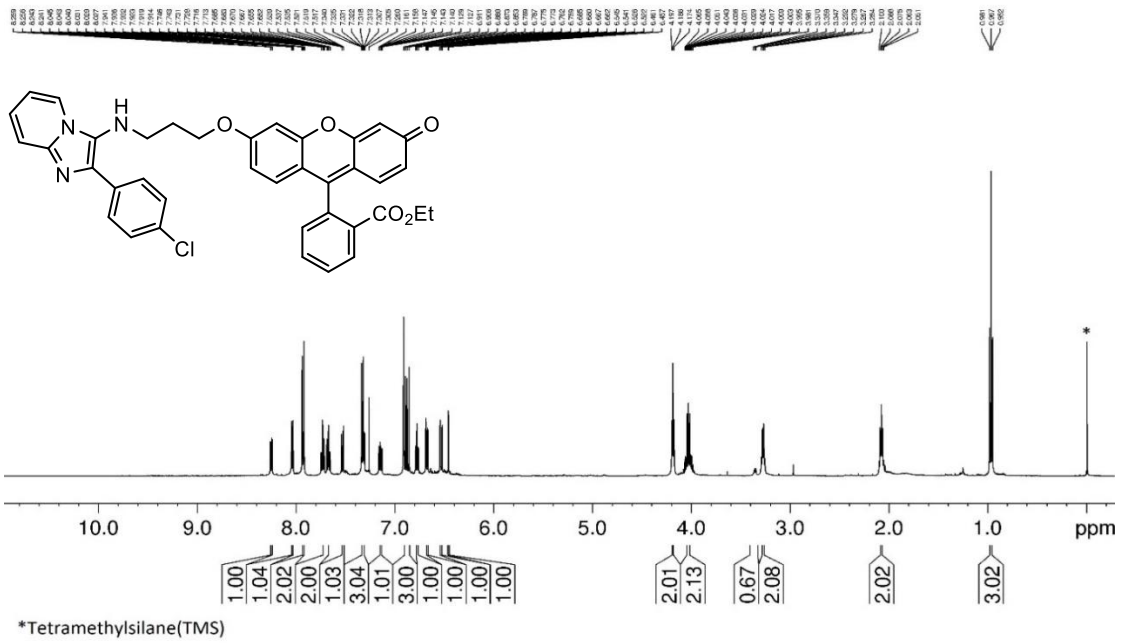




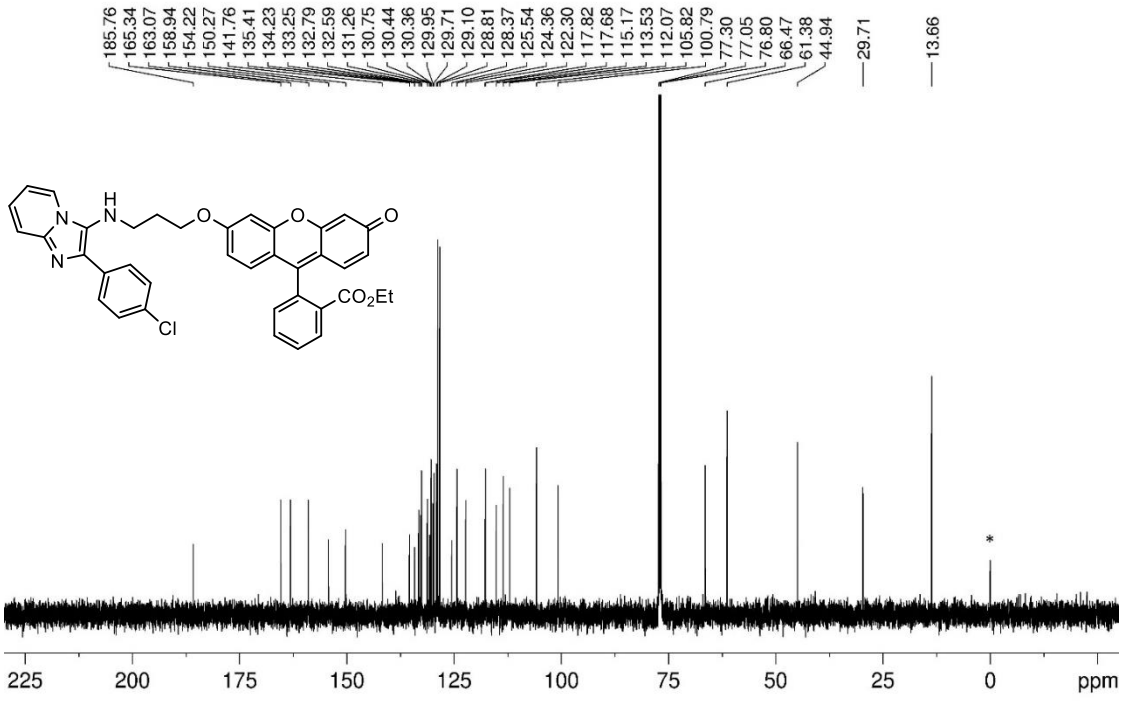
Ethyl 2-(6-(2-(2-isocyanoethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (3c)



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

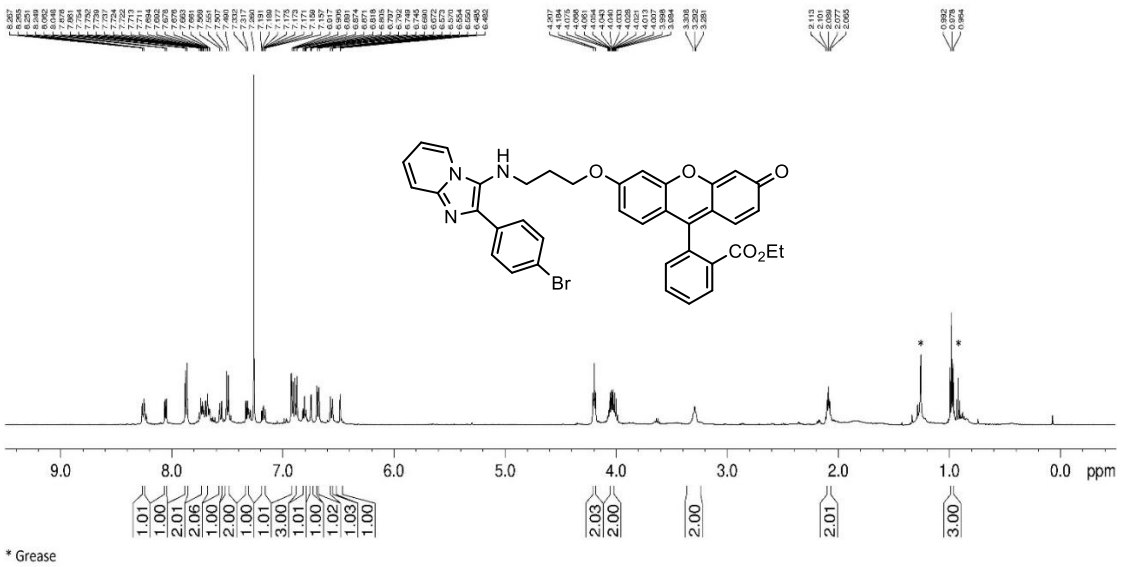


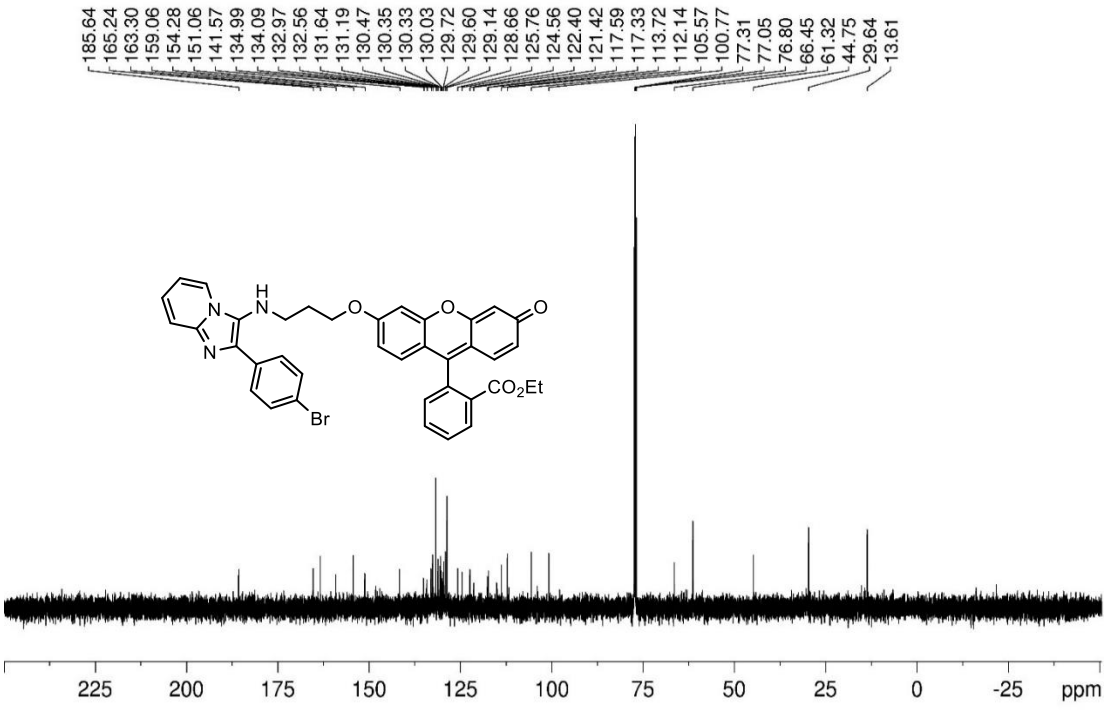
*Tetramethylsilane(TMS)



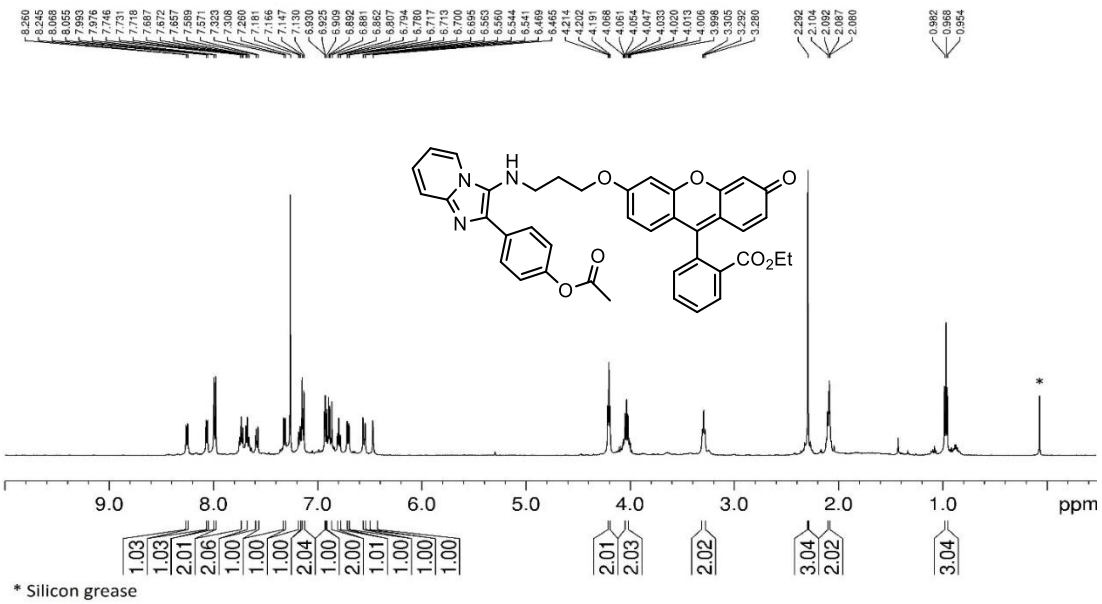
*Tetramethylsilane(TMS)

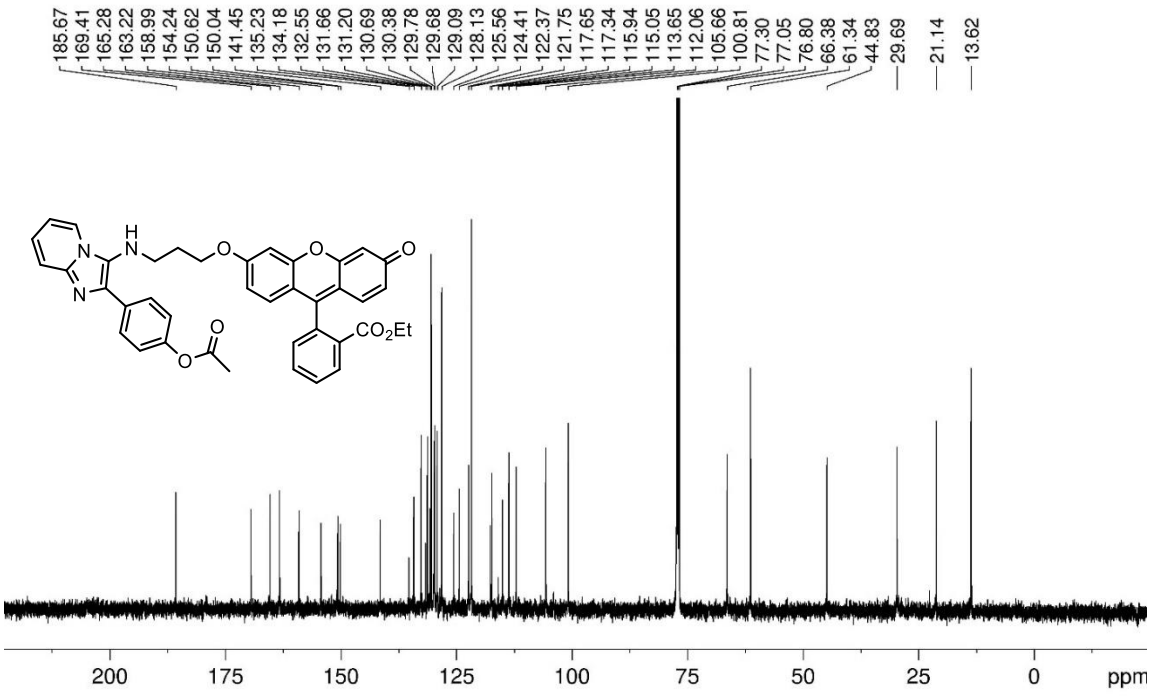
Ethyl 2-(6-(3-((2-(4-bromophenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4b)



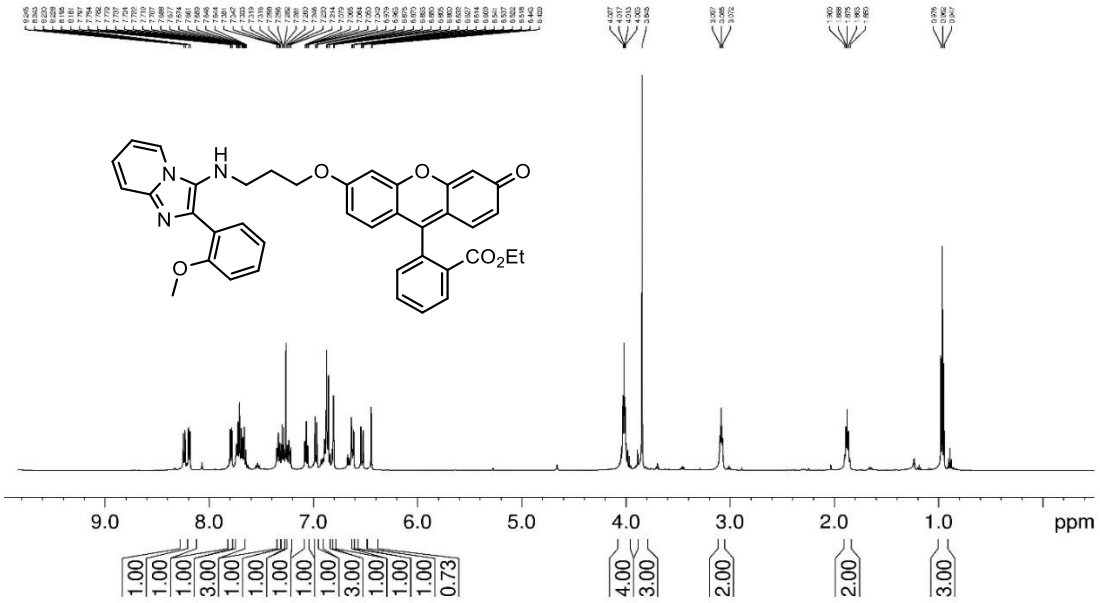


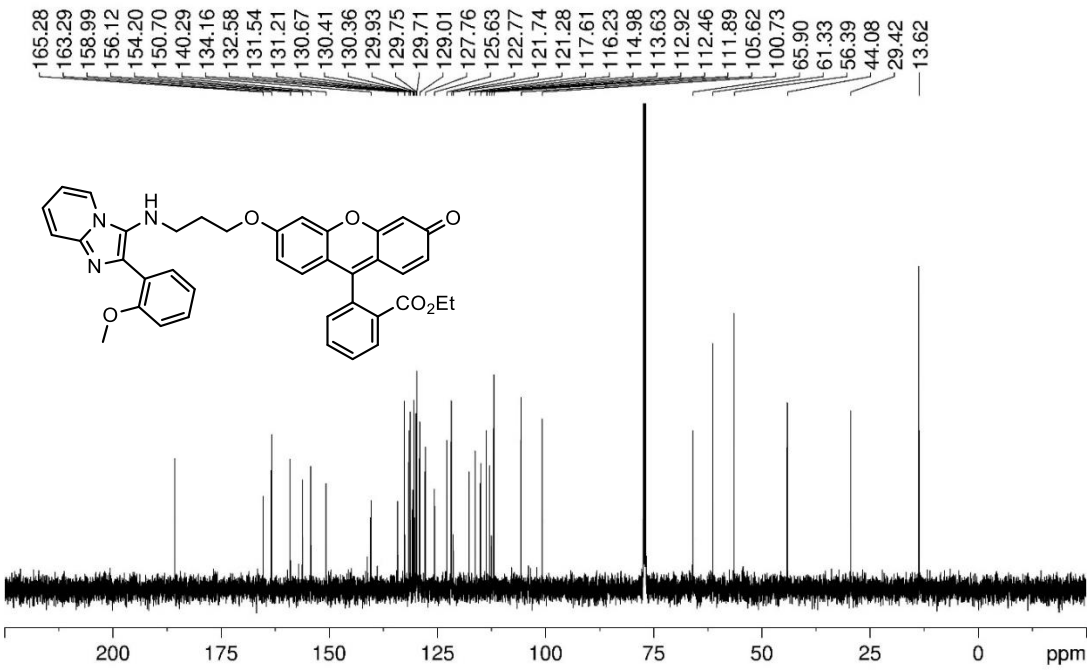
Ethyl 2-(6-(3-((2-(4-acetoxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4c)



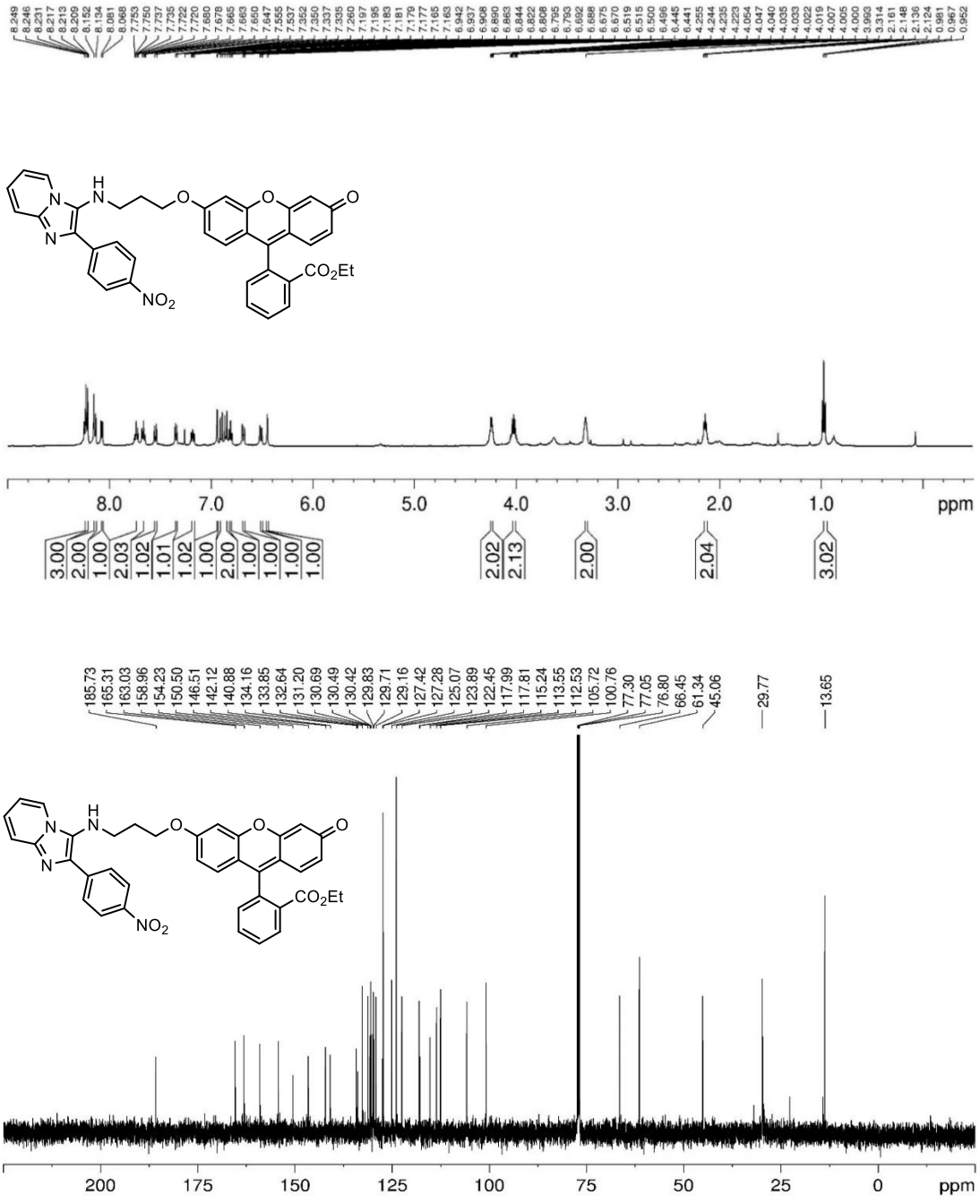


Ethyl 2-(6-(3-((2-(2-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4d)

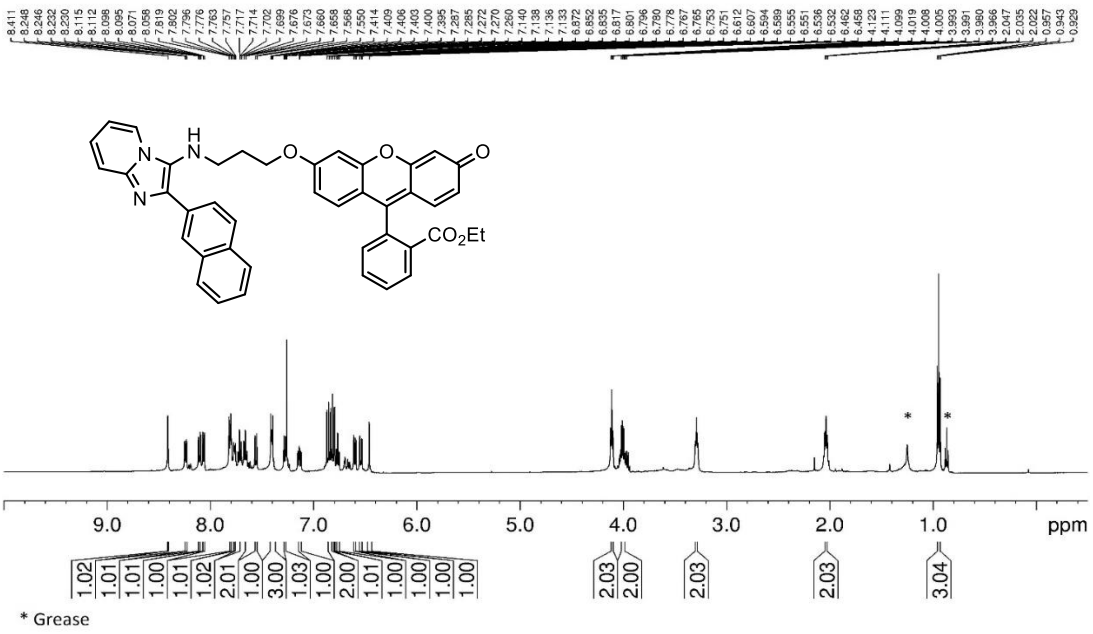


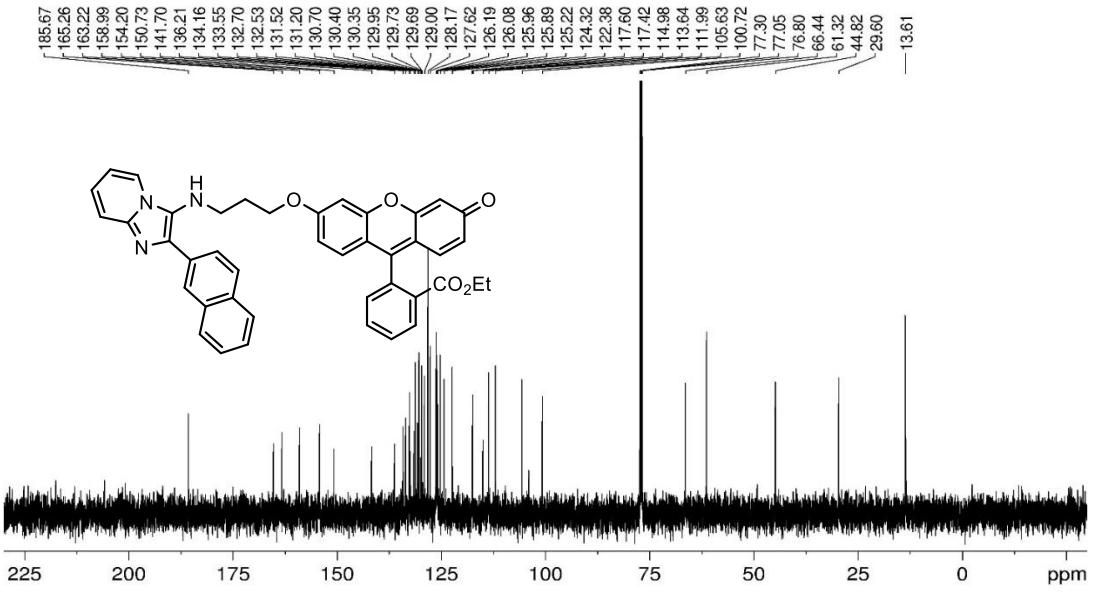


Ethyl 2-(6-(3-((2-(4-nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4e)

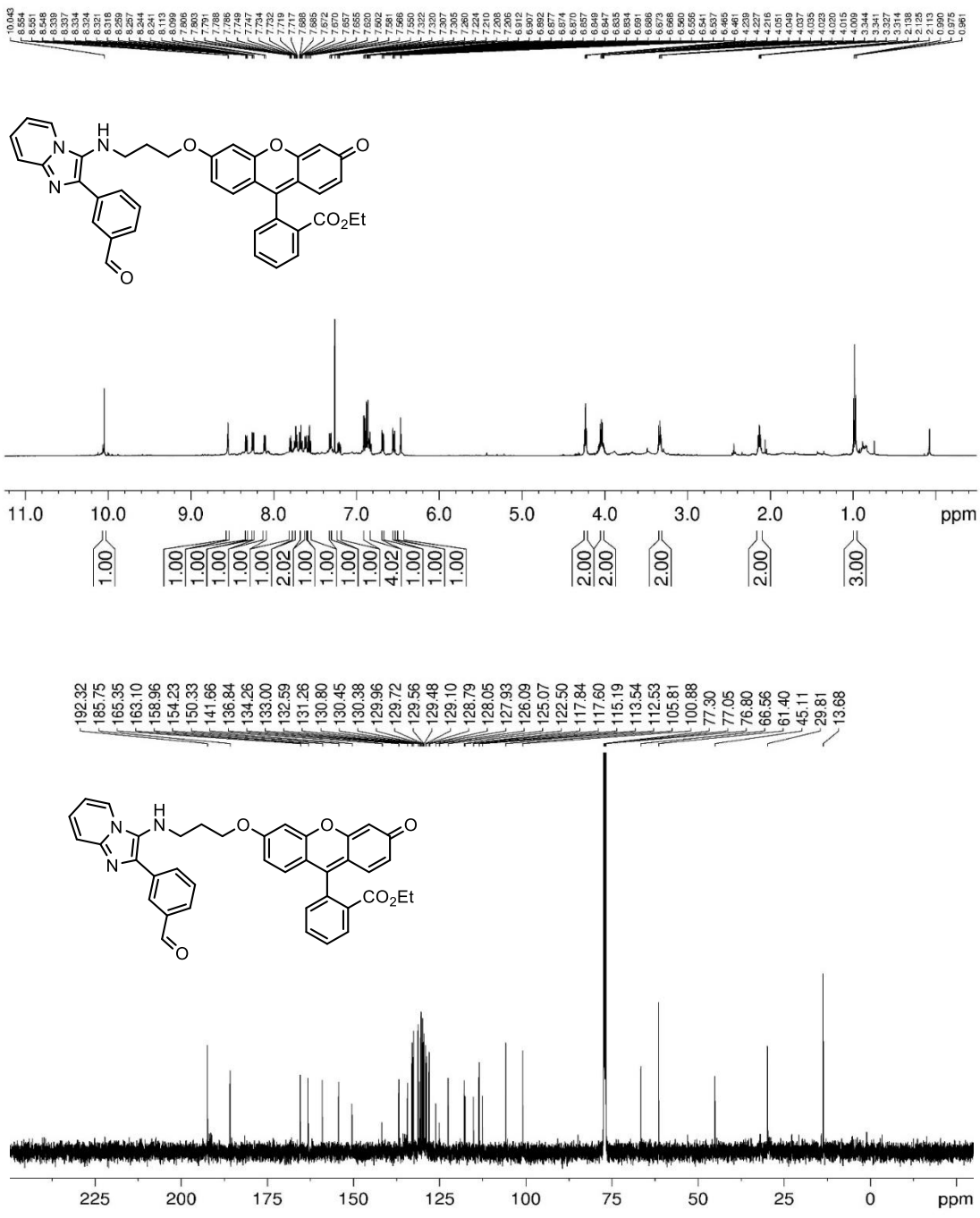


Ethyl 2-(6-(3-((2-(naphthalen-2-yl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4f)

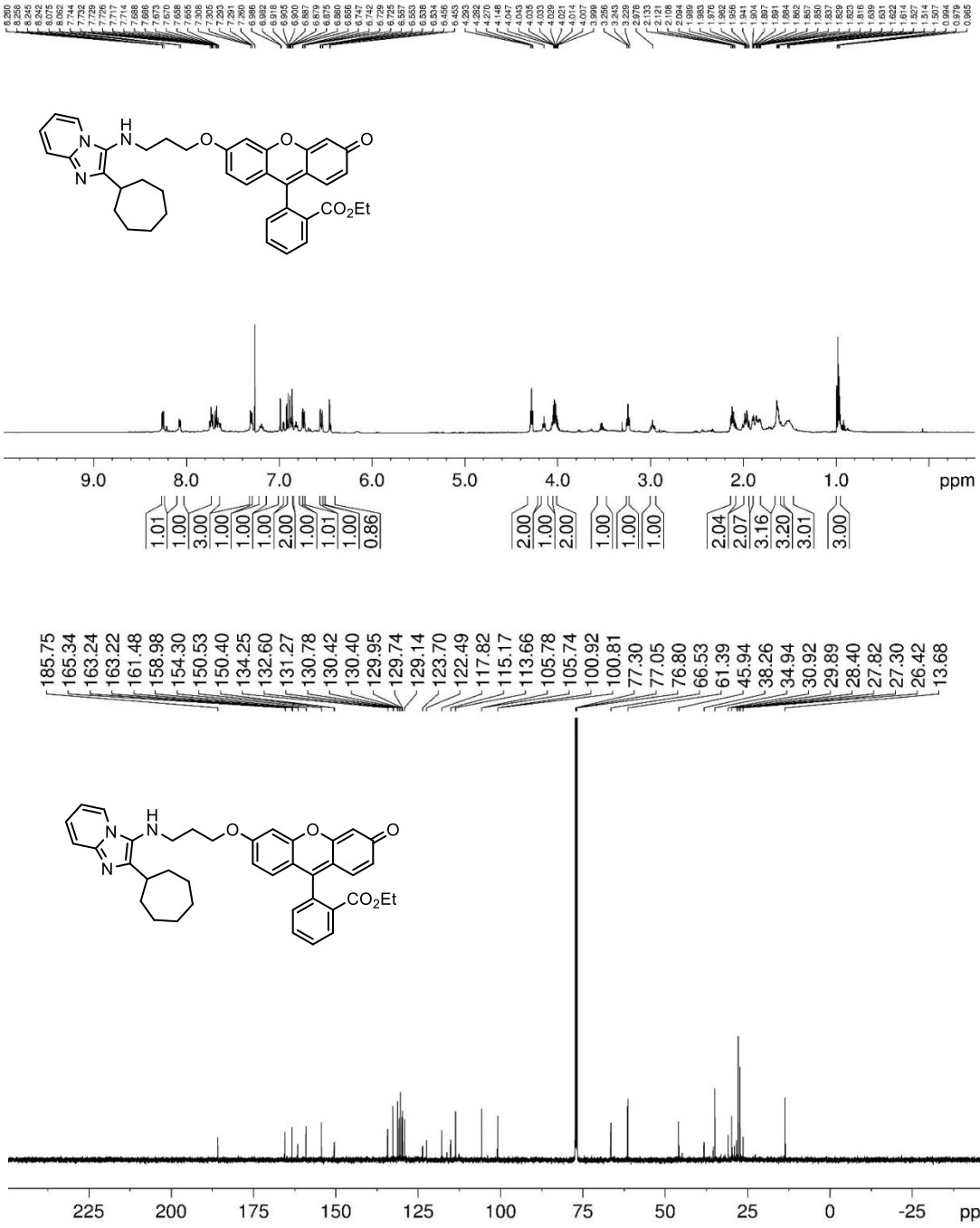




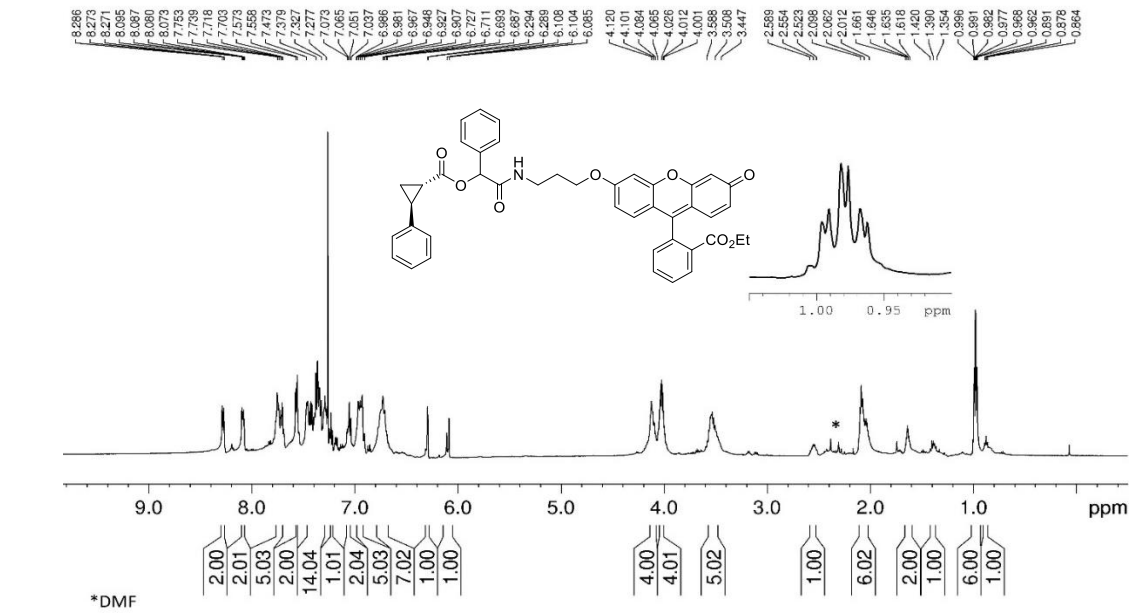
Ethyl 2-(6-(3-((2-(3-formylphenyl)imidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4g)

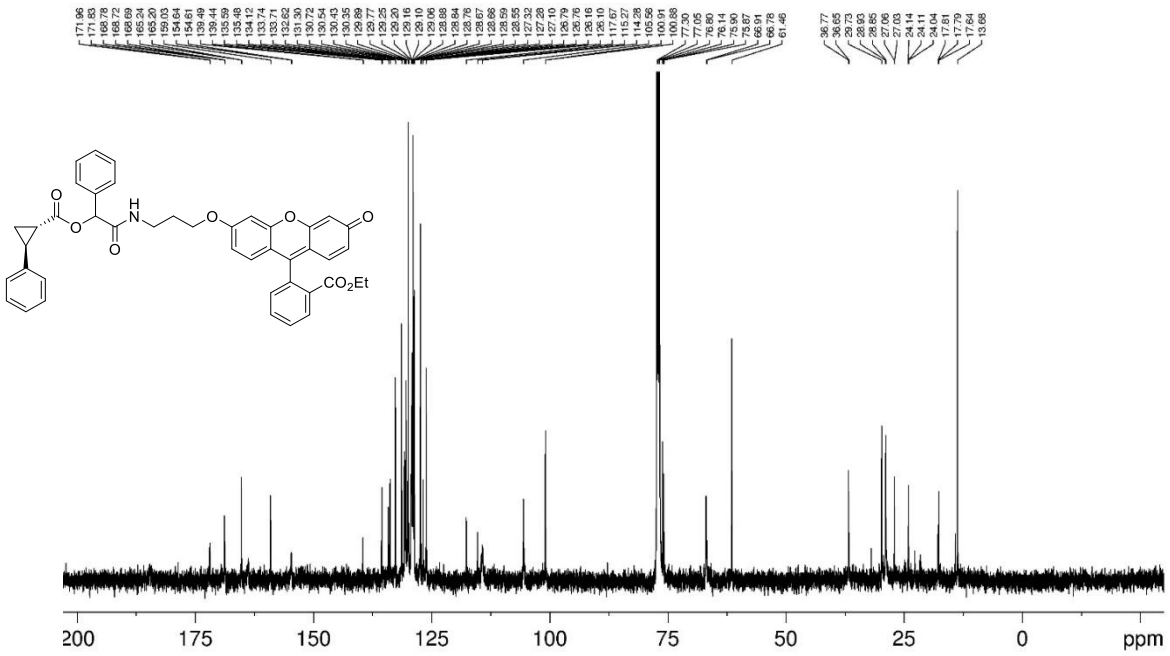


Ethyl 2-(6-(3-((2-cycloheptylimidazo[1,2-a]pyridin-3-yl)amino)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (4h)

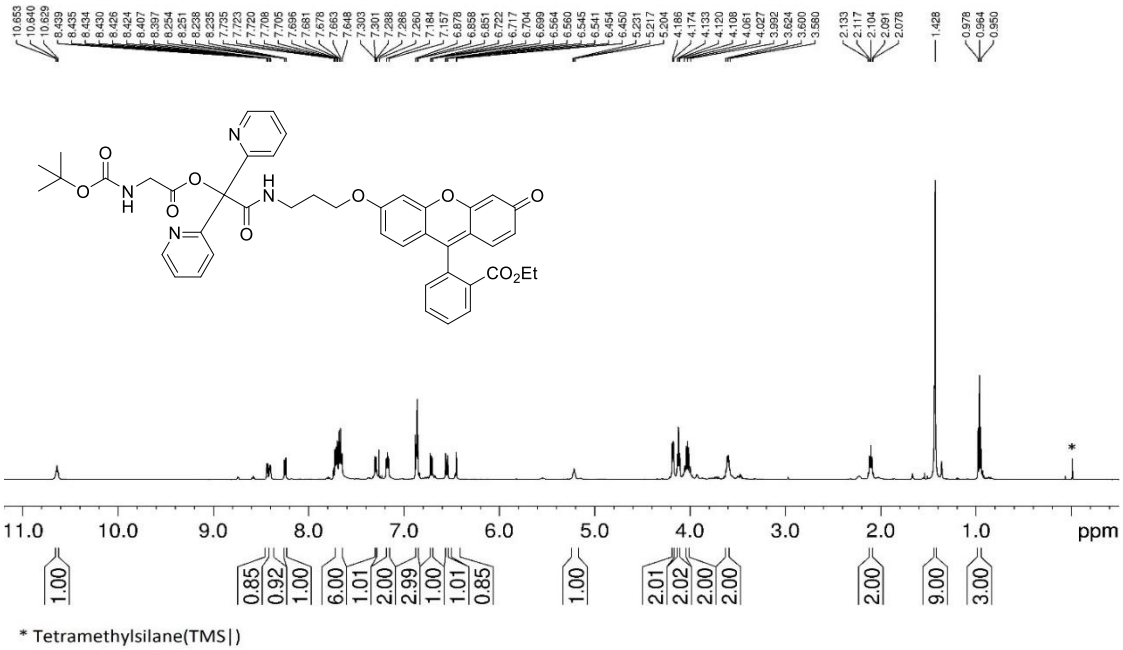


Ethyl 2-(3-oxo-6-(3-(2-phenyl-2-(((1*S*,2*S*)-2-phenylcyclopropane-1-carbonyl)oxy)acetamido)propoxy)-3*H*-xanthen-9-yl)benzoate (5a)

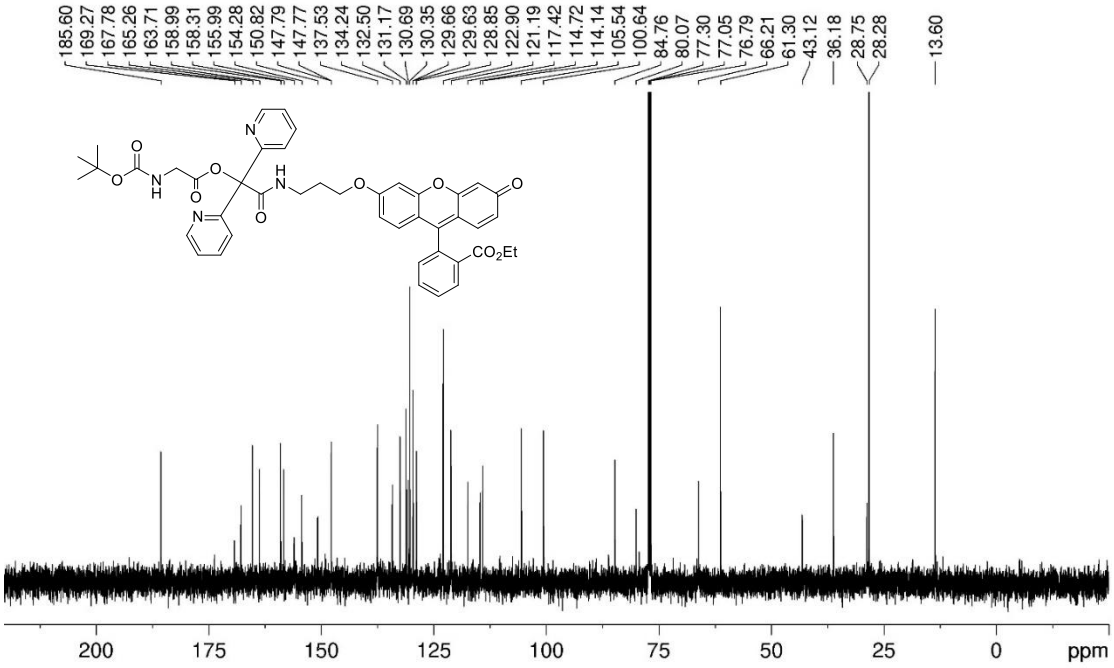




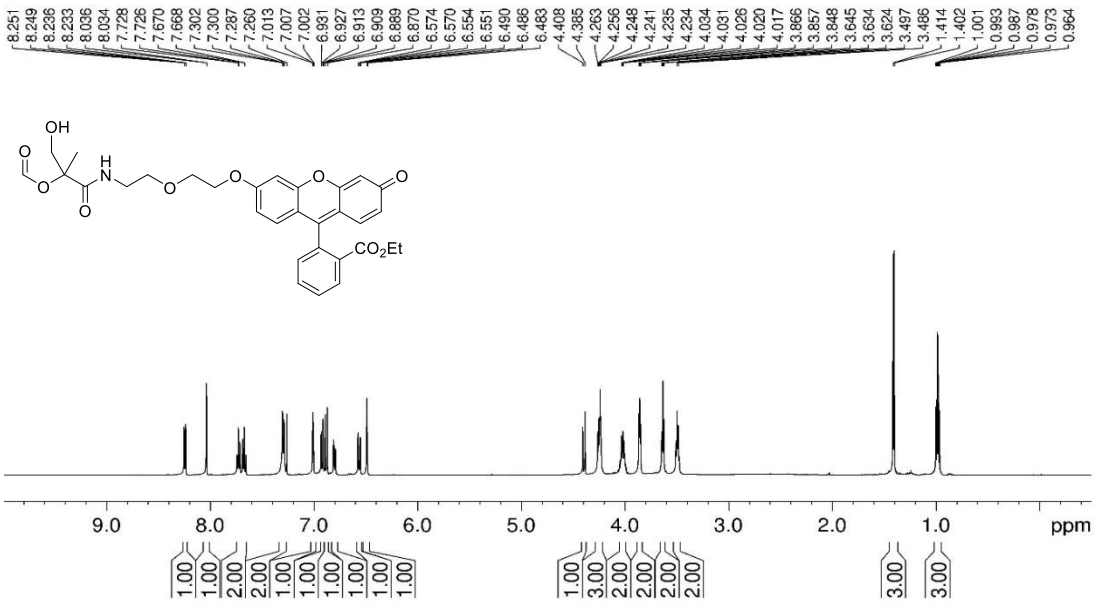
Ethyl 2-(6-((2,2-dimethyl-4,7,10-trioxo-9,9-di(pyridin-2-yl)-3,8-dioxa-5,11-diazatetradecan-14-yl)oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5b)

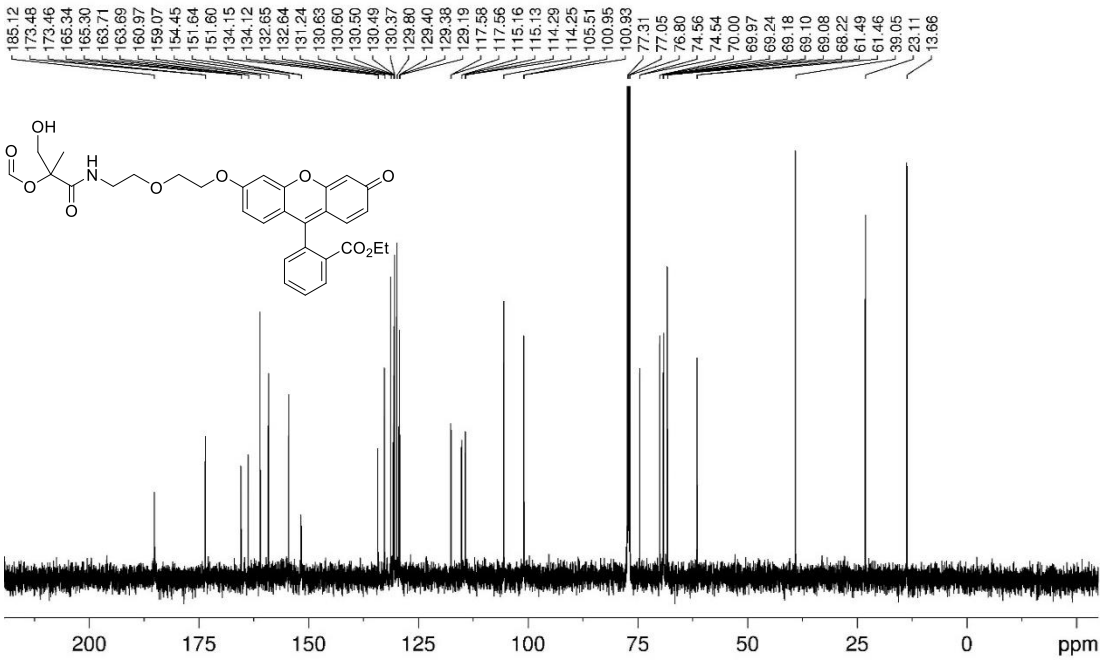


* Tetramethylsilane(TMS|)

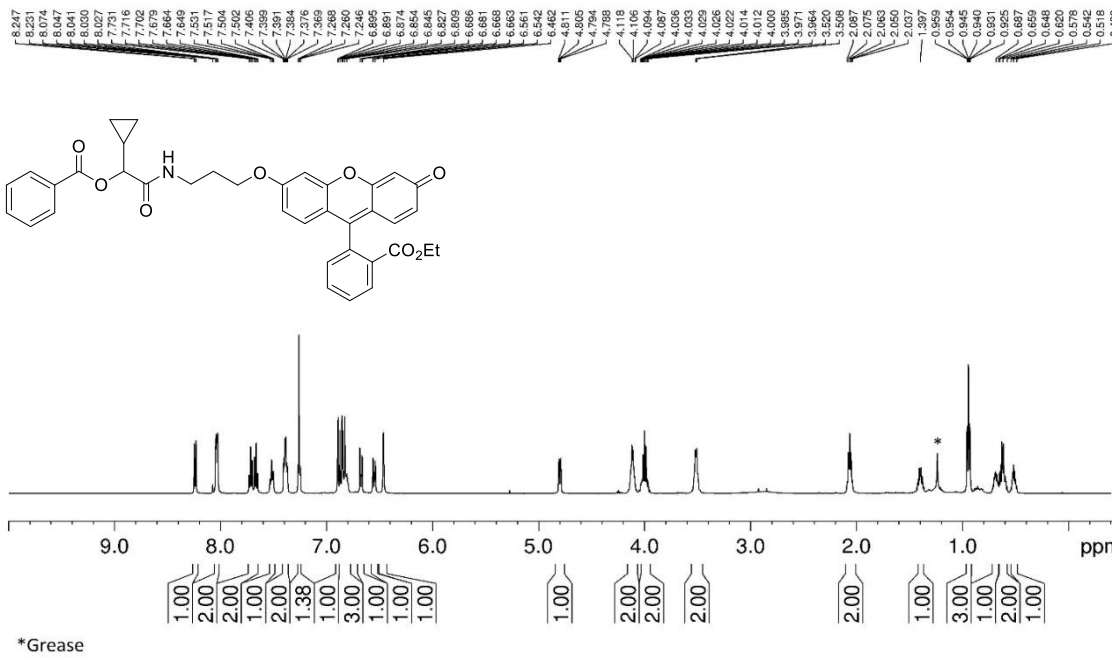


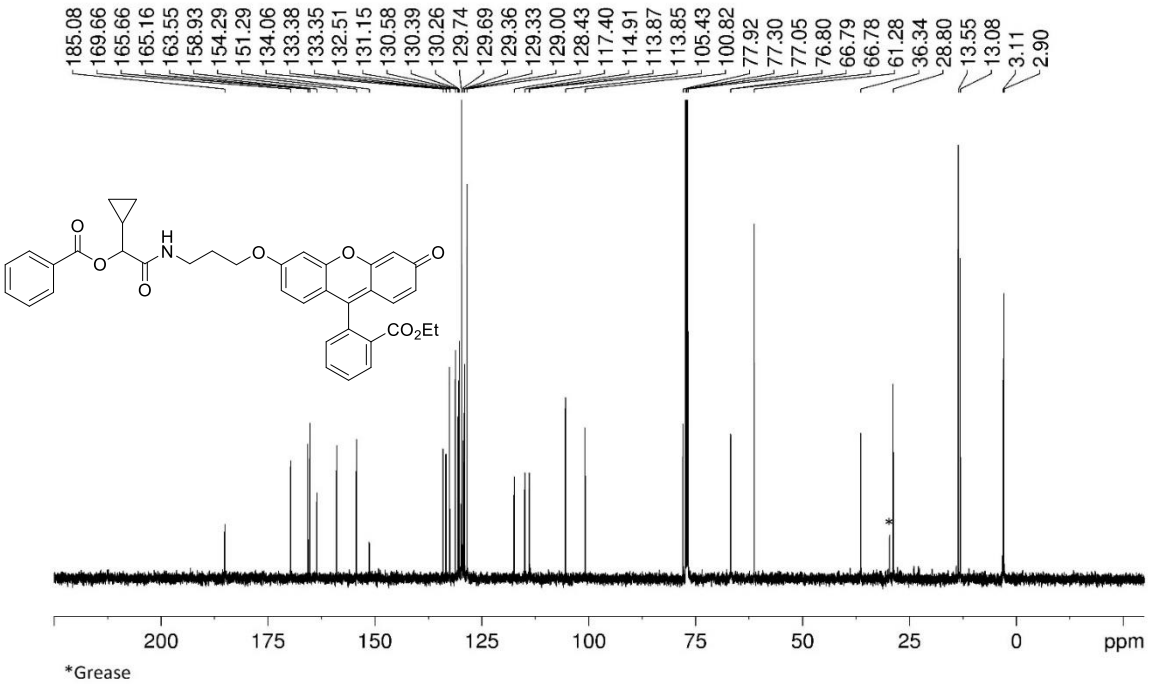
Ethyl 2-(6-(2-(2-(2-(formyloxy)-3-hydroxy-2-methylpropanamido)ethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5c)



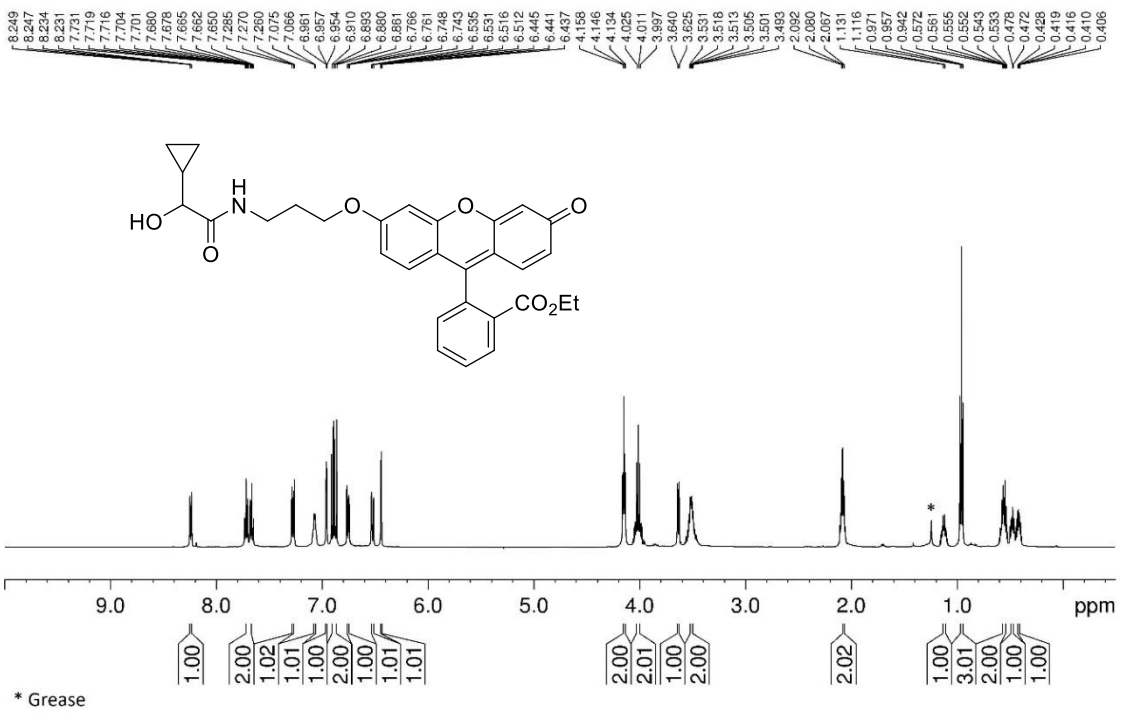


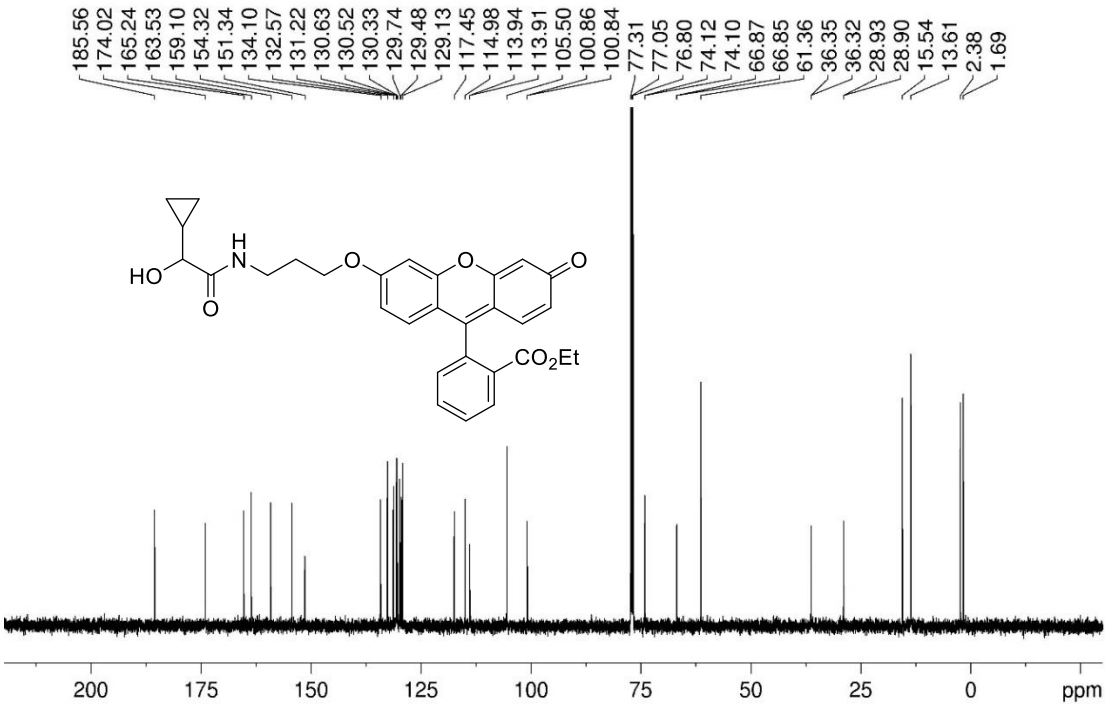
Ethyl 2-(6-(3-(2-(benzoyloxy)-2-cyclopropylacetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5d)



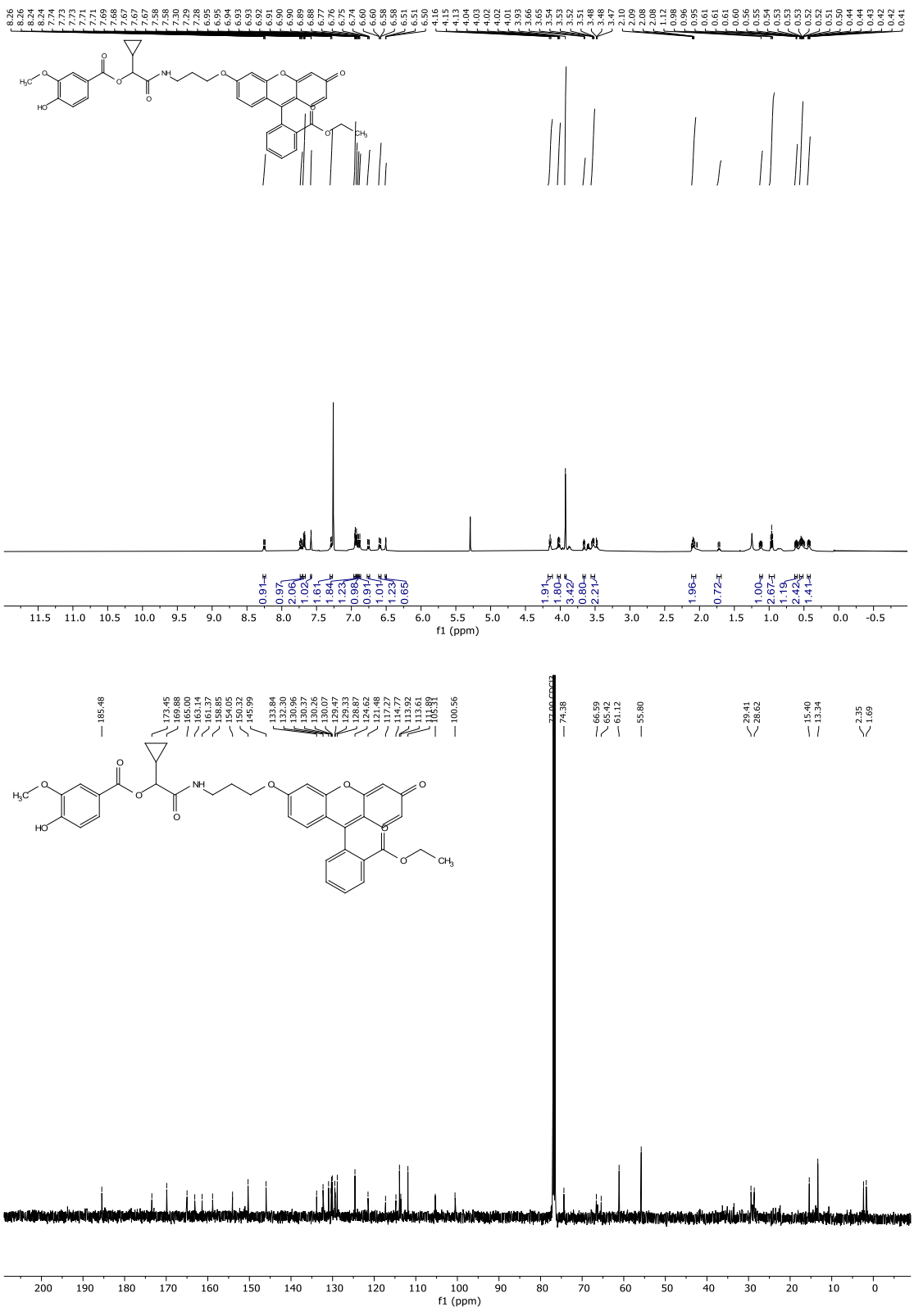


Ethyl 2-(6-(3-(2-cyclopropyl-2-hydroxyacetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (5e)

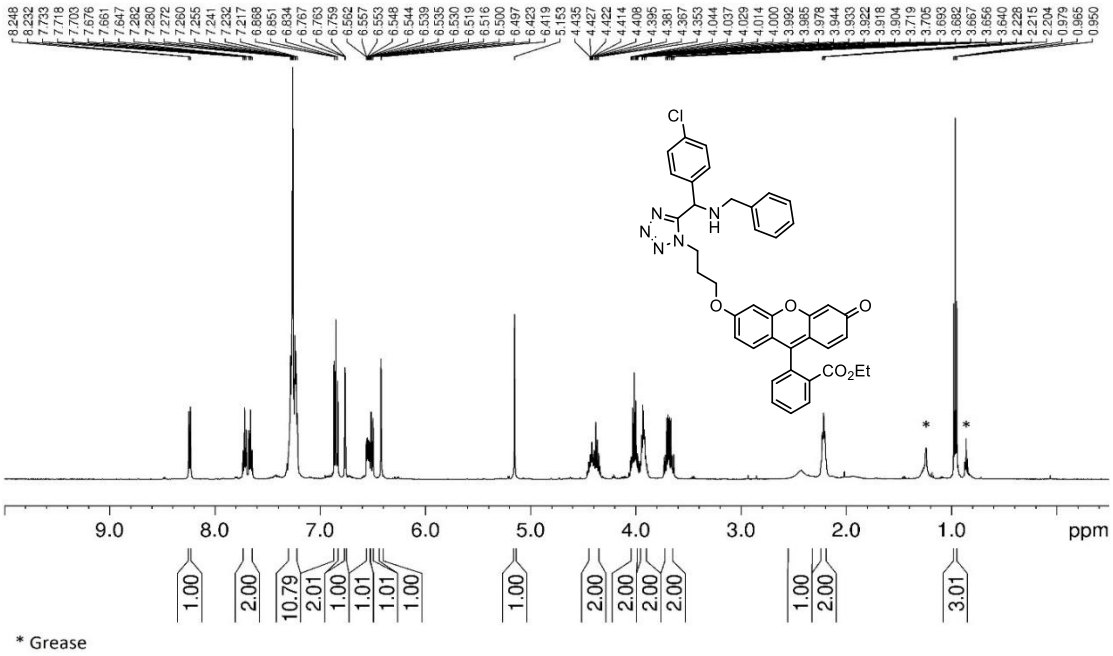




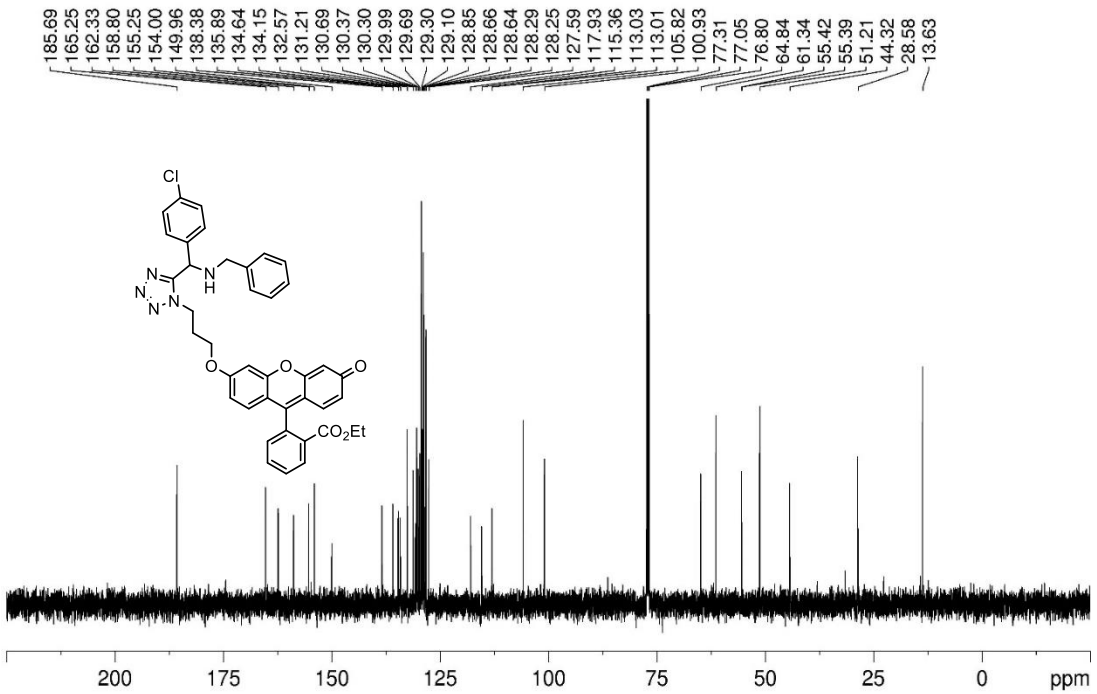
2-((3-((9-(2-(ethoxycarbonyl)phenyl)-3-oxo-3*H*-xanthen-6-yl)oxy)propyl)amino)-2-oxoethyl 4-hydroxy-3-methoxybenzoate (5f)



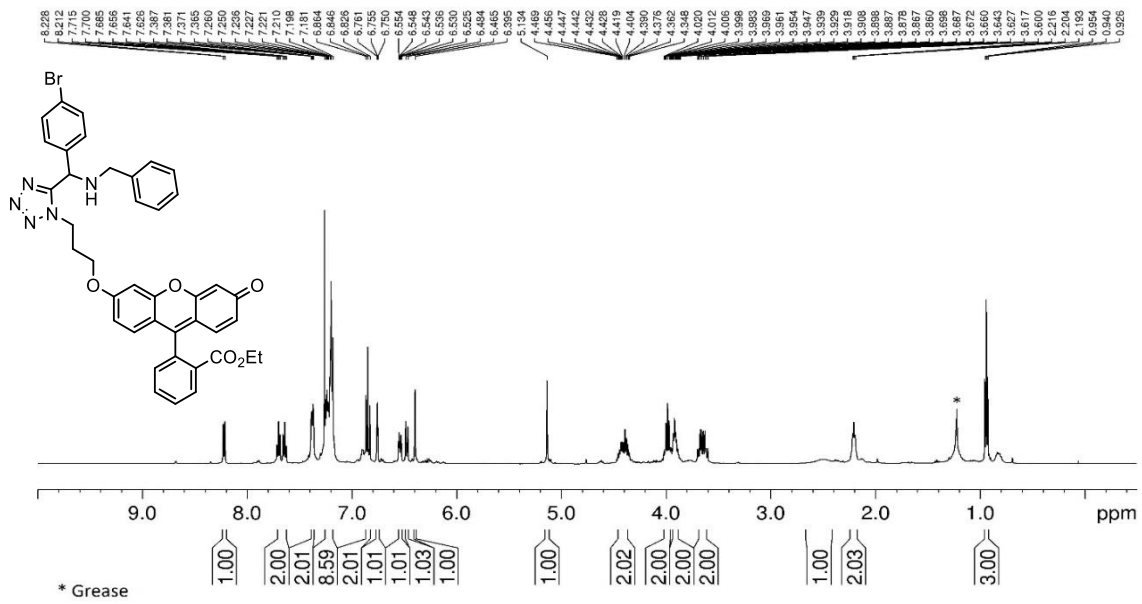
Ethyl 2-(6-(3-(5-((benzylamino)(4-chlorophenyl)methyl)-1*H*-tetrazol-1-yl) propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6a)

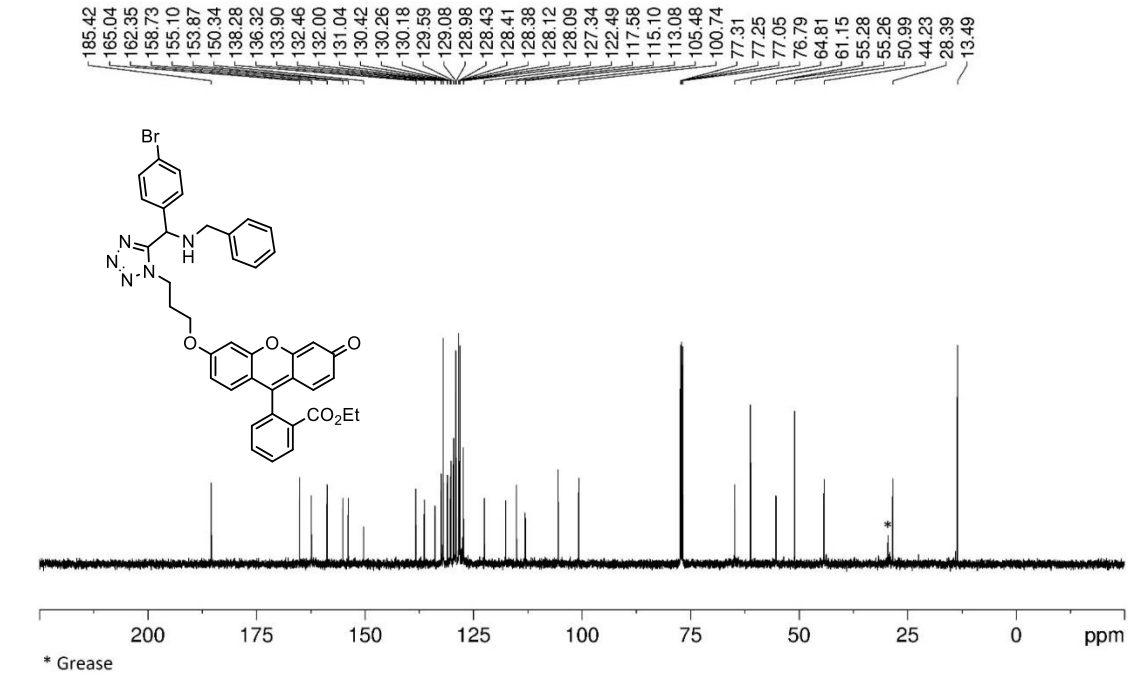


* Grease

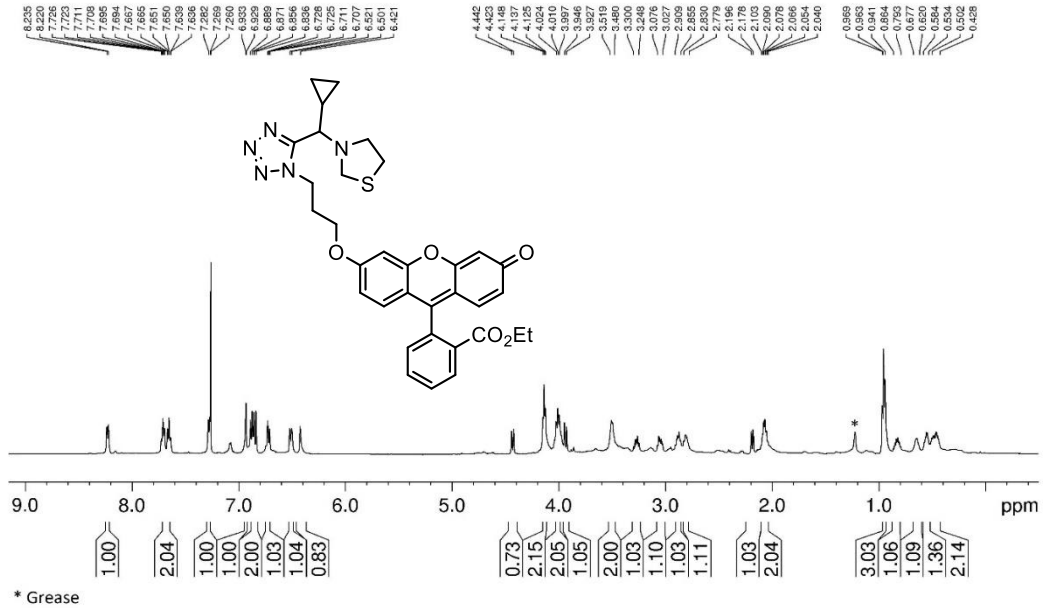


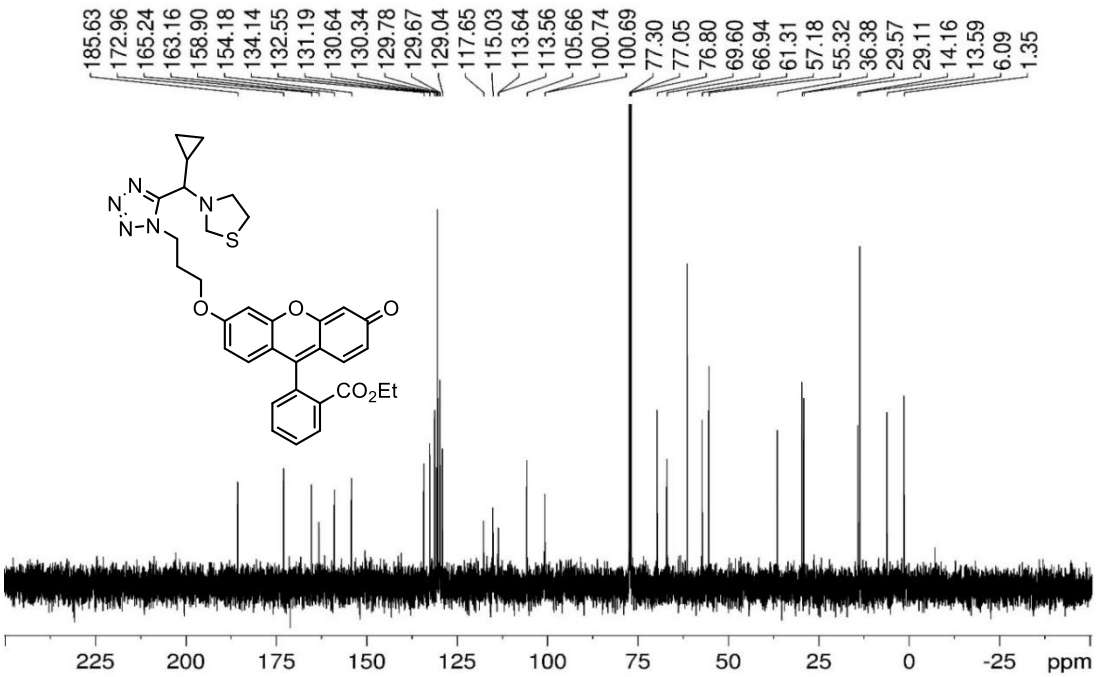
Ethyl 2-(6-(3-(5-((benzylamino)(4-bromophenyl)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6b)



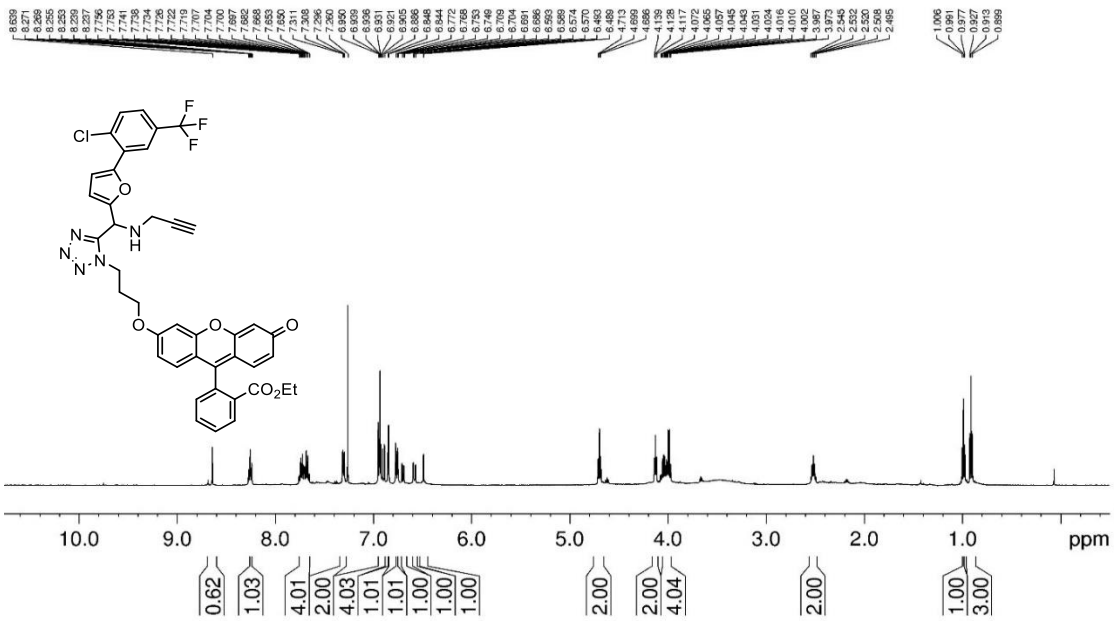


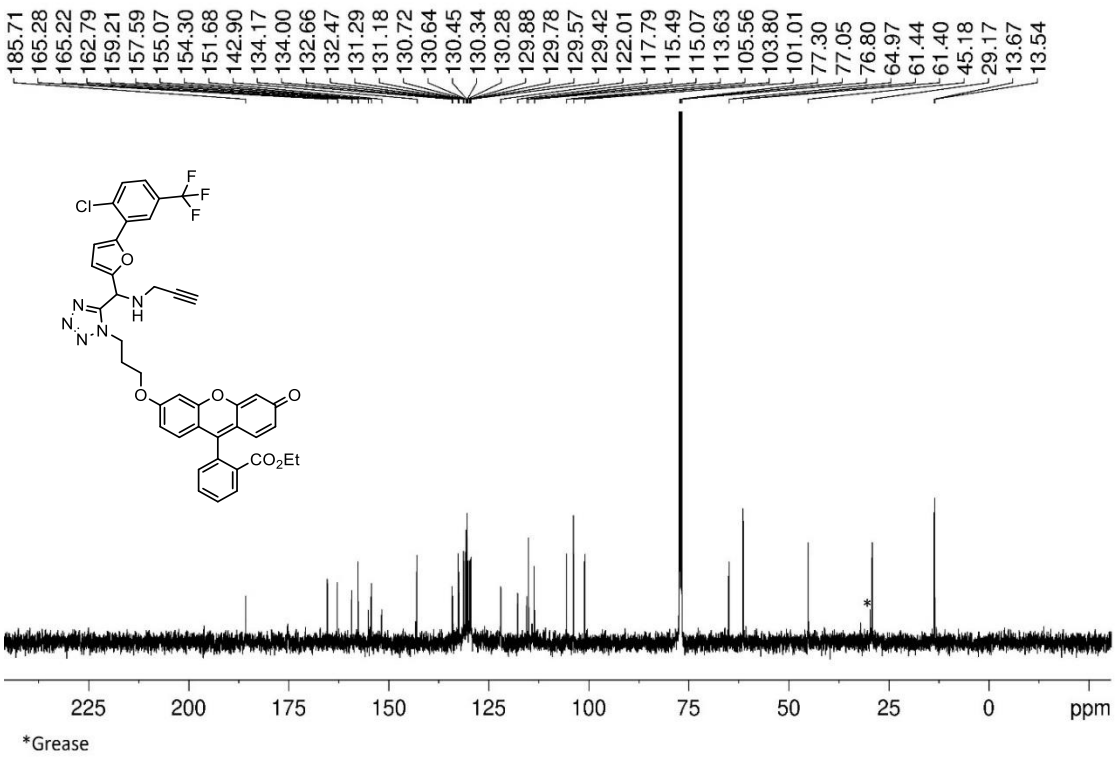
Ethyl 2-(6-(3-(5-(cyclopropyl(thiazolidin-3-yl)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6c)



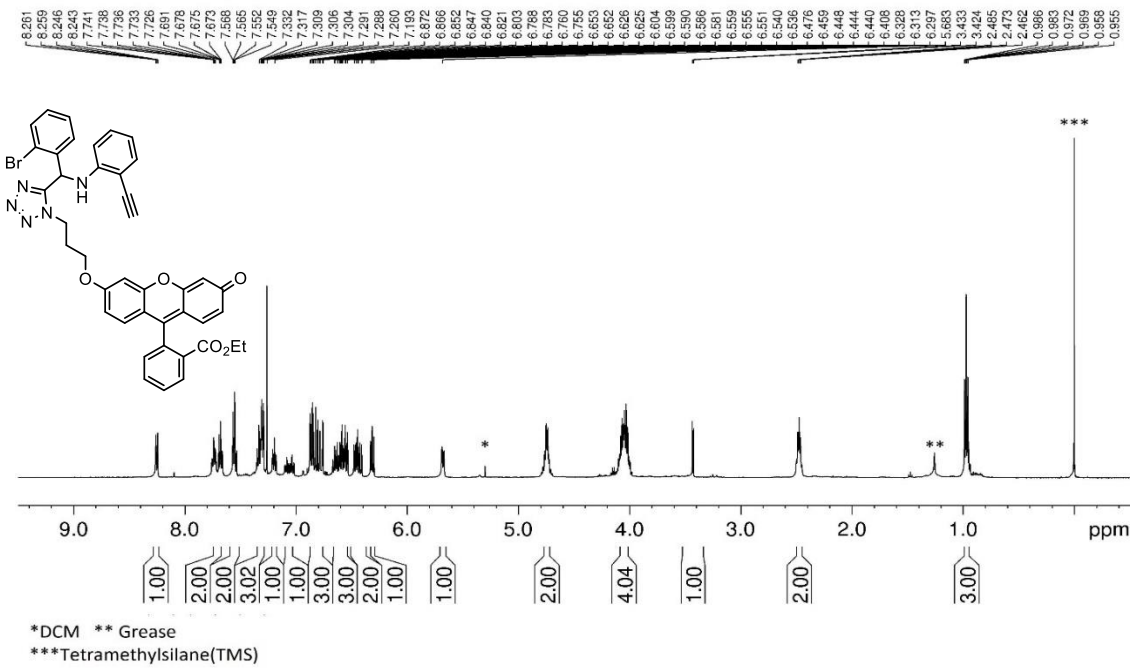


Ethyl 2-(6-(3-(5-((5-(2-chloro-5-(trifluoromethyl)phenyl)furan-2-yl)(prop-2-yn-1-ylamino)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6d)

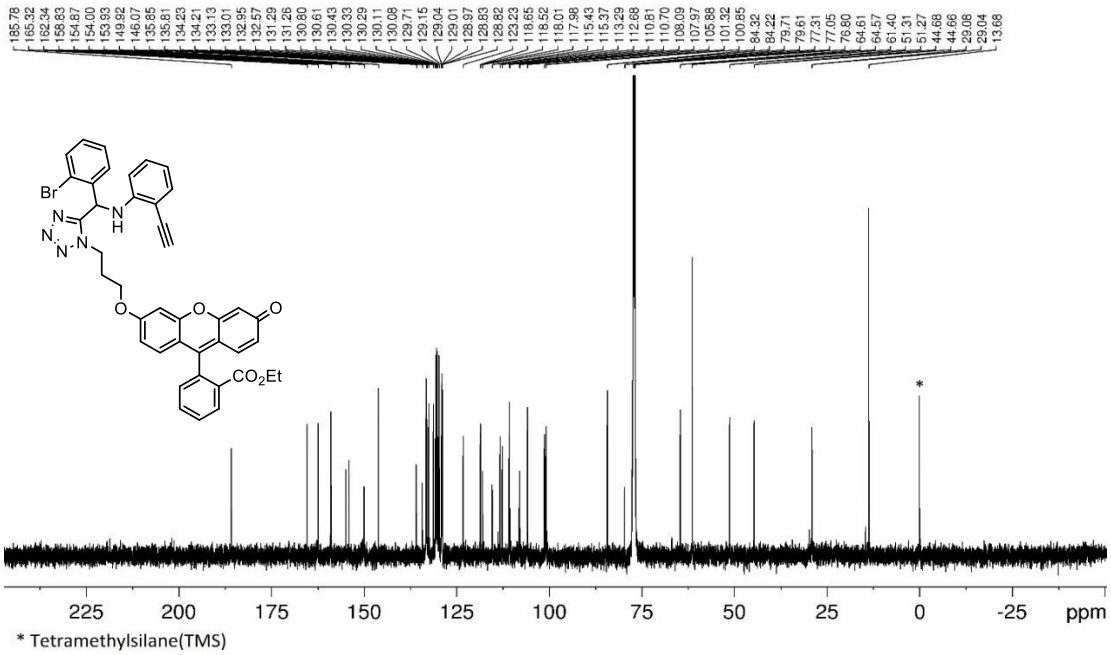




Ethyl 2-(6-(3-(5-((2-bromophenyl)((2-ethynylphenyl)amino)methyl)-1*H*-tetrazol-1-yl)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6e)

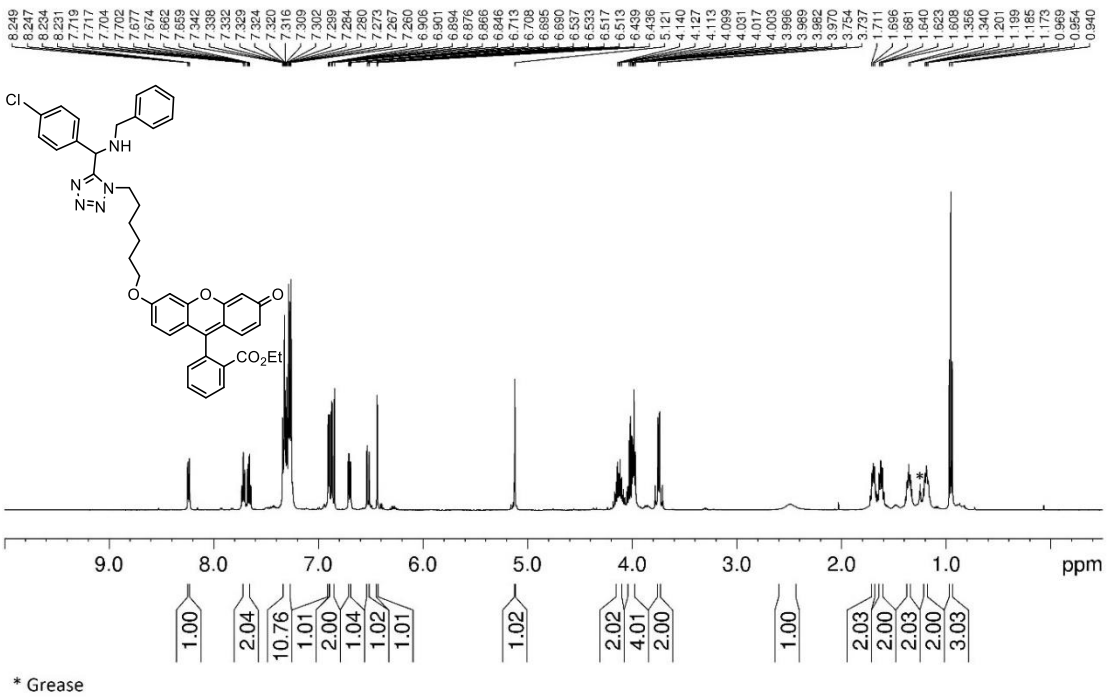


*DCM ** Grease
***Tetramethylsilane(TMS)



* Tetramethylsilane(TMS)

Ethyl 2-(6-((6-(5-((benzylamino)(4-chlorophenyl)methyl)-1*H*-tetrazol-1-yl)hexyl)oxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6f)



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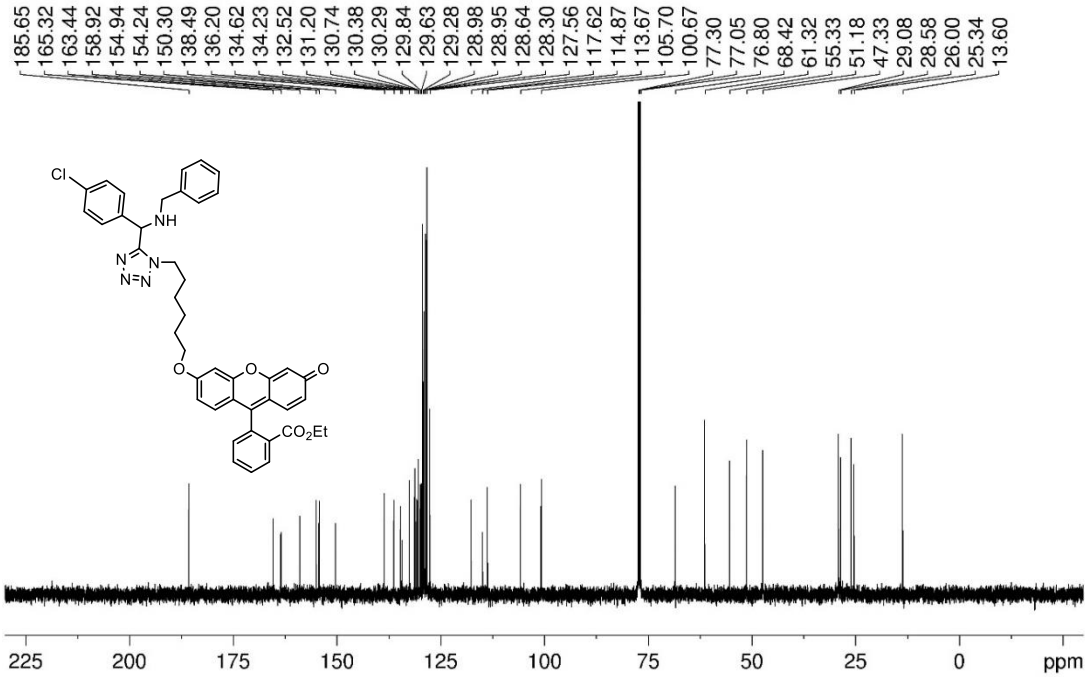
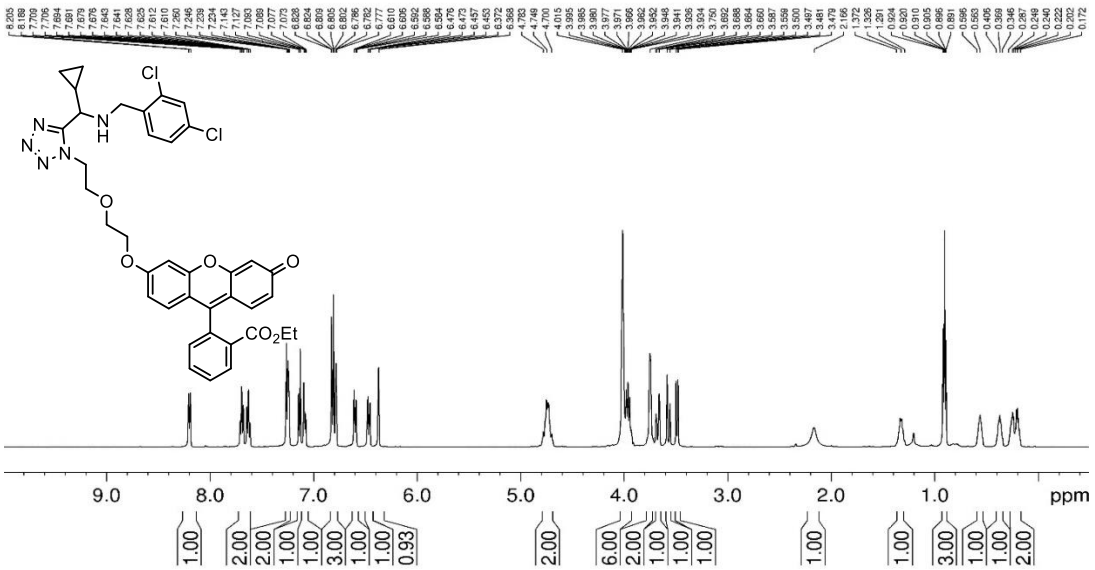
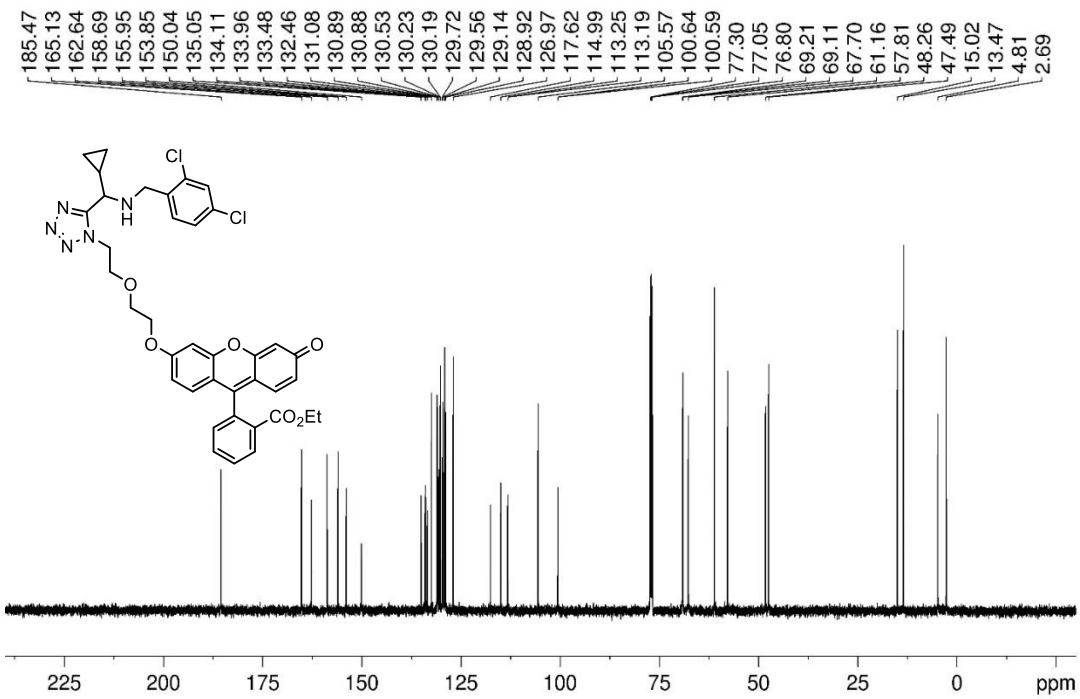
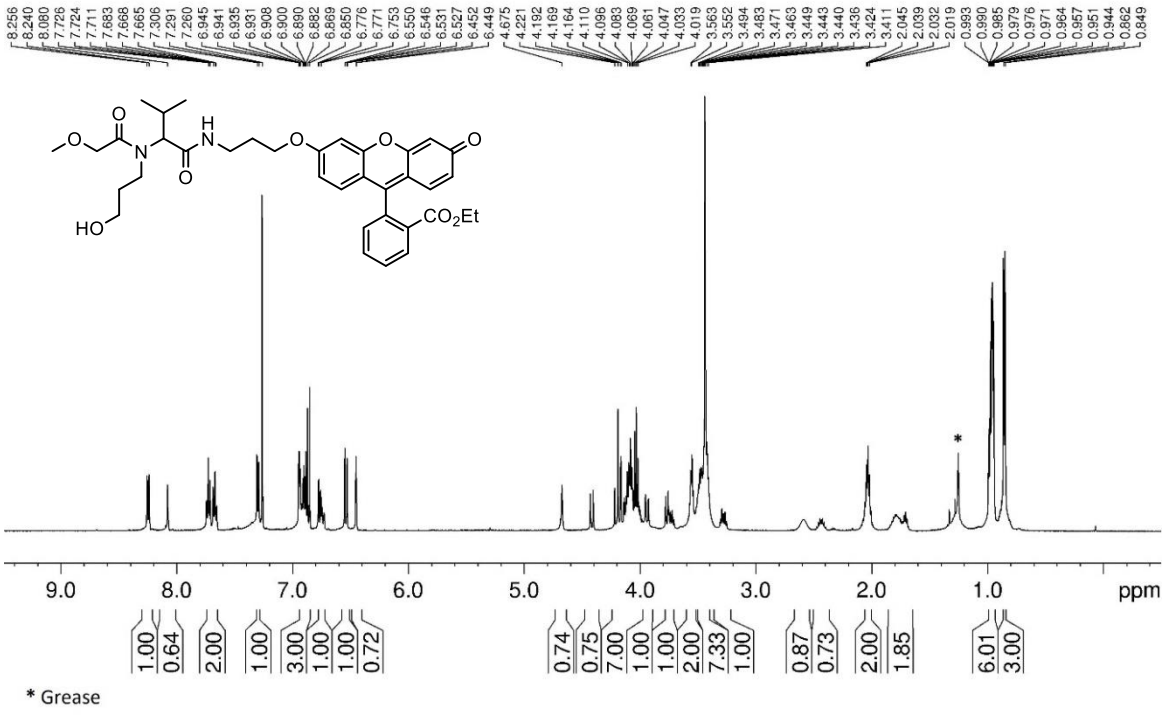


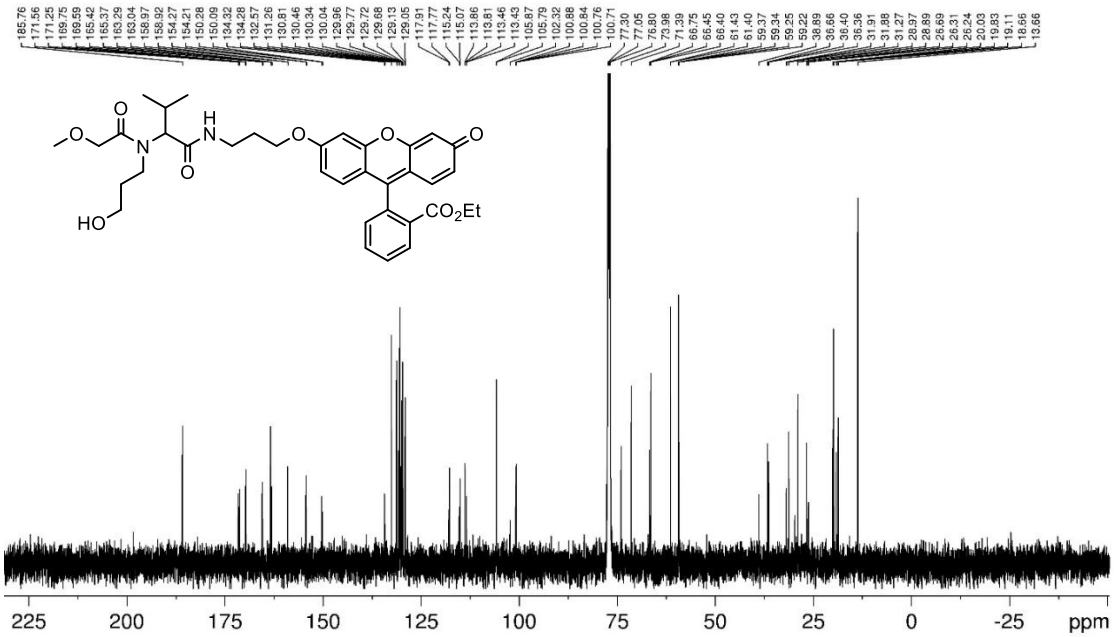
Ethyl 2-(6-(2-(2-(5-(cyclopropyl((2,4-dichlorobenzyl)amino)methyl)-1*H*-tetrazol-1-yl)ethoxy)ethoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (6g)



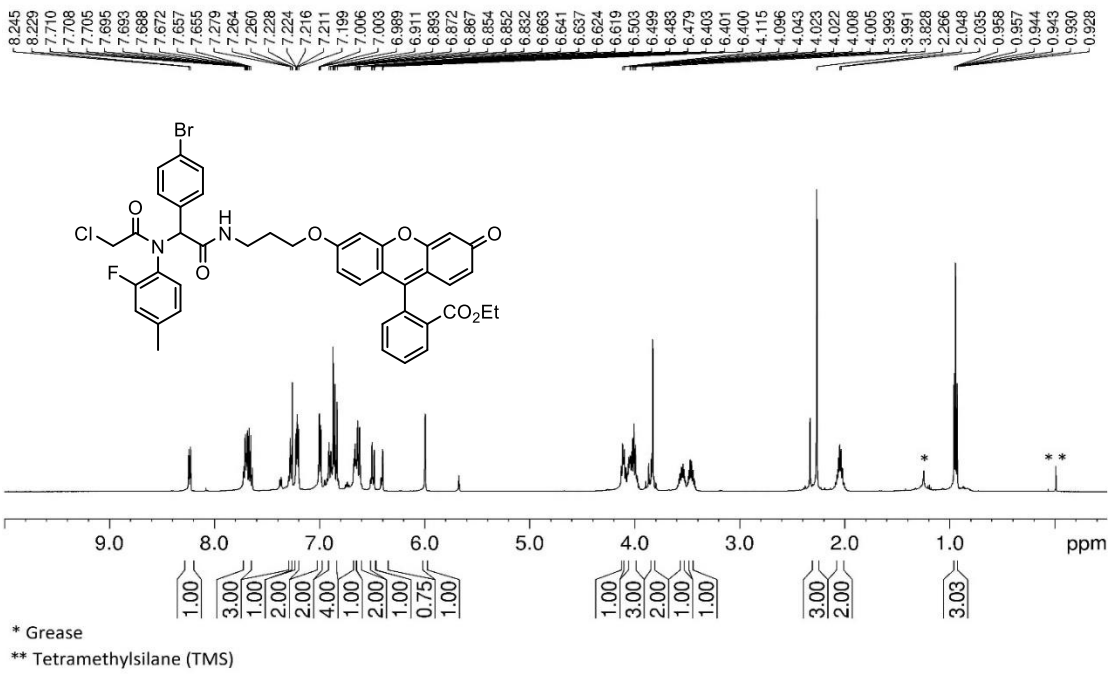


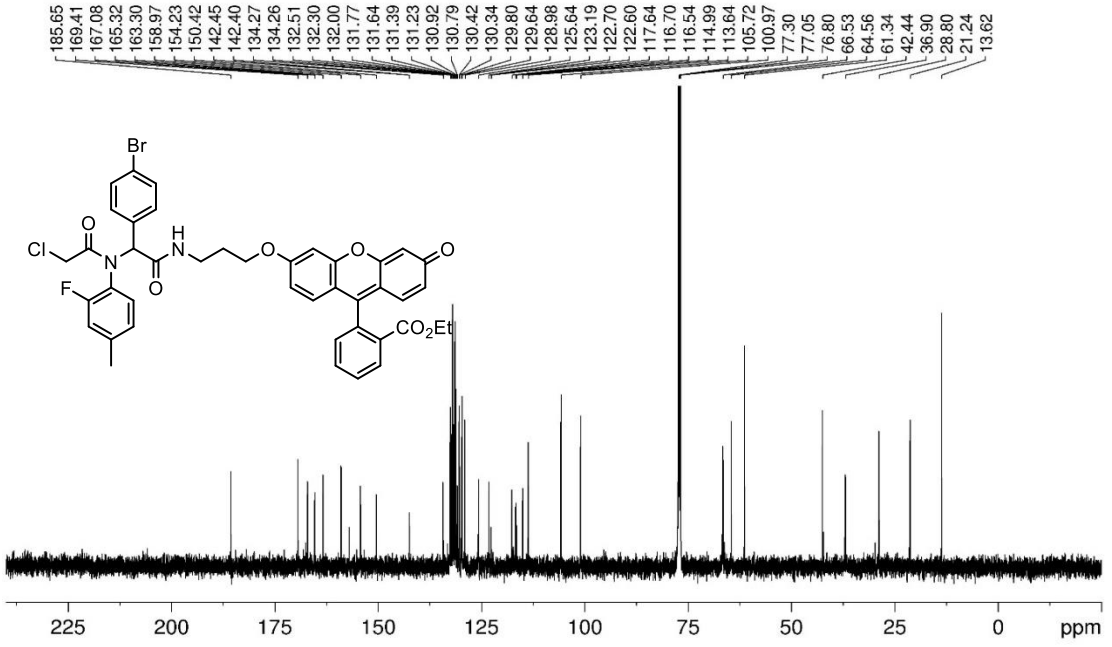
Ethyl 2-(6-(3-(2-(*N*-(3-hydroxypropyl)-2-methoxyacetamido)-3-methyl butanamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7a)



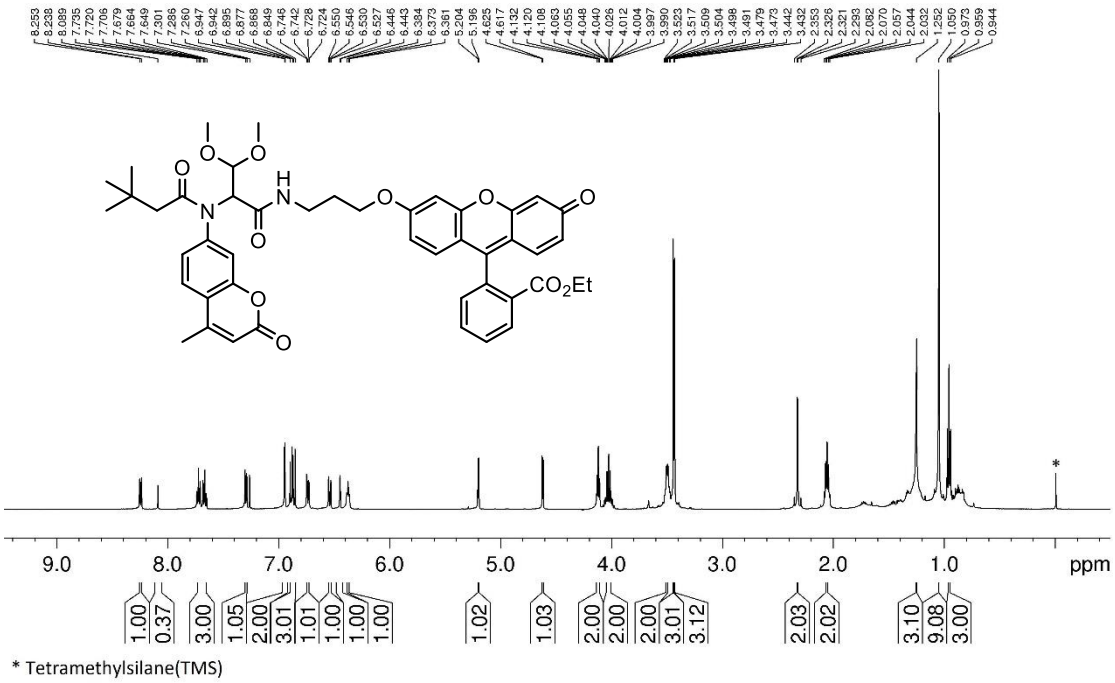


Ethyl 2-(6-(3-(2-(4-bromophenyl)-2-(2-chloro-*N*-(2-fluoro-4-methylphenyl) acetamido)acetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7b)

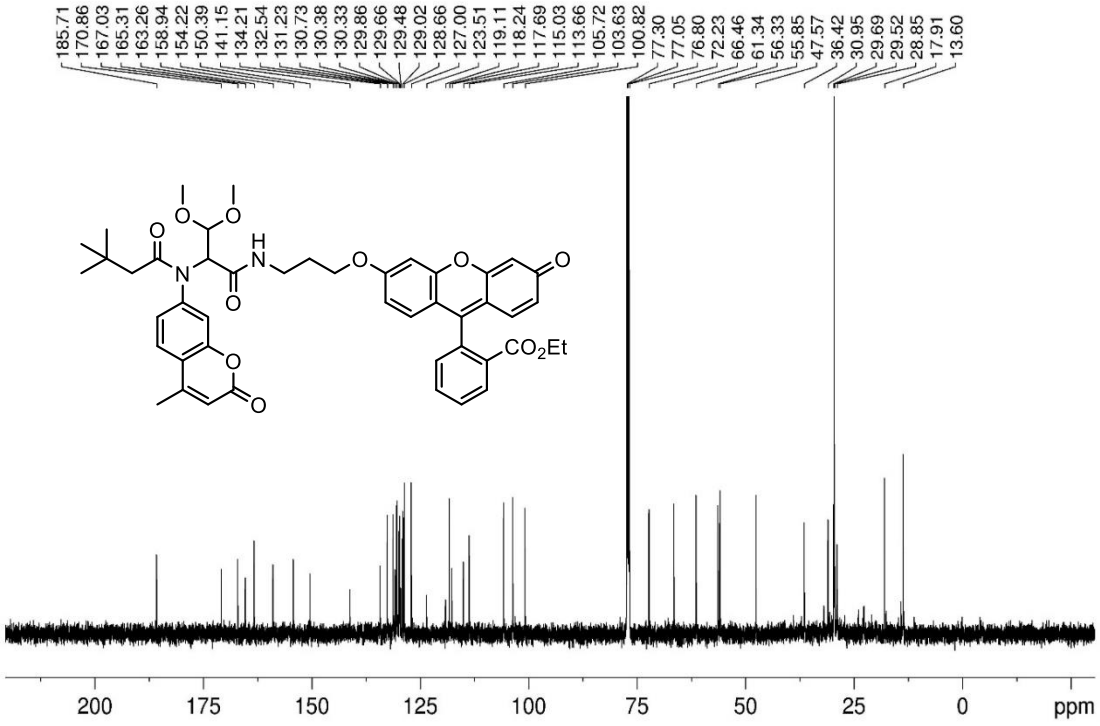




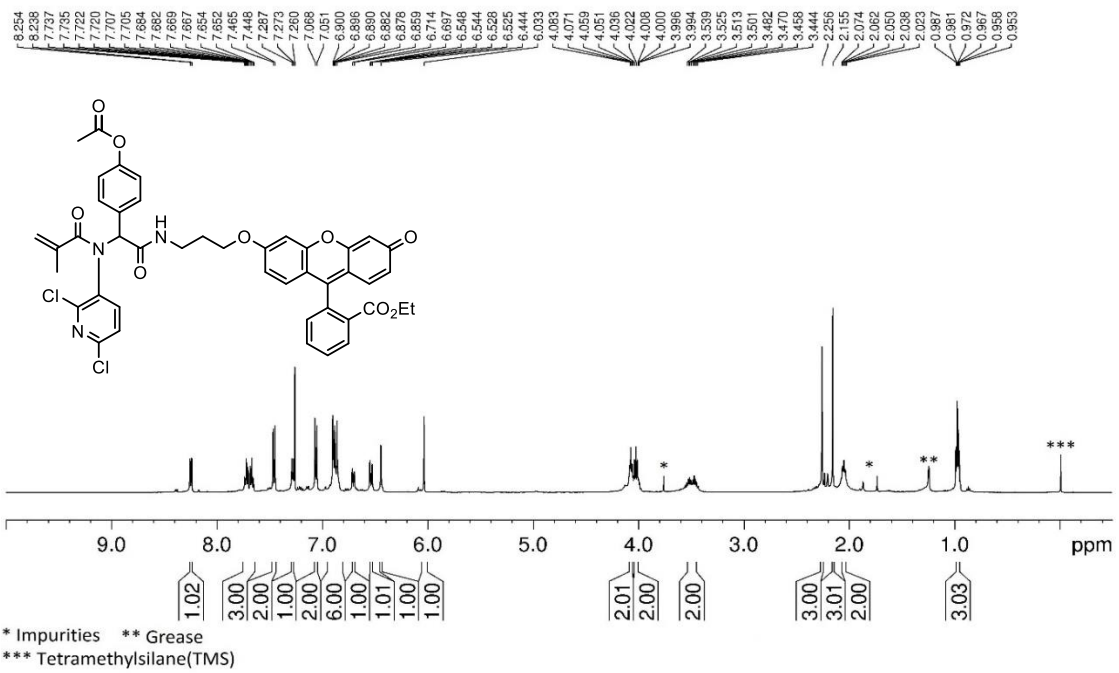
Ethyl 2-(6-(3-(2-(3,3-dimethyl-*N*-(4-methyl-2-oxo-2H-chromen-7-yl)butanamido)-3,3-dimethoxypropanamido)propoxy)-3*H*-xanthen-9-yl)benzoate (7c)

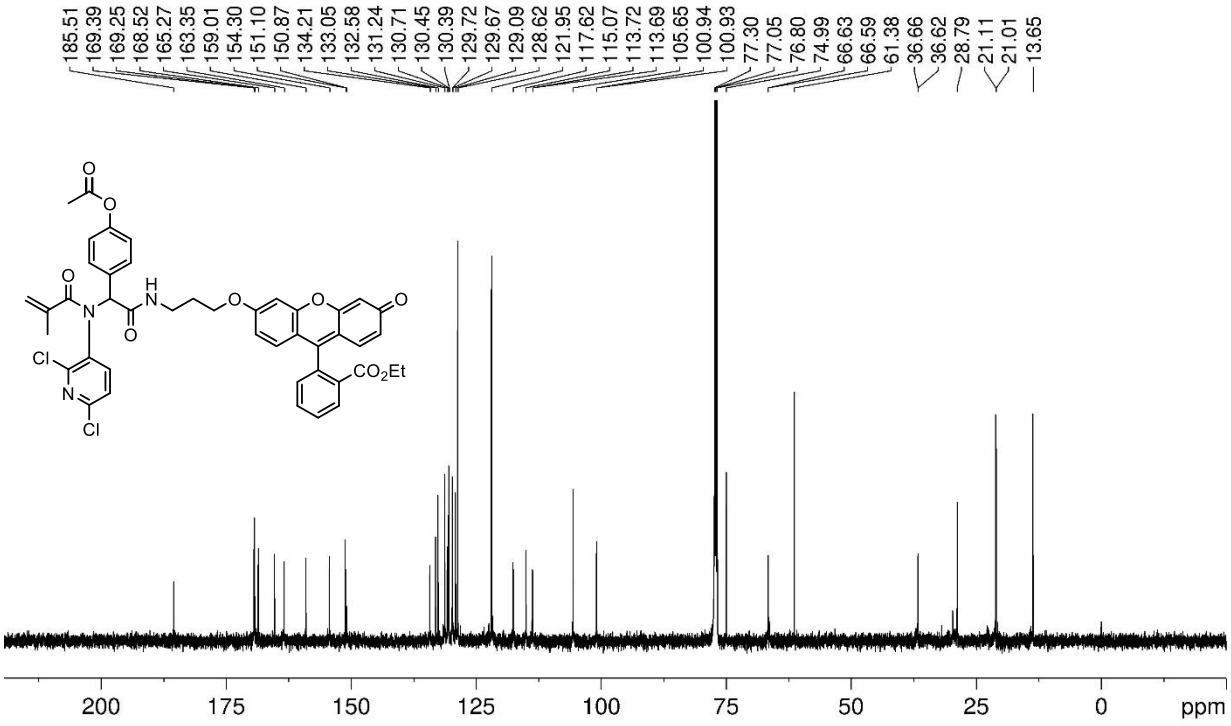


* Tetramethylsilane(TMS)



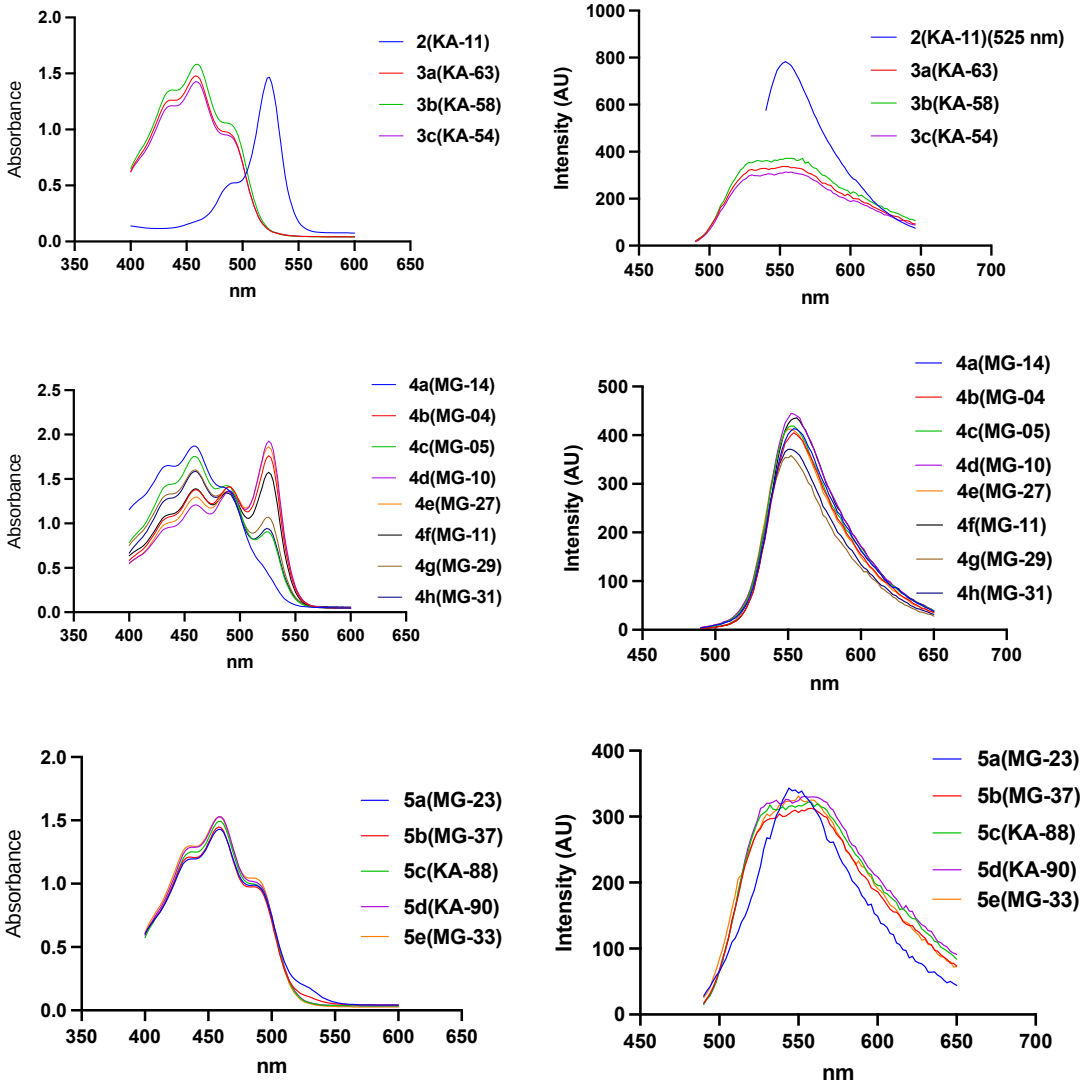
Ethyl 2-(6-(3-(2-(4-acetoxyphenyl)-2-(*N*-(2,6-dichloropyridin-3-yl) methacrylamido)acetamido)propoxy)-3-oxo-3*H*-xanthen-9-yl)benzoate (7d)





4. Procedure for absorption-fluorescence measurements

Three standard solutions were prepared for all compounds. The first solution S_1 had a concentration of 0.1 M and a volume of 100 µL. The second solution S_2 was prepared by diluting S_1 and had a concentration of 2 mM and a volume of 0.1 mL. The third solution S_3 was prepared by diluting S_2 and had a concentration of 200 µM and a volume of 200 µL. The weighed amount of each compound was dissolved in 100 µL of DMSO resulting in the production of S_1. For the production of S_2, 2 µL of S_1 were taken, which were diluted to a final volume of 0.1 mL. To produce S_3, 20 µL was taken from S_2 and diluted to a final volume of 200 µL DMSO. The S_3 solution of each compound was then used to obtain the emission and absorption spectra. The wavelength of maximum absorption was first determined by scanning the 400-600 nm region. Then, the solution of each compound was irradiated with 460 nm or 525 nm wavelength radiation and the fluorescence spectra were obtained.



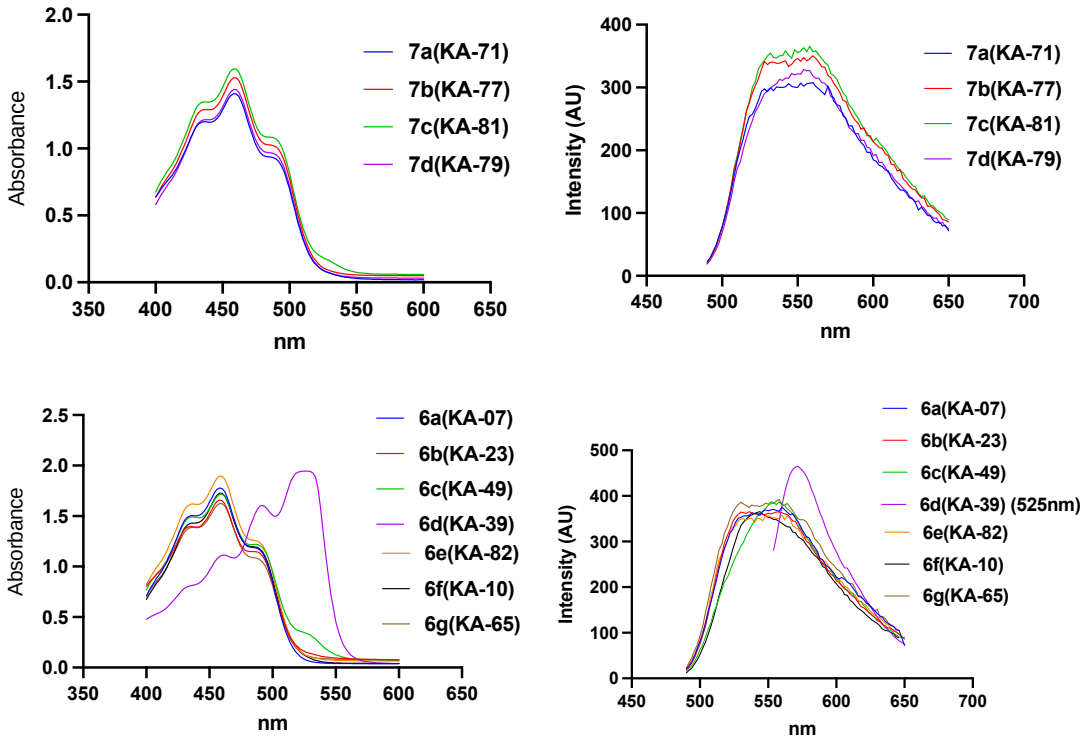
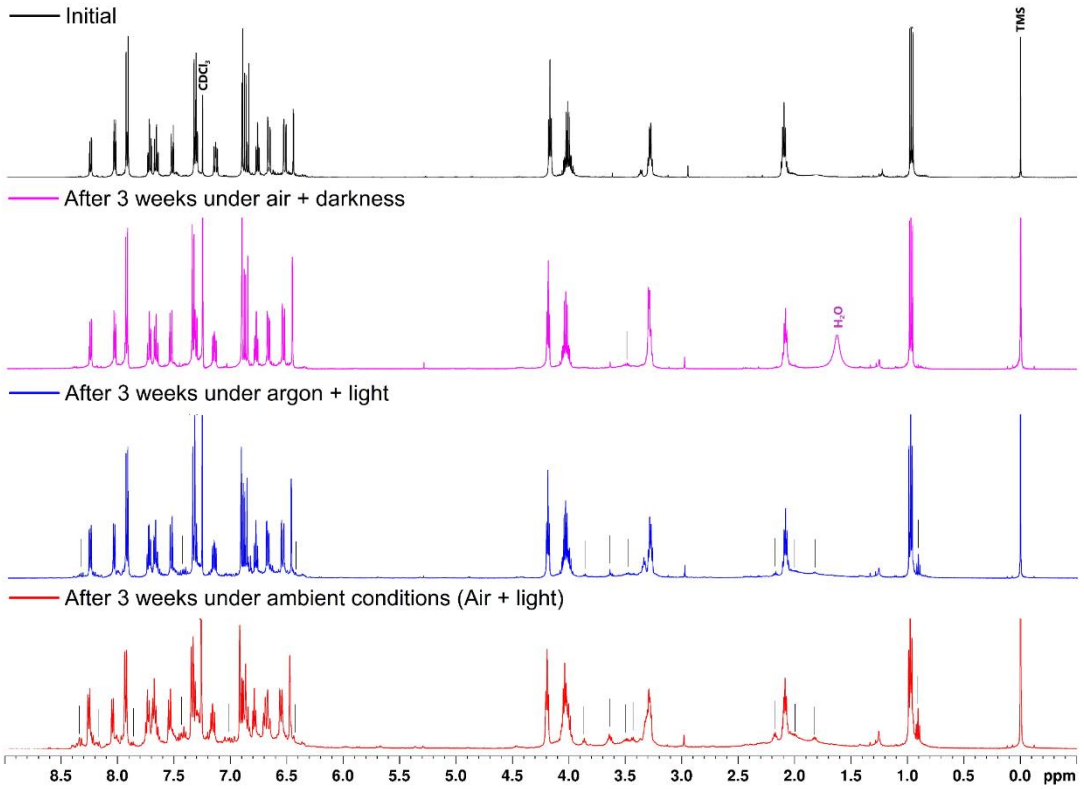


Figure S1. Absorbance and emission spectra of the newly synthesized derivatives.

A



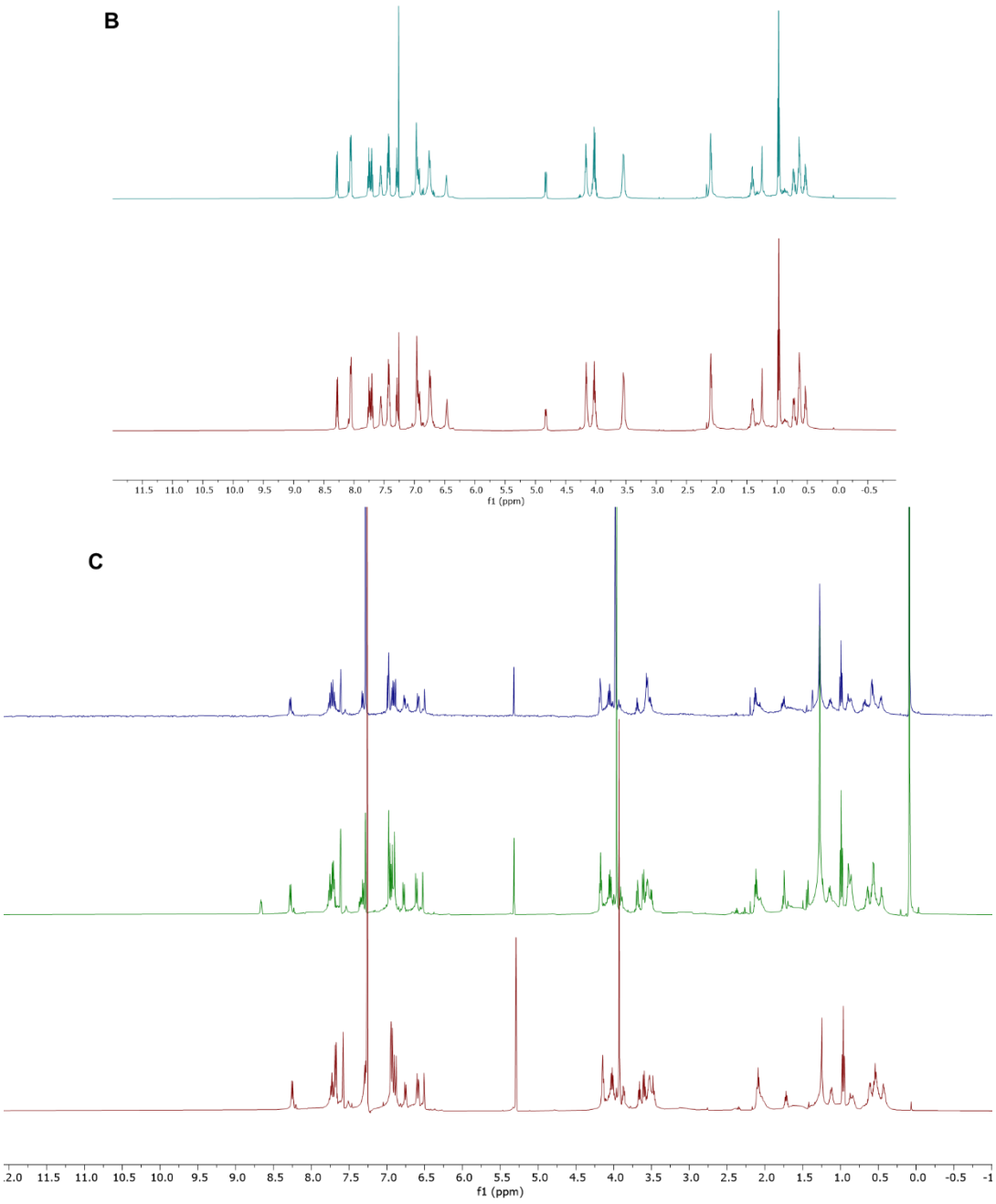


Figure S2. (A) Stability experiments of compound **4a** after 3 weeks' exposure in different conditions (light/dark and ambient/inert atmosphere). The NMR peaks that exhibit changes have been marked for easier interpretation; (B) Stability experiments of compound **5d** after 1 h UV (365 nm) irradiation (cyan) *vs* no irradiation (red); (C) Stability experiments of compound **5f** after 1 h irradiation at 500 nm (blue), 1 h UV (365 nm) irradiation (green) *vs* no irradiation (red). The peaks of vanillic acid have been appeared during UV irradiation.

5. Expression and purification of 15-LOX-1

Newly transformed BL21 (DE3) *E. coli* cells with the recombinant pET26b plasmid carrying the ALOX15 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 1L 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-15LOX1-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 min, 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 min 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 min, 4 °C). The supernatant was incubated with a pre-equilibrated NEBExpress® Ni Resin column for 1 h 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 °C. The purified protein was aliquoted and stored at -20 °C.

6. 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-15LOX1 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 min with an interval time of 20 sec. Only the linear part was used for the determination of the enzymatic activity, typically extending over the first 1-5 min 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 RT, 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	-

7. 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 RT, 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: 5µM, 10µM, 20µM and 40µM. 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 rt, 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.

Table S1. IC_{50} values of the synthesized compounds. *All IC_{50} experiments were performed in triplicates (n=3) and the standard error is reported.*

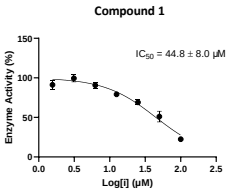
Entry	Compounds	IC_{50} (µM)
1	**1**	44.8 ± 8.0
2	**2**	23.5 ± 3.8
3	**3a**	74.0 ± 12.7
4	**3b**	29.3 ± 7.9
5	**3c**	20.8 ± 4.7

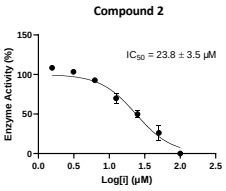
Table S2. IC_{50} values of the newly synthesized compounds. *All IC_{50} experiments were performed in triplicates (n=3) and the standard error is reported.*

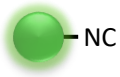
Entry	Compounds	IC_{50} (µM)		Entry	Compounds	IC_{50} (µM)
1	**4a**	78.7 ± 20.0		**14**	**5f**	43.3 ± 7.5
2	**4b**	NM*		**15**	**6a**	52.1 ± 6.9
3	**4c**	>100		**16**	**6b**	54.8 ± 7.9
4	**4d**	NM		**17**	**6c**	>100
5	**4e**	>100		**18**	**6d**	20.6 ± 2.7
6	**4f**	>100		**19**	**6e**	NM
7	**4g**	>100		**20**	**6f**	53.4 ± 15.3
8	**4h**	>100		**21**	**6g**	>100
9	**5a**	NM		**22**	**7a**	27.3 ± 3.9
10	**5b**	NM		**23**	**7b**	NM
11	**5c**	NM		**24**	**7c**	NM
12	**5d**	43.7 ± 3.2		**25**	**7d**	>100
13	**5e**	5.7 ± 0.6				

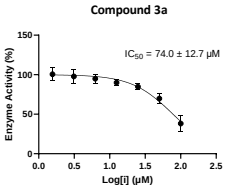
**NM: not measured due to poor solubility in assay buffer.*

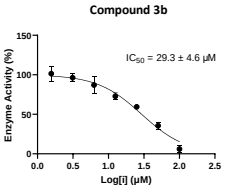
IC_{50} Graphs

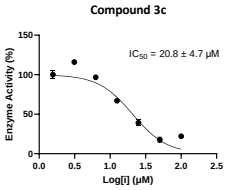

Compound 1
IC50 = 44.8 ± 8.0 μM
Enzyme Activity (%)
Log[i] (μM)

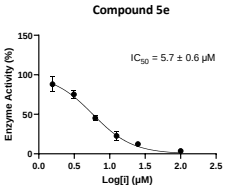

Compound 2
IC50 = 23.8 ± 3.5 μM
Enzyme Activity (%)
Log[i] (μM)

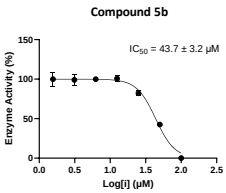

NC

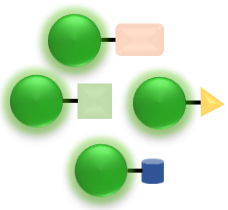

Compound 3a
IC50 = 74.0 ± 12.7 μM
Enzyme Activity (%)
Log[i] (μM)

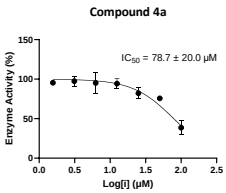

Compound 3b
IC50 = 29.3 ± 4.6 μM
Enzyme Activity (%)
Log[i] (μM)

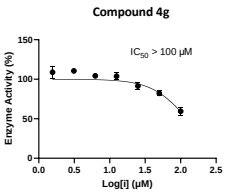

Compound 3c
IC50 = 20.8 ± 4.7 μM
Enzyme Activity (%)
Log[i] (μM)

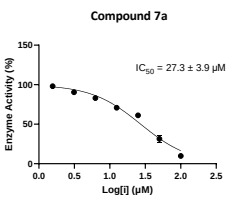

Compound 5e
IC50 = 5.7 ± 0.6 μM
Enzyme Activity (%)
Log[i] (μM)

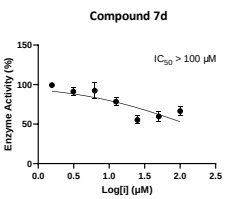

Compound 5b
IC50 = 43.7 ± 3.2 μM
Enzyme Activity (%)
Log[i] (μM)

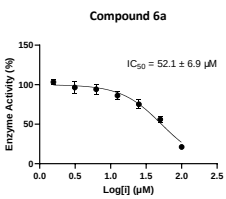


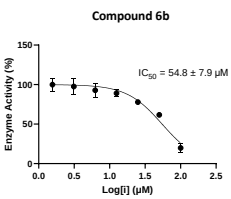

Compound 4a
IC50 = 78.7 ± 20.0 μM
Enzyme Activity (%)
Log[i] (μM)

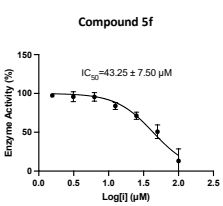

Compound 4g
IC50 > 100 μM
Enzyme Activity (%)
Log[i] (μM)

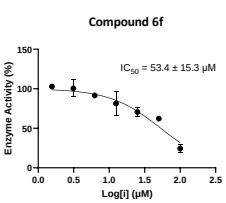

Compound 7a
IC50 = 27.3 ± 3.9 μM
Enzyme Activity (%)
Log[i] (μM)

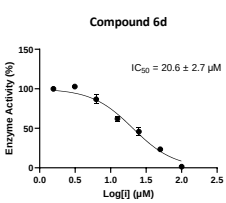

Compound 7d
IC50 > 100 μM
Enzyme Activity (%)
Log[i] (μM)


Compound 6a
IC50 = 52.1 ± 6.9 μM
Enzyme Activity (%)
Log[i] (μM)


Compound 6b
IC50 = 54.8 ± 7.9 μM
Enzyme Activity (%)
Log[i] (μM)


Compound 5f
IC50=43.25 ± 7.50 μM
Enzyme Activity (%)
Log[i] (μM)


Compound 6f
IC50 = 53.4 ± 15.3 μM
Enzyme Activity (%)
Log[i] (μM)


Compound 6d
IC50 = 20.6 ± 2.7 μM
Enzyme Activity (%)
Log[i] (μM)

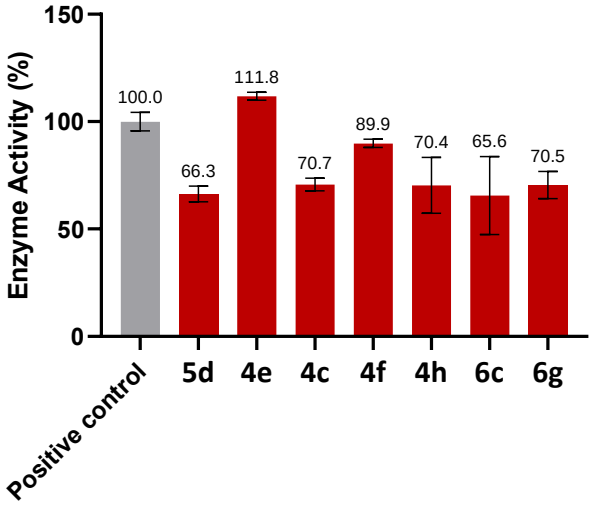


Figure S3. Inactive compounds screened at 50 µM. Cut-off is set at 50% of the enzyme activity. *Inhibitory screening experiments were performed in triplicates (n=3) and the standard error is reported.*

Enzyme Kinetics

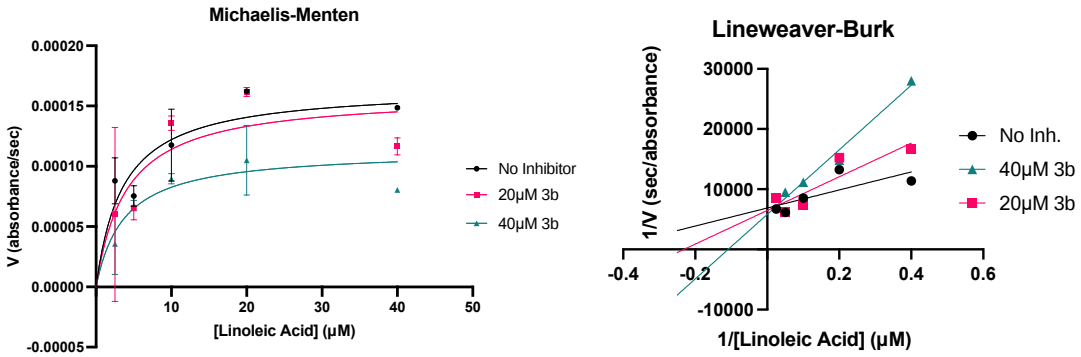


Figure S4. Steady-State kinetic characterization of 15-LOX-1 in the presence of different concentrations of compound **3b**. (A) Michaelis-Menten representation; (B) Lineweaver-Burk representation. *Enzyme kinetic experiments were performed in triplicates (n=3), and the standard error is reported.*

Table S3. Enzyme kinetic parameters for inhibition of 15-LOX-1 by compound **3b**.

3b (µM)	K_m^{app} (µM)	V_{max}^{app} (absorbance/sec)
0	3.5	$1.6 * 10^{-4}$
20	4.0	$1.5 * 10^{-4}$
40	5.9	$1.5 * 10^{-4}$

These are the best fit values calculated by Prism 8

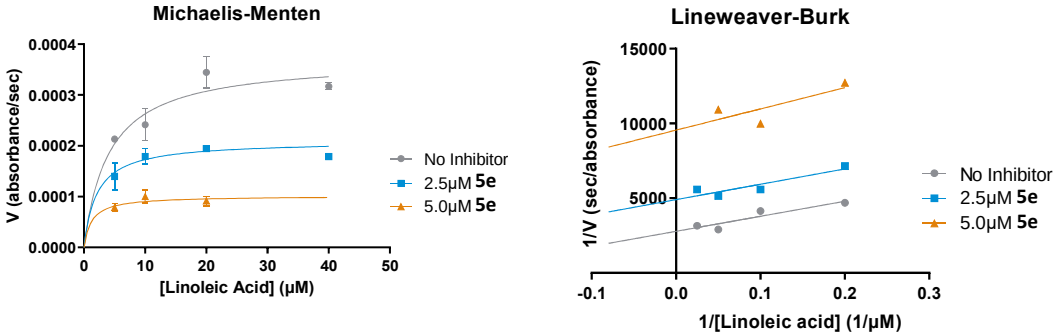


Figure S5. Steady-State kinetic characterization of 15-LOX-1 in the presence of different concentrations of compound **5e**. (A) Michaelis-Menten representation; (B) Lineweaver-Burk representation. *Enzyme kinetic experiments were performed in triplicates (n=3), and the standard error is reported.*

Table S4. Enzyme kinetic parameters for inhibition of 15-LOX-1 by compound **5e**.

5e (μM)	**K_m^{app} (μM)**	**V_{max}^{app} (absorbance/sec)**
0	4.0	$3.7 * 10^{-4}$
2.5	2.1	$2.1 * 10^{-4}$
5.0	1.2	$1.0 * 10^{-4}$

These are the best fit values calculated by Prism 8

8. Molecular docking studies

The following crystal structure of rabbit reticulocyte 15-lipoxygenase was used for docking: 1LOX. Missing residues were reconstructed using CHARMM-GUI,[2] which model and add missing regions. The resulting structure was then refined via energy minimization in UCSF ChimeraX[3] to optimize geometry and resolve steric clashes, ensuring a stable and accurate starting conformation for docking studies. Subsequently, the structure was using AutoDockTools 1.5.6.[4] The grid box was defined to include the whole protein structure. 5e input file was prepared, including energy-minimization, in Chemdraw. Flexible torsions were then added using AutoDockTools. Docking simulations were performed using AutoDock Vina 1.2.5.[5] The visualization of the results was done using UCSF ChimeraX and computational analysis and data visualization was performed on custom Jupyter Notebook[6] on the Google Colab framework,[7] utilizing widely used, published tools and libraries for data processing, statistical evaluations, and molecular dynamics analysis.

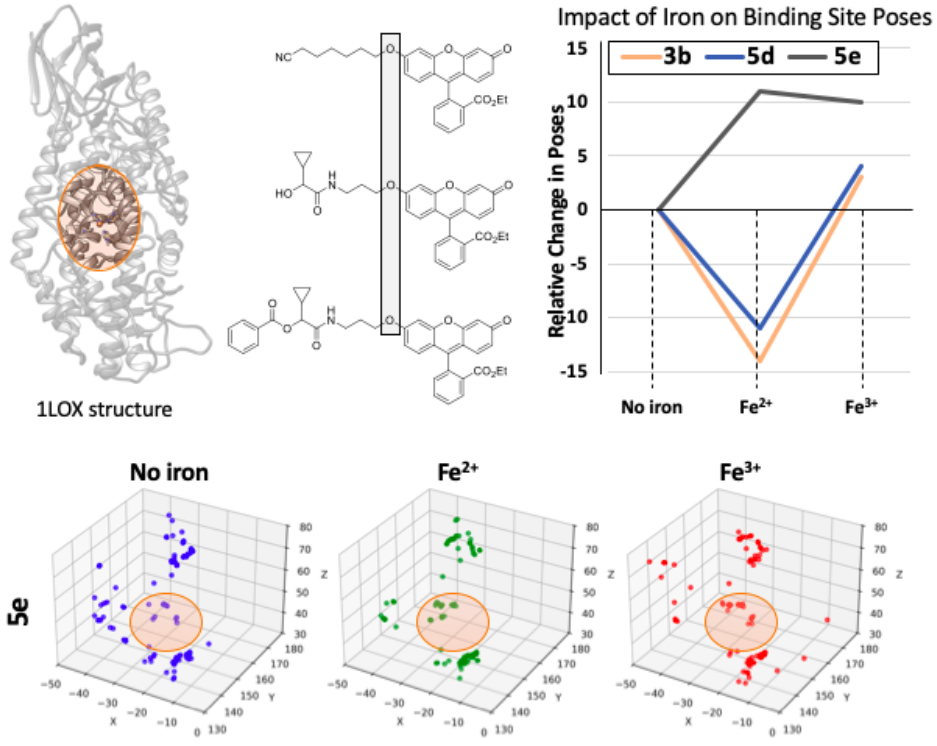


Figure S6. The impact of iron binding site poses. The number of 5e docking poses inside and outside the active site of 15 lipoxygenase-1 in presence or absence of (Fe^{2+} and Fe^{3+}).

9. Metal and pH screening

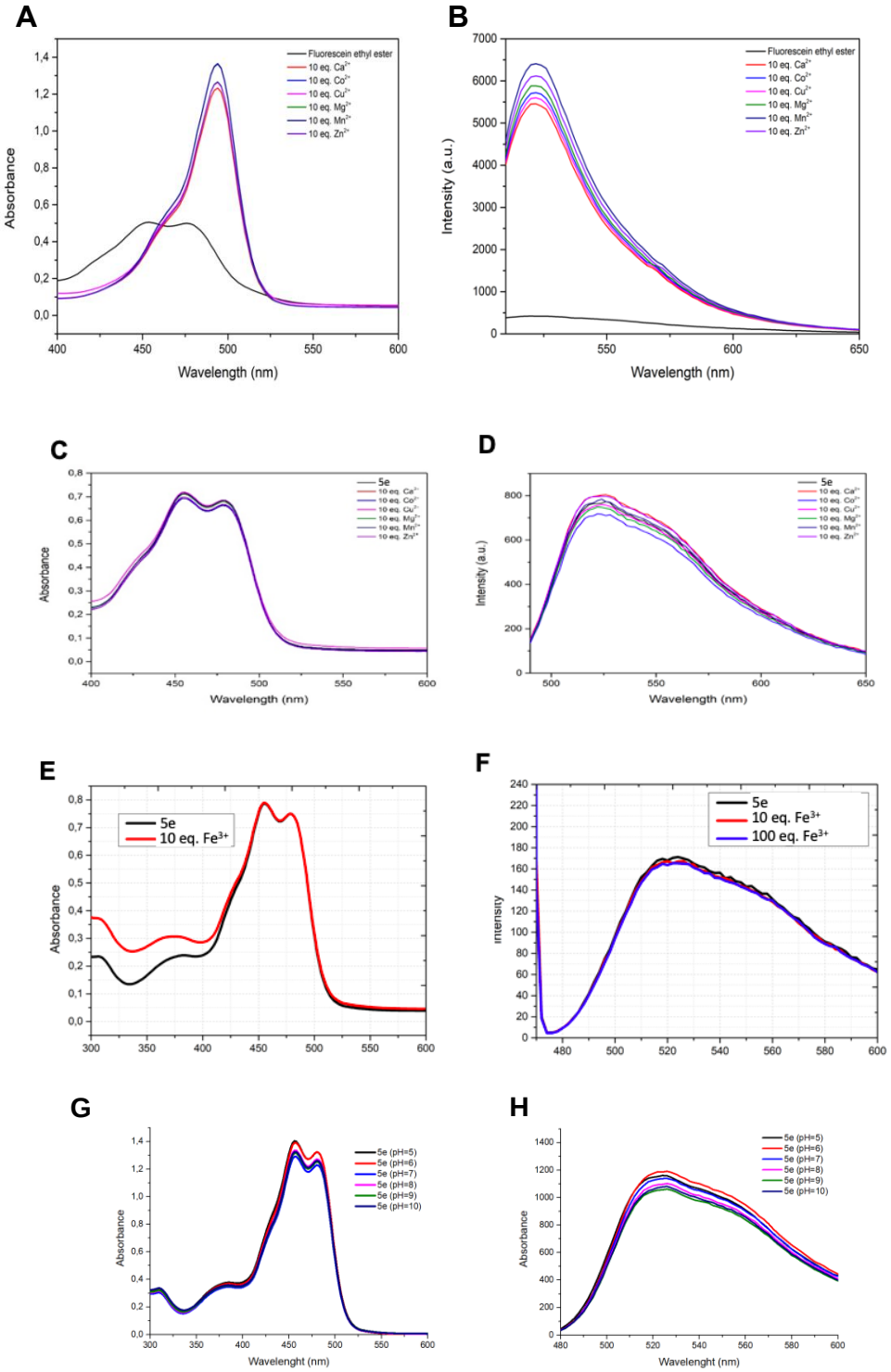


Figure S7. (A) Fluorescein ethyl ester absorbance spectrum in the presence of 10 equivalents of various bivalent metals; (B) Fluorescein ethyl ester fluorescence spectrum in the presence of 10 equivalents of various bivalent metal (excitation wavelength at 493 nm). (C) **5e** absorbance spectrum in the presence of 10 equivalents of various bivalent metals; (D) **5e** fluorescence spectrum in the presence of 10 equivalents of various bivalent metal (excitation wavelength at 456 nm); (E) **5e** absorbance spectrum in the presence of 10 equivalents of Fe^{3+}; (F) **5e** fluorescence spectrum in the presence of 10 and 100 equivalents of Fe^{3+} (excitation wavelength at 456 nm). (G) **5e** absorbance spectrum in five different pH buffers (50mM HEPES, 50mM NaCl, pH=5-10); (H) **5e** fluorescence spectrum in five different pH buffers (50mM HEPES, 50mM NaCl, pH=5-10, excitation wavelength at 456 nm).

10. General methods for cell and imaging studies

Cell culture

Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. RAW 264.7 (RRID:CVCL_0493, ATTC) 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 **5e**, at the indicated concentrations, dissolved in complete medium without phenol-red (*in situ* labelling). Confocal images of equatorial regions of the cells were collected over a period of 20 minutes. Wherever indicated, cells were washed with PBS (thrice) before addition of fresh complete medium without phenol-red and imaging on a confocal microscope.

Imaging in fixed cells

RAW 264.7 cells were seeded on 6-well plates containing uncoated glass coverslips with 1.0 x 10^5 cells per sample. Cells were grown for one day. The medium was replaced with medium containing 5 µM **5e** and incubated for 1 hour in the incubator. Cells were then washed with PBS (once) and subsequently fixed with ice-cold 4% paraformaldehyde for 15 minutes on ice and 15 minutes at RT. Cells were washed with PBS (thrice), mounted on microscope slides using mounting medium with DAPI (Vector laboratories) and imaged on a confocal microscope.

Imaging-based competition and irreversibility assays in live cells

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.

For the competition assay, the next day the medium was replaced with medium containing ThioLox at the indicated concentration or DMSO and incubated for 1 hour in the incubator. Cells were then washed with PBS and subsequently treated either with 5e (1 or 5 µM) or DMSO dissolved in complete medium without phenol-red. After 16 minutes, live cells were imaged on a confocal microscope.

For the irreversibility assay, the next day the medium was replaced with medium containing either DMSO or ThioLox (50 µM) and incubated for 1 hour in the incubator. The medium was the replaced with fresh medium containing 5e (5 µM) with either DMSO or ThioLox (50 µM) and incubated for 1 hour in the incubator. Subsequently, the medium was replaced again with medium either DMSO or ThioLox (50 µM) and incubated for 1 hour in the incubator. Finally, cells we replaced the medium with complete medium without phenol-red and live cells were imaged on a confocal microscope.

For both assays, experiments were performed in triplicates.

Super-resolution imaging

Laser scanning confocal microscopy was carried out using a Zeiss LSM 900 with the Airyscan 2 Super-Resolution module with the pinhole 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 analyzed in ImageJ/Fiji.[8] Specific analyses are described below.

Imaging analysis

Imaging analysis of all data was done using ImageJ/Fiji. Intensity distribution histograms were generated by extracting fluorescence intensity values of pixels contributing to cell labeling, creating a frequency distribution for each concentration condition. Images were manually thresholded to extract values only from cells. Intensity profile values were extracted using the built-in Plot Profile plugin along a defined line across the cell. The mean intensity and corresponding cell area from thresholded images were extracted using the Measure plugin, and these values were used in downstream quantification, such as in box-plot generation for competition experiments

Statistical analysis

Pre-processing of image data was performed using ImageJ/Fiji. Images were pre-processed either manually (thresholding for competition experiments) or using a custom macro involving contrast enhancement, local contrast optimization, filtering, and segmentation using watershed (for irreversibility assay). Regions of interest (ROIs) corresponding to cells were automatically generated, and intensity measurements were extracted for each ROI. The total area and raw integrated density values were calculated for each image and used for downstream quantification.

All data were visualized using GraphPad Prism 5.0 or Microsoft Excel. Boxplots were generated in GraphPad Prism, where the box represents the interquartile range (25th–75th percentile), the line inside the box represents the median, and the whiskers indicate minimum and maximum values. Diverging bar plots were created in Excel using normalized intensity values.

No data transformation or outlier exclusion was applied unless stated otherwise. Sample sizes (n) are indicated in the respective figure legends. Experiments for which statistical analysis was performed were conducted with three independent replicates (n = 3).

Statistical comparisons in GraphPad Prism were conducted using one-way ANOVA followed by Dunnett's multiple comparison test when comparing multiple groups to a control. For pairwise comparisons, statistical analysis was performed using Welch's t-test (two-sided, assuming unequal variances) implemented in Python (SciPy ttest_ind function). The default significance threshold (alpha) was 0.05. P values were reported

as exact values or summarized using the standard asterisk notation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). No multiple testing correction beyond Dunnett's or Welch's adjustment was applied.

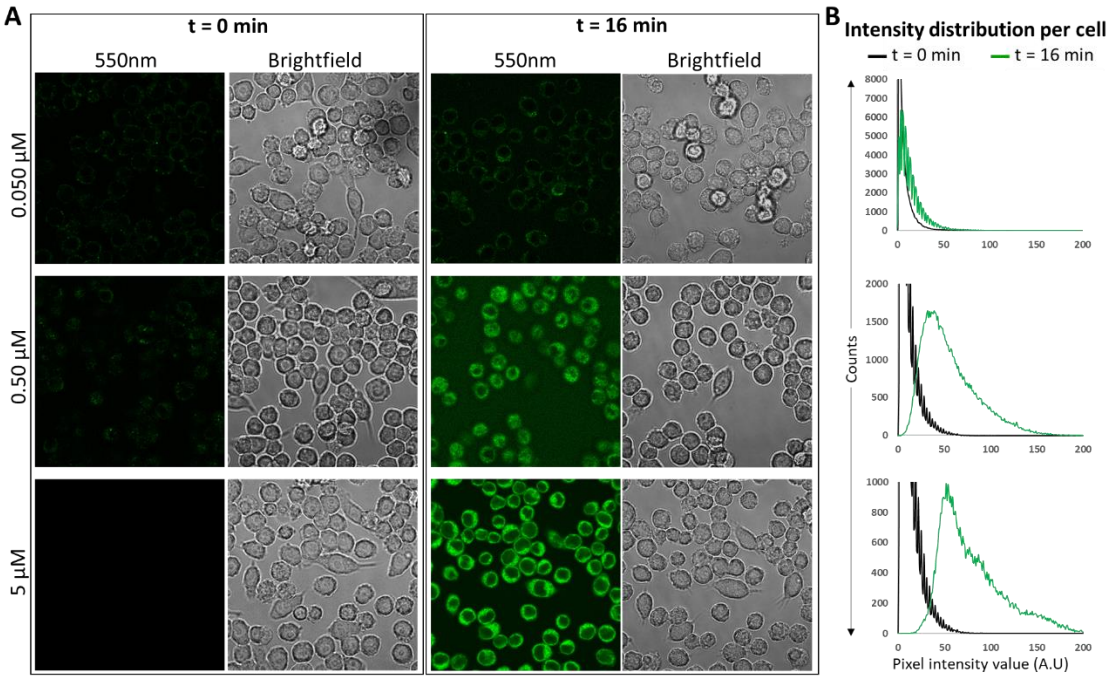


Figure S8. 5e is a highly cell permeable molecule. (A) Representative images of the imaging-based assay used to evaluate cell permeability of **5e** in live cells. Images were recorded for three **5e** concentrations, as indicated, in different time points. Left panel show images at time 0 minutes (prior **5e** addition) and right images show labelling of cell when it had reached saturation. (B) Histograms of the corresponding experiments in (A) showing how the intensity distribution differs from background (t = 0 mins) as well as between the different 5e concentrations. A single replicate was performed (n = 1).

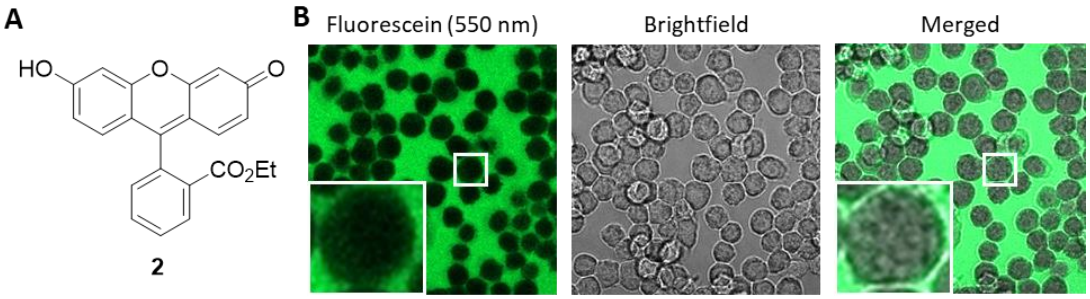


Figure S9. The ester fluorescein **2** is cell impermeable. (A) Structure of compound **2** used as a control in the imaging-based cell permeability assay; (B) Images of RAW 264.7 live cells treated with free fluorescein. The compound was unable to cross plasma membrane in the time course of our assay. A single replicate was performed (n = 1).

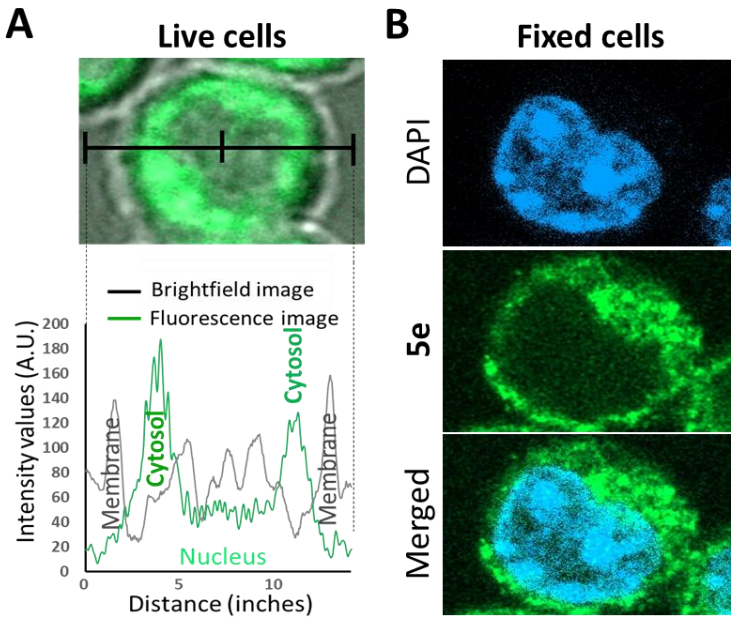


Figure S10. 5e localizes in both the cytoplasm and the nucleus of RAW 264.7 cells. (A) Zoomed-in image of live cells treated with 5 µM of **5e** after 16 minutes (merged of fluorescence and brightfield image). Intensity profile across the cell (at the position indicated by the black line in the image) in both the fluorescence and brightfield image (plot below) show that while **5e** localized predominantly in the cytosol, some nuclear accumulation is also observed (as opposed to the cell membrane); (B) Nuclear staining by DAPI in fixed cells prelabelled with **5e**. Live cells labelled with **5e** (5 µM) were fixed using paraformaldehyde and mounted on a coverslip using a DAPI-containing mounting medium. Images were recorded using a confocal microscope. A single replicate was performed (n = 1).

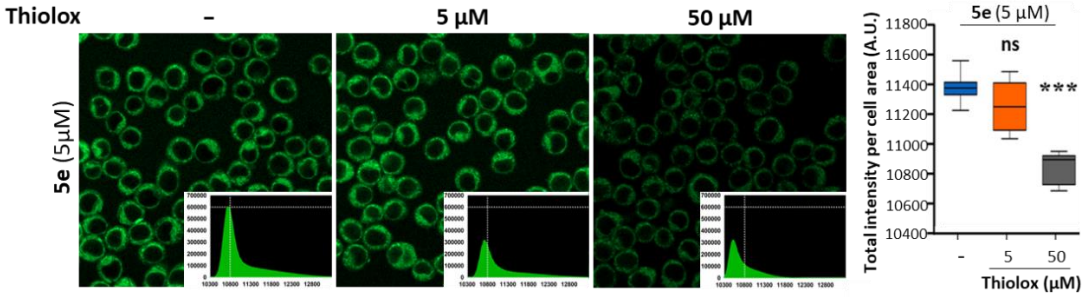


Figure S11. 5e labelling in live cells is reduced by Thiolox pre-treatment. Representative images of **5e**-labelled live cells pretreated with either DMSO (left image) or equimolar (middle) and 10-times (with respect to **5e**, right image) Thiolox concentration. Insets (histograms) show how the intensity distribution of **5e** changes when cells had been pre-treated with Thiolox. The ranges on both x and y axis of the histograms were kept the same in all three conditions; (B) Box-plot of the quantified intensity per cell area from experiments using 5 µM **5e** with or without competition by Thiolox. The box represents the interquartile range (25th to 75th percentile), the line inside the box represents the median, and the whiskers represent the minimum and maximum values. Data represent three independent experiments (n = 3). Statistical significance was assessed using a one-way ANOVA with Dunnett's multiple comparison test. Significance is indicated by stars (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

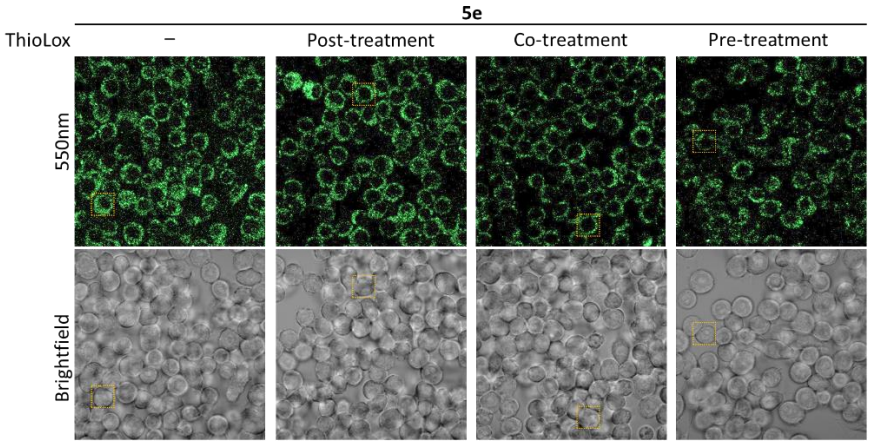


Figure S12. Full cell images of cells shown in Figure 5G. Dotted yellow boxes indicate cells used in Figure 5G. Top row shows fluorescence images and bottom row brightfield images.

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